TREATISE ON FISHERIES HARVEST AND POST-HARVEST TECHNOLOGIES

(17 January - 06 February, 2023)

ICAR-CENTRAL INSTITUTE OF FISHERIES TECHNOLOGY
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Chapter 1

Fishing vessels of India

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Introduction

The fishing vessels can be classified into (i) artisanal fishing vessels, (ii) traditional fishing vessels, (iii) motorized vessels, (iv) mechanized vessels, (v) Fishing related vessels.

Artisanal fishing vessels: Small-scale, low-technology, low-capital, low- energy, relatively small fishing vessels, making short fishing trips, close to shore by individual fishers of coastal or island ethnic fishers and mainly for local consumption. In practice, definition varies between countries- India wooden dugout canoes, coracles and catamaran are artisanal crafts.

Fig.1 Artisanal coracle-reservoir/ river fishing

Fig.2 & 3 Artisanal fishing vessel-Nicobar & Wooden Cattamaran

Traditional fishing vessels: These are vessels using traditional methods for fishing. There is no deck equipment such as winch. No insulated/cold storage is available onboard. No wheel house and accommodation is provided in these vessels. In general simple traditional fishing carried out from these vessels.

Fig.4 Traditional fishing boat - Andamans

Motorized vessels: Motor is used for the propulsion of these vessels. Fig.5 shows motorized fishing boat used in marine fishing. 2 hp to 65 hp inboard and outboard engines are used here.

Fig.5 Outboard motor fitted vessel for marine fishing.

Mechanized fishing: Uses engine power for cruising and fishing activities. These vessels use mechanical/hydraulic/electric power for fishing gear handling. Has insulated/cold storage/freezer storage onboard. Accommodation/galley/toilet facilities are available for

multiday fishing. Also, communication, lifesaving, fire control, light and sound signals, etc. are required in these boats.

TABLE 12. **FISHING CRAFTS IN THE FISHERY** (excluding Lakshadweep and Andaman & Nicobar Islands)

State	Mechanized								Motorized			Non-motorized	Total
	Trawlers	Gillnetters	Dolnetters/ Bagnetters	Liners	Ring seiners	Purse-seiners	Others	Total Mechanized	Inboard	Outboard	Total Motorized		
West Bengal	2,004	1,764	191	31	0	0	24	4,014	6,564	0	6,564	476	11,054
Odisha	1,390	358	0	0	0	0	0	1,748	2,443	3,235	5,678	1,256	8,682
Andhra Pradesh	1,176	0	0	0	0	0	0	1,176	3,146	8,932	12,078	6,965	20,219
Tamil Nadu	5,278	441	0	16	219	0	7	5,961	8,945	22,334	31,279	6,115	43,355
Puducherry	223	0	0	0	78	0	0	301	387	975	1,362	656	2,319
Kerala	2,654	417	0	2	646	81	0	3,800	0	13,868	13,868	4,016	21,684
Karnataka	3,071	40	0	0	0	669	0	3,780	304	5,575	5,879	2,225	11,884
Goa	600	0	0	0	0	209	49	858	5	937	942	182	1,982
Maharashtra	3,408	584	1,637	0	0	230	8	5,867	5,979	809	6,788	2,865	15,520
Gujarat	9,905	2,602	1,554	0	0	0	0	14,061	3,541	9,284	12,825	756	27,642
Daman & Diu	1,063	342	14	0	0	0	0	1,419	95	301	396	177	1,992
Total	**30,772**	**6,548**	**3,396**	**49**	**943**	**1,189**	**88**	**42,985**	**31,409**	**66,250**	**97,659**	**25,689**	**1,66,333**

(CMFRI-2016)

Types of mechanized fishing vessels: Following types of commercial fishing are used in India.

- Trawler
- Stern trawler
- Seiner
- Purse seiner
- Ring Seiner
- Gill netters
- Dol Netters
- Liners
- Hand liner
- Long liner
- Pole and liner
- Trollers
- Multipurpose fishing vessels

<u>Trawler</u>

Uses trawl gear for catching fish from the sea. This vessel has a main engine fitted with reversible reduction gear box for propulsion. The trawl winch powered by the main engine handles the trawl gear. The gallows fitted in aft is used for shooting and hauling the gear as well as storing the otter boards after the fishing.

Fig.6 Commercial Trawler, otter boards seen hanging on the gallows and winch in the last Fig.

Seiner

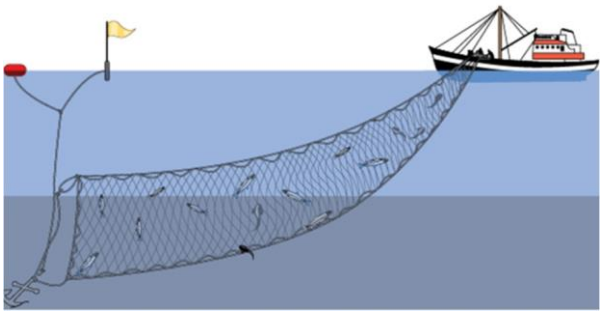
These vessels use surrounding seine nets. They comprise a large group ranging from open boats and canoes up to large ocean going vessels. They are used to catch pelagic species. Relatively high maneuverability is required for operation of the surrounding and seine nets.

To assist in fish school spotting observation crows nests are fitted on forward or on the mast. The equipment of seiners consists usually of a power block and a net drum for hauling and stowing the net aboard and one or more winches for setting and hauling operations. In small boat and canoe type seine netting, all operations are generally performed by hand. For removing of fish collected in the purse, a brailer is provided. OBM and IBM type Ring Seiners shown in Fig.7 below.

Fig.7- Small boats are OBM fitted and large one has IBM.

Gill Netters

Boats and canoes use gill net in inland and marine waters. The decked small gill netters fish in coastal waters and medium sized vessels operate gillnets in offshore. Deep sea gillnetters have their wheelhouse in the aft. On small vessels setting and hauling operations are performed by hand. Larger vessels are often equipped with hydraulic net haulers as seen below.


(a)

(b)

Fig.8 (a) Gill netting (b) Hydraulic winch used for deep sea gill netting

Liners

These vessels use lines and hooks with or without bait or lure. Depending on the method of fishing with lines, area of operation and species to be caught, liners comprise vessels of all size classes. Containers or tanks for storing the bait are kept on main deck. Sufficient deck area for attaching the bait to the hooks and a convenient place for preparing the lines for setting and hauling are typical features for line fishing vessels. Fig.9 (a) shows a long liner and (b) shows the main line hauler and (c) the line setter.



(a) (b) (c)

Pole and line vessels

These vessels are used primarily for catching of tuna and skipjack, the fishermen stand on the railing or on special platforms and fish with poles, to which a line with hook is attached. Tanks with live bait and a water spray system for fish attraction are typical features of these vessels. Because live bait is used to attract fish, the fishing method is also known as live-bait fishing. Fig.10 shows a pole and line vessel used in Lakshadweep.

Fig.10

Dol netter

Dol nets are fixed bag nets which are tied to the poles or ropes anchored at the sea bottom and kept afloat by floats. In the Saurashtra coast heaps of stones are used as anchors. Below figure shows a Dolnetter.

Fig.11

Trollers

Equipped for catching pelagic fish swimming close to the surface these vessels tow a number of lines fitted with lures. The lines are attached to trolling booms which are raised and lowered by topping lifts and fore and aft stays. Manual, hydraulic or electrically powered reels (gurdies) are frequently used to haul in the lines. According to area of operation, vessels may be laid out with wheelhouse and mast either forward or in the after part of the vessel.

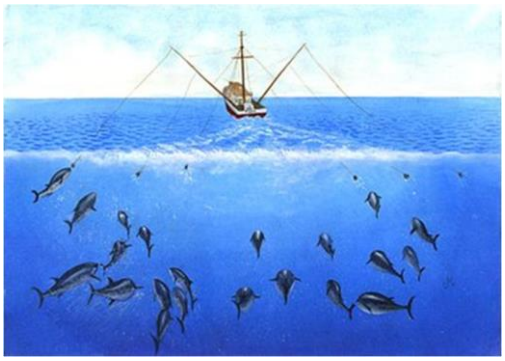


Fig.12 Troller

Multipurpose vessels

These are vessels which are equipped for alternative use of two or more different fishing gear without major modifications to the vessels' outfit and equipment. The simplest examples of this concept are traditional open craft which operate one of the surrounding net types of gear, e.g., purse seine, during the seasonal appearance of pelagic species and handlines for demersal fish

during the remainder of the year - no special features or equipment are used and the appearance of the craft is unchanged. Other examples of combinations in common use are gillnetter/longliner, trawler/gillnetter, trawler/purse seiner etc., with a variety of other gear being used in cases where gear and equipment investment is not high and layout changes minimal, e.g., a gillnetter may use hand lining, trolling and trap fishing when seasonal variations are appropriate.

The deck equipment used in fishing vessels

- Long lining- Line hauler & setter
- Trawling- Trawl winch, gallows, mast & derrick
- Gill netting – Net hauler
- Purse seining – Power block, line spooler, brailer
- Pole and lone vessel – Pole and line, water sprayer

Fishing related vessels

Following are the vessels elated to fishing activities.

Fishery Research Vessels, Training vessels and Marine Ambulance

Fishery Research Vessel: Research vessels are mainly engaged experimental fishing using various gear experiments. The size of fishery research operation and on research programmes. The vessels are usually fitted for the operation of two or more fishing gear. Special winches for taking samples and apparatus for measurements of environmental characteristics are provided. The cabin comprises space for laboratories and accommodation for scientific staff. Store rooms for instruments and samples are also provided. Fig.13 is the picture of F.V.Sagar Harita research vessel of CIFT.

Fig.13

Novel features	L= 19.75m, Breadth=6.5 m Depth =2.8m, V = 10 knots
Bulbous bow	Reduces resistance and improves fuel efficiency
Larger fuel tank (14000L capacity)	For greater endurance at sea
RSW tank (4-5m^3)	Quick and better quality fish preservation
Solar panels (20m^2)	Navigational lighting, wheel house, mess lighting,fan
Hydraulic longline winch	Reduces operation constrains by one third
Split trawl winch	To save deck space
Multi stage Gillnet drum	Reduces the human effort
Stainless roller at stern	For easy hauling of net
Net drum	For neat storage of gear
Freezer-cold store-RSW tank in a row	For easy handling and quality assurance of catch
FRP wheel house construction	For increased stability and carrying capacity with vessels of similar size also reduces the resistance
Efficient propulsion system	Increased thrust, maneuverability and energy efficiency during fishing operations
Bilge keel	To reduce rolling and improved sea keeping characteristics

Fishery training vessels

These vessels are used for training future fishermen and students in navigation, seamanship, fishing operations and fish handling. They are mostly typical fishing vessels with additional accommodation for trainees. Fig. 14 M.V.Prashikshini training vessel of CIFNET


Fig 14

Marine Ambulance

For sea rescue marine ambulances are used. These boats require high speed and essential mecical facilities. Fig.15 shows a marine ambulance used in Kerala for the rescue of fishermen.

Fig.15

Chapter 2

Fishing gear materials: properties & identification

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Introduction

Netting materials for fabrication of fishing gear are either of textile or non-textile origin. The raw material for fish netting consists of fibres which can be distinguished into two groups: natural fibres and man-made fibres. Different kinds of fibres originating from plant and animal body parts have been used for production of textiles and other products are termed as natural fibres. Traditional fishing gears used earlier, till 1950s were mainly with natural fibres such as cotton, manila, sisal, jute and coir. Natural polymers and synthetic polymers constitute man-made fibres. Natural polymers are manufactured by the alteration of natural polymers like cellulose and protein while synthetic polymers are obtained by synthesis or chemical process. Man-made fibres derived from cellulose eg: rayon, are susceptible to microbial deterioration while synthetic fibres are very resistant to biodeterioration. In the late 1950s, with the introduction of man-made synthetic fibres, natural fibres used for the fishing gears have been substituted by these synthetic materials. This transition was mainly due to the highly positive properties of these fibres such as highly non-biodegradable nature, high breaking strength, better uniformity in characteristics, high abrasion resistance, low maintenance cost and long service life.

Synthetic fibres

Synthetic fibres are produced entirely by chemical process or synthesis from simple basic substances such as phenol, benzene, acetylene etc. The chemical process involves the production of macromolecular compounds by polycondensation or polymerization of simple molecules of a monomer. The raw materials are petroleum, coal, coke and hydrocarbon. Depending on the type of polymer, synthetic fibres are classified into different groups and are known by different names in different countries. Altogether seven groups of polymers are developed; most important polymer/synthetic fibres used in fishing gears are polyamide (PA), polyester (PES), polyethylene (PE) and polypropylene (PP). Other synthetic fibres, which are less widely used and generally restricted to Japanese fisheries, are polyvinyl alcohol (PVA), polyvinyl chloride (PVC) and polyvinylidene chloride (PVD). Aramid fibres, ultra high molecular weight polyethylene (UHMWPE) and liquid crystal polymer are later additions to this group.

***Polyamide (PA)*:** Polyamide, a synthetic polymer, popularly known as nylon, invented in 1935 refers to a family of polymers called linear polyamides. Nylon consists of repeating units of amide with peptide linkages between them. Depending on the raw material and method of making two types of nylon viz., PA 6 and PA 66 are available for fibre applications. PA 66, widely used for fibres is made from adipic acid and hexamethylene diamine while PA 6 is built with caprolactam. With regard to the fisheries, there is no difference between PA 66 and PA 6, while in India, for fishing purposes PA 6 is used. The softness, lightness, elastic recovery, stretchability and high abrasion and temperature resistance are superior properties inherent to nylon. However, high moisture absorption along with dimensional instability and requirement of UV stabilization are its disadvantages. On wetting, nylon loses up to 30% of tensile strength and 50% of tensile modulus.

Polyolefins: Polypropylene (PP) and Polyethylene (PE) are often collectively called "polyolefines". Polyolefin fibres are long-chain polymers composed (at least 85% by weight) of ethylene, propylene or other olefin units. Polyolefin fibres are made by melt spinning. They do not absorb moisture and have a high resistance to UV degradation.

***Polyethylene* (PE):** PE fibre is defined as: “fibres composed of linear macromolecules made up of saturated aliphatic hydrocarbons”. PE fibres, used for fishing gear, are produced by a method developed by Ziegler, in the early 1950s.The monomer ethylene, the basic substance of polyethylene, is normally obtained by cracking petroleum. Linear polyethylene or high-density polyethylene has high crystallinity, melting temperature, hardness and tensile strength. In India, PE is used for manufacture of netting and ropes.

***Polypropylene* (PP):** PP fibre is defined as: “fibres composed of linear macromolecules made up of saturated aliphatic carbon units in which one carbon atom in two carries a methyl side group”. This is an additive polymer of propylene. PP was commercialized in 1956 by polymerizing propylene using catalysis. Though PP netting and ropes are available, in India, PP is mainly used for ropes.

***Polyester* (PES):** The principal PES fibres are made from polymerization of terephthalic acid and ethylene alcohol. It was first synthesized by Whinfield and Dickson of Great Britain in 1940-41 and named the fibre "Terylene”.

Recent advances in synthetic fibres

Introduction of synthetic materials with high tensile strength properties has made it possible to bring out changes in the design and size of fishing nets. As the fishing industry became highly

competitive, the search and research for new generation materials which give better strength for less thickness resulted in invention of new materials. Aramid fibres, Kevlar, UHMWPE, biodegradable plastic, etc. are recent introductions to the fishing gear material sector. These materials have advantages, especially less drag which results in fuel efficiency. The performance of UHMWPE webbing and rope in the Indian context is being studied by ICAR-CIFT. Among the new fibre types, only Sapphire and UHMWPE are used on a commercial basis for fishing gear viz., trawls and purse seines in Australia and Alaskan waters. Sapphire is also used on a limited scale in large mesh gillnets targeting large pelagic in Maharashtra region of India.

Ultrahigh molecular weight polyethylene (UHMWPE): UHMWPE is a type of polyolefin synthesized from monomer of ethylene processed by different methods such as compression molding, ram extrusion, gel spinning, and sintering. Polyethylene with an ultrahigh molecular weight (UHMWPE) is used as the starting material. In normal polyethylene, the molecules are not orientated and are easily torn apart. The fibres made by gel spinning have a high degree of molecular orientation with very high tensile strength. The fibre is made up of extremely long chains of polyethylene, which attains a parallel orientation > 95% and a level of crystallinity of up to 85%. The extremely long chains have molecular weight usually between 3.1 and 5.67 million while HDPE molecule has only 700 to 1,800 monomer units per molecule.

UHMWPE, also known as high modulus polyethylene (HMPE) or high-performance polyethylene (HPPE) is a thermoplastic. It has extremely low moisture absorption, very low coefficient of friction, is self-lubricating and is highly resistant to abrasion (10 times more resistant to abrasion than carbon steel). This is available as Dyneema and Spectra produced by two different companies. Commercial grades of dyneema fibres SK 60 and SK 75 are specially designed for ropes, cordage, fisheries and textile applications.

UHMWPE is 15 times stronger than steel and up to 40% stronger than Kevlar. UHMWPE netting is 3 times stronger than nylon with the same dimension, and increases the net's strength while the abrasion resistance increases the net's life. Netting can be used for trawl nets, purse seine nets and aquaculture nets. Nylon purse seines last for about 2-3 years while UHMWPE netting ensures 2-3 times more life for the net. The netting twines made with dyneema fibre can be reduced by upto a factor of 2 on thickness (diameter basis) and on weight basis by a factor of 4. This allows fishing vessels to increase their catch potentially by as much as 80% by trawling faster or using larger nets, or to reduce fuel consumption. Besides, less deck space is required

due to lower bulk volume of the net. Purse seines made of dyneema would facilitate 40% increase in sinking speed due to better filtering and reduced drag. Larger net for the same weight can be made. The net has better durability with negligible wear & tear.

Ropes made from UHMWPE have a higher breaking strength than that of steel wire ropes of the same thickness, but have only one-tenth the weight. Fishing uses for these high-strength polyethylene ropes include warp lines, bridles and headlines. By using UHMWPE ropes, the frequent oiling & greasing required for wire ropes can be avoided which would facilitate a clean and safe deck and free the crew from greasing the rope frequently. It also helps in a clean catch devoid of oil and grease contamination.

Sapphire: Sapphire PE netting manufactured from specialized polymers available in twisted and braided form is suitable for trawl nets and for cage culture. It has the highest knot breaking strength, knot stability and dimensional uniformity. Braided twine having compact construction restricts mud penetration and provides lesser drag. Sapphire is used on a limited scale for fabrication of large mesh gillnets targeting large pelagics in Maharashtra region of India. Sapphire ultracore is a knotless HDPE star netting with an outer layer of heavier sapphire ultracore which features strands of marine grade stainless steel as an integral part of the netting twine. The stiffness and cut resistance enable it to be used as a predator protection net cum cage bag net where the predation problem is very high.

Aramid fibres: Aramid fibres are fibres in which the base material is a long-chain synthetic polyamide in which at least 85% of the amide linkages are attached directly to two aromatic rings. Two types of aramid fibres are produced by the DuPont Company: Kevlar (para-aramid) and Nomex (meta-aramid), which differ primarily in the substitution positions on the aromatic ring. Generally, aramid fibres have medium to very high tensile strength, medium to low elongation-to-break, and moderate to very high modulus.

KEVLAR® polyphenylene terephthalamide (PPTA): A polymer containing aromatic and amide molecular groups is one of the most important man-made organic fibres ever developed. Because of its unique combination of properties, KEVLAR® is used in the fishing sector as netting, fishing rod and fishing line. Fibres of KEVLAR® consist of long molecular chains produced from poly (p-phenylene terephthalamide). The chains are highly oriented with strong interchain bonding, which result in a unique combination of properties. The strength to weight ratio of Kevlar is high; on a weight basis, it is five times as strong as steel and ten times as strong

as aluminum. It has high tensile strength at low weight, low elongation to break, high toughness (work-to-break), and excellent dimensional stability. In sea water, ropes with KEVLAR® are up to 95% lighter than steel ropes of comparable strength.

Liquid Crystal Polymer Fibre: Vectran®, a high-performance thermoplastic multifilament yarn spun from Vectra® liquid crystal polymer (LCP), is the only commercially available melt-spun LCP Fibre in the world. Vectran fibre is five times stronger than steel and 10 times stronger than aluminum. Vectran fibre is 4 times stronger than polyethylene fibre or nylon fibre. The unique properties that characterize Vectran fibre include: high strength and modulus; high abrasion resistance; minimal moisture absorption; and high impact resistance. Although Vectran is lacking UV resistance, this limitation can be overcome by using polyester as a protective covering. It is very suitable for trawl nets and ropes.

Fluorocarbon fibre: Fluorocarbon fibre is a new material that can be used in angling and high-speed jigging lines. It has very high knot strength, almost invisible in water, has high breaking strength and abrasion resistance.

Properties

Synthetic netting materials are generally resistant to biodeterioration. This is the major advantage of synthetics over natural fibres and it is the prime requisite for a fibre for consideration as a fishing gear material. Besides, synthetic fibres have high breaking strength, better uniformity in characteristics, long service life and low maintenance cost. However, unlike natural fibres, they are prone to degradation under sunlight at a much faster rate. For quality evaluation and selection of appropriate material for different gears, knowledge on various properties of netting yarn are required. The numerical values of these properties are determined through standard test procedures. As far as the fishing gear purpose is concerned, properties which are of importance are as follows.

Diameter

The diameter of netting material is an important factor influencing the fishing gear performance. Thickness and rigidity of the material influences the resistance of fishing gear to water flow and hence the power required or the speed obtained in towing gears are depended on it. Diameter of a material is dependent on the type of polymer, type of yarn, size of yarn, specification and construction. Diameter is usually determined as the distance between the two edges of the yarn/twine measured on a travelling microscope and expressed in mm.

Linear density

It is the mass per unit length of the material. The mass in g of 1000 m length of a material is expressed as R tex and mass of 9000 m of the material as R denier. For the same kind of material, lower Rtex means thinner material and generally costs less while buying on a mass basis.

Twist

The number of turns or twists imparted to a twine per unit length is important as it influences many properties especially the breaking strength, diameter, linear density, resistance to abrasion and general wear and tear of the twine. As the amount of twist increases the breaking strength also increases upto a critical degree of twist beyond which it would weaken the twine. The stability of a twine depends on the correct amount of twists per unit length. The twine has an inner/strand/primary twist and outer/secondary/twine twist. Balance between these two twists ie: primary twist for making strands from yarns and secondary twist to make twine from strands is important. The twist can be in two directions, viz., left hand (S twist) or right hand (Z twist). In S twist, the slope of the twisted product follows the direction of the central portion of the letter `S'. Similarly, in Z twist, it follows the central portion of `Z'. Generally, the yarns and strands are twisted in the opposite directions for stability. In a double twisted twine, the direction of twist can be SZS or ZSZ for yarn, strand and twine respectively.

Breaking load

The breaking load of a material denotes the ability of a material to withstand the strain. It depends on the type of polymer, type of yarn, degree of twist and thickness of the material. Knotting also causes reduction in the breaking strength. This is dependent on the type of polymer, type of yarn and knot, twine construction and also on the degree of stretching. A length of yarn is extended until it reaches the load at rupture by a suitable apparatus, The Universal Testing Machine, that records the applied force. Breaking load is expressed in Newton (N).

Elongation

Elongation is the increase in the length of a specimen during a tensile test and is expressed mostly in percentage of the nominal gauge length. It involves a reversible and an irreversible elongation. Irreversible or permanent elongation is the part of the total increase in length which remains after the removal of the stress. Elongation is also tested in UTM and sample preparation procedure is similar as above described in breaking load.

Abrasion Resistance: The resistance of netting materials to abrasion, i.e., abrasion with hard substances such as boat hull, sea bottom and net haulers, or abrasion between yarns/twines is important in determining the life of a net. The resistance to abrasion depends on the type of fibre, thickness and construction of the material. Polyamide has the maximum abrasion resistance, followed by PP, PES and PVC. For testing the abrasion resistance, the principle is to apply a certain number of frictions or abrasion cycles and measuring the remaining breaking load of abraded material expressed as the percentage of initial breaking load.

Weathering Resistance: Even though all fibres, irrespective of natural or synthetic are prone to degradation on exposure to weathering, the problem is severe with synthetic fibres. The main factor responsible for weathering is the sunlight, i.e., the ultra violet part of the sun's radiation. Different synthetic fibres show variation in their susceptibility to and rate of deterioration by sunlight depending on the type of polymer and fibre. The rate of deterioration is generally assessed by the loss in breaking strength. Weathering resistance is measured by exposing the material to natural sunlight. Weathering resistance can also be studied in controlled conditions in the laboratory by using Weather O meters with artificial light sources such as fluorescent arc, UV arc, Carbon arc, Mercury arc and Xenon arc. Xenon arc is a good substitute as it approximates solar radiation and gives very steady illumination. Weathering studies in Xenotest take only one-seventh to one tenth of the time than the samples exposed to natural conditions.

Tests for identification of synthetic fibres

Different groups of synthetic fibres can be identified by various methods.

Water test

Identification of synthetic fibres can be started with this test. In a short piece of netting yarn, tie a simple overhand knot and put the piece into a vessel filled with water. Air bubbles in the material must be squeezed out by hand underwater. Based on water test, netting materials can be classified into two groups; (1) synthetic fibres which float in water (PE & PP) (2) fibres which sink (all other synthetic fibres).

Burning test

In the burning test, the nature of burning and smoke in the flame as well as after leaving the flame are observed. The netting material can be brought near to the flame and after removal from the flame, observe the smell of smoke and the residue. Synthetic fibres shrink and melt in the flame, the melting substance drips from the flame mostly forming a bead or a hard irregular

residue. The changes in different synthetic fibres during burning test is given in table 1

Solubility test

Solubility test is a relatively simple chemical test. Fibres of the sample to be tested should be in a loose form. The netting yarn must be untwisted and the fibres can be cut into small pieces of 1cm length. Coarse material like split fibres and especially monofilaments should be cut to very small pieces. Take 10-15ml of the solvent into the test tube and put the sample pieces into it. The results of the reactions are shown in table 2.

Table 1. Burning characteristics of synthetic fibres

Material	PA	PES	PE	PP
In flame	Melts, burns with light flame, white smoke, melting drops fall down.	Melts, burns with light flame, sooty black smoke, melting drops fall down.	Shrinks, curls, melts and burns with light flame, drops of melting fall down.	Shrinks, melts and burns with light flame melting drops fall down.
After leaving the flame	Stops burning, melting drops can be stretched into fine thread.	Stops burning, melting bead may be stretch into fine thread.	Continues to burn rapidly, hot melting substance cannot be stretched.	Continues to burn slowly, hot melting substance can be stretched.

(Source: Klust, 1982)

Table 2. Identification of synthetic fibres by solubility test

Reagent	Type of fibre			
	PA 6	PES	PE	PP
Hydrochloric acid/HCL (37%) 30 minutes at room temperature	+	0	0	0
Sulphuric acid/H_2SO_4 (97-98%) 30 minutes at room temperature	+	+	0	0
(1) Dimethylformamide/HCON $(CH_3)_2$ 5 minutes boiling	+	+	0 (2)	0 (2)
Formic acid/HCOOH (96-100%) 30 minutes at room temperature	+	0	0	0
Glacial acetic acid/CH_3-COOH 5 minutes boiling	+	0	0	0
Xylene/C_6H_4 $(CH_3)_2$ 5 minutes boiling (inflammable)	0	0	+	+
Pyridine 30 minutes at room temperature	0	0	0	0

(Source: Klust, 1982)

+ = soluble, 0 = not soluble, (1) = Dimethylformamide is decomposed by exposure to light even when stored in a brown bottle, needs to be stored away from light preferably in a cool place, (2) Destroyed but not soluble

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Chapter 3

Marine mammal interactions in fishing systems and their mitigation

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Introduction

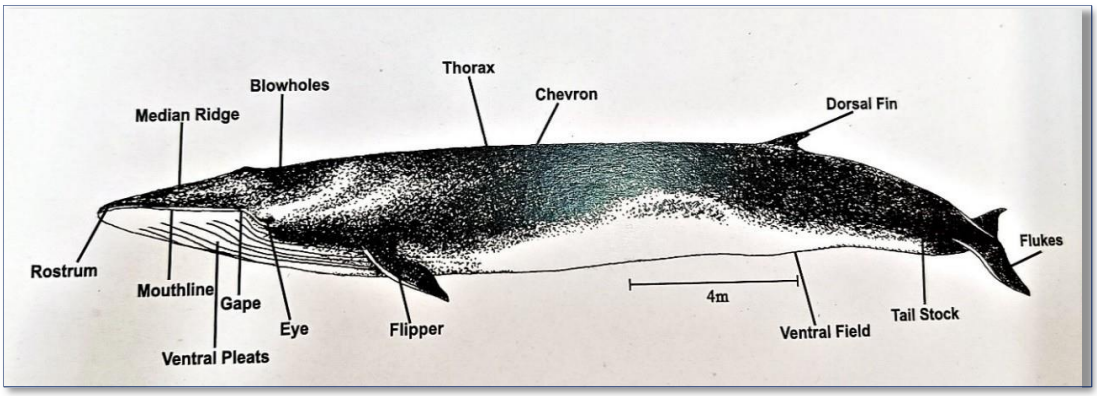
The ocean covers 70% of the earth's surface and is the largest living space that accommodates verities of flora and fauna. Marine biodiversity is very complex and special. It ranges from single-celled or microscopic communities to the gigantic blue whale. The known species diversity in the ocean is less than 13% of all living species currently described. The oceanic environment plays a direct and indirect role in human life by regulating the earth's system, providing social and economic goods and services, supply of living and non-living resources, etc. Fisheries is one of the major resources which play an important role in ocean biodiversity, growth, and development of many countries and also ensure the food security of millions of coastal communities (Srinivasan et al., 2010; FAO, 2011). Unlike other natural resources, they are renewable (capable of growth) if managed properly. Due to several factors, fishery resources are difficult to manage effectively (Munro and Scott, 1985). There are several issues associated with the management of fishery resources, which include over-exploitation of targeted and non-targeted species, ecosystem degradation, ghost fishing, pollution, as well as the carbon footprint of the fishing operations (Ardill and Gillett 2011). The interaction of marine megafauna with fisheries is one of the recent critical issues addressed by fishery managers and marine biologists around the globe. The incidence of protected marine species in the Indian gillnet fishery is estimated as 0-3number per operation (Koya et al., 2018). Fisheries is the major reason for the non-natural mortality of large marine vertebrates such as marine mammals, turtles, sharks, rays, skates, etc. among these, marine mammals are the charismatic animals that exert a major influence on the marine food web, structure, and function of the marine ecosystem. Many marine mammals are categorized as protected species. In a complex fishery with verities of vessel gear combinations, the chances of mammal interaction with the fishery are very high. The balance between the conservation of vulnerable species and the responsible utilization of fishery resources is a challenging topic. Which need to be taken care rightly with proper management measures.

Biological features of marine mammals

Mammals are highly developed animal groups that stand on the apex of the animal kingdom. They have a diverse distribution with suitable adaptation to living in the respective geographical realms. Mammals who live in the aquatic environment are Morphologically and anatomically adapted for life in water. Hydrodynamic body, modified appendages for reducing drag and maximizing propulsion, efficient respiratory system with high oxygen retention, better thermoregulatory mechanisms, specialized sensory and communication mechanism etc. Making them unique from other groups of mammals. 'Marine mammal' is a general term to address the members of 5 different groups Viz Cetaceans, sirenians, pinnipeds, sea otters, and polar bears. A common feature for all marine mammals is that they spent their entire life in the ocean or nearby related ecosystems and derive all of their food from aquatic habitats (Jefferson et al 1993).

Table: 1 Classification of Marine mammals

Kingdom: Animalia	
Phylum: Chordata	
Class: Mammalia	
Order: Cetacea	Whales, Dolphins and porpoises
Order: Sirenia	Dugongs and Manatees
Order: Carnivora	Pinnipids (Seals, Sea lions and walrus) and other marine carnivore (Polar bear, Otters)



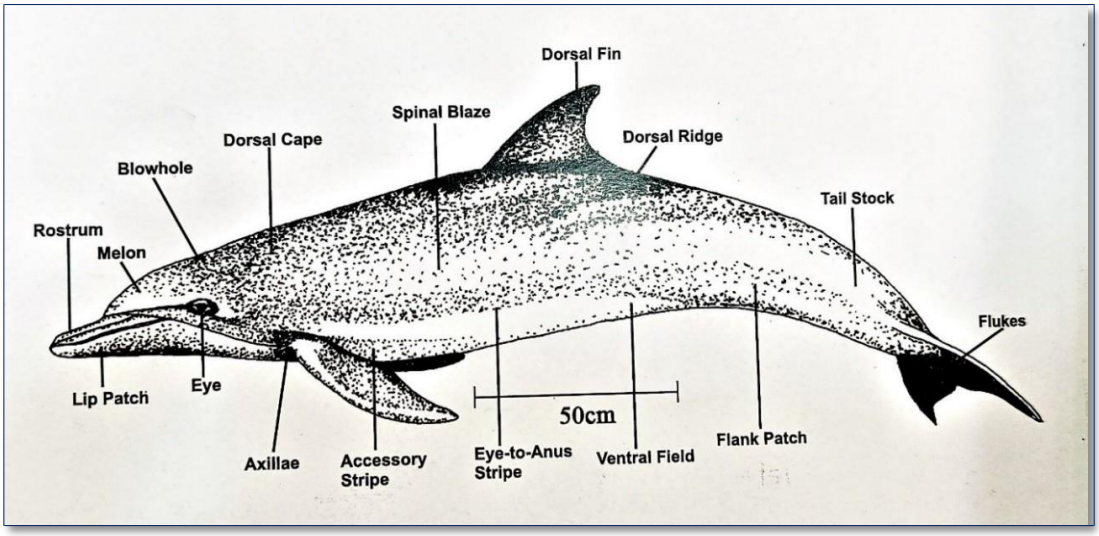


Fig: 1 Morphological features of a typical cetaceans (Vivekanandan and Jeyabaskaran, 2012

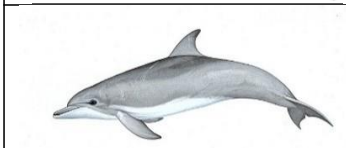
Marine mammals of India

Worldwide 130 species of marine mammals are identified from various oceanic regions viz. tropical, subtropical, temperate, and polar regions (Jefferson et al 2008). Indian seas accommodate verities of marine mammals belonging to two orders, cetaceans and sirenians. Which includes baleen whales, toothed whales, dolphins porpoises, and dugongs. (Vivekanandan and Jeyabaskaran,2012). Out of 130 species reported worldwide, 25 species of cetaceans are reported from Indian waters of which five are Mysticeti (Baleen whales) and the rest are Odontoceti, which includes Delphinidae, Physeteridae, Kogiidae, Ziphiidae, Phocoenidae and Platanistdae (Kumaran, 2002). Only one species of sirenian (Dugong dugon) is reported. All the marine mammals of India are protected by law and positioned under Wildlife (Protection) Act, 1972. Out of which three species Gangetic dolphin (Platanista gangetica), Irrawaddy dolphin (Orcaella brevirostris), and dugong (Dugong dugon) are protected under Schedule I, and the rest are placed under Schedule II. As per the act, Schedule I and Part II of the Schedule provide absolute protection. Capture, use, and trade of animals under this schedule prescribed the highest penalties. India is the first country in the world to have cetacean fauna as a National aquatic animal. Gangetic dolphin Platanista gangetica gangetica is declared as the National Aquatic Animal by the Prime minister of India in the First Meeting of the National Ganga River Basin Authority (NGRBA) on the 5th of October 2009.

Interaction of marine mammals with fishing systems

Cetaceans coming under the family Dephinidae shows more interaction with coastal fisheries in India. Dolphins are members of this family. Active movement, overlapping with the feeding and activity zones of other commercially targeted nektonic groups are some of the reasons for this higher interaction. While analyzing the depth-wise and zone-wise distribution of marine mammals in India, there are several species with active distribution in the coastal fishing zones. Four species of dolphins viz Stenella longirostris (Spinner dolphin) Tursiops aduncus (The Indo-Pacific bottlenose dolphin), Delphinus capensis (The long-beaked common dolphin) Sousa chinensis (The Indo-Pacific humpback dolphin) are the four major dolphin species abundant in the coastal waters (Jayapraksh et al., 1995). (Table: 2).

Table: 2: Major dolphin species with higher interaction with fishing systems

	Species
	*Stenella longirostris*
	*Tursiops aduncus*
	*Delphinus capensis*
	*Sousa chinensis* -

The mammal-fishery interactions are of several kinds viz. biological/ ecological and direct/operational interactions (Wickens, 1994). In mammal-fishery interaction, most of the interactions are reported as predatory type. Fish is one of the most important diets of marine mammals and many are competing with fishermen for the catch. Some of the dolphins forage exclusively on fish (Barros & Wells, 1998, Panicker and Sutaria, Sule et al 2015).

Major pelagic fishing systems with marine mammal interaction

Indian fisheries are multispecies-multi gear in nature and characterized by a heterogenous fishery management system (Najmudeen and Sathiadhas, 2008). Fishing is carried out with more than 20 gear and vessel combinations. Out of the various gear operated, gillnets and seine nets are more vulnerable to marine mammal interaction (Cockcroft & Krohn, 1994; Perrin et al., 1994; Archer et al., 2001; Wise et al., 2001; Read et al., 2006, Joseph et al., 2021).

Gillnets have either a single shot/unit of the net or a number of units tied end to end to form a full fleet of length ranging from 600 to 16500 m with a hung depth of 3 to 20 m. Based on mesh size, Indian gillnets are classified into small meshed nets with 14 to 45 mm mesh size and large meshed nets with 45 to 500 mm mesh size which target verities of species viz. sardine, mackerel, anchovy, seer fish, shark, tuna, pomfret, hilsa, barracuda, billfish, carangid, perch, elasmobranch. etc. Fishing is normally conducted at a depth of 20-1000 m. The study by ICAR-Central Institute of Fisheries Technology, Cochin from 20 major fishing harbors along the Indian coast reports, coastal gillnets are more prone to cetacean interaction. As gill nets are stretched wall of the net with very low visibility in the water, the net will obstruct the movement of animals that comes in the range of operation and lead to entanglement. Depredation may another reason for the interaction. There are many reports that marine mammals feed on fish caught by fishing gear (Gonener and Ozdemir, 2012). While examining the inshore and offshore gill net bycatch composition, finless porpoise, humpback dolphin, and Indo-Pacific bottlenose dolphins are caught in the coastal gillnet targeting tuna and seer fishes. Whereas spinner dolphins, Risso's dolphin, and dwarf sperm whales are the species reported from the offshore drift gillnet (Anderson et al. 2020, Yousuf et al. 2009)

Marine mammal interaction in gillnets

Almost 84% of the global cetacean bycatch is reported from gill net fishery (Read et al., 2006)

Most of the fishery-mammal interactions are reported during the late 1980s (Northridge, 1984). due increase in incidental catch of protected species in gill nets, the operation of gillnets in the high seas is banned by many countries by laws (He, 2006). In India, technological advancement and modernization in the gillnet sector resulted in an increase in the quantum of gillnets taken for operation even in distant and oceanic waters (Thomas,2019). These escalations in the size of the gillnets increased the chances of marine mammal encounters. The modernization also resulted in the shifting of area operation of gillnets from coastal waters to deep sea so there has

been a change in the species composition of cetacean bycatch in drift gillnets also (Anderson, 2014). Almost three decades of observation from Indian waters report 98.8 % of mammal mortality reported were due to entanglement in gillnets (Jeyabaskaran et al. 2016). Among the major fishing systems operated along the Indian coast, cetacean interaction is reported maximum from gillnets (57.7%), particularly in the small meshed gillnets operated in the coastal waters. Joseph et al. (2021). In India finless porpoise, humpback dolphin, and Indo-Pacific bottlenose dolphins must have been caught in tuna/seer gillnets operated in inshore waters while spinner dolphins, Risso's dolphin, and dwarf sperm whales among other species dominated the offshore drift gillnet bycatch (Anderson et al. 2020, Yousuf et al. 2009). The annual cetacean mortality caused by the Indian gillnet fishery is estimated in the range of 1000-10,000/year (Lal Mohan. 1994, Yousuf et al. 2009, Kumaran, 2002) and most of the moralities are associated with the pelagic fishery of yellowfin tuna (Thunnus albacares), sharks, and seerfish (Scomberomorus commerson and S. guttatus).

Marine mammal interaction in seine nets

In India, after gillnets, seine nets are most prone to marine mammal interaction (Joseph et al, 2021). Unlike gillnet fishery, seine fishery in India is highly regional and restricted to the southern coast. surrounding nets are mainly employed to catch the shoaling pelagic fishes like sardines, mackerel, tuna, etc. Purse sines and Ring sines (mini purse seines) are the two major seine nets in India. Ring seines, otherwise known as mini-purse seines, are a group of lightly constructed seines adapted for operation in the traditional motorized sector. The total length of a seine net ranges from 600-1000 m with a depth of 83-100 m and its operation is confined to a depth of less than 75 m. The operation of a seine net consists of all the aspects of hunting, scouting the fish, chasing, and interception of the fish school, etc. cetaceans are the major bycatch reported in seine fisheries, especially the members belonging to the family Delphinidae (dolphins) are more vulnerable to fishery interaction.

The dolphin species which has more access to coastal waters showed more interaction with fishing systems. 84.8% of the interaction with fishing systems was exhibited by four species *Stenella longirostris, Tursiops aduncus, Delphinus capensis,* and *Sousa chinensis*, which are abundant in the coastal waters (Table.2). (Joseph et al. 2021, Raphael et al., 2017; Edwin et al., 2017; Koya et al., 2018). Larger herd size, active swimming behavior, and sharing of common ecological niche with the fishes which are targeted by seine fishing makes dolphins more

susceptible to capture and entanglement in the fishing nets. There are reports of targeted capture and landing of dolphins from seine nets in India (Jayaprakash et al. 1995, Yousaf et al., 2009). In 1984, almost 42 common dolphins, Delphinus delphis were landed in Kochi by 12.5m purse seiner and animals were sold to the local market for 27.5 INR/specimen. Similarly, in 1995 and 2009, finless porpoise, Neophocaena phocaenoides were landed by purse seines from off the Mangalore coast of Karnataka and the Gulf of manner region. Dolphin fishery interaction in India is mainly associated with small pelagic fishery (especially oil sardines) in near-shore shallow waters (Yousaf et al., 2009). The majority of the cetaceans-seine net interactions were reported from the states like Kerala, Karnataka, and Goa where the higher landing of small pelagic are reported. (Prathibha et al., 2018, Yohannan and M. Sivadas, 2003, Joseph et al.2021, Yousaf et al., 2009, Edwin et al.2017, Raphael et al., 2017, Prajith et al 2014).

Depredation is another reason for the mammal-seine net interaction. Cetaceans especially dolphins considered fishing nets as an easily accessible and available source of food. when the catch concentrates on the bund area of the net, dolphins approach the net and remove the catch by biting and tearing off the net. Removal or damage of the harvested catch in commercial or recreational fishing by predators which leads to the damage of the fishing gear is referred to as depredation. Depredation directly causes economic loss by damaging fishing gear. The indirect loss is by reducing the quality of the catch.

Mitigation

The mitigation measures to minimize the marine mammal fishery of active and passive types. Making alterations in the structural features, increasing the visibility of fishing gear by means of using thick twines, incorporating add-on reflectors, and colouring the netting panels are the major passive methods. Whereas mechanical sound generation using crackers, explosives, gunshots, etc. are come under the active type (Jeffersons and Curry, 1996). Indian fishermen follow both active and passive mitigation measures to deter marine mammals from the fishing operation and to safeguard their catch and gear. which can be further classified as indigenous and modern methods. The major indigenous mitigation strategies adopted by the Indian fishermen to minimize cetacean bycatch/interaction are the selection of suitable grounds, structural modifications in the gear, sound generation using crackers, vessel chasses, use of boat noises making a loud noise, throwing bait to distract the mammals and jumping into the water to scare them. While practicing these indigenous methods, fishers are cautious to avoid injury to

the animals. They even patrol the fishing ground with small boats and alter the attention of mammals with the help of objects like tyres, boat anchors, stones covered in plastic bags, etc. The major modern mitigation methods are the use of acoustic deterrent devices like pingers. Besides this several government agencies and research institutes of the country are engaged in various outreach programs to create awareness among fishers about the protected marine species and their importance in the ecosystems.

Dolphin wall nets (DWN)

Dolphin wall nets are the indigenously fabricated wall of nets that creates a barrier between the seine net and the dolphins during fishing operations. The net is 1000-1500m long with plastic cans as float and large steel rings as sinkers. The DWN is an innovation from the side of local fishers of Kerala, the Southernmost state of India which reduce the operational damages and results in catch loss during fishing. besides this, unknowingly the net ensures the protection of mammals from incidental catch and mortality (Prajith et al. 2014)

Medina panels

This is a panel of relatively small mesh netting (50 mm or less) sewn into the purse seine at the distance of about 1/3 of the float line length from the bunt-end tip, to surround the apex of the backdown area where porpoises are most likely to come in contact with the net. Usually, it is one or two strips deep and 330 m long. The longer the Medina panel the more effective it is, specially fitted into the net throughout the bunches area and as near the bunt as practical. The system is named after the Californian skipper who first used it. (FAO 2022)

Pingers

Sound has a significant role in the lives of marine mammals and sound is the prime mode of information transformation used for communication. As an adaptation to living in a vast aquatic environment, the acoustic system of marine mammals is well developed. Understanding this advantage of communication mechanisms using sound, the use of aquatics pingers is the most suitable and efficient mechanism to distract cetaceans from the fishing gears (Fig. 3). Pingers sometime referred to as net alarms are one of the best options to reduce injury and mortality in marine mammals. Dolphin pingers are devices that produce ultrasound which alert and keep the dolphins and porpoises away from the nets. Pinger is designed to work by emitting a sound wave signal beyond 70 kHz that is known to be in the best hearing range of most dolphin species. The signal acts as an alarm; in some cases, the pinger stimulates dolphins to use their

echolocation, which alerts them to the presence of the pingers and fishing nets. This sound wave is not audible to human beings, but it creates a disturbance to dolphins and alerts dolphins to the presence of nets. Pingers are efficient to minimize cetacean interaction both in gillnets and seine nets.

Fig:2 A typical banana pinger used in gill nets and seine nets

Conclusion

A global survey by the United Nations in 2005 reports that 70% of dolphin species are at risk due to various human activities. Removal of the apex predators like cetaceans by incidental or purposeful killing may lead to an imbalance in the ecosystem. Marine mammal fishery research in India is still in the infancy stage. Most of the studies are based on stranding events. Research to formulate suitable mechanisms to reduce or avoid mammal interaction with the fishing system is the need of the hour. Marine cetaceans' ecology, behavior, and biology need a better understanding. To reduce marine mammal incidental catch and kill, there should be a management system or consortia which comprises government agencies, academicians, researchers, and fishermen. Understanding the fishermen's perception is essential while formulating the research. A refinement in the existing indigenous mitigation measures by the application of a suitable scientific approach with the involvement of fishermen is needed. Besides this awareness about the importance of marine mammals and another megafauna should be created among fishers, the general public, school students, etc. through various outreach and extension programmes.

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Chapter 4

Fisheries associated Environmental burdens and Energy use

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Introduction

Fishing involves the dissipation of energy to accomplish its primary activity i.e., harvesting of fishery resources. While the active cost of fishing is less understood, and consequently receives less attention than the direct impact on fishery stocks and marine ecosystems. Fisheries sector is highly external energy dependent sector and depend mainly on fossil fuels. It is precisely the availability of fossil energy that enables fisheries to continue even when stocks are in decline. Subsequently, analyses of energy in terms of fuel consumption in fisheries, and changes in energy use over time, can also provide a powerful tool to know the stock health in fisheries sector. Global greenhouse gas emissions would be significantly higher if inland fisheries had to be replaced with other forms of animal protein production. From an energetic perspective, fishing is a set of different process (from fabrication of craft/gear to landing of catch) in which different forms of energy are dissipated in order to capture fish and shellfish. However, as of now very few fishery-specific energy analyses have systematically attempted to account for energy use. Inland fisheries are a low carbon footprint food source compared to marine fisheries. Inland fisheries often use non-mechanized gear that does not require fuel (consumed by boats using active fishing gear in major marine fisheries). The three dominant forms of energy dissipated to these ends include animate, wind, and fossil fuel energy. Animate Energy- Animate energy is common to all fisheries irrespective of their technological sophistication. In traditional artisanal fisheries sector, human muscles are still source of the energy used for propulsion scouting, deploying/hauling the gears and catch handling. Wind Energy- For as long as people have sailed, it is likely that wind energy has been used to support fishing activities. Wind energy not only allowed fishing vessels to be propelled but it facilitates other supporting activities too. Specifically, various trawl or dragger fisheries in which gear are towed were almost all first developed within the context of sail fisheries. Fossil Fuels- Fossil fuels are dominant form of source of energy used in fishing. In the early 1900s Gasoline and diesel based internal combustion engines were first adapted for use on fishing boats. After 2nd world war the size of the global fishing fleet increased along with engine power. In other hand relatively, small

engines are also introduced into small-scale fisheries round the globe. These both trend of increasing dominance and size of engines, resulted surprising enhanced fossil fuel consumption for the world's fish harvest sector. Fossil fuels produce carbon dioxide in atmosphere which leads to 'greenhouse effect' and other toxic pollutants which are harmful to the environment and human kind. Greenhouse effect leads to irreversible climatic and oceanographic changes.

Environmental burdens due to construction of fishing vessel

Fishing is one of the energy intensive methods in food production. Energy inputs can be indirect or direct in which indirect energy are linked with building and maintaining fishing vessels and gears. In contrast, direct energy inputs are typically those required to propel fishing vessels and deploy fishing gears mainly in the form of fossil fuels. Combustion of fuels and the release of greenhouse gases to the atmosphere cause environmental impact like climate changes, ocean acidification etc. Fuel consumption rate varies widely according to gear type, materials, shape, fishing practice etc. Selection and deployment of energy efficient harvesting technologies suitable for target resources by modifying the existing gears, adoption of alternative fuel-efficient gears are the prime options for fuel conservation. Several technologies evolved over the years in the fishing industry which have improved the fish catch as well as the effort and the related inadequate practices leading to damage to the ecosystem and these ecological impacts were well explained in much of the literature. Hence, this chapter mainly dealt with the environmental impacts of boatbuilding materials and emissions from fishing. Energy analysis are pertinent in relation to fisheries due to the accepted importance of fuel consumption in fishing operations and related environmental impacts. In view of the budding significance of energy use and its impacts on environment, energy inputs in marine fishing and post-harvest operations have been reported by several authors in recent years.

In fishing boat construction, the common materials used in India include wood, fibre reinforced plastic, aluminium, steel, plywood, ferrocement, etc. While selecting a material for boat construction some basic factors to be considered are type, size, speed, the shape of the vessel, availability and suitability of the material, and economic and environmental viability. The performance and efficiency of a boat are directly dependent on the choice of the boat-building material which also has a direct impact on the environment. By taking these facts into account, a boat designer can select the best possible alternative for building a boat of high efficiency and durability. A fishing boat is made up of different components and their construction is a complex

process. Certain quantities of greenhouse gases (GHGs) are produced in the process of manufacture, transportation, and utilization of these components, which can be converted in terms of equivalent CO2. Every ocean has marine debris, and more than 60% of it is plastic that comes from the fishing industry, offshore platforms, recreational shipping etc. At present, the larger class of fishing vessels are made of steel while vessels belonging to the medium and lower categories mostly use wood for construction. Fiberglass, ferrocement, and aluminium are the new substitutes for conventional boat building materials as these can improve the lifespan of the boats. However, traditional fishing boats still play a vital role in this era. Despite its obvious advantages, all boat-building materials are susceptible to the effects of the marine environment, for example, glass fibres are the most selected material for boat construction, which are vulnerable to the effects of sunlight in marine conditions. Fiberglass-reinforced plastic (FRP) is a polyester resin-based composite, reinforced with fine strands of glass filaments. Glass fibre is prone to osmosis, and gelcoat gets faded in sunlight resulting in the attack of UV radiation. FRP fragments have a higher density than seawater and will tend to concentrate nearshore. The polyester resins or epoxy resins in the FRP undergoing physical & chemical degradation led to the release of microplastics which affects the environment. Marine organisms consume these plastic particles and end up in the human food chain causing severe health issues. Additionally, the deteriorating and peeling paint with high concentrations of tributyltin and lead from the abandoned boats may provide a long-term environmental issue

Fishing and Energy use

Commercial fishing operation mainly utilizes fossil fuels which result in the emission of greenhouse gases. The active cost of fishing is less understood and consequently receives less attention to GHG emissions than the direct impact on fishery stock and marine ecosystem. Similarly, in the harvest process, several reoccurring inputs are required for every fishing operation, viz. fuel, lubricant, ice, freshwater, etc. These inputs have their own carbon footprint value for construction/extraction/process, especially fuel contributes more than 95% out of all the components. Despite the fact that the prevailing pre-harvest phase of marine capture fisheries lacks general detail and standardization about LCA/carbon footprint studies; such studies and their findings can be useful in formulating constructional and operational recommendations to improve the environmental performance of fisheries, under the context of an ecosystem approach to fisheries along with future certification and different eco-labelling of

fisheries. Studies related to pre-harvest, harvest, and post-harvest fisheries LCA/carbon footprint analysis would be more appreciated by policymakers for the regulation of fishing boat yards and other related fishing ventures. Based on behaviour and habitat, there are different methods of fish harvest and on the basis of their operation, the quantum of fuel and energy requirement also varies. As per the study by Parker et al., 2018, the world fishing fleet burned about 40 billion liters of fuel and emitted 179 million tonnes of CO2 equivalent and other GHGs to the atmosphere. Overcapacity and irresponsible use of fossil fuels leads to increased levels of fuel consumption in fishing contributing to climate change in the long run. India contributes 134 million metric tonnes (2.7%) of CO2 emission due to total marine capture fisheries, against 90 million metric tonnes (3.9% of global production) of fish production. The emissions due to fishing were not given importance as compared to other sectors for emission in India, however, the contribution of the fisheries sector is negligible which roughly may be <1% of global GHG emission. The other associated important environmental parameters by which the health of the environment, humans, and resources can be evaluated due to the fishing process are; terrestrial acidification, formation of fine particulate matter, Water consumption, Ionizing radiation, ozone formation, human carcinogenic toxicity, fossil resource scarcity, mineral resource scarcity environment deterioration, human health, resource depletion, and stratospheric ozone depletion, etc.

Different types of vessel and gear combinations are used for fishing to exploit various fish stocks. The important fishing practices are trawling, gillnetting, longlining, dol netting, purse seining, etc. One major reason for the substantial increase in eq. CO2 emission by the construction process is the increase in the number and efficiency of fishing boats otherwise called overcapacity, which need more inputs and equipment, resulting in more eq. CO2 emission.

Energy spent in different fishing operations

Based on behaviour and habitat, there are different methods of fish harvest and on the basis of their operation the quantum of fuel and energy requirement also varies. According to the study of globally large-scale industrial fishing sector consumed about 14 -19 million t and small-scale fishing sector consumed about 1-2.5 million t of fuel oil. The production of fish per tonne of fuel was 2-5 t in the industrial sector and 10-20 t in the small-scale sector. In energy context some of the important fishing methods are listed below:

Trawling: Trawling is one of the most energy intensive fishing methods. It consumed nearly 5 times more fuel compared to longlining and gillnetting (passive fishing methods) and over 11 times to purse seining for every kilogram of fish produced. Reports suggested from south-west coast of India have shown that trawling consumes 6.5 times more fuel compared to purse seining and 1.8 times more fuel than gillnetting, to produce one kg of fish. For large trawlers, 90% fuel consumption accounts during active trawling operation. Percentage of fuel cost in the operational expenditure of trawlers may vary between 45% and 75%, depending on engine power and duration of voyage.

Gillnetting/longlining: Gillnetting and longlining are the passive type of fishing where the gross energy requirement is comparatively lower than trawling. These passive gears are either fixed or drifting in water column which do not require energy for operation process except hauling where it is done by mechanical means. Among the operational inputs, fuel contributed 95% of the gross energy requirement. A study suggested that the larger mechanized boats emitted 1.18 t CO2/t of fish caught, and the smaller motorized boats (with outboard motor) 0.59 t CO2/t of fish caught. Among the mechanized craft, the trawlers emitted more CO2 (1.43 t CO2/t of fish) than the gillnetters, bagnetters, seiners, liners and dolnetters (0.56–1.07 t CO2/t of fish).

Purse seining: Purse seining is one of the most aggressive and efficient commercial fishing methods for capture of shoaling pelagic species. It is a fishing technique which targets pelagic shoaling fishes. Before actual operation the shoal detection needs more fuel for fish scout, once shoal gets detected the encircling, capture and hauling process is follow-up. Purse seine operations are relatively energy efficient and greenhouse gas (GHG) emissions for small scale mechanized purse seine operation is low compared to trawling, gillnetting and lining operations. Some of the energy conserving fishing practices such as large-scale purse seining became possible only with the introduction of synthetic netting material.

Traps and pots:

Traps or pots are gears in which fish are retained or enter voluntarily and will be hampered from escaping. They are designed in such a way that the entrance itself became a non-return device, allowing the fish to enter the trap but making it impossible to leave the catching chamber. It can be baited or non-baited. Generally passive fishing gears like gillnets and trammel nets, tangle nets, longlines, trap nets and pots, and other lift nets consuming very little power in fishing and in some cases no mechanical energy. Although travelling, setting and retrieval of gear may use

some energy, target stocks are attracted by bait or are carried to the gear or encounter it by chance and are trapped.

Energy intensive illegal fishing: There are several fish harvest practices which require more energy; light fishing is one of them. Fishing using lights has been practiced from historic times, a classic example is 200-years old Chinese dipnet, which use lights (earlier hurricane lamp and now CFL lamps) to attract fish to the net. Chinese dipnets are mostly animate energy based sustainable fishing operation. More than half of the purse-seine vessels, stick-held dipnet and squid jigging boats use artificial light. Report of the ICES-FAO Working Group on Fishing Technology and Fish Behaviour (WGFTFB), 2012, suggests that roughly global marine catches using lights is 1.09 million tonnes (1.6% of global catches) in 2010. Roughly 16% of the light fishing catches comprise of squids, and the remaining >80% are fish species. Since light source requires electrical energy which is being produced by main engine/auxiliary engine; this practice is energy intensive. At present in India the light fishing is banned.

Small scale fisheries: Small scale fisheries involves a range of practices, but are typically traditional activities using less capital and comparatively simple gear, commonly with small fishing vessels, making short fishing trips close to shore. Globally 57% of vessels are motorized, of which 79% (2.1 million vessels) are less than 12 m overall. Due to the small size of vessel, the area of operation is limited and operations are mainly on daily basis, which accounts for an average of 1–3 tonnes of fish per person annually. Small scale fisheries require less capital investment and energy for operation. Among all fish harvesting systems, mechanized trawling is the most energy intensive operation and traditional non-motorized gillnetting is the most energy efficient having the lowest gross energy requirement. Out of non-motorized systems, stake nets have comparatively high energy intensive. Among motorized operations, ring seines have a lower gross energy requirement per ton of fish landed. Fishing operations requires scouting of shoal/search of fishing ground which may be distantly located have relatively high gross energy requirement per t of fish landed.

Estimates of fuel use and cost

Annual fuel use is about 50 million m3, 1.2% of total global oil consumption. With marine fish and invertebrate landings at 80.4 million tonnes, global average fuel-use intensity was 620 litres (527 kg) per live weight tonne, or about 1.9 tonnes of catch per tonne of fuel. Fishing vessels released some 134 million kg of carbon dioxide (CO2) into the atmosphere at an average of 1.7

kg of CO2 per tonne of live-weight landings. They further noted that these were likely to be serious underestimates, as they did not account for freshwater fisheries or for substantial IUU catches. Global fisheries were estimated to use 12.5 times the amount of fuel energy as their edible-protein energy output, which, although significantly inefficient, compared well with a number of other animal-protein production systems. In context of Indian marine capture fisheries, the substantial increase in fossil fuel noticed due to increased fishing effort and efficiency during the last five decades. Which has resulted in, equivalent to CO2 emission of 0.30 million tonnes (mt) in the year 1961 to 3.60 mt in 2010. Roughly for every tonne of fish caught, the CO2 emission has increased from 0.50 to 1.02 t during above said period.

Conservation of fuel as a part of responsible way of fishing

In fish different fish harvesting system different approaches to energy conservation could be one of the ways to conservation of natural asset as well as environment safe. Energy security and conservation have great significance on account of responsible fishing and also to meet the demand-supply gap of fossil fuel. Thus, considering non-renewable nature of fossil fuel, limited availability and effects of its use on environment should be addressed in holistic way. Trawling consumes 0.8 kg of fuel while longlining and gillnetting consumes between 0.15 and 0.25 kg of fuel and purse seining requires 0.07 kg of fuel, to catch one kilogram of fish. Hence most potential for fuel conservation exist in trawling. In trawling typically, a substantial portion of the time is spent on towing the gear. During the tow, resistance of the vessel is insignificant compared to the resistance of the gear. The gear resistance therefore has a large effect up on overall fuel economy. Fuel cost can be over 50 percent of the total expenses on a fishing trip. According to a study, fuel consumption due to floats, sweeps, warp, otter boards, foot rope and webbing are 3%, 4%, 5%, 20%, 10% and 58% respectively. Some of the preventive measures can save fuel in trawling operation viz. Use of knotless netting, thinner twine, large meshes, cambered otter boards, optimal angle of attack of otter boards, slotted otter boards, multi-rig trawling, pair trawling etc. The fuel consumption significantly increases at maximum speed of vessel, this is because of increase in wave breaking resistance. Facts established that reduction of 10-20% speed can lead to save fuel by 35 to 61% fuel. Generally, two-stroke outboard engine have high fuel consumption compared to 4-stroke petrol outboard engines. Turbo-charged diesel engines are about 15% more fuel efficient than normally aspirated engines., which have a much better fuel economy and emission standards, are also being introduced in small-scale fisheries.

Summary

In modern fisheries, the major direct and indirect energy inputs can be systematically analyzed using process analysis and input-output techniques. Mostly direct fuel inputs are used primarily for vessel propulsion. On average direct fuel energy inputs account for between 75 and 90% of the total energy inputs, irrespective of the fishing gear used or the species targeted. The remaining 10 to 25% generally depends on vessel construction and maintenance, and the provision of labour, fishing gear, bait, and ice if used which depends on the character of the fishery and the scope of the analysis conducted. The secondary energy-consuming activities, which include onboard processing and storage are negligible compared to primary energy consumption in terms of fuel burned. The study of environmental burden is important in relative resource-use analysis and greenhouse gas (GHG) impacts in climate change mitigation. It has got emphasis due to the high instability in fossil fuel costs which has potentially lasting impacts on the economic performance of various fishing systems. The effects of fishing and its implications on ecosystems, especially from the boat-building sector or the usage of energy, fuel, and emissions, were not particularly addressed and are anticipated to have significant effects on ecological sustainability and food security globally.

Chapter 5

Line Fishing in India: Status and Conservation Challenges

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Introduction

Hook and line gears consist of a minimum of two parts, a hook that is attached to a monofilament line and artificial or natural baits used to lure fish to the hook. This type of gear is one of the most common fishing gears used by both artisanal and mechanized sectors. Hook and line is one of the best methods of fishing with regards sustainability as this method has little impact on the surrounding environment and the catch can be selective. For example, any fish too small, or not the right species can be placed back into the water, without harm. These gears make it possible to operate in places with rocky or uneven bottom where it is impossible to deploy gears like ring seine or trawls. Hook and line gear can be classified based on the method of operation. They are hand lines, troll lines, long line, jigging line, and pole and line fishing.

a. Hand lines

Hand line fishing, or hand lining, is a fishing technique where a single fishing line is held in the hands. One or more fishing lures or baited hooks are attached to the line. Hand lining is among the oldest forms of fishing and is commonly practised throughout the world today. This may be used to capture of all kinds of demersal fishes from motorized as well as mechanized vessels. The gear can be described as hook, fishing lure (or a fishing jig), sinker and float are generally attached to the line. Many hand lines use swivel to prevent excessive fouling and kinking of the line. Sometimes rollers are hauled over rollers on sides of vessel.

Generally, the gear is made up of polyamide (PA) braided, twisted or monofilament line. Diameter of line used for fishing is varying highly from 0.2mm to 1cm. On the contrary, in Ratnagiri, hand line with hook made up of PA monofilament twine with diameter varying from 0.23 to 1 mm and length ranging from 5 to 16 m was operated. In Car Nicobar, 3 to 20 m long monofilament line was used for construction of hand line. Whereas in north east India, a rod can be seen tied with indigenous fiber or cotton thread or nylon twine and the end was fixed to a hook.

b. Troll lines

These are lines with baited hooks that are dragged behind vessels called trollers. Trolling is primarily used for surface and subsurface fish. Splashing or rippling of water produced by an object has led to some improvement in the hook and line techniques. It is practiced in Androth island of Lakshadweep (Pillai et al., 2006) for catching tuna (Vinay et al., 2017). Troll lines vary from region to region but use both natural and artificial baits. A trolling line consists of a line with natural or artificial baited hooks and is trailed by a vessel near the surface or at a certain depth. Several lines are often towed at the same time, by using outriggers to keep the lines away from the wake of the vessel. The lines are hauled by hand or with small winches. A piece of rubber is often included in each line as a shock absorber. Trolling speeds vary depending on the target species, but generally are between 2.3 and 7 knots. Troll lines may be set for fish close to the surface or the lines can be weighted for fish at selected depths. Lines may be hauled in by hand or by mechanical means (i.e., hydraulics). At the end of each line there are a variety of embellishments – spoons, spinners, and feathered jigs, in addition to baitfish.

c. Longlines

Long lining can be used to target both pelagic and demersal fish with the lines being rigged and set at a position in the water column to suit the particular species. A basic long line consists of a long length of line, light rope or more common now is heavy nylon monofilament, the 'main line', this can be many miles in length depending on the fishery. To this main line, multiple branch lines with baited hooks on (snoods) are attached at regular intervals. This rig is set either on the seabed (demersal) or in midwater (pelagic) with a 'dhan' bouy at either end, and allowed to fish for a period.

Longlines can be further classified as 1. Set longlines: These are stationary lines that are anchored to the vessel, the seafloor or to an anchored buoy. Setting can be practised either horizontally or vertically. 2. Drift longlines: these are attached to floats that drift freely with the ocean currents.

d. Jigger lines

These are a specialized type of vertical line, fitted with specialized ripped hooks, used primarily in the southern hemisphere Squid fisheries and some northern Cod fisheries. Multiple hooks are evenly spaced along the main line, which is hauled in using jerky vertical movements. This movement simulates the realistic movement of common prey species of the targeted species. In

squid fishery, lights are used to attract the squid towards the surface. As the line is jerked vertically, the squid attack the hooks and are either caught by the mouth or the body. Jigger lines are typically used by specialized jigger vessels, but may also be operated from other types of boats. Jigger lines are generally of two types hand operated and automated jigging machines. Hand operated jigger line employs a reel or drum on which the jigging line is rolled over. Multiple jigs are attached to the jigging line and the reel is released by rotating the reel or drum. In automated jigging machine the machine has two drums and one drum is driven electrically. The machine lowers and retrieves the line in predetermined speed. A wire mesh frame is positioned in such a way to collect the squids falling off the jigs slides directly into boxes on deck.

e. Pole and line gear

The gear consists of a hook and line attached to a pole. Both artificial and natural fish are used to lure the prey. Poles are commonly made out of wood or fiberglass and can be operated by hand or mechanized. Albacore Tuna and other Tuna species are commonly caught by the pole and line method in commercial fisheries. Pole and line fishing can occur from the surface to great depths, the only limiting factor is the amount of line used. Pole and line fishing is extensively used in some areas such as Lakshadweep island of India, Japan, Maldives, Sri Lanka, California and Hawaii for catching skipjack and other species such as frigate mackerel, little tuna and bonitos. Bamboo poles are traditionally used of size ranging from 2.4 to 2.7m in length. A 75-90cm line is fastened to this pole. A 60cm wire leader bearing lure is attached to the end of this line to which a barbless hook is attached. Small fishes of weight 15-20kg are hauled by a single fisherman. In case of larger fish, a single leader is attached to two lines from two poles. The vessels use live bait and water shower to mimic shoal of small fishes to attract the tuna.

Targeted species in hook and line fishery

Hook and line fishing is highly targeted fishing practice which manages to land high value fishes. Though a high variability in value of fish is observed, Indian waters have shown less diversity in hook and line fishing. Out of five methodologies discussed here, four of them except jigging has targeted tunas invariably all around the coast. More details on targeted species are given gear wise as follows.

f. Long lining

In the Indian seas, longline fishery is mainly targeting yellowfin and bigeye tunas. As reported elsewhere, the bycatches, especially sharks constitute a major portion of the longline catch in the Indian waters also. Mechanized sectors of Kerala, Tamil Nadu, and Andhra Pradesh rely on longlining for high value fishes like tuna, marlin, sail fish and sharks. In Kerala, landings from hooks and lines fishery contribute about 3.3% of the total fishery. Seerfish landings registered an upward trend with 83.3% increase from 2010 to 2011, out of which 54.7% was contributed by longline in Kerala. During 2011, 50.8% of elasmobranch catch was contributed by line fishing and grouper contributed about 15% by longline. In Tamil Nadu, 10.6% of seerfish, 1.2% of tuna and 4.2% of elasmobranchs were contributed by hook and line. In Visakhapatnam, annual catch of tuna recorded by hooks and lines was 2714 t during 2011 constituting dominant species, *Thunnus albacares* (53%), *Katsuwonus pelamis* (31%) and *Euthynnus affinis* (16%). According to CMFRI (2012), a total of 29 longliners are operating in Kerala coast, 380 in Tamil Nadu and 21 in Andhra Pradesh during 2010.

g. Handlining

A very popular method for catching big demersal fishes like emperor fishes (Lethrinids) and snappers (Lutjanids) in the coastal areas of Indian waters. Bottom handlining was carried out with ‘vallams’ towed by mother ships to the fishing grounds close to the continental slope. Recently handlines are found to be operated by Thoothoor fishermen all around Indian coast. Deep sea going fishermen of Toothoor operates handlining for Kalava fish (*Epinephelus sp.*) from December to April from mechanized boats. Species like *Selar crumenophthalmus, Decapterus sp., Auxis rochei, Auxis thazard, Epinephelus areolatus E. bleekeri, E. cholorostigma E. tauvina, Thunnus albacares* contributes major catch from Indian coast.

h. Pole and line fishing

The pole and line fishing technique supplies 11% of global tuna and is considered as a best practice due to its high selectivity and low environmental impact. 10% of the Indian Ocean tuna catch comes from small-scale pole and line fisheries operating out of the Maldives and Lakshadweep islands, landing a majority of skipjack tuna (Katsuwonus pelamis) amongst yellowfin (*Thunnus albacares*), bigeye (*Thunnus obesus*), kawakawa (*Euthynnus affinis*) and *Auxis* spp. These fisheries utilize small planktivores from island lagoons and reefs as live-bait

to target oceanic skipjack resources, thereby reducing the pressure on the sensitive coral reefs of their atoll ecosystems.

i. Troll lining

This method is practiced to a lesser extent in India. An established fishery of pole and line is practiced at Androth island of Lakshadweep. Troll line contribute only 3.3% of tuna landing of Lakshadweep. The catch is dominated by yellow fin tuna, frigate tuna, little tuna, skipjack tuna and other species like shark, seer fish and sword fish. Other than India, Maldives and Sri Lanka have troll line fisheries for tuna species.

j. Jigging

This methodology is solely aimed to catch cephalopods based on their feeding behavior all around the world. Countries like Japan, China Sea, New Zealand, Peru, Korea, Malaysia and Vietnam operate automated squid jigging for a wide range of cephalopods (*Todarodes pacificus, Ommastrephes bartramii, Loligo bleekeri, Photololigo edulis, Nototodarus sloanii, Dosidicus gigas, Uroteuthis duvauceli, Sepioteuthis lessoniana, Sepia aculeate, Sthenoteuthis oualaniensis*). Fish jigging is prasticed along North Pacific coast of Japan for Mackerel (Scombridae) and Hairtail (*Trichiurus lepturus*). Automated squid jigging is in experimental stage in India (Mohammed, 2016) whereas a few places like Ratnagiri, Vizhinjam, Kanyakumari, Palk Bay, Tuticorin and Gulf of Mannar- motorised crafts operates hand jigging seasonally. The catch mainly comprised of *Sepia pharaonis*, *Loligo duvauceli*, *Sepia aculiata* and *Sepioteuthis lessoniana*.

Hook and line fishing: bycatch scenario

Since the numbers of species caught are less in a single operation, average mortality rate is assumed to be less than other fishing methods considering population parameters. Line fishing catches desired fishes during operation and unlike trawls, it avoids contact with the sea bottom hence it is assumed that very few species are affected other than targeted species. In a multispecies fishery like India, bycatch reduction has always been challenge. Since the selectivity of line fishing is prominent, concern for bycatch is considerably less alarming.

The trolling method is used all around the world in fisheries targeting tuna, salmon (Salmo spp.), barracuda (*Sphyraena barracuda*) and others, with incidental capture of seabirds reported. In the Mediterranean, small Maltese vessels undertaking trolling for tuna, Bream (*Dentex dentex*) and other predatory fish killed 35 birds. Unpublished information in several countries reported

captures of shearwaters (*Puffinus carneipes* and *P. pacificus*), Yellow-nosed albatrosses, Australian pelicans (*Pelecanus conspicillatus*) and boobies (*Sula* sp.) either by taking hooks or by colliding with gear and becoming entangled. Studies indicated minor implications when targeting Yellowfin tuna but major concerns (catch rate of 0.41 birds/day) when targeting Bigeye tuna. Many authors suggested that capture in this trolling occurs commonly and needs to be better studied, particularly when the vessels troll lines slowly.

Handlines are used to catch different species of tunas all around the Pacific Ocean, Indian Ocean, Red Sea, Mediterranean and Atlantic Ocean, frequently around FADs. Handlines are also reported to be a selective fishing method. But high levels of incidental capture mortality of birds (0.61 birds/day) were reported in Atlantic.

Surface long lines for dolphinfish practiced in the Atlantic had a high bycatch of seabirds (0.147 birds/1000 hooks). However, the traditional pelagic longline captures seabirds during winter months, while the surface longline for Dolphinfish takes place during summer in the Atlantic. A range of characteristics including low depth, deployment during daylight hours, and use of small hooks make it particularly dangerous for seabirds by being available throughout fishing and not only during deployment as in the longline for Swordfish and tuna. Catch rate of sea turtles was also high in the surface longline for Dolphinfish (1.08 turtles/1000 hooks) comparable to rates reported in the pelagic longline fishery for Swordfish in the SW Atlantic of 0.68–2.85 turtles/1000 hooks.

Sharks and cetaceans cause significant damage worldwide in pelagic longline fishery operations. Damages are in the form of bite-offs, loss of gear, catch displacement, reduced gear efficiency, and depredation of the catch. The experimental longlines operated in Indian waters showed a very high shark catch during the post-monsoon season in the Bay of Bengal.

Conservation of non-targeted resources

Major bycatch in line fishing are turtles, seabirds, sharks and non-targeted fishes. The most discussed case is the incident of turtles in tuna long line. There are many methods adopted by sector all around the world for the conservation of these resources. Methodologies developed specifically for each organism. These methodologies are listed below:

a. **Avoid hotspots:** Hotspots are the location where the unwanted species are caught in large quantities. There is currently no quantification of what constitutes a hotspot. This would be

left to the fishermen to determine if they are fishing in an area that is resulting in the incidental capture of sharks, sea turtles, sea birds, marine mammals or unwanted fishes.

b. **Set operational depth to deeper or shallow waters**: This may work in case of shark species which swim to the surface waters. Setting line deeper than 100m will avoid most of the species and only yellow fin tuna may come in contact.

c. **Use circle hook with offset:** Circle hooks have a rounded shape with a point oriented toward the shank, which is different than the J hook that has a point oriented parallel to the shaft. Circle hooks are wider and therefore more difficult for sea turtles to become hooked on. The offset creates a larger gap between the point and the shank hence the turtles can escape from accidental hooking. Similar to other species, circle hooks are wider and more difficult for some marine mammals to bite and become hooked on. Bill fishes are also known to escape from circle hooks without incidents of hooking. Use of wider circle hooks in place of narrower J and tuna hooks to reduce turtle bycatch rates and mortality in longline fisheries has also been found to reduce seabird bycatch rates by about 80%.

d. **Line weighting:** Weights are added to the branch line so hooks are quickly deployed to the target fishing depths. This reduces bycatch of seabirds by moving the baited hooks out of the diving range of seabirds. The effectiveness of line weighting depends on the distance between the weight and the hook (a short distance accelerates the initial sink rate) and the amount of weight added (greater weight accelerates the subsequent sink rate). This mitigation measure must be used in conjunction with properly deployed streamer lines or night setting in case of seabird interaction. Using weight or lead swivels of minimum weight 45g within 1m of the hook may reduce sea turtle interaction also.

e. **Use of finfish bait:** Using finfish instead of squid for bait has been shown to reduce sea turtle interactions. This may be more effective for leatherback sea turtles compared to other species. Using finfish instead of squid for bait has been shown to reduce interactions with some but not all shark species

f. **Night setting**: Night setting is the practice of setting and hauling fishing gear between dusk and dawn. No modifications to fishing gear are needed and this has been proved to avoid sea bird interaction to logline.

g. **Shorter soak time:** This reduces the amount of time the gear is in the water, reducing potential interactions. It also may reduce mortality in incidentally captured turtles because

they remain hooked for a shorter period of time Adequate soak time reductions would be species/fishery specific. The challenging part is to determine soaking time for specific species with experimental fishing.

h. **Streamer line (tori or bird scaring line):** This is a line with streamers that is towed from a high point as the baited hooks are deployed (usually near the stern). An aerial segment with streamers suspended at regular intervals is formed as the vessel moves forward, creating drag on the streamer line. The mitigation measure works by maintaining the streamer line over the sinking baited hooks, therefore preventing seabirds from attacking the bait and becoming hooked.

i. **Conduct fleet communications:** This will allow fishermen and policy makers to determine where marine mammal sightings may have occurred and move fishing locations when interactions occur

j. **Prohibit the use of wire leaders and shark lines:** Shark lines are attached to the floats and stay above mainline of logline. Wire leaders prevent sharks from being able to bite through and escape after accidental capture. Shark lines may attract more sharks to the fishing gear.

k. **Removing the first and/or second hooks closest to the float in each basket:** The hooks closest to the float fish in shallower water and therefore have a higher likelihood of incidentally capturing sea turtles.

l. **Hook-shielding devices:** These are devices that encase the point and barb of baited hooks. This prevents seabird attacks during the setting process. Hooks are released after the hook has reached a minimum of 10m depth or has been in the water for a minimum of 10 minutes. The Hook Pod and Smart Tuna Hook are two devices assessed as having met ACAP (Agreement on the Conservation of Albatrosses and Petrels) performance requirements.

m. **Use 'weak' hooks:** These are specially designed hooks that break or bend when certain amount of pressure is applied, allowing incidentally captured species the ability to escape. Mostly used in case of marine mammal incidents as they are stronger than fishes.

n. **Restrict the use of light sticks:** This may reduce billfish interactions by lessening the ability to see baited hooks. Turtles are also found attracted to light sticks.

o. **Use of monofilament for the mainline and branch line:** Monofilament line reduces the risk of entanglement compared to multifilament lines. Monofilament is less flexible, making it easier to release entangled sea turtles (i.e. reduces knotting of the line).

p. **Time/area closures:** Time-area closures and restrictions on the timing of setting could further reduce seabird bycatch as these factors have been observed to have significant effects on seabird catch rates

q. **Cover the point of the hook:** This will reduce the ability of sea turtles to bite and become hooked.

r. **Avoid using light sources:** This may reduce sea turtle interactions by lessening the ability of turtles to see baited hooks.

s. **Fisheries certification:** It is important to recognize and reward good fishing practices in the marketplace. Among the most popular seafood certification organizations is the Marine Stewardship Council. The Council certifies fisheries based on the sustainability of fish stocks, the level of environmental impact (one of the parameters is that the fisheries should have negligible/low levels of bycatch), and whether the fishery is being effectively managed. A fishery that comes close to meeting these criteria of sustainability is the pole and line skipjack tuna fishery in Lakshadweep. However, it is important to recognize the dynamic nature of what constitutes bycatch and evolve incentive systems which recognize the moral, social, and economic implications of bycatch along with its ecological impacts. It is equally important to understand that certification alone is not likely to bring about major improvements in the conservation of bycatch species. So far certification has primarily been effective in raising awareness among consumers (Ward, 2008). Its shortcomings are that it is seen primarily to market opportunities, and has rarely, if ever, helped the recovery of depleted species.

Line fishing methods especially longline and pole and line widely used in Indian waters has advantages in biological and economical aspects as discussed earlier. Considering the current production from line fishing where tuna is targeted, production level has to fill in the huge gap with estimated potential of tuna from coastal fishing and island fishing. However, it is also to be noted that line fishing has the clear drawback of needing to use additional biological resources in the form of bait especially live bait for pole and line fishing. The large-scale development of the line fishery is one of the means of optimizing exploitation of resources from Indian waters. At the same time, it is necessary to understand that development of the fleet must not only be aimed at increasing size but also at increasing efficiency.

Conclusion

Many targeted resources are seasonal which affects the market for the species. Constant support from the Government as a policy or direct allowance of incentives can support the sector to a great extent. The sector still requires research and awareness among the consumer as well as the fisherfolk to attract towards this fishery as many of the resources are non-conventional. Hopefully, the sector is expected to achieve its full potential through constant support from legislation as well as research.

Chapter 6

Fish Processing Methods

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Introduction

Research on food processing have attracted more due to huge demand in supply of healthy and safe food products. Health, nutrition and convenience are the major factors driving the global food industry in this era. Fish products have attracted considerable attention as a source of high amounts of important nutritional Components like high-quality protein, essential vitamins, minerals and healthful polyunsaturated fatty acids to the human diet. As a result of this the fresh fish and seafood's rank third among the food categories with the fastest overall growth worldwide, next to drinkable yogurt (18 %) and fresh soup (18 %). Consumption of both freshwater and seawater fish is expected to increase in the future. As fish is highly nutritious, it is also highly susceptible to spoilage, due to intrinsic and extrinsic factors. Proper processing and packaging help in maintaining the eating quality of fish for extended period. Worldwide, an array of processing and packaging methods are followed. This ranges from a simple chilled or ice storage, salted and drying to most recent and advanced high pressure and electromagnetic field applications, which attracts opportunities from both small scale and industrial level entrepreneurs. Fish products in live, fresh chilled, whole cleaned, fillets steaks, battered and breaded products, variety of dried products, smoked fish, fish sausage and traditional products are the range of low-cost processing methods which can be readily adopted by small-scale fishers. The processing methods like canning or heat processing, freezing, vacuum and modified atmosphere packaging, analogue products, high pressure processing, pulsed light processing, irradiation, electromagnetic field etc are the processing methods which requires higher investments can be adopted by large scale entrepreneurs, apart from the above-mentioned processing methods.

Benefits of Processing

- Converts raw food into edible, usable and palatable form
- Helps in preservation and storage of perishable and semi-perishable agricultural commodities

- Helps in avoiding glut in the market and reduces post-harvest losses and make the produce available during off-season
- Generates employment
- Development of ready-to-consume convenient products which saves time for cooking
- Helps in improving palatability and organoleptic quality of the produce by value addition and helps in inhibiting anti-nutritional factors
- Helps in easing marketing and distribution tasks
- Enables transportation of delicate perishable foods across long distances
- Makes foods safe for consumption by controlling pathogenic microorganisms
- Modern food processing also improves the quality of living by way of healthy foods developed for special people who are allergic to certain ingredients, diabetic etc., who cannot consume some common food elements
- Food processing can also bring nutritional and food security
- Provides potential for export to fetch foreign exchange

Aim of Preservation/ Processing

Based on the perishability and the extent of preservation required, foods may be classified as:

1. ***Perishable foods*:** Those that deteriorate readily (Seafood, meat, fruits and vegetables) unless special methods of preservation are employed.
2. ***Semi-perishable foods***: Those that contain natural inhibitors of spoilage (root vegetables) or those that have received some type of mild treatment which creates greater tolerance to the environmental conditions and abuses during distribution and handling (such as pickled meat and vegetables).
3. ***Non-perishable foods*** *(shelf-stable)*: Those that are non-perishable at room temperature (cereal grains, sugar, nuts). Some have been made shelf stable by suitable means (canning) or processed to reduce their moisture content (dried fish and shellfishes, raisins). Food preservation in the broad sense, refers to all the measures taken against any kind of spoilage in food.

Live Fishery Products

There is a great demand for live fish and shellfishes, the world over. These products fetch maximum price compared to all the other forms of value-added products as it maintains the

freshness. The candidate species for live transportation include high value species, cultured grouper, red snapper, seabreams, seabass, red tilapia, reef fish, air breathing fishes, shrimp, crabs, lobster, clams, oyster and mussels. These are normally transported in air cargo maintained at low temperature in order to lessen the metabolic activities of the animals.

Chilled Fishery Products

Chilling is an effective method of maintaining the freshness of fish products. This normally involves keeping fishes in melting ice or slurry ice to maintain the fish temperature around 1- 4 ºC, which delays the enzymatic action and microbial activity, thereby extending the shelf life of the products. Traditionally, chilling is carried out using melting ice, either flake ice or crushed block ice. Of late, slurry ice has been introduced for chilling. A wide range of fish and shellfish products varying from whole, headless, peeled gutted, headless gutted fish, fillets, steaks, loins, cubes can be preserved by chilling. Shelf life of fishes from different environment has been studied by the Division extensively. Shelf life of 12-15 days has been achieved for seer fish and black pomfret. Indian Mackerel and Indian oil sardine had very short shelf life in ice (3-7 days), due to rancidity and belly bursting. Tilapia from freshwater and brackish water showed significant difference in shelf life when stored in ice. The former kept longer (14-15 days) than latter (8-10 days).

Frozen Fishery Products

Freezing is an age-old practice to retain the quality and freshness of fishery products for a long time. This involves the conversion of water present in fishery products to ice i.e., a phase change from liquid to solid phase takes place in freezing. This retards the microbial and enzymatic action by reducing the water available for their action. This involves exposing fish products to very low temperature (< -35° C) to enable freezing of free water and maintained at -18° C till it is consumed. Plate freezing, air blast freezing, cryogenic freezing and individual quick freezing are the methods adopted by the industry to preserve food products.

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. However, the fish canning industry in India is declining due to the high cost of cans. Recent innovations like polymer coated Tin Free Steel (TFS) cans provide a cheaper alternative. Studies conducted at CIFT showed that polyester-coated TFS cans are used for processing ready to serve fish products, which can be stored at room temperature for long periods. The industry can utilize these cans for processing ready to eat fish and shell fish products for both domestic and export markets. This will help in reviving the canning industry in India.

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

Dried and Salted Fishery Products

Drying is probably one of the oldest methods of food preservation. It consists of removal of water to a final desired concentration, which in turn reduces the water activity of the product, thereby assuring microbial stability and extended shelf-life of the product. In some cases, common table salt (Sodium chloride) is also used to prolong the shelf life of fish. Salt absorbs much of the water in the food and makes it difficult for micro-organisms to survive.

Smoked Fishery Products

Smoking is one of the most widely used traditional fish processing methods employed in many countries to preserve fish. The preservation effect of the smoke is a result of drying of the product during the smoking as well as due to smoke particle absorption into the flesh. The smoke particles, mainly phenolic compounds, carbonyl and organic acids, being absorbed by the product, inhibit bacterial growth on the surface of the product. The smoke particles also have a

positive effect on the taste and colour of the product and in many instances, smoking is normally practiced to improve these sensory characteristics.

Hot and liquid smoked fish chunks and masmin chunks

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 (Chang et al., 1996).

The production of battered and breaded fish products involves several stages. The method varies with the type of products and pickup desired. In most cases it involves seven steps. They are portioning/forming, pre-dusting, battering, breading, pre-frying, freezing and, packaging and cold storage. 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 involves fully automated batter and breading lines which start from portioning and end with appropriate packaging of the product. A variety of battered and breaded products can be prepared from shrimp, squid, clams, fish fillets, minced meat from low-cost fish etc. A brief profile of some important battered and breaded products is given below.

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.

Shrimp products

Battered and breaded shrimp can be prepared from wild as well as from farmed shrimp in different styles and forms. The most important among them are butterfly, round tail-on, peeled and deveined (PD), nobashi (stretched shrimp) etc. The products from farmed shrimp have indicated longer shelf life, 16-18 months compared to those from wild variety 12-14 months at –20 º C

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.

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.

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.

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.

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. Depending on the type of raw material, fish mince can have a frozen storage life up to 6 months without any appreciable quality deterioration. Generally minced fish is frozen as 1-2 kg blocks at -40 ^{0}C in plate freezers and stored in cold store at -18° C.

Surimi

Surimi is stabilized myofibrillar protein obtained from mechanically deboned flesh that is washed with water and blended with cryoprotectants. 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.

Fiberized products

Fiberized products are in great demand among the surimi based imitation shellfish products. The ingredients used in the formulation of fiberized 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. Sausages prepared from rohu mince treated with potassium sorbate had a shelf life of 16 days at refrigerated temperatures.

Emerging technologies for value addition of fish

Retort pouch processing:

As in canning, retort pouch food is sterilized after packing, but the sterilizing procedure differs. The pouches are processed in an over pressure retort. The time and temperature will be

standardized depending on the product. With the availability of retort pouches, it can function as an excellent import substitute for metallic cans**.** Besides, cost reduction retort pouch packages have unique advantages like boil in bag facility, ease of opening, reduced weight and do not require refrigeration for storage. Processed food products can be kept for long periods at ambient temperature. The energy saving is more in processing in flexible pouches compared to cans. On a comparison of total costs, including energy, warehousing and shipping, the pouch looks even more favourable. There is 30 to 40% reduction in processing time compared to cans, solids fill is greater per unit, empty warehousing is 85% smaller and weight of the empty package is substantially smaller.

Extrusion:

In order to improve the utilization of underutilized fisheries resources, there is a need to minimize the post-harvest losses, develop innovative processing technologies and utilize processing waste for industrial and human use. One such technology, which will be suitable for utilization of low value fish or by catch, is extrusion technology. Use of fish mince with cereals for extrusion process will enable production of shelf-stable products at ambient temperature. Extrusion cooking is used in the manufacture of food products such as ready-to-eat breakfast cereals, expanded snacks, pasta, fat-bread, soup and drink bases. The raw material in the form of powder at ambient temperature is fed into extruder at a known feeding rate. The material first gets compacted and then softens and gelatinizes and/or melts to form a plasticized material, which flows downstream into extruder channel. Basically, an extruder is a pump, heat exchanger and bio-reactor that simultaneously transfer, mixes, heats, shears, stretches, shapes and transforms chemically and physically at elevated pressure and temperature in a short time. At times, the extrusion cooking process is also referred as High Temperature Short Time process. In extrusion process gelatinization of starch and denaturation of protein ingredient is achieved by combined effect of temperature and mechanical shear. The conversion of raw starch to cook and digestible materials by the application of heat and moisture is called gelatinization. During extrusion the conditions that prevail are high temperature, high shear rate and low moisture available for starch may lead to breakdown of starch molecules to dextrins.

Irradiation

Irradiation is a physical treatment that consists of exposing foods to the direct action of electronic, electromagnetic rays to assure the innocuity of foods and to prolong the shelf life.

Irradiation of food can control insect infestation, reduce the numbers of pathogenic or spoilage microorganisms, and delay or eliminate natural biological processes such as ripening, germination, or sprouting in fresh food. Like all preservation methods, irradiation should supplement rather than replace good food hygiene, handling, and preparation practices.

Three types of ionizing radiation are used in commercial radiation to process products such as foods and medical and pharmaceutical devices (International Atomic Energy Agency (IAEA), radiation from high-energy gamma rays, X-rays, and accelerated electrons.

- Gamma rays, which are produced by radioactive substances (called radioisotopes). The approved sources of gamma rays for food irradiation are the radionuclides cobalt-60 (^{60}Co; the most common) and cesium-137 (^{137}Cs). They contain energy levels of 1.17 and 1.33 MeV (^{60}Co) and 0.662 MeV (^{137}Cs).
- Electron beams, which are produced in accelerators, such as in a linear accelerator (linac) or a Van de Graaff generator at nearly the speed of light. Maximum quantum energy is not to exceed 10 MeV.
- X-rays or decelerating rays, which can be likewise produced in accelerators. Maximum quantum energy of the electrons is not to exceed 5 MeV

Different forms of irradiation treatment are raduarization (for shelf-life extension), radicidation (for elimination of target pathogens) and radappertization (for sterilization). Radiation processing is widely used for medical product sterilization and food irradiation. Moreover, the use of irradiation has become a standard treatment to sterilize packages in aseptic processing of foods and pharmaceuticals. Irradiation produces some chemical changes, which, although lethal to foodborne bacteria, do not affect the nutritional quality of the food but lead to the production of small amounts of radiolytic products. Gamma irradiation has been considered as an interesting method of preservation to extend the shelf life of fish and also to reduce qualitatively and quantitatively the microbial population in fish and fish products. Irradiation doses of 2–7 kGy can reduce important food pathogens such as *Salmonella*, Listeria, and Vibrio spp., as well as many fish-specific spoilers such as *Pseudomonaceae* and *Enterobacteriaceae* that can be significantly decreased in number.

Microwave processing:

The applications of microwave heating on fish processing include drying, pasteurization, sterilization, thawing, tempering, baking etc. Microwaves are electromagnetic waves whose

frequency varies within 300 MHz to 300 GHz. Microwave heating is caused by the ability of the materials to absorb microwave energy and convert it into heat. Microwave heating of food materials mainly occurs due to dipolar and ionic mechanisms. Water content in the food material causes dielectric heating due to the dipolar nature of water. When an oscillating electric field is incident on the water molecules, the permanently polarized dipolar molecules try to realign in the direction of the electric field. At high frequency electric field, this realignment occurs at a million times per second and causes internal friction of molecules resulting in the volumetric heating of the material. Microwave heating also occur due to the oscillatory migration of ions in the food which generates heat in the presence of a high frequency oscillating electric field. Studies showed that chemical changes involved during different microwave cooking practices of skipjack tuna and will retain omega-3 fatty acids compared to frying/canning. Microwave blanching can be carried out for color retention and enzyme inactivation which is carried out by immersing food materials in hot water, steam or boiling solutions containing acids or salts. Microwave drying is used to remove moisture from fish and fishery products. Microwave drying has advantage of fast drying rates and improving the quality of product. In microwave drying, due to volumetric heating, the vapours are generated inside and an internal pressure gradient is developed which forces the water outside. Thus, shrinkage of food materials is prevented in microwave drying. One of the disadvantages of microwave drying is that excessive temperature along the corner or edges of food products results in scorching and production of off-flavours especially during final stages of drying. Microwave combined with other drying methods such as air drying or infrared or vacuum drying or freeze drying gave better drying characteristics compared to their respective drying methods or microwave drying alone.

Ohmic heating:

Ohmic heating is an emerging technology with large number of actual and future applications. Ohmic heating technology is considered a major advance in the continuous processing of particulate food products. Ohmic heating is direct resistance heating by the flow of an electrical current through foods, so that heating is by internal heat generation. Ohmic heating is defined as a process wherein electric current is passed through materials with the primary purpose of heating the object. During ohmic heating, heating occurs in the form of internal energy transformation (from electric to thermal) within the material. Therefore, it can be explained as an internal thermal energy generation technology and it enables the material to heat at extremely

rapid rates from a few seconds to a few minutes. Ohmic heating have a large number of actual and potential future applications, including its use in blanching, evaporation, dehydration, fermentation, extraction, sterilization, pasteurization and heating of foods. The microbial inactivation due to ohmic heating can be explained by the presence of electric field. The additional effect of ohmic treatment may be its low frequency (usually 50e60 Hz), which allows cell walls to build up charges and form pores. As a main consequence of this effect, the D value observed for the microbial inactivation under ohmic heating is reduced when compared to traditional heating methods. More research is needed to completely understand all effects produced by ohmic heating to food products, effects of applied electric field, the applied electric frequency during ohmic heating over different microorganisms and foods, cold spot determination etc.

Accelerated Freeze Drying

Accelerated freeze-drying is now being increasingly used for the preservation of high value food products. The product has the advantages like absence of shrinkage, quick re-hydration up to 95%, minimum heat induced damage etc. In India this technique is now applied for processing shrimp, squid rings etc. The possibilities for various ready-to-eat products based on fish and shellfish employing this technique are immense. In this, there is a speeding of the freeze-drying process, as a result of modification in the heating mechanism. Food is arranged in single layers between metal sheets or grids held in a tray. This is kept between the heating plates. When the required pressure and temperature is attained in the chamber, fluid contained within the hollow plates is heated to temperature of 60 to 100° C. The heat is conducted through the metal mesh, and trays to the product while allowing the water vapour to escape through the mesh channels to the side of the heating plates from where it is removed. Otherwise, the pressure at the food surface would increase and the ice will melt. When the ice is melted from the surface the pressure is applied to the plates using a hydraulic mechanism so that the mesh will be pressed against the surface of the fish giving more direct heat contact to the product. At the same time the temperature of the heating material is reduced since, after sublimation the surface temperature of the fish will be the same as that of heating plates (Balachandran, 2001). This method appeared to reduce the freeze-drying time appreciably from 10-12 hours to 6-7 hours, depending on the thickness of the food, temperature and pressure, and hence it is termed as accelerated freeze drying.

Infrared and Radiofrequency Processing Technologies:

Electromagnetic radiation is a form of energy that is transmitted through the space at an enormous velocity (speed of light). The heat generation in material exposed to EMR could be due to vibrational movement (as in case IR) or rotational movement (as in case of RF and MW) of molecules. Application of EMR heating is gaining popularity in food processing because of its definite advantages over the conventional processes. Faster and efficient heat transfer, low processing cost, uniform product heating and better organoleptic and nutritional value in the processed material are some of the important features of EMR processing. In conventional heating system like hot air heating, the heat is applied at the surface which is carried inwards through conduction mode of heating. In case of EMR/dielectric heating, the waves can penetrate the material to be absorbed by inner layers. The quick energy absorption causes rapid heat and mass transfer leading to reduced processing time and better product quality.

The main advantage of electromagnetic heating over conventional electric and gas oven-based heating is its high thermal efficiency in converting the electrical energy to heat in the food. In ordinary ovens, a major portion of the energy is lost in heating the air that surrounds the food, fairly a good amount escapes through the vent, besides being lost through the conduction to the outside air. In contrast, almost all the heat generated by electromagnetic radiations, which reaches the interior of the oven, is produced inside the food material itself. According to the reports the energy efficiency of EMR based systems is 40-70%, as compared to approximately 7-14% in case of conventional electric and gas ovens.

High pressure processing:

High pressure processing (HPP) is an emerging and innovative technology that has a great potential for extending the shelf-life with minimal or no heat treatment. It is also effective in preserving the organoleptic attributes of many foods. High pressure Processing is a non-thermal technology in which the food product to be treated is placed in a pressure vessel capable of sustaining the required pressure and the product is submerged in a liquid, which acts as the pressure transmitting medium. Water, castor oil, silicone oil, sodium benzoate, ethanol or glycol may be used as the pressure transmitting medium. The ability of the pressure transmitting fluid to protect the inner vessel surface from corrosion, the specific HP system being used, the process temperature range and the viscosity of the fluid under pressure are some of the factors involved in selecting the medium.

Ultrasound Processing:

Ultrasound refers to sound that is just above the range of human hearing, i.e., above frequency of 20 kHz. Ultrasound when propagated through a biological structure induces compressions and depressions of the medium particles imparting a high amount of energy to the material. The sound ranges for food applications employed can be divided into two, namely, low energy, high frequency diagnostic ultrasound and high energy low frequency power ultrasound. Low energy applications involve the use of ultrasound in the frequency range of 5-10 MHz at intensities below 1 W/cm^2. Ultrasonic waves at this range are capable of causing physical, mechanical, or chemical changes in the material leading to disrupting the physical integrity, acceleration of certain chemical reactions through generation of immense pressure, shear, and temperature gradient in the medium. Ultrasonics has been successfully used to inactivate *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes*, *Staphylcoccus aureus* and other pathogens.

Bio preservation:

Bacteriocins are a heterogeneous group of antibacterial proteins that vary in spectrum of activity, mode of action, molecular weight, genetic origin and biochemical properties. Various spices and essential oils have preservative properties and have been used to extend the storage life of fish and fishery products. Natural compounds such as essential oils, chitosan, nisin and lysozyme, bacteriocins have been investigated to replace chemical preservatives and to obtain green label products.

Application of enzymes:

Enzymes have been used for the production of various cured and fermented fish products from centuries. Because of their appreciable activity at moderate temperature, products and process have emerged that utilizes enzymes in a deliberate and controlled fashion in the field of food processing. Cold active enzymes including elastase, collagenase, chymotrypsin extracted from Atlantic cod were used in various food processing applications. The other applications of cold active enzymes include caviar production, extraction of carotenoprotein etc. Treatment with protease under mild treatment conditions extending for a few hours can result in the recovery of the proteins from fish frame or shrimp shell waste. The role of transglutaminase in surimi production is well established. The gel strength of surimi can be improved by the application of extracellular microbial transglutaminase. Lipase extracted from Pseudomonas spp. can be used to produce PUFA enriched cod liver oil. Enzymatic de-skinning of fish fillets was done by partial

denaturation of skin collagen using a gentle heat treatment followed by immersion in enzyme solution for several hours at low temperature (0-10° C). De-skinning of tuna, Herrin, Squid were also carried out by using different enzyme technology.

Conclusion

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.

Chapter 7
Basics of thermal processing technology

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Introduction

Processing and preservation of food is an important activity 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 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 1):

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 1. 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℃, 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 follows 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.

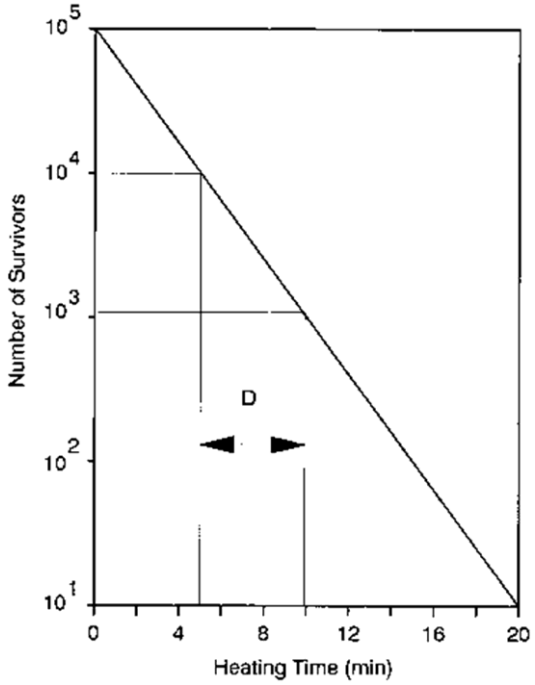

Fig 1. Survivor curve

Table 2. 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 bacteria/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 semi-logarithmic 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 semi-log 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* .

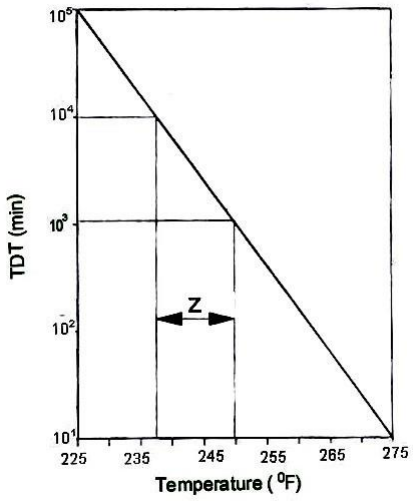

Fig. 2 TDT Curve

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:

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

where, t = time required to achieve commercial sterility

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

$F_0 = mD_{121.1°C}$

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

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

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

Thus, an F_0 value of 3 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 center 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 – 1400º C 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 from 0.19 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, e.g., 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 magnesium is the most commonly used material. Alloyed aluminium is first given an anticorrosive treatment; usually anodizing 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 polypropylene, 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

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℃ (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

Chapter 8

Non-thermal fish preservation techniques

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Non-thermal preservation of food

Conventional thermal processing results in some undesirable changes in food, such as loss of nutritional components that are temperature-sensitive, change in the texture of food due to heat, and changes in the organoleptic characteristics of food. Non-thermal food processing simply refers to methods where the food materials receive microbiological inactivation without the direct application of heat. They are relatively young technologies, which use mechanisms other than conventional heating to reduce or eliminate microorganisms. Hence it offers an alternative to conventional thermal processing.

1. High pressure processing

- High Pressure Processing is also known as high hydrostatic pressure (HHP) or ultra-high pressure (UHL) processing.
- It is a non-thermal, cold pasteurization technique, which generally consists of subjecting food, previously sealed in flexible and water-resistant packaging, to a high level of hydrostatic pressure (pressure transmitted by water) up to 600 MPa / 87,000 psi for a few seconds to a few minutes (1 – 20 min).
- HHP utilizes a very common medium, i.e., water, to apply the pressure on the product to be treated.
- HHP transmits isostatic pressure (100–1000 MPa) instantly to product at low temperature and might have comparable preservation effect as thermal processing through inactivating undesirable microorganisms and enzymes.
- An HPP unit consists of a pressure compartment in which food is kept and water is introduced into the chamber. Food is then pressurized using this water.

Major applications in seafood

1. Post pack lethality intervention for RTE seafood
   - *Cold post-packaging pasteurization*: For shelf-life extension, keeping freshness, maintaining higher sensorial qualities, functional properties and improving food safety.

2. Low pressure process application
    - *Mollusc shucking*: In HPP, the muscle, which is responsible for closing the shell, will not be able to contract and the oyster will open. This exposes the meat for easy extraction, resulting in a significant yield increase.
    - *Crustacean meat extraction*: In HPP, meat of crustaceans such as lobster or king crab will contract and detach from the shell, facilitating extraction with yield of almost 100 %.

2. Pulsed electric field (PEF) processing

- PEF is an efficient non-thermal food processing technique using short, high voltage pulses.
- It is used for inactivation of spoilage and pathogenic microorganisms in various food products. Electric pulses are applied for destroying harmful bacteria in food.
- Microbial inactivation is achieved by dielectric breakdown of the bacterial membranes
- Food material is placed between electrodes. The field intensity is typically 20–80 kV cm^{-1}) and the exposure time is a few milliseconds or nanoseconds.
- It enhances the shelf life of the food without quality loss.
- The PEF mechanism is called ***electroporation***. Very short electric pulses of high voltage are applied to the food. Small pores are formed in the cell membrane of the food by the electric pulses without damaging the cell compounds, such as vitamins.
- Pulsed electric field is generally used for liquid food or semi-solid food that can flow easily.

PEF device

- A typical PEF device consists of a food treatment chamber, a control system, and a pulse generator.
- The food is kept in the treatment chamber in between two electrodes generally made of stainless steel.

Applications of PEF in fisheries field

- PEF improves water holding properties of fish (submitting the fish muscle to PEF made its structure more porous)
- PEF technology improves extractive effectiveness to obtain protein from mussel (Improved extraction yield of protein)

- It can be used as a pre-treatment for drying
- PEF can be used to valorize by-products from fish processing industries.
- High-intensity PEF has been identified as an improved a method to extract calcium & chondroitin sulphate from fishbone.
- PEF has been tried for extraction of collagen from fish waste.
- PEF enzymatic-assisted extraction has been used for isolation of the abalone viscera protein.
- PEF can be used as a pre-treatment for fish waste for enhancing the yield of the extraction process.

3. Irradiation/Radiation processing

- Refers to the process by which an object is exposed to radiation (A deliberate exposure to radiation)
- Irradiation is a process of applying low levels of ionizing radiation to food material to sterilize or extend its shelf life.
- Radiation inactivates food spoilage organisms, including bacteria, moulds, and yeasts.
- It is effective in lengthening the shelf-life of fresh fruits and vegetables by controlling the normal biological changes associated with ripening, maturation, sprouting, and finally aging.
- Radiation also destroys disease-causing organisms, including parasitic worms and insect pests, that damage food in storage.
- Irradiation is harmful or noxious to humans. However, the dose for seafood pre-treatment is low, therefore making it safe for consumption. Food irradiated under approved conditions does not become radioactive.

Agri-food applications of irradiation

- *Radicidation and Radurization***:** Refer to these applications of less than 10 kGy doses.
- Radurization: Application of an ionization dose sufficient to preserve the quality of food by ensuring a substantial reduction in the number of spoilage bacteria.
- Radicidation: Application to the food of a dose of ionization sufficient to reduce the specific number of viable pathogenic bacteria to a level such that they are not detectable by any known method. This term also applies to the destruction of specific parasites.

- *Radappertization*: Application of high dose (10 to 60 kGy) of ionization to food in order to reduce the number and/or activity of living microorganisms so that none (except viruses) is detectable by any recognized method. Such radio-sanitized products can then be stored for up to 2 years at room temperature in sealed plastic packaging**.**

Table 1: Dose requirement in various applications of food irradiation

Dose Level	Dose	Applications
Low	<1 kGy	▪ Inhibition of sprouting of potato, onion and other tubers ▪ Insect disinfestation in stored grain, pulses and their products, dried fruits such as dates and figs ▪ Destruction of parasites in meat and meat products
Medium	1–10 kGy	▪ Shelf-life extension of fresh meat, poultry and seafood by elimination of vegetative bacteria responsible for spoilage ▪ Elimination of pathogenic organisms from meat, seafood and poultry ▪ Treatment for quarantine purposes of fruits and vegetables
High	>10 kGy	▪ Hygienization of spices, vegetable seasonings, etc. ▪ Sterilization of food for special requirements ▪ Shelf stable foods without refrigeration

4. Ultraviolet (UV) Radiation

- A very economical non-thermal technology
- Non-heat technique for decontamination for improving both the shelf-life and safety of foodstuff.
- It is basically used to reduce the microbial load on the surface of food materials that are indirectly exposed to radiation, because of its low depth of penetration.
- UV radiation is a form of energy considered to be non-ionizing radiation having in general germicidal properties at wavelengths in the range of 200–280 nm (usually termed UV-C).
- UV irradiation has demonstrated to be effective not only in reducing microbial load but also inactivating enzymes activity in plant products.

Applications in the fisheries sector

- For food products, UV-C light technology application has been mostly confined to liquids and free-flowing foods.

- UV light is used in the fish industry to decrease the microbial load and increase the shelf life of fish, reduce the microbiological load in fish meal, disinfect working surfaces, and to sterilize the water in aquaculture and wastewater facilities.
- However, to achieve a more effective reduction in bacterial load, the studies indicate that UV light should not be used as a stand-alone strategy, but integrated with other technologies.

5. Pulsed Light (PL) Preservation

- Pulsed light (PL) is an alternative technique to continuous ultraviolet treatment for solid and liquid foods.
- PL consists of successive repetition of high-power pulses of light/short time high-peak pulses of broad-spectrum white light.
- Comparatively, PL has a thousand times strength greater than the normal UV light which is quite continuous.
- Pulsed xenon UV uses the full spectrum of ultraviolet light to disperse germ-killing energy.
- The light spectrum includes wavelengths from 180 to 1100 nm with a considerable amount of light in the short-wave UV spectrum.
- Similar to other non-thermal food processing technologies, PL also has potential in the inactivation or elimination of microbes in food.
- Specific examples of foods processed by PL include fish, vegetables, fruits, and meat.
- PL can be used alongside other novel technologies as a hurdle in the inactivation of microbes on the surfaces of foods.

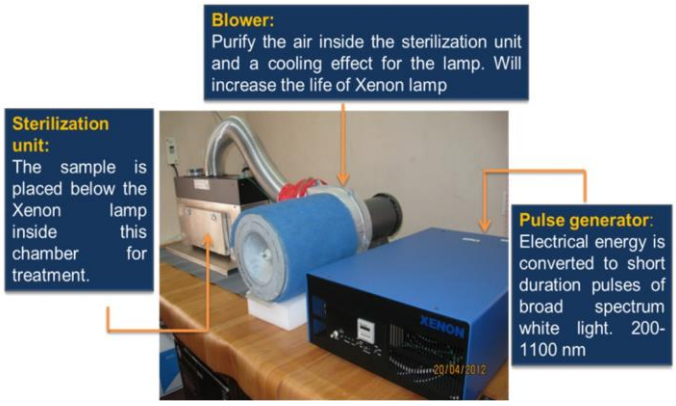

Figure 1: Pulsed Light Equipment of CIFT

6. **Ultrasound (US) processing**

- US is a compressional wave with a frequency of over 20 kHz.
- US is sound wave bearing certain frequency that is more than the normal human hearing frequency, which is more than 20 kHz.
- The frequency of US used in the food industry for microbial inactivation ranges from 20 kHz to 10 MHz.
- The bactericidal action of US is mainly due to the cavitation process, in which microbubbles are produced and collapsed within a liquid medium.
- During the cavitation process, the temperature can increase to as high as 5500 °C and the pressure can increase up to 100 MPa, resulting in localized microbial sterilization.
- The bactericidal mechanisms of ultrasound include breakage of cell walls, disruption and thinning of cell membranes and free radical activity due to the collapse of cavitation bubbles.

Method of application of ultrasound

- *Ultrasonic horn*: Horn is dipped in the liquid solution or juice and is treated with certain treatment frequency.
- *Ultrasonic bath*: Food material or packaged food is kept and the sound waves are generated in a bath that creates ultrasound effect and brings about desired changes in food.

Applications in the seafood industry

Freezing

- Improves freezing by better preservation of the microstructure; Requires less time and small crystal size; Improved diffusion & Rapid decrease in temperature.

Thawing

- Reduction in thawing time; Preserve colour; Inhibits lipid oxidation; Improved product quality & Reduced product dehydration.

Brining/Pickling

- Low water activity and longer shelf life; Require less sodium chloride & Uniform distribution of salt in less time.

Drying

- Intensification of mass transfer; Shorter processing time; Enhanced organoleptic properties & Increased drying rate due to less resistance.

7. Cold Plasma (CP) Technology

- Plasma: Fourth state of matter after solid, liquid, and gas.
- When the energy of gases crosses a certain value, it results in the ionization of gas molecules. Ionization of gas molecules gives rise to plasma.
- Two types
  - -Thermal plasma
  - -*Cold plasma* (non-thermal)
- Cold plasma is a non-thermal treatment that works in the temperature range 25–65º C.
- Cold plasma has high antimicrobial activity and efficient enzyme inactivation capacity.
- The composition of the plasma reactive species largely depends on the composition of gas which is ionized.
- The gases commonly used for the generation of plasma include argon, helium, oxygen, nitrogen and air.

Cold plasma generation

- The gases are subjected to any of the types of energy like thermal, electrical, magnetic field, etc., to generate plasma containing positive ions, negative ions, and reactive species like ozone and singlet oxygen.
- Methods
  - -Radio frequency plasma
  - -Dielectric barrier discharges
  - -Gliding arc discharge
  - -Microwave
  - -Corona discharges
- Cold plasma is an ionized gas generated through gas ionization under corona discharge, dielectric barrier discharge, microwaves or radiofrequency waves.

Advantages & Applications

- Reduction of the microbial load in food or on the surface of food. All kinds of microbes are said to be inactivated by cold plasma technology, including viruses, fungi, and bacteria.
- Enhance the physical and chemical properties of food constituents like lipids and proteins.
- Sterilization of food processing equipments.
- Inactivation of food spoilage enzymes.

- Treatment of food packaging material. Cold plasma can serve for in-package sterilization.
- Treatment of wastewater.
- Cold plasma is produced at near ambient temperature and does not depend on high temperature for microbial inactivation.
- Since the temperature used is ambient, there are no chances of thermal damage to heat-sensitive food material.
- It has continually been referred to as an eco-friendly technique since, besides having minimal changes on the food matrix, its application does not result to the generation of toxic residuals/wastes.

8. Ozone treatment

- Ozone is a colorless gas with a typical odor.
- It contains three molecules of oxygen and is chemically written as O_3. It is formed when molecular oxygen (O_2) combines with singlet O.
- Ozone is a very reactive gas, and it is very much unstable and cannot be stored and needs to be produced on the spot when needed.
- Ozone is extensively employed as an effective antibacterial against many bacteria in food. Due to its high oxidizing potential and the ability to attack cellular components, ozone has broad-spectrum of disinfection.
- Ozone treatment is a chemical method of food decontamination that involves exposing contaminated foodstuffs (fruits, vegetables, beverages, spices, herbs, meat, fish, and so on) to ozone in aqueous and/or gaseous phases.

Effect of ozone on microbes

- Ozone alters the permeability of cells by damaging the microbial cell membranes.
- Ozone is also known to damage the structure of proteins, leading to the malfunctioning of microbial enzymes, which affects the metabolic activity and finally results in microbial cell death.
- Chemical composition, pH, additives, temperature, initial bacteria population, and ozone contact time with food and food surface type are factors determining the efficiency of ozone treatment on microbial reduction in seafoods

Other methods

Acidic Electrolyte Water

- Electrolyte water (EW) is made from water without the addition of any hazardous chemicals except sodium chloride.
- EW is known as either a sanitizer (EW containing HOCl, an acidic electrolyte water) or a cleaner (EW containing NaOH, an alkaline electrolyte water.
- The simplicity of EW production and application is the foremost reason for its popularity.
- In numerous fields such as medical sterilization, agriculture, food sanitation and livestock management, EW is gaining attention because of its antimicrobial properties.

Dense phase carbon dioxide (DPCD)

- DPCD processing utilizes the liquefied carbon dioxide and performs at mild temperature and relatively low pressure, about one tenth of the pressures for HHP.
- It is applied to cold pasteurize and extend the shelf life of product without heating.
- Carbon dioxide is a nontoxic, non-flammable and low-cost gas; in the supercritical state, the fluid CO_2 rapidly penetrate porous materials due to its low viscosity ($3–7 \times 10^{-5}$ Pas) and surface tension. This penetration is accompanied with pH decrease, bicarbonate ion generation and cell disruption, which contribute to the microbial and enzyme inactivation.

High voltage electrical discharge (HVED) processing

- Different from PEF in electrode geometry, shape of pulses and mode of actions, HVED generally consists a needle electrode and a grounded one (normally flat geometry) or wire plane.
- Though the advantages of PEF and HVED are promising, the release of metals from the corrosion or migration of electrode materials should be concerned and investigated in the future applications.

Conclusion

The demand from consumer for safe and nutritious food products has promoted the rapid development of non-conventional processing technologies. With non-thermal treatments, consumers get high quality, healthy, and safe food products. But there are two sides of the coin: with advantages come some disadvantages as well. If food is exposed for a longer period or treated at a higher intensity, these non-thermal technologies may lead to some undesirable changes in food, such as oxidation of lipids and loss of colour and flavour. But these technologies have many advantages compared to thermal processing. After overcoming the limitations properly in a planned manner, non-thermal technologies will have a broader scope for development and commercialization in food processing industries.

Chapter 9

Hygienic handling, chilling and freezing of fish and shell fish

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Food hygiene relates to "all the conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain". The production of safe and quality fish and its products requires effective hygienic practices throughout the food chain from fish harvest to consumption. These hygienic measures aim at preventing or reducing fish contamination and microbial growth.

Fish is a food commodity loaded with important components such as proteins, omega-3 fatty acids, vitamins and minerals which are incredibly vital for the functions of body. However, seafood is simultaneously highly perishable and hence requires effective preservation protocols to maintain its quality and safety. Ensuring the quality of fish begins with harvest and extends throughout the post-harvest chain. Fish being highly delicate, critically requires efficient cold chain management throughout the supply chain to guarantee quality of the fresh, chilled, frozen or processed fishery products. Along the cold chain, right from the harvesting routed through onboard storage, landing centre, transportation, till domestic/export/retail marketing, the qualitative loss account for 2 -5 % while quantitative loss ranges from 3 - 17 %, being maximum during harvesting. Therefore, strengthening of the harvest practices by proper measures as well as post- harvest infrastructure facilities such as cold storage facilities, ice plants, freezing/processing units, roads and transportation, modern and hygienic wholesale and retail market outlets etc., as well as effective marketing system in identified areas are the key requirements for the development of this sector. There are, however, several constraints in handling the fish; the important among them are the bacteriological, chemical and physical processes that cause degradation of fish. Proper handling and preservation can increase its shelf life and retain its quality and nutritional attributes. The objective of handling, processing and preservation is to control or reduce the spoilage process so that the final product is wholesome and safe for the consumer. Fish and fishery products brought to market in a well-preserved condition will generally command higher prices, both at wholesale and retail levels, and thus give better returns to the fishing operation.

Effective handling and transportation can help to deliver the fish in the same condition as it is at the time of catch within the limits of practicability under good commercial practice. For this, the general and important rules to be followed include: Maintaining the fish at low temperature throughout the post-harvest chain by proper icing; Avoid mishandling of the fish; Sorting of fish, catch wise (species-wise, size-wise); Use of clean containers/surface for the holding/transportation of fish; Use of good quality water and ice; Personnel hygiene at every handling stage.

Hygienic onboard fish handling

Handling of fish starts the moment they are harvested and refers to the conditions that fish are subjected to, after harvest till conveyed on-shore. Careful and hygienic handling of fish onboard the fishing vessel can ensure enhanced longevity of fish. These mainly include proper vessel design and maintenance, cleanliness of vessel premises, workers hygiene and maintenance of cold chain. For this:

1. Vessels must be designed and constructed so as to protect fish from contamination by bilge-water, sewage, smoke, fuel or other objectionable substances.
2. Equipment, materials, surfaces and surface coatings that come into contact with fish and fishery products must be corrosion-proof, durable, non-toxic as well as easy to clean and disinfect.
3. Fishing vessels should be designed and equipped with suitable holds, tanks or containers to preserve fresh fish and fishery products throughout the fishing period.
4. Chilling devices must allow easy monitoring of temperatures.
5. Ensuring availability of potable water for washing and cleaning of fish and fishery products retained on board as well as for ice that is used to chill the samples.
6. All vessels must be kept free of pests using pest control devices.
7. Sorting and heading and/or gutting of fish must be carried out hygienically as soon as practicable after capture and the eviscerated products must be washed immediately and thoroughly with either potable water or clean seawater.
8. Crew members must maintain a reasonable standard of hygiene and prevent contamination of fish or fishery products and where appropriate, wear suitable protective clothing, head covering and footwear.

Hygienic fish handling in harbour/market

In the seafood supply chain, the first trade/sale point is the landing centres or harbours. These primary markets are the most crucial location which ensures the economic returns of fishermen as well as the availability of quality fish along the entire value chain. These domestic markets plays a very crucial role in the development of fisheries sector in the country as about 85% of the total fish landing is distributed through domestic markets. They play a major role in strengthening the nutritional and food security. Ensuring hygienic handling practices in domestic market helps to minimize post-harvest losses and leads to food safety. Following minimum basic requirements can ensure good hygiene in domestic market:

- Cleanliness of the market premises
- Availability of potable water, ice facility and cold storage facilities
- Hygienic stalls with proper roofing and flooring and portable display unit with facility for cutting and storage of fish
- Maintenance of proper hygiene by workers
- Proper drainage and waste management system
- Transportation facilities that ensure maintenance of cold chain
- Communication facilities
- Restroom and toilet facilities

Hygienic fish handling in processing units

Seafood Processing units are powerful economic drivers that has a major role in determining the domestic as well as international trade of aquatic produce. They mainly focus on value addition approaches of the fish thus improving the market value of the products. Following hygienic practices in these units will ensure improved fish quality which in turn is critical to increase marketing opportunities.

- Appropriate design and layout comprising sufficient working space under adequate hygienic conditions, an area for machinery, equipment and 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 portable 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.

Strictly following these simple but important hygienic practices can definitely ensure high quality and safe fish to the consumers.

Low temperature preservation

Proper preservation of fish assumes greater importance so that this nutritious source is delivered in prime quality to the seafood consumers. Among the various preservation methods available, low temperature preservation viz., chilling as well as freezing has attracted interest of many researchers on account of its minimal changes in the texture and other characteristics of fish upon proper processing and storage.

Chilling

Shelf stability of fish is very important for ascertaining its availability to a wide range of customers across the globe. This can be assured only by proper handling and preservation techniques. Among the various preservation techniques, chilling assures effectiveness in delaying bacterial growth and prolong the shelf life of fish. Although chilling is effective in delaying the spoilage, it will not inhibit the spoilage completely as the enzymes and bacteria will be active at the chilled temperature. The objective of chilling is to cool the fish as quickly as possible to as low a temperature as possible without freezing. The storage life of chilled fish in different forms of ice like flake ice, slurry ice, ozone-slurry ice range from almost 4 to 20 days depending on the species. Studies have indicated that for every 10^0C reduction in temperature,

the rate of deterioration decreases by a factor of 2-3. Hence higher and faster rate of temperature reduction upon capture assures better and prolonged stability of the seafoods.

The most common and cheapest means of chilling seafood is icing. Other means of chilling include: Air chilling; Use of alternative methods like chilled water viz., Refrigerated sea water (RSW), Chilled sea water (CSW), Chilled fresh water (CFW); Chilling of fish by dry ice (solid carbon dioxide), liquid nitrogen, cold ammonia or other refrigerants, etc. Chilling is a relatively short-term means of preservation when compared to other techniques like freezing, canning, salting or drying etc.

Icing is widely employed for chilled storage of marine as well as fresh water fishes as well as shell fishes. Fishes are kept in a chill store in insulated boxes with proper icing prior to pre-processing. The major advantage of using ice for chilling the fish is its high latent heat of fusion which facilitates the removal of large amount of heat from the object to be cooled. During transition from ice to water, 1 kg of ice absorbs 80 kcal of heat and this will be sufficient to cool about 3 kg of fish from ambient temperature of 30^{O}C to 0^{O}C. Hence theoretically about 30% of ice is needed to bring down the temperature from ambient conditions to 0^{o}C. However, ice is needed to maintain the temperature as well as to accommodate the heat from the environment and hence in tropical conditions, a 1: 1 fish to ice ratio is ideal for ice storage. Icing of fish is very easy as it does not involve sophistication or high level of skill. Further it's easy availability is an added advantage. However, due to lack of knowledge icing is not properly practiced during fish handling and preservation. The proper use of ice can substantially reduce post-harvest losses and improve the quality of fish. In general, icing of fish is done in three stages during the post-harvest supply chain: on board fishing vessel immediately after harvest; after landing in the landing centre or before transportation; during retail sale. For icing to be effective, standard protocols like use of good quality ice, cleaning, dressing and sorting of fish for icing, proper layering of ice and fish etc. should be ensured.

Ice is available in several forms such as blocks, plates, tubes, shells, soft, chip and flakes. To ensure maximum contact of ice with the fish, proper selection of the size of ice particles and good stowage practices are needed. Flake ice is the most popular form of ice for industrial use because of its cooling efficiency. It is also relatively dry and will not stick together to form clumps when stored. Cooling capacity is more for flake ice due to a large surface area for heat

exchange. On being smaller in size and less thickness with smooth edges, it also causes minimum damage to the flesh.

Shelf life of iced fish

Shelf life of food is defined as the maximum length of time a given product is fit for human consumption. It is the time period during which the food can be stored and displayed whilst still maintaining an acceptable quality or specific functionality. For fish, shelf life is the time from when it is taken from the water until it is no longer fit to eat. Shelf life of chill stored fish range from 4 to 20 days. The stability of fish is dependent on various intrinsic as well as extrinsic factors. Various research carried out in this aspect has derived at a few general observations which reports that in ice storage:

- Non-fatty fishes can be kept longer than fatty fishes
- White fleshed fishes can be kept longer than dark fleshed fishes
- Freshwater fishes can be kept longer than marine fishes
- Tropical fishes can be kept longer than temperate fishes
- Smaller species can be kept longer than big fishes
- Flat fishes can be kept longer than round fishes
- Thick skinned fishes can be kept longer than thin skinned fishes

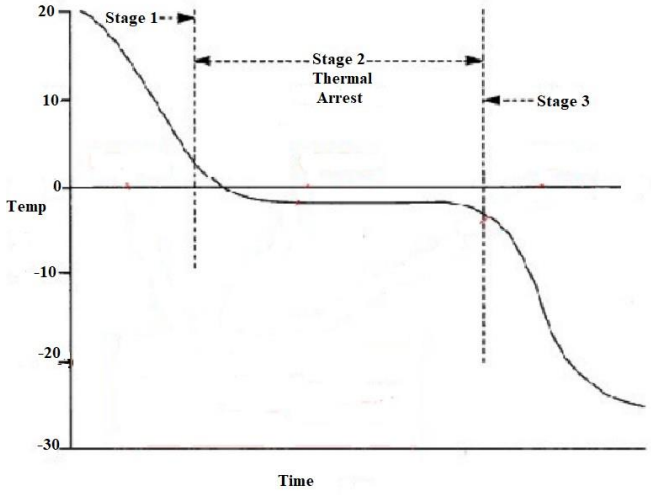
Quality Changes in fish during the chilling/icing

- *Weight loss:* The fish chilled with ice shows gradual weight loss upon storage. Losses which occur in iced fish are largely or entirely due to formation of free liquid drip. This is on account of quality changes viz., protein denaturation associated with the chilling and associated storage. Drip water carries with it a considerable percentage of soluble proteins, salts, other flavouring and nutritive components of the fish.
- *Discolouration:* Improper icing/chilling results in bruising, damage and consequent discolouration of flesh. Improper and delayed gutting of fish facilitate the powerful digestive enzymes to attack the viscera and belly walls resulting in belly burn or disruption at iced temperature which also cause discolouration. It is well known that pelagic fishes with filled digestive tract may develop torn or burst bellies well before the signs of spoilage sets in.

- *Rancidity:* In case of fatty fishes, even at low temperature of 0 to 2^{o}C, rancidity may develop on account of fat oxidation and the rancid flavour becomes a limiting factor affecting its keeping quality during storage.
- *Shrinkage:* Shrinkage is a common phenomenon in fish packed with ice, particularly in the upper layers. The shrinkage in lean fishes are higher than that of fatty fishes as the subcutaneous layer of fat serves to reduce the evaporation of tissue moisture.
- *Weight gain:* Fish stored in refrigerated and chilled seawater exhibits the tendency to gain weight and uptake salt thereby limiting the application of this chilling system in seafoods.

Freezing

Low temperature preservation like freezing is the best method to retain the quality and freshness of fish and fish products for a long time. Freezing reduces the spoilage activity and extends the shelf life of the product. It represents the main method of processing fish for human consumption, and it accounted for 55.2% of total processed fish for human consumption and 25.3% of total fish production. Freezing involves the cooling down of food materials from ambient temperature conditions to a temperature below the freezing point. Generally, the freezing process has three stages; first stage (pre-freezing stage) corresponds to removal of heat from the food, when the temperature is reduced from ambient to freezing point. The second stage which is the freezing stage, is the period of transformation of water to ice through the whole mass of food. The second stage is also referred to as the zone of maximum crystallization. Between the first and second stages there is a transitory super cooling period when the temperature falls below the freezing point which is not observed in all cases. In the third stage nearly 75% of the water in the muscle turns into ice which leads to further rapid drop in temperature, as the thermal diffusivity of ice being much higher than water.



Freezing Curve of fish

As the water in fish freezes out as pure crystals of ice, the remaining unfrozen water contains higher concentration of salts and other compounds which are naturally present in the fish muscle. The increasing concentration of the salts will depress the freezing point of the unfrozen water. Hence unlike pure water, conversion to ice will not occur at 0^0C but proceeds over a range of temperature. Thus, even at -30^0C, a portion of water in the fish muscle will remain in unfrozen state. Slow freezing produce ice crystals of comparatively larger size and few in numbers which may cause rupture of the cell walls and result in fluid loss and textural changes on defrosting. In contrast fast or quick freezing produce large number of small and uniform crystals, thus reducing the possibility of shrinkage or rupture.

The drip loss on thawing of fish occurs mainly due to denaturation of protein during freezing which result in the loss of water binding capacity of the protein. The optimum range of temperature for denaturation is -1^0C to -2º C; thus, in order to reduce the thaw drip to minimum, the time spent in this temperature zone should be minimum. If the temperature of fish/fishery product is reduced from 0^0C to -5º C in 2 hours or less, then it can be termed as a quick frozen product. During freezing process, the temperature of the fish should be lowered to - 30º C such that the thermal centre of the fish attains - 20º C prior to its removal from the freezer. The time taken to lower the temperature of the thermal centre to - 20º C is termed as the freezing time. Based on this, most of the commercial freezers operate at temperatures of -35º C to - 40º C. The major factors which affect freezing time include: Freezer type, Freezer operating temperature, Refrigeration system and operating condition, Air velocity in an air blast freezer, Product

temperature, Product thickness, Product shape, Product contact area and density, Product packing, Species of fish

Freezing systems

Freezing techniques have evolved with different modes of operation and the first man made freezing system was reported to be freezing using ice-salt mixture; followed by the developments in mechanical refrigeration. Mechanical refrigeration can broadly be classified into two: direct and indirect system wherein the direct system, the refrigerant absorbs heat directly from the material to be cooled while in indirect/ brine system, the refrigerant absorbs the heat that brine absorbs from the material to be cooled.

Based on this mode of operation, they are further classified as:

- *Freezing in Air*
- *Indirect contact freezing*
- *Spray or Immersion freezing*
- *Cryogenic freezing*

Air freezing

Seafoods can be frozen in air at temperatures ranging from –18° to – 40° C.

Sharp Freezing

Sharp freezers are cold storage rooms especially constructed to operate at and maintain low temperatures. Freezing time generally ranges from 3-72 hours or more depending on the conditions and the size of product. In this method, the product to be frozen is placed in a very cold room, maintained at temperatures in the range of –15°C to –30°C. In this system, the air within the room will circulate by convection, with little or no provision for forced convection. Hence foods placed at these low temperatures are frozen comparatively slow, taking several hours or even days for complete freezing.

Air blast freezing

In an air blast freezer, fish is frozen by circulation of a stream of high velocity cold air either in a batch or continuously, typically in a duct or tunnel at -18 to -34°C or lower, moving counter current to the product at a speed of 1-20 meter/sec.

Continuous air blast freezers/tunnel freezers: In this type of air blast freezer, the fish are conveyed through the freezer (trolleys or they may be loaded on a continuously moving belt or conveyor) usually entering at one and leaving at the other.

Batch air blast freezers: Batch air blast freezers use pallets, trolleys or shelf arrangements for loading the product. The freezer is fully loaded, and when freezing is complete, the freezer is emptied and reloaded for a further batch freeze.

Air blast freezing is economical and is capable of accommodating products of different sizes and shapes. However, it can result in excessive dehydration of unpackaged products if conditions are not carefully controlled, as well as undesirable bulging of packaged products which are not confined between flat rigid plates during freezing.

Modern designs of belt freezers are mostly based on the spiral belt freezer concept. In these freezers, a conveyor belt that can be bent laterally is used. The design consists of a self-staking and self-enclosing continuous belt for compactness and improved air flow control. The number of tiers in the belt stack can be varied to accommodate different capacities and line layouts. The products are placed on the belt outside the freezer where it can be supervised. Both packed as well as unpacked products are frozen and the freezer gives a large flexibility both with regard to product and freezing time. Both horizontal and vertical air flow can be applied and the latter is observed to be more efficient.

Fluidized bed freezing is a version of air blast freezing wherein marine products like small sized prawns, uniform sized fillets etc. can be frozen by passing through meshed belts where they are fluidized by a stream of forced cold air moving upward through the bed at a rate sufficient to partially lift or suspend the particles. Freezing by this method is rapid and a minimum air velocity of 2 meter/sec. or more is necessary to fluidize the particles and an air temperature of - 35°C is common. The bed depth depends on ease of fluidization and this in turn depends on size, shape and uniformity of the particles. A bed depth of slightly more than 3 cm is suitable for small prawns where as a depth of 20 to 25 cm can be used for non-fluidizable products such as fillets. Fluidized bed freezing has proven successful for many kinds and sizes of food products. The best results are obtained with products that are relatively small and uniform in size. Some fluidized-bed freezers involve a two stage freezing technique wherein the first stage consists of an ordinary air-blast freezing to set the surface of the product and the second stage consists of fluidized bed freezing. The advantages of fluidized bed freezing include more efficient heat transfer and more rapid rates of freezing and less product dehydration and less frequent defrosting of the equipment. Dehydration loses of about 1% have been reported during fluidized bed freezing of prawns. The short freezing time is apparently responsible for the small loss of

moisture. The major disadvantage of fluidized-bed freezing is that large or non-uniform products cannot be fluidized at reasonable air velocities.

Contact Plate Freezing

Plate freezers consist of a vertical or horizontal stack of hollow plates, through which refrigerant is pumped at - 40º C. Fish products can be frozen by placing them in contact with these metal plate surface cooled by expanding refrigerants. This equipment consists of a stack of horizontal or vertical 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. In this method the packages must be of uniform thickness. A packaged product of 3 to 4 cm thickness can be frozen in one to two hours when cooled by plates at -35°C. Freezing times are extended considerably when the package contains a significant volume of void spaces. Double contact plate freezers are commonly used for freezing foods in retail packages. This equipment may be batch, semi-automatic or automatic. Advantages of this type of equipment include good economy and space utilization, relatively low operating costs compared with other methods, little dehydration of the product and therefore minimum defrosting of condensers, and high rates of heat transfer.

Spray or Immersion freezing

Immersion freezing is a method of commercially preparing frozen foods so that the product remains suitable for consumption over a long period of time. The process helps to lock in moisture as well as maintain the flavour and taste of the processed food. 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. 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. The major advantages of liquid immersion freezing are rapid heat transfer, lower operating and investment costs and easy adaptability to continuous operations. Quick freezing preserves the texture of tissues more successfully and causes less dehydration during the freezing process. However, it is difficult to derive freezants with suitable properties.

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. The rate of freezing obtained with cryogenic methods is much greater than that obtained with conventional air-blast freezing or plate freezing, but is only moderately greater than that obtained with fluidized bed or liquid immersion freezing. Currently liquid nitrogen is used in most of the cryogenic food freezers. Usually, liquid nitrogen is sprayed or dribbled on the product or alternatively very cold gaseous nitrogen is brought into contact with the product. Freezing with carbon dioxide as well as using freon are all other means employed. Carbon dioxide is absorbed or entrained by the product in this method. This entrapped CO_2 should be removed before it is packaged in an impervious material. Further used of refrigerants like freon, though economic is being withdrawn by the industry on account of the concerns with regard to its role in ozone depletion.

Advantages of cryogenic freezing include: improved baseline production rates by reducing the amount of time required to remove heat from a product; marked increase in product yield due to less product dehydration; improved product safety and minimum product degradation due to the short freezing time; better texture retention due to formation of smaller internal ice crystals; low labour costs through reduced product handling and quicker cleanup and consistent production rates.

Crusto Freezing is a combination of cryogenic freezing system and air blast freezing system. The equipment utilizes the possibility of a fast and efficient crust freezing of extremely wet, sticky products which can then be easily handled in a spiral belt freezer or a fluidized bed freezer without deformation or breakage.

Quality changes during freezing and frozen storage

The quality of frozen-thawed cooked fish is influenced by a number of factors including species, composition, size, harvesting conditions, elapsed time between harvest and freezing, the state of rigor and quality when frozen and the details of freezing process and frozen storage. The major problems encountered during the freeze-processing of fish are oxidative deterioration, dehydration, toughening, loss of juiciness, and excessive drip. Effective pre freezing and freezing techniques are available for controlling many of these problems. Reasonable control of toughening and loss of juiciness can be accomplished by storing fish for a minimal time and / or at temperatures at -18°C or lower. Undesirable oxidative changes in fish can be minimized by (1) eliminating oxygen (2) avoiding contamination with heavy metals (oxidative catalysts) (3) adding antioxidants and (4) by using low storage temperature. Dehydration can be avoided by applying glaze and suitable protective coatings.

Cooling seafoods is among the most effective methods for preserving their quality. From a choice refrigerants, it can be chilling which facilitates short term preservation to freezing at sub-zero temperatures leading to extended storage life for months and even years, depending on temperature employed. Application of these preservation techniques with standard operating protocols can ensure superior quality seafoods to the customers.

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Chapter 10

Cured and dried fish products

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Curing is a process by which the fish is preserved by sun drying, salting, smoking, artificial drying etc. This can be done either by any single method or a combination of these methods. Simple sun drying was the widely practiced traditional method of fish preservation. By this, preservation was achieved by removing the water 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 is one of the oldest techniques for preserving fish, and it is a traditional processing method in many parts of the world. Salting is a simple method of fish preservation. Often, salting is used in combination with drying and smoking. However, if the salting process is carried out incorrectly, due to the use of poor-quality starting materials, that is, stale fish, or, the addition of insufficient salt, the product can spoil and will be lost.

Drying is the removal of water from fish, and like salting, is a very common method of preserving fish, particularly in tropical countries. When sufficient water has been removed fish will be preserved because water is essential for bacteria and enzymes to survive and work to spoil fish. Drying is often used in combination with salting and or smoking for additional preservation.

How does salt preserve fish?

Salt is a valuable agent in helping to prevent spoilage. Salt preserves by extracting water. This is called dehydration (drying), and happens because water from inside the fish is drawn out into the strong salt solution outside the fish. As the water moves out, the salt moves in, penetrating deep into the flesh of the fish. Water is essential for bacteria (germs) to grow, so if the water is removed, bacteria cannot grow. Furthermore, the spoilage bacteria do not like salty conditions. The more salt in the fish, the more they dislike it. It is important to use clean, dry salt for preserving fish. Dirty salt should not be used and if the salt is wet, it must first be dried. There are some special bacteria that like to live in salt-- these are called the salt-loving bacteria or

halophiles. They require salty conditions to grow and can easily be identified in salt because of their pink or red colour. These specialised bacteria can spoil fish, producing unpleasant smells.

Salting methods

There are two methods of salting fish-- wet salting and dry salting.

Wet salting: The principle of wet salting is to keep the fish for a long time in a solution of salt and water, otherwise known as 'brine'. Brining and pickle curing are the two methods used for wet salting. Which method is used depends on whether the product will be further processed by drying or smoking, or just preserved with salting. Brining requires the water used to be saturated with salt. To make the brine, mix four parts of clean water and one part of salt (for example, 10 litres (2 gallons) of clean, fresh water to 2.7 kg-3.6 kg (6-8lbs of dry salt) in a clean, large plastic drum. Keep adding salt to the water, until no more salt will dissolve.

The next step depends on what kind of fish you want to salt. If the fish is large, it is best to cut off the head, and gut and clean the fish before soaking it in the brine. Large fish must be cut open, and it is preferable to take out the backbone. Fish which are covered in a heavy coating of scales must be scaled. In places where the flesh is thick, slashes must be made so that the salted brine can penetrate the flesh. Very large fish should be cut into thin fillets. If the fish is small, it can be soaked after it has been gutted and gilled. After the fish has been prepared according to its size, it must be cleaned and put in the brine. Stir the mixture every 20 to 30 minutes. Brining will take as little time as 30 minutes for light salting, or up to 24 hours for medium salting. Fish for drying, smoking and canning are usually brined prior to processing.

Dry salting or Kench curing: In this method the fish is salted, but the juices, and brine (pickle) are allowed to drain away. For 2 parts of fish, 1 part of salt is needed. Layers of fish are separated by layers of salt and placed into a wooden box that has slats cut out of the sides, enabling the draining of juice. It is important to layer the fish with the first layer being flesh-side upwards, and the next layer of fish being flesh-side down. The final layer should be salt. The box has a lid placed on top of the stack and weighted to press the fish down. This encourages faster salt penetration and water removal. Salting time varies from three days to a week, depending on the type and size of fish. This method cannot be recommended for general use in the tropics as the fish are not covered by the brine or pickle and are therefore more susceptible to spoilage and insect attack. Exposure to the air and the presence of salt also encourages the rate of fat oxidation

which gives rise to discoloration and the characteristic rancid flavour. Recommended fish are barracuda, parrotfish, snapper and shark.

In dry salting, the size of salt crystals is important. Fine crystals tend to dissolve too quickly and are dragged down and drained, whereas large crystals dissolve very slowly and there is a risk of deterioration. Fine crystals and larger crystals should be combined. The fine crystals will dissolve quickly and salt will penetrate the flesh immediately. The large ones will dissolve slowly to maintain the salt's action during the whole time of salting. To store dry salted fish, first brush off all excess salt, and place fish neatly in a strong plastic bag. Seal the bag, and keep it somewhere cool, and away from sunlight. The fish should be inspected at regular intervals. If there has been a period of damp weather, and the dried fish show signs of moisture, they should be given a few hours of air drying. If signs of rust and mold appear, the fish should be scrubbed in a light salt brine containing some vinegar, then spread out to dry in the air for a day or two. The product should last for many months.

Pickle salting: Pickle curing is a type of wet salting where the fish is layered by granular salt (fish-to-salt ratio of 1-part fish to 0.3 or 0.4 parts salt by weight) which, dissolves in the surface moisture of the fish forming solution which penetrates into the fish removing moisture from the fish. The fish is allowed to remain in this self-brine. If the self-brine is not sufficient, saturated brine is added to immerse the fish. For salting, keep the fish in to flesh to flesh and skin to skin form. After each layer of fish sprinkle on a thin layer of salt. Make sure you finish with a layer of fish, skin upwards, and a final layer of salt. Water from the fish will quickly start to form. The surrounding salt will dissolve in this water. This is called the pickle. It is retained inside the container and will eventually cover all the fish. Place a clean piece of wood weighed down with clean stones on the top of the fish until salting is completed. This will take 36 to 40 hours for small, whole fish and three to four days for large pieces of fish. Wet salted fish should be consumed within 2 months of storage at an ambient temperature. It will keep for several months if stored in a cool place.

Mona curing: Mona curing is mainly adopted for medium to small size fishes. Before salting, the intestine and entrails are removed by pulling out through the gill region without split opening the fish. The flesh is not exposed during salting thereby causing less contamination and the

product has a shelf stability of about two months. The yield obtained by this method is about 70%.

Pit curing: In this method, fish is mixed with salt (4:1) and placed in pits dug on beaches. The pits may be lined with palymrah / coconut leaves. After 2-3 days of maturation, the fish is taken out for marketing in wet condition and packed in bamboo baskets and transported to markets without drying. The quality of fish cured by this technique is poor with a shelf stability of upto three weeks only.

Colombo Curing: Colombo curing is similar to pickling process which is widely practiced in Sri Lanka. A piece of dried Malabar tamarind (*Garginia cambogea*) is kept in the abdomen portion of the gutted and cleaned fish which is further stacked in airtight wooden barrels filled with brine. Fishes cured by this method has a shelf life for upto 6 months.

Type of salted fish products:

- Heavy salted fish- salt content of fish muscle is above 20g/100g water phase
- Medium salted fish - salt content of fish muscle is above 10g/100g water phase and is lower or equal to 20g salt /100g water phase
- Light salted fish - salt content of fish muscle is above 4g/100g and is lower or equal to 10g salt /100g water phase

Signs of spoilage in salted fish

1. **Reddening or pink**: The fish takes on a red colouring. This is caused by red halophilic bacteria. These organisms are usually found in solar salt.

2. **Dun**: Dun is characterised by a peppering of light brown or fawn spots on the fish. This is mainly caused by growth of halophilic mould called *Sporendonema epizoum*. During the initial stages of appearance of moulds on the fish, it is possible to remove them manually. In advanced stages it penetrates into the flesh. To avoid the mould growth, it is necessary that the fish be dried, packed and stored properly to avoid uptake of moisture. It is caused by the use of impure salt and unsanitary practices during preparation.

3. **Souring**: Fish which has soured has a bitter taste. Souring is due to improper salting which results in the uneven distribution of salt throughout the muscles of the fish.

4. Salt burn: The fish is extremely dry and cannot be rehydrated. This happens when too much fine salt has been used. This salt draws out the surface moisture so rapidly that protein in the fish becomes solid, which stops the fish from taking in water later on. A mixture of large and small grain sizes is recommended for dry salting of fish

5. Sliming: The surface of the fish acquires a slippery coating of slime. This usually occurs in brined fish because of inadequate salting, lack of freshness of fish, and other factors.

6. Case hardening: Under certain conditions, where the constant rate drying is very rapid due to high temperature and low relative humidity, the surface of the fish can become 'case hardened' and the movement of moisture from the deeper layers to the surface is prevented. This can result in a fish which is dry at surface. However, the centre remains wet and hence spoils quickly.

7. Rancidity: This is caused by the oxidation of fat, which is more pronounced in oil rich fishes like mackerel, sardine 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 hydroxyl acids, which impart the characteristic odors. At this stage the colour of the fish changes from yellowish to brown referred to as rust. This change results in an unpleasant flavour and odour to the product, leading to consumer rejection.

8. Insect Infestation: Spoilage due to insect infestation occurs during initial drying stages as well as during storage of the dried samples. The flies which attack the fish during the initial drying stage are mainly blowflies belonging to the family Calliphoridae and Sarcophagidae. These flies are attracted by the smell of decaying matter and odours emitted from the deteriorating fishes. During the glut season when the fish is in plenty and some are left to rot, these flies come and lay their eggs. These eggs develop into maggots, which bury within the gill region and sand for protection from extreme heat. and develop mainly when conditions are favourable. The most commonly found pests during storage are beetles belonging to the family Dermestidae. The commonly found beetles are *Dermestes ater, D. frischii* and *D. maculates*

9. Fragmentation: Denaturation and excess drying of fish results in breaking down of the fish during handling. Fish can become brittle and liable to physical damage when handled roughly. Insect infestation is also a reason behind fragmentation in dried samples. It is necessary that fresh fish be used as raw material to ensure a good finished product.

Prevention of spoilage in cured fish products

Raw material

- ✓ Fish must be as fresh as possible. Fatty fish is best wet salted, while lean fish is best dry salted. Take care not to damage fish during handling.
- ✓ Salt must be clean and dry. Note: mix of 1/3 small crystals:2/3 large crystals for dry salting. Fine crystals are usually better for wet salting
- ✓ Use clean water

Processing methods

- ✓ Ensure that hands, clothing, cooking utensils and work surfaces are perfectly clean.
- ✓ Take note of the time required for each step of the salting process.
- ✓ Pay attention to the amount of salt or brine/fish weight ratio.
- ✓ Containers must be clean, and possess a secure lid.

Handling of finished products

- ✓ Dry salted fish can be enclosed in clean/dry plastic bags or wrapped and secured inside dry banana leaves.
- ✓ The salted fish must be stored in a clean, and if possible, cool place. Keep it away from dust, insects, rodents and direct sunshine. Not only is it important to obtain a good product, but it is also important to keep it in good condition.

Indian Standard Specification for Common Salt for Fish Curing

Physical properties - The material shall be crystalline, white, pale pink or light grey in colour, free from visible contamination with clay, grit and other extraneous adulterants and impurities.

Microbial quality: Salt shall be free from halophilic microorganisms, the most common of them being the red halophilic bacteria.

Particle Size: The material shall be between 2.36 mm and 5.00 mm in size.

Moisture Content: The moisture content of the material shall be not more than 6.0 percent by mass

Table 1: Requirements of common salt for fish curing

Sl.No	Characteristics	Grade 1	Grade 2
1.	Matter, insoluble in water, percent by mass, Max	0.5	1.0
2.	Sodium chloride (as NaCl), percent by mass, Min	98	96
3.	Calcium and magnesium (as Ca), percent by mass, Max	0.5	-
4.	Soluble iron compounds (as Fe), parts per million, Max	10	20
5.	Matter soluble in water other than NaCl, percent by mass, Max	1.5	3.0
6.	Copper (as Cu), parts per million, Max	1	1

Drying phases of fish

1. Initially, water on or near the surface of the fish evaporates. The rate of drying depends on: i) surface area of the fish (size), ii) speed of air movement over the fish, iii) relative humidity of the air.

2. The second phase occurs when the surface of the fish has evaporated. The drying rate in this phase depends on: i) the nature of the fish. Fat in fish flesh retards water movement; ii) fish shape. The thicker the fish, the longer the time of drying; iii) temperature, iv) water content Drying will proceed more rapidly at higher temperatures; The higher the water content, the longer the time of drying

Methods of Drying

Drying of fish is most often done using sun drying or mechanical dryers. Sun drying depends heavily on the natural weather conditions since the fish is dried by heat from the sun and the air current carries the water away. Here there is no control over the operations and many a time the losses cannot be substantiated. Hence it is necessary that the operations be controlled to get a product, which has an extended shelf life, but at the same time the texture, taste and flavour is maintained. It is here that artificial driers where processing parameters are controlled gain a lot of importance. Such processes are carried out in a controlled chamber or area. Such products

have advantages over sun-dried products since they have better keeping quality and longer shelf life.

In mechanical driers, removal of water from the fish is achieved by an external input of thermal energy. This is an expensive method since there is need for fuel for heating and maintenance of the temperature. The drying chamber consists of a long tunnel in which the washed and cleaned fish is placed on trays or racks. A blast of hot air is passed over the material to be dried. After the required degree of drying the product is removed from drier and packed. These can be broadly classified into two types. In one type, the heat is transferred into the product through a hot gas, usually air. Eg. Kiln dryers, cabinet dryers, tunnel dryers and fluidized bed dryers. In the second type, the heat is transferred into the product through a solid surface, which may also be used as the cabinet for the product to be dried. E.g., drum dryer, vacuum dryer

Signs of spoilage in dried fish

1. **Case hardening**: The fish has a chalk-white appearance, and is hard and brittle. This is caused by over-rapid drying, which leads to drying out of the outside of the fish while the inside is still moist.

2. **Mould growth**: The growth of black, blue and green moulds on dried fish is evident. This is due to the high moisture content of the fish either because it was not dried properly or, because it took up moisture from the air and became sufficiently wet to let mould grow.

3. **Reddening**: As with spoiled salted fish, reddening may also occur in spoiled dried fish. Reddening is caused by the red halophiles (salt-loving bacteria) which grow on the dried fish when impure salt contaminated with these bacteria is used

Quality standards for dried fish products- Bureau of Indian standards (BIS)

Sl. No	Product	Moisture (%)	Sodium chloride (%)	Acid insoluble ash
1.	Dry salted cat fish	35	25 (min)	1.5
2.	Dry salted Dhoma	35	10-15	2
3.	Dry salted Horse mackeral	35	25-30	1.5

4.	Dry salted Thread fin	40	25	1.5
5.	Dry salted leather jacket	35	25-30	1.5
6.	Dry salted Mackeral	30	25-30	1.5
7.	Dry salted Jew fish (Ghol)	40	25	1.5
8.	Dry salted seer fish	35	25-30	1.5
9.	Dry salted shark	35	25-30	1.5
10.	Dry salted Tuna	35	20-25	1.5
11	Dried salted Anchovy	-	15 (Max)	1.5

Prevention of spoilage in dried fish

In order to prevent spoilage, care and attention must be used during

1. **Processing**: The fish used must be fresh, prepared correctly according to size, and dried under the required climatic conditions. Using drying racks above the ground will protect against pests.
2. **Transport**: When the fish has dried, it can be packaged into clean plastic bags, or dry secured banana leaves and transported. If the fish is to be sold, it can be displayed inside rat-and-insect-proof boxes, covered with mosquito netting and placed on a table. In this way, the product can be viewed by customers, without being handled.
3. **Storage:** The dried fish must be stored in a place that is free of insects and rodents. The best type of store house is raised on stilts above the ground, in a well-ventilated, shady spot.

Microbiology requirement for salted fish /dried fish

Parameters	**Limit**
Total plate count	Not more than 5 laksh/g
E. coli	Not more than 20/g
Staphylococcus aureus	Not more than 100/g
Salmonella & Shigella	Absent in 25g
Vibrio cholerae	Absent in 25g
Vibrio parahaemolyticus	Absent in 25g

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Chapter 11

Battered and Breaded Fish Products

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Battered and breaded products are convenient products having high demand among consumers as it is available for consumption in 'ready to eat' and 'ready to fry' forms. They are also known as coated products or enrobed products. As the name implies, in the coated products the meat protein component represents the core which is usually surrounded by a cereal base that forms the outer coating. In simple terms, coated products are those products in which one food material is coated with another stuff. The coating is referred to as the batter and/or bread crumbs adhering to the product after cooking. The coating increases the bulkiness of the product thus profit also increases. Consumers relish such products because of their taste and crispy texture. Ready-to-eat and ready-to-cook/fry products can be taken up well in the domestic market and frozen ready-to-cook coated products can target distant markets. The process of manufacturing of battered and breaded products can be simple home-style as well as fully automated production lines depending on the investment.

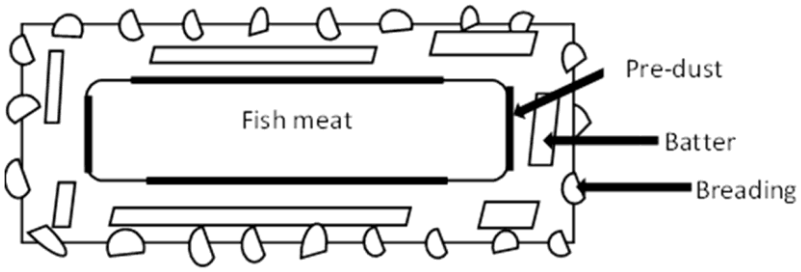

Fig. 1. A cross sectional view of battered and breaded fish products

Functions of coating

- Coating enhances the appearance of the product
- Enhance taste characteristics by providing food products with more crispy texture
- Improves the nutritional value
- Provide more desirable colour

- Act as moisture barrier & minimise moisture loss during frozen storage and microwave reheating
- Act as sealant by preventing natural juices from flowing out and seal the flavour inside

Coating process

Fig. 2. Process involved in the production of coated products

The fig. 2 represents the steps involved in the development of battered and breaded products.

1. **Portioning**

The process of **portioning** is also known as forming where the substrate is cut or shaped most economically way with minimum cutting loss to lessen processing loss in the succeeding stages. The surface area of the cut portions is an important factor to focus upon. The shapes of the portions can be oval, round, fillet shape, finger shape, triangle, ball shape, bell shape, boot shape etc. Frozen fish fillets moulded in the form of blocks is one of the common substrates for coated products.

2. Pre-dusting

Pre-dusting refers to the process of dusting the fish portions with raw flour before they are dipped into the batter. Pre-dust is the first layer applied before battering and breading. It forms an intermediate layer between the fish portion and the subsequent batter. The pre-dusting flour adheres to the surface of the portions by absorbing free water at the surface. Generally, the same flour used to make the batter is applied as pre-dust. Very fine bread crumbs alone or in combination with flour can also be used. Pre-dusting is carried out for the proper and uniform adhesion of the batter to the portion. Along with the flour, salt, spices, seasonings and flavourings can also be added to make the pre-dust. It is better to add the flavour components to the pre-dust as it can protect the volatile flavour components by embedding them under the coating layers and the amount of flavouring agents required will be less compared to seasoning the outer layers. The pre-dusting material should have good flow characteristics to minimize clump formation.

This process is not required in every product, the choice can be made depending upon the wetness of the surface, extracted proteins on the product surface and availability of the equipment in case of the automated line. If pre-dusting is required, preconditioning of the product surface is essential as a frozen surface or the presence of ice crystals will interfere with the binding of the fine flour particles to the product.

3. Battering

A **batter** can be defined as a suspension of dry ingredients used for coating the products. In short, it is the wet coating used before the process of breading. It is usually a liquid mixture composed of water, flour, starch and seasonings in which fish portions are dipped before the frying process. The function of the batter is glue or bind the product with the entire outer layer of crumbs. The main ingredients used in different types of batter include wheat flour, corn flour, proteins, gums and leavening agents. All these ingredients are not present in all batters. The starch component of the flour helps in better adhesion and the addition of modified starch can enhance the adhesion to the product. Starch also improves texture of the fried products. **Proteins** such as wheat proteins, egg proteins, dairy proteins and soy proteins etc. can be added for better adhesion and proper texture of the product. **Gums** are added to the batter for maintaining batter viscosity and water holding. Hydrocolloids like xanthan, guar and modified cellulose gums are used for this purpose. **Leavening agents** are used in the tempura batter to produce air spaces within the coating layer which subsequently gives proper crispiness to the fried products. Sodium bicarbonate is the most commonly used leavening agent in the batter for this purpose. When it gets hydrated, carbon dioxide is released and get entrapped in the batter in turn assisting to increase the volume of the final product. Furthermore, the created air spaces can influence the light reflection from the surface of the product which improves the colour and appearance.

Additionally, **flavouring agents** such as spices, salt and sugar can be added to the batter. Pepper is one of the common ingredients usually added to the batter. Natural colouring agents such as paprika can be used to enhance the red colour of the outer layer. Batter viscosity is an important parameter to give much importance as it influences the amount of batter remaining on the product and gets a consistent amount of pickup. There are three categories of batter such as adhesion batter, cohesion batter and tempura batter.

Adhesion batter: These batters are developed to adhere to the product. They are usually starch based having high solid content and low viscosity. The adhesion batter is generally made up of corn starch or modified corn starch. Adhesion batters are generally applied as a thin coat which can adhere well to the surface of the product. As it forms a thin coat, the applied batter gets dried up quickly and a significant amount stays on the product which acts as a good base for glueing the bread crumbs.

Cohesion batter: This batter is used for forming a shell or envelope around the product and acts as the base for cementing the breading. Cohesion batters are thicker flour-based batters compared to adhesion batters. They contain a medium amount of solid content compared to other batters. As it is more viscous and forms a thicker layer, the drying time required is more.

Tempura batter: This kind of batter is for making a puffed layer with a lot of air spaces around the product. Tempura batters are a type of cohesion batter with a leavening agent. In this case, the process of battering is not usually followed by breading. The products will be crispier. These kind of batters are having high solid content that usually made from a mixture of flour and starch. The viscosity is high for tempura batters.

Whenever required the dry ingredients of the batter can be mixed, further, it can be diluted with water in the ratio of 1:2 (Batter mix to water) before breading. Care should be taken to prepare a homogeneous batter without clump formation. The batter should be prepared with chilled water and while processing it should be kept in ice (5–10°C) to maintain its viscosity.

4. Breading

Generally, bread-based crumbs are used for coating followed by the process of battering. Instead of bread crumbs small potato chips or puffed grains like rice can also be used. Typically, the layer of breading is cereal-based crumbs that are baked or dried and crushed to fine, medium or large crumbs. The sticky batter helps the dry crumbs to adhere properly to the product. The process of breading is followed to enhance the appearance, and texture along with the increase in volume and weight of the product. There are different kinds of bread crumbs simple to very structured baked ones. The particle size, area-to-volume relationship, browning rate, moisture absorption, colour and texture and oil absorption are some of the important characteristics of bread crumbs that influence the functional characteristics.

Different types of breading are as follows.

1. **Flour type:** This type of crumb is available in powder forms that are more economical compared to other types. These are used when the products are intended for deep frying. In this case, the coating matrix will be very dense and a low browning rate on the surface is expected. The pick-up and weight gain in the final product will be relatively less.
2. **Traditional/ cracker type:** This type of bread crumbs are usually white or coloured crumbs having a flat-like flake structure with minimal or no crust on the surface. This type of crumbs can be made easier with the least expenses. It forms an even surface on the product as it has a fine granulation and the rate of browning achieved during frying is low. This type of crumbs is used for making fully-fried or oven-heated types of products. Moreover, they can be used in pre-dust in combination with different flours. The much denser flakes give a crunchy texture to the final product after frying.
3. **Home-style or American type:** This type of crumb has a distinct crust that gives a nice highlighting during the frying that resembles the crumbs usually consumers prepare at home. A medium to high browning rate can be achieved using these crumbs. Compared to the other types, American-style bread crumbs gives a crispier texture to the product as they have an open structure. To get more pickup, medium to large quantities may require while coating. The price of American-style crumbs is much higher than flour and cracker type but they are cheaper compared to Japanese crumbs.
4. **Japanese-style:** These types of crumbs are having an elongated spindle shape**.** It is the most expensive type of crumb usually used in full-fat fried or oven-heated high-value products. They are produced by electrical induction heating rather than the conventional baking process to have a fairly open/porous texture. The process helps to form a very light-density crumb without having a crust and also it is possible to produce large- sized crumbs without having a hard texture. Japanese-style crumbs are produced as white or coloured crumbs. Pickup of the product can be controlled from medium to high and the degree of browning to medium light to dark during frying.
5. **Frying**

   The battered and breaded products are deep-fried or flash fried after breading. The purpose of frying is to develop a golden brown colour on the surface of the product and to solidify the coating system for better adherence to the product for preventing breakage further. In the

process of flash frying, or par frying the product is fried for a very short time of 20-30 seconds (for less than 1 minute) at 190°C to cement the breading to the surface to prevent breakage and also to develop a nice brown colour. Whereas in deep frying the product is fried for a longer time at 180–200°C to get a fully cooked product. If the product is only par-fried, it must be fully cooked and if the product is deep-fried it should be reheated/ warmed before consumption.

For every coated product the pickup should be calculated as (final weight of the product-initial weight of the portion)/final weight of the product X100. Generally, the product is considered fritter in case it is having a pickup of more than 30%.

Battered and breaded fish products

Few examples for fish based battered and breaded products are as follows:

Fish cutlets

Fish cutlets are prepared by mixing cooked meat with vegetables, salt and spices followed by shaping the mix, battering and breading. The cutlets are available in different shapes like round, oval, heart shape, triangle etc. It can be stored in the freezer after coating or after flash frying.

Fish Finger

Fish fingers or portions or sticks are regular-sized portions cut from rectangular frozen blocks of fish flesh. Unlike other coated products, the fish finger is a product having 100% fish meat. Generally, after coating, it is flash-fried and stored under frozen conditions. The fish finger can be made from fillet and fish mince.

Fish Balls

Fish balls are also ready-to-eat coated products prepared from the mince of a low-cost fish. It is generally prepared by mixing the fish mince with starch, salt and spices. Small balls of around 2cm thickness are prepared from the mix and cooked in 1% boiling brine. The cooked balls are then battered and breaded after cooling.

Coated Shrimp

Coated shrimp can be prepared in different product styles like peeled and deveined, butterfly, round tail- on, cooked and peeled, nobashi etc. Both farmed and wild-caught shrimps can be used for the preparation of coated shrimps. After coating it with batter and bread crumbs it can be flash fried or deep fried.

Coated squid rings

To prepare the squid rings, cleaned squid tubes are cut into the form of rings followed by cooking in brine to make it tender before battering and breading. The flash-fried coated rings can be packed properly and stored in the freezer.

Storage

Battered and breaded products can be stored after coating or after flash frying preferably in thermoformed trays in frozen condition. These products can be stored for six months in the freezer and two weeks in the refrigerator.

Defects in battered and breaded products

1) **Excess/insufficient pickup:** This can be observed as a thick or thin coating over the products. The defect occurs when the batter viscosity is too high or too low.
2) **Uneven coating:** The use of low viscous batter is the major reason for uneven coating in the products. This will lead to insufficient breading adhering to the product. The presence of ice crystals or partial freezing can also cause uneven coating.
3) **Marriages or doubles:** It is the defect where fried products are found sticking to each other. The use of sticky batters is one of the reasons for this kind of defect.
4) **Flares and Tails:** This happens when an excess amount of batter sticks to the fried product. The use of highly viscous batter can result in this kind of defect. Excess quantities of breading attached to the products can also result in flares and tails.
5) **Dark colour:** This defect can be seen in products if proper frying temperature and frying time are not followed. Frying at a high temperature can darken the oil and also the product. Frying for too long can burn the surface of the coated products.
6) **Ballooning:** In this defect, the coating layer of the product gets cracks followed by falling off the breading after frying. This occurs when the outside coating gets hardened faster during flash frying without allowing the water vapour to escape from the product. Proper batter viscosity should be maintained to avoid this defect.
7) **Shelling:** In this defect, a hard shell is formed around the products as the hot water vapour is trapped inside the product while frying. This usually happens when thick tempura batter is used in the product. The viscosity of the batter can be maintained properly to avoid this defect. The high amount of pre-dust deposition and too much water on the substrate can be the other reasons for shelling.

Chapter 12

Protein and protein derivatives from aquatic food processing waste

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Introduction

In today's scenario, a large portion of the global population very much aware of health benefits one can achieve through consumption of aquatic food products. Particularly, fish and shellfish are highly nutritious and delicious. The demand for fish is ever increasing. On the other hand, aquatic animals like fish and shellfish are highly perishable compared to meat from land animals due to near neutral post mortem pH, low glycogen reservoir, low connective tissue content and high moisture content. Immediately after harvesting of fish (immediately after death), it undergoes various bio-chemical and microbiological changes which lead to spoilage. Hence, fish is essentially processed and preserved to make the fish available in edible condition. As a result of processing, a greater portion of raw material is discarded as waste which is biochemically equivalent to edible portion. In this chapter, the potentiality of fish waste as secondary raw materials, protein richness, waste generation during industrial processing and technology availability in producing protein and derivatives with ICAR-Central Institute of Fisheries Technology (ICAR-CIFT, Cochin) is briefed.

Secondary raw material

Aquatic food processing discards are now called as secondary raw material because of their potential for the production of high value products. For any country, to develop a systematic way to utilize or to set up an industry, the information on amount of waste generated would be the first aspect to be searched. Unfortunately, even in well developed countries, the data on waste generation from fish processing sector is not available, due to the complexity in obtaining such information. The available data are derived from the information on export quantity. However, it is essential to have information part wise, as many of the high value ingredients are derived from the specific parts (organs). The properties of derived high value products depend on the parts from which they are derived. For example, the properties of gelatin from fish skin, scale and bone are different.

Factors influencing the amount of waste generated

Fish processing sector generate two types of waste i.e. solid waste and liquid waste. Often the effluents undergo various treatments prior to discharging. Most often, the environmental issues are emerging when these discards are not properly handled/disposed particularly the solid waste creates the problem. The amount of waste generated from fish and shellfish depends on certain inherent aspects and processing related *parameters.*

Fish related parameters

- ✓ Species
- ✓ Size/Age group
- ✓ Biological nature (size of head, length of intestine, shorter fins etc.,)
- ✓ Body shape (Cylindrical, flat etc)

Process related parameters

- ✓ Style of dressing
- ✓ Style of product
- ✓ Skill of handling person
- ✓ Skill on handling the machines involved and their design
- ✓ Intended use
- ✓ Quality of raw material

Obtaining the information on waste generation is quite difficult with reference to above parameters. Hence, generating a data base for the commercially important processed fish is essential and highly useful for any nation which aims in industrial development in this sector.

Quantification of Secondary raw material of aquatic origin from India – A case study

During the financial year 2015-16, India has exported 9,45,892 MT of Seafood worth US$ 4.7 Billion (Rs. 30,420.83 crores). The quantity of export is roughly less than 1% of Indian total fish production. Today, the Indian seafood are tasted in 106 countries in the world and major markets are SE Asia, EU, USA, Japan, China and Middle East. India secured the position as a largest exporter of shrimp to USA, the 2nd largest exporter of shrimps to Europe and the 4th largest exporter of shrimps to Japan. The demand for Indian seafood products across the global consumers is increasing and the phase of Indian seafood business changes day by day. The

resource and infrastructure of the Indian seafood industry has witnessed a tremendous growth in the recent past. India has an installed processing capacity of 23,000 M.T with 506 state-of-the-art processing plants, out of which over 62% of them are EU approved plants. Almost every plant has put in place HACCP and other Quality control system on par with the best in the world to ensure highest quality output.

Table 1. Amount of waste generated (%) during industrial processing of seafood

Products	Waste Generated (%; w/w)
Shrimp products	50
Fish fillets	65
Fish steaks	30
Whole and gutted fish	10
Surimi	70
Cuttle fish rings	50
Cuttle fish whole	30
Cuttle fish fillets	50
Squids whole cleaned	20
Squid tubes	50
Squid rings	55

In the present article, for estimating the approximate raw material could have been used and waste could have been generated in the processing industry, the waste percentage was considered conservatively. The presented value of waste generation is only from industrial processing sector and excluded the waste generation during house hold preparations. Hence the countries estimate for the fish by product generation will be definitely pretty higher than the represented figure.

Table 2. Approximate estimation of fish by products generated in the processing industry

Product	Quantity (ton)	Approximated waste percentage	Raw material quantity (ton)[1]	Quantity of waste generated (ton)[2]
Frozen shrimp	373866	40%	623110	249244
Frozen fin fish	228749	50%	457498	228749
Frozen cuttlefish	65596	50%	131192	65596
frozen squid	81769	50%	163538	81769
Dried items	43320	20%	54150	10830
Live items	5493	00%	5493	0

Chilled items	33150	20%	41437.5	8287.5
Others	113949	10%	126610	12661
Total	945892		1603028.5	657136.5

[1, 2]The presented values are approximate estimation, not the actual figures.

Protein content in secondary raw material

The discards from fish/shellfish contain protein in the range of 9-27% depends on the waste parts. The tissue proteins for example the meat from head and filleting frames contains major muscle protein fractions like myosin, actin, troponin, tropomyosin etc. The skin, scale and bone contains the protein namely collagen (an integral protein moiety of connective tissues). Shrimp shell waste contains carotenoproteins.

Table 3. Categorization of seafood discards

Based on the site	**Based on physical state of waste**		**Based on the aquatic animal**	**Based on the richness of bio-chemical constituent**	**Based on the complexity**
	Solid waste	**Liquid waste (effluents)**			
• On board waste • Industrial waste • Landing center waste • Retail waste • Waste from domestic preparation	• Dark meat • Head • Skin • Scale • Fins • Frames • Visceral mass (including Air bladder and liver) • Gills • Crab shells • Shrimp head and shells • Cuttle fish bone • Squid pen • Ink sac • Cuttle fish skin Shells from oyster, mussels and clams	• Effluents consist of blood, slime, mucus, wash off (Processing units effluents and peeling shed effluents) • Surimi wash water	• Fin fish waste • Shellfish waste • Crustacean waste • Cephalopods waste • Mollusk waste	• Waste rich in protein • Waste rich in lipid • Waste rich in minerals • Waste with special molecules	• Simple waste (Scale, skin, shrimp cuticle) • Complex waste (Head waste, visceral waste, shrimp head, Squid and cuttlefish waste)

Table 4. Protein content in major fish waste parts

	Waste Parts	Protein (%)
1.	Head	11-13
2.	Back-bone/ frame	10-15
3.	Cut-offs	12-22
4.	Skin	8-12
5.	Milt	14-27
6.	Viscera	9-23
7.	Shrimp head waste	9-14%

(Source**:** Rustard, 2007

Handling of secondary raw material

Considering the importance of secondary raw material generated in seafood processing industry, the hygienic handling of raw material to be given due importance. Without proper utilization of secondary raw material, sustainability in fish processing sector will be impossible. The following points may be followed to maintain the quality based on the intended use.

- Collection of waste
- Sorting of waste parts wise and based on quality
- Washing in chilled water/chlorinated water
- Packing in a suitable packaging material
- Preservation based on the intended use (Chilling, freezing, salting and drying or any other chemical treatment)

Proteins from secondary raw material and the possible industrial products

Fish processing discards are rich in fish muscle proteins (Myosin, actin troponin, tropomyosin etc.), connective tissue proteins (Collagen and its derivative gelatin), fish enzymes, hemoproteins and carotenoproteins. The relevant industrial products which exploit the above mentioned proteins are fish protein concentrate, surimi from frame meat, fish meal, shrimp head meal, squid meal, dried fish scale and dried fish skin.

Table 5. The protein components from secondary raw material and the relevant possible industrial products

Proteins from secondary raw material	Protein rich industrial products from secondary raw material
• Fish muscle proteins (Myosin, actin troponin, tropomyosin) • Collagen • Gelatin • Fish enzymes • Hemoproteins • Carotenoproteins	• Fish protein concentrate/fish protein powder • Surimi • Fish meal • Shrimp head meal • Clam meal • Squid meal • Dried fish scale • Dried fish skin

Fish protein concentrate

Fish protein powder (FPP) is a dried fish product, meant for human consumption, in which the protein is more concentrated than in the original fish flesh. Different methods for the separation of meat from fish are employed, such as washing meat with water for two to 3 cycles and concentrating, solubilization of muscle by pH adjustment and iso-electric precipitation, solvent extraction to method to remove the fat, cooking and drying, and a combination of various methods. The raw material such as fish filleting frames, head waste, tuna red meat and belly flaps can be used to produce fish protein concentrate

Earlier studies conducted on rat have shown that fish proteins have greater cholesterol lowering ability (Ammu et al., 1989) and can protect the animal against lipid peroxidation. Fish protein reduces serum cholesterol, triglycerides and free fatty acids and increases the proportion of HDL cholesterol. In general, protein supplements claim to help weight loss and muscle building. Fish protein supplement have shown beneficial effects on blood levels of glucose and LDL-cholesterol as well as glucose tolerance and nutritional composition of body in overweight adults (Vikoren et al., 2013). In another study, dietary scallop protein completely prevented high-fat, high-sucrose-induced obesity whilst maintaining content of lean body mass and improving the lipid profile of plasma in male C57BL/6J mice (Tastesan et al., 2014).

Fish Collagen

Collagen is a structural protein found mainly in the skin and bones of all animals. Collagen is the most abundant protein originating from the animal source, comprising approximately 30% of total animal protein. It is composed of three α-chains which are intertwined to form a triple-

helix. It is present in the connective tissue matrix that makes the framework of skin, bones and joints, cornea, blood ducts, and the placenta. There are many types of collagen, but 90% of our body's collagen protein is Type-I collagen. It is found to be rich in amino acids such as glycine, valine, alanine, proline and hydroxyproline (Burghagen, 1999). Glycine constitutes one third of the total amino acid content of collagen followed by hydroxyproline and proline, which account for another one-third. Owing to this structural uniqueness of collagen molecule, there is increasing interest for the direct consumption of collagen in the form of their easily digestible derivatives. Worldwide, this interest has been taken-up by the nutraceutical industry, especially in developing countries.

Currently, collagen is used in many pharmaceutical and cosmetic products, due to its structural role and better compatibility with human body. It is commonly used in the cosmetic industry for the production of skin lotions as it forms a superior protective film to soothen and hydrate the skin. Such potential of collagen has tremendous bearing on anti-aging treatment. Apart from that, collagen has a wide range of applications in the field of cosmetic and burn surgery, especially as dermal fillers in the reconstruction of skin and bone. Collagen gels have potential clinical importance in the preparation of 'artificial skin' used in treating major wounds. Injectable collagen hydrogels have been successfully used for soft-tissue augmentation, drug delivery carriers and hard-tissue augmentation. Microfibrous collagen sheets are used as promising drug carriers for the treatment of cancer. It is also an essential component in diverse orthopedic and dental treatments. Further, collagen is recently projected as a joint mobility supplement.

Fish Gelatin

Gelatin is a soluble polypeptide obtained by denaturing the insoluble collagen. Procedures to derive gelatin involve the breakdown of cross-linkages existing between the polypeptide chains of collagen along with some amount of breakage of intra-polypeptide chain bonds. Tissues that contain collagen are subjected to mild degradative processes, i.e., treatment using alkali or acid followed or accompanied by heating in the presence of water, the systematic fibrous structure of collagen is broken down irreversibly and gelatin is obtained. It is the only protein based food material that gels and melts reversibly below the human body temperature (37°C). Gelatin possesses unique and outstanding functional properties and can be obtained in reasonable cost, make it one of the most widely used food and pharmaceutical ingredient.

Fish skins and bones can be utilized to produce gelatin, thus contributing to solve the problems of waste disposal with the advantage of value addition. The main drawback of the fish gelatins are the gels based on them tend to be less stable and have inferior rheological properties compared to mammalian gelatins. It may be noted that fish gelatin has its own unique properties like better release of a product's aroma and flavor with less inherent off-flavor and off-odor than a commercial pork gelatin, which offer new opportunities to product developers.

Fish enzymes

Fish visceral waste can serve as a source of large amount of enzymes which have potential applications in different sector starting from laundry application to pharmaceutical applications (Simpson and Haard, 1987). The nature of fish visceral enzymes is different from the enzymes found in the digestive system of terrestrial animals. Hence, they can be exploited for certain distinct applications. Fish pepsins can act even at low temperature and higher pH optimum than the pepsins from terrestrial source. Moreover, fish pepsins do not undergo autolysis at low pH (Raa, 1990). The differences in the properties of pepsins from fish and other sources could be attributed to the difference in the sequence and composition of aminoacids (Gildberg and Overbj, 1990). Fish enzymes can be used as processing aids in the following applications

- Protein hydrolysates production
- In production of caviar from a variety of fish species
- for removal of squid skin
- for cleaning of scallop
- for descaling of fish
- coagulation of milk
- Cheese production

Hemoproteins

Hemoproteins are complex proteins, composed of a protein molecule and a non-protein compound (prosthetic group). Hemoglobin and myoglobin belongs to the category of hemoproteins involves in transport of oxygen in the blood and tissues of animals, respectively. The heme portion can be recovered from blood as well as muscles discards. The recovered material may be used iron supplement or as a chemical substrate for production of the cooked

cured-meat pigment. During the production of hydrolyzates from meat, hemin could be recovered as by-product.

Carotenoproteins

Carotenoproteins and carotenoids are other classes of compounds found in the flesh and skin of fishes and in the exoskeleton of shellfish. They are not synthesized in their body. They are acquired through their food chain (Haard, 1992). Similar to hemoproteins, Carotenoids are also composed of a protein moiety and a non-protein prosthetic group. Isolation of carotenoproteins and carotenoids from shellfish processing discards has been reported (Long and Haard, 1988). Inclusion of caratenoids pigments in feed formulations of some of the aquacultured fishes and ornamental fishes shows the importance of these compounds in industrial applications (Shahidi et al., 1993).

Protein derivatives from secondary raw material

Fish protein hydrolysates (Bioactive peptides)

Apart from being highly nutritious, fish muscle proteins can be made use for preparing fish protein hydrolysates which comprises of bioactive peptides with valuable nutraceutical and pharmaceutical potentials. Fish protein hydrolysates (FPH) are the mixture of amino acids and peptides obtained by digesting proteins from fish meat or fish processing waste with proteases.The size of these peptides may range from 2 to 20 amino acid residues with the molecular masses of <6000 Da and are highly bioactive. The food derived peptides can be used as functional food ingredients or as nutraceuticals to benefit the human health and prevent disease. In this context, large pharmaceutical companies are more interested to invest in bioactive peptide research to open therapeutic prospects.

Application of fish protein hydrolysates

Nutritional application

The proximate composition of fish protein hydrolysate would vary with the raw material (head, bone, skin, viscera), type of process, type of drying, extent of hydrolysis and any other pre-treatment of raw material. The chemical composition of food materials has an important role on human health in supply of essential nutrients for maintaining prosperous health. Chemical composition of fish protein hydrolysates is important in nutrition perspective of human health.

Table 6. Proximate composition of fish protein hydrolysate

Waste Parts	Protein (%)
Moisture	< 10 %
Protein	60-90 %
Fat	<5 %
Ash	0.45-27%

(Source; Chalamaiah et al., 2010)

Amino acid composition of protein hydrolysates from different raw material produced using different enzyme source under different hydrolysis conditions expected to have variation. In general, required essential amino acids are abundant in FPH with richness in glutamic and aspartic acid content. FPH do also have non-essential amino acids. Presence of aromatic amino acid in fish frame protein hydrolysates has been reported. Studies have clearly shown that FPH from fish meat/fish waste could be an ideal source of essential amino acids (Chalamaiah et al., 2010).

Nutraceutical applications

There are fish protein hydrolysate products/peptides specifically marketed as health supplements in developed countries. These products are proven to have specific health role other than the nutritional benefit. Protein hyd5rolysates or peptides present in the hydrolysate have demonstrated to have antioxidant, anti-obesity, immune modulation, anti-coagulation, anti-microbial, anticancer and antihypertension etc. (Elavarasan et al., 2014; and Elavarasan et al., 2016).

Table 7. Commercially marketed fish protein hydrolysate products as Nutraceuticals

Product brand name	Particulars	Nutraceutical applications	Country
PROTIZEN®	Produced by enzymatic hydrolysis of white fish proteins	It is “mood food” and dietary supplement to fight against stress and its symptoms (weight disorders<comma> work pressure<comma> sleep troubles<comma> concentration difficulties and mood troubles).	UK
Amizate®	Produced from Atlantic salmon fish proteins by autolysis	Sports nutrition (supports the body’s muscle anabolism and metabolic recovery).	North America

Nutripeptin®	Manufactured by enzymatic hydrolysis of Cod fish fillet/muscle protein	It helps in the blood glucose stabilization<comma> and weight management.	UK and USA
Seacure®	Prepared by hydrolyzing deep ocean white fish proteins	Dietary supplement helps to support the cells in the gastrointestinal tract and regulate bowel functions.	US and Canada
Vasotensin®	Produced from Bonito (*Sarda orientalis*) by thermolysin hydrolysis	It supports healthy vascular function for optimal blood flow and healthy blood pressure levels.	US and Japan
LIQUAMEN®	Prepared from *Molva molva* by autolysis	Dietary supplement that helps in reducing oxidative stress<comma> lowering glycemic index and anti-stress.	UK
Stabilium® 200	Prepared from *Molva dypterygia* by autolysis	Supports the body's response to stress and provides nutritional support for memory and cognitive function.	UK
PEPTACE®	Produced from Bonito (*Sarda orientalis*) by thermolysin hydrolysis	It lowers the blood pressure by inhibiting ACE enzyme.	US and Japan
SEAGEST®	Prepared by hydrolyzing deep ocean white fish proteins	It supports the structure of the intestinal lining and promotes intestinal health.	US
MOLVAL®	Produced from North Atlantic fish *Molva molva* by enzymatic hydrolysis	Dietary supplement recommended for cholesterol equilibrium<comma> stress control and promotes good cardiovascular health.	UK

(Source: Chalamaiah et al., 2010)

Fish protein hydrolysate as a functional ingredient

Fish protein hydrolysates are soluble in wide range of pH which is an ideal characteristic helps to use in wide range of products. Protein hydrolysates have improved water holding, oil binding, emulsifying and foaming properties. However, the key factor which determine the functional properties is degree of hydrolysis. In general, extensive hydrolysis leads to loss of functionality. There is a critical degree of hydrolysis at which protein hydrolysates should be prepared with reference to particular function to be used as a functional ingredient (Elavarasan et al., 2016; Gajanan et al., 2017).

Fish protein hydrolysate as a feed ingredient and other applications

Fish protein hydrolysates (FPHs) have been used in aquaculture feeds in order to enhance the growth and survival of fish. Studies have shown that FPH has boosted the growth performance and immunological status of many culture species. The amino acid composition and the peptides present in hydrolysate are responsible for the improved growth and immunological status. FPH is also being used as a source of protein in poultry feed formulation and in pet animal foods. Other applications include FPH as a plant booster, ingredient in microbiological media and as a cryo-protectant in fish mince/surimi.

Collagen peptide/gelatin hydrolysate

Collagen peptide is alternatively known as 'collagen hydrolysate', 'gelatin hydrolysate' and 'hydrolysed collagen'. Since collagen and gelatin are high molecular weight proteins of approximately 300 kDa, it is difficult for digestion and hence becomes unavailable to human body for their biological functions. Consequently, in recent years, much attention has been paid to the development of small molecular weight peptides from the native collagen with improved biological activities. This can be achieved by the process of hydrolysis in which the native collagen/gelatin molecules are cleaved to small fragments. The hydrolysis process leads to formation of fragmenting from the collagen of about 300 kDa to small peptides having an average molecular weight of less than 5 kDa. The visible consequence of this hydrolytic transformation is the complete dissolution of resultant peptide mixture in cold water, which further widens the application prospects of collagen peptide.

Small peptides are desirable for nutraceutical and pharmaceutical applications, whereas large peptides are desirable for the functional modification of food products. Standardisation of collagen production technology is a stepping stone in the nutraceutical and health food industry. From a nutritional perspective, peptides are more bioavailable than proteins or free amino acids and at the same time, less allergenic than their native proteins (Otani et al., 1990). Apart from that collagen peptides are shown to promote the absorption of vitamins and minerals. Hence, recently combined formulations of collagen peptide with minerals and vitamins are coming up in the market. Apart from their nutritional benefits, bioactive collagen peptides possess a wide range of physiological functions including antihypertensive, antioxidative, anticancer, immunomodulatory, antimicrobial, mineral binding, antithrombotic and hypocholesterolemic effects (Gomez-Guillen et al., 2011). Enzymatically hydrolyzed collagen have shown better

biological activities compared to the peptides derived from fish muscle protein with antioxidants and antihypertensive agents.

Table 8. The protein derivatives from secondary raw material and the possible industrial products

Protein derivatives from secondary raw material	**Protein derivatives based industrial products from secondary raw material**
• Fish protein hydrolysate • Collagen peptides • Gelatin hydrolysate	• Fish silage • Flavorings • Collagen peptides • Gelatin hydrolysate • Fish protein hydrolysate • Shrimp protein hydrolysate • Fish waste paste • Cuttlefish and squid by-products paste

Conclusion

Globally, the aquatic food waste (secondary raw material) has been identified as source of high value functional ingredients. On the other hand current exploitation of aquatic food waste is happening as high volume low value products for example fish silage, fish meal, squid meal, shrimp head meal etc. The major high value protein based product from fish waste is collagen and its derivatives. The way the fish waste utilized in India needs a rattled shift in order to realize the full potential of seafood processing waste generated in India.

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Chapter 13

Marine Nutraceuticals from seafood waste

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Introduction

The marine ecosystem is still an underexploited reservoir of several bioactive compounds, having significant therapeutic and prophylactic role against a number of common lifestyle diseases. With the growing public consciousness of the health benefits of fish and seafood in general, the health food platform is now to set for the development of mainstream nutraceutical formulations. The current nutraceutical industry is familiar with a small number of marine-based nutraceuticals. Fish oil (mainly omega-3 polyunsaturated fatty acids), algal oil, shark liver oil and squalene, chondroitin salts, collagen, gelatin, collagen peptide, chitin, chitosan as well as their monomers and oligomers, peptides and related compounds, vitamins (A, particularly its precursor β-carotene, D and E), seaweed (macroalgae) and its components, protein hydrolysates and other products have become a topic of great interest for both pharmaceutical and health food industries.

It is estimated that fish processing waste after filleting accounts for approximately 75% of the total fish weight. About 30% of the total fish weight remains as waste in the form of skins and bones during preparation of fish fillets. Bio conversion of these wastes is an environmentally friendly and profitable option for the utilization of fish waste. Some viable options for generating wealth from waste through nutraceutical products are discussed in this chapter.

Options and opportunities

Generally, two different methods, mass transformation and sorting, have been developed to improve the economic value of fish wastes. Mass transformation involves the conversion of fish waste into a single product. Typical examples of transformed fish waste include fishmeal, fish oil, fertilisers, and hydrolysates such as protein hydrolysate. Alternatively, sorting involves utilising various fish body parts such as bones, guts, and fins separately to enhance their economic value. For example, sorting enables the production of specialised products such as liver oil, gelatine, omega-3, protein containing sports food and drinks, calcium, cosmetics, and pharmaceuticals. Wider acceptance and adoption of both methods could lead to significant

reductions in wastes going to landfill and reduce the damaging impact of fish wastes on the environment.

Fish protein hydrolysate:

Fish protein hydrolysates are obtained by the controlled hydrolysis of fish protein either by employing acid, alkali or commercially available proteolytic enzymes. Hydrolysates find application as milk replace and food flavouring. Enzymes like papain, ficin, trypsin, bromelein and pancreatin are used for hydrolysis. The process consists of chopping, mincing, cooking and cooling to the desired temperature, hydrolysis, sieving, pasteurizing the liquid, concentrating and drying (by vacuum or spray drying). The fish protein hydrolysate has desirable functional properties with potential applications as emulsifiers and binder agents; and can be used in place of diary based and plant-based protein hydrolysates as well as protein powders currently available in market place (Binsi et al., 2016). The yield of hydrolysate is a critical parameter which decides the economics of operation. The yield is primarily dependent on factors such as enzyme-substrate ratio, temperature, ph, hydrolysis period, enzyme used etc.

The peptides formed by the hydrolysis of fish proteins are proven to have bioactive properties like antihypertensive, antithrombotic, immune modulatory and antioxidative properties. Also, they are good source of nutritional and functional properties. A variety of nutraceuticals from FPH are commercially produced and are available in international markets. Oyster peptide extract developed by ICAR-CIFT possessed antioxidant and anti-inflammatory activities. Similarly, hydrolysate made from squilla meat effectively reduced oil absorption in breaded and battered products, when incorporated in the batter mix.

In the industrial process of preparation of hydrolysates enzyme hydrolysis process is followed. Papain, bromelein, pepsin, ficin and trypsin are used for hydrolysis. Most hydrolysates are bitter in taste. Hence flavouring agents are like cocoa, malt and sugar are used during the fortification in food preparation to mask the bitter taste. Protein hydrolysate has special application in sports medicine because its consumption allows amino acids to be absorbed by the body more rapidly than intact proteins, thus maximizing nutrient delivery to muscle tissues. Bioactive peptides are generally short peptides (3–20 amino acids) derived from proteins that can exert biological activities over and above their expected nutritional value. From a nutritional perspective, these peptides are more bioavailable than proteins or free amino acids and at the same time, less allergenic than their native proteins. Apart from their nutritional benefits, bioactive peptides exhibit a wide range of physiological functions including antihypertensive, antioxidative, opioid agonistic, anticancer immunomodulatory, antiproliferative,

antimicrobial, prebiotic, mineral binding, antithrombotic, hypolipidemic and hypocholesterolemic effects. These beneficial properties of fish protein hydrolysates may be due to the unique combination or high proportions of certain amino acids such as arginine and taurine with low levels of branched-chain amino acids found in fish meat.

***Fish collagen/gelatin/collagen peptides*:**

Collagen is the major structural protein in the connective tissue. Collagen extracted from fishes can be used in cosmetics, foods, biomedical applications etc. CIFT has developed the method for the preparation of absorbable surgical sutures from fish gut. Gelatin is the hydrolysed form of collagen with applications in development of bio degradable packaging, food and pharmaceuticals. Both collagen and gelatin are high molecular weight proteins of approximately 300 kDa, hence a considerable proportion is unavailable to human body for biological functions. Consequently, in recent years, much attention has been paid to the development of small molecular weight peptides from the native collagen with improved biological activities. This can be achieved by the process of hydrolysis in which the native collagen/gelatin molecules are cleaved to small fragments of less than 5 kDa. Currently, collagen peptides are being incorporated in a wide array of food products including protein bars, cereal bars, protein drinks, smoothies, yogurts, cold desserts, soups, cured meats etc. Nowadays, collagen/gelatin peptides have gained increasing attention as these peptides exhibit various biological activities such as antioxidant, anti-hypertensive, anti-human immunodeficiency virus, anti-proliferative, anticoagulant, calcium-binding, anti-obesity, anti-diabetic activities and postponement of age-related diseases. ICAR-Central Institute of Fisheries Technology (Cochin, India) has standardised a protocol for the extraction of collagen peptide from fish scale and bone. Further a nutritional mix based on collagen peptides was developed with a protein content of 78%. The product is mainly intended for middle aged and old people, ladies and sports-persons who needs a regular supply of collagen for healthy joints and bones. It may also be beneficial for patients suffering from osteoporosis and long-term- nursing home residents where there is a possibility of development of pressure ulcers.

Collagen peptide from fish scale and Nutritional mix formulated by CIFT

***Chitins*:**

The shrimp processing industry in India churns out more than 2 lakh tones of head and shell waste per annum, which can be economically converted to chitin and its derivatives. Chitin is the most abundant polymer next to cellulose. It is a linear polymer of N acetyl-D-glucosamine. Glucosamine hydrochloride can be produced from chitin by hydrolysis. Glucosamine hydrochloride and sulphate are at present marketed as food supplement for the treatment of osteoarthritis. It also possesses other beneficial actions in wound healing and skin moisturization. The deacetylated chitin is known as chitosan. Chitin and chitosan have various applications in agriculture such as in germination of seeds and enhanced protection against pathogenic organisms in plants and suppress them in soil to induce chitinase activity and protenase inhibition, antivirus activity, in micro encapsulation fertilizers and insecticides. The delivery of drugs and the interactions with living tissues seem to be the major topics of current research on chitosan. Other areas of interest are the antimicrobial action, nerve regeneration, cartilage and bone regeneration, skin and bone substitutes, oral delivery for wound healing etc. Carboxy methylation of chitosan imparts water-solubility to chitosan. ICAR-CIFT has recently standardised the methodology for production of chitin, glucosamine hydrochloride, chitosan and carboxymethyl chitosan. Similarly, collagen-chitosan film from fish waste, developed by CIFT has wide applications in wound dressing and dental surgery. The antioxidant chitosan derivative developed by CIFT recently was found to be useful in microencapsulating vitamins and ß carotene, so as to give a novel delivery system. Similarly, a biocompatible and biodegradable wound healing formulation, composed of microencapsulated curcumin and hydrogel composite (Succinyl chitosan-fish collagen-poly ethylene glycol) developed at ICAR-CIFT, showed significantly enhanced rate of collagen deposition and hydroxyproline content in wound tissue

on 14th day of post wounding as compared to control and standard. Apart from that, free radical mediated grafting of gallic acid, ferulic acid, vanillic acid and coumaric acid onto chitosan were optimised. All the derivatives showed good antioxidant and antimicrobial activities.

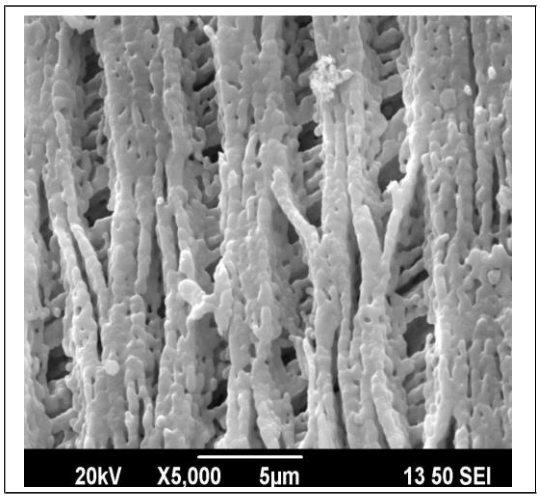
***Fish calcium*:**

In marine ecosystem, there is a large amount of calcium, mainly in the form of calcium carbonate and calcium phosphate, distributed as skeletal elements of teleosts, exoskeletal elements of molluscs or as coral deposits. Every year a considerable amount of total fish catch is discarded as processing left overs and these include trimmings, fins, frames, heads, skin and viscera. The bone fraction, which comprises approximately 15-20 % of the total body weight of fish has high calcium content. Calcium and phosphorus comprise about 2 % (20 g/kg dry weight) of the whole fish. Generally, fatty fish have lower ash levels compared to lean species. The filleting wastes of tuna and other bigger fishes are very good sources for calcium when the quantity of calcium is concerned. Also, the bone structure differs between species since a large number of teleosts have acellular bone (bone without enclosed osteocytes). Cellular bones are confined to only a few fish groups, e.g. Salmonidae. The higher surface to volume ratio in acellular fish bone is likely to increase the calcium availability compared to cellular bone. The ash content is highest in lean fish species with acellular bones. Apart from that exoskeleton of mollusks and coral deposits are excellent source of calcium. However, the calcium forms these deposits are mainly in the form of calcium carbonate. Central Institute of Fisheries Technology, Cochin has optimized the process to extract from fish bone which is mainly treated as processing discards during filleting operation of larger fishes, viz tuna, carps etc. The calcium powder was supplemented with vitamin D which is known to enhance absorption and bioavailability of calcium in the body. *In vivo* studies conducted at CIFT in albino rats have shown that fish calcium powder supplemented with vitamin D has improved the absorption and bioavailability.

Calcium extracted from Tuna bone

***Hydroxyapatite (HAp)*:**

Hydroxyapatite is the major mineral component of bone tissue and teeth, with the chemical formula of $Ca_{10}(PO4)_6(OH)_2$. The composition Hap derives from biological sources differs from that of synthetic hydroxyapatite, due to the presence of several ionic substitutions in the lattice, such as CO_3, F, Mg^{2+} and Na^+. It is a member of the calcium phosphate group with 1.67 stoichiometric of Ca/P ratio. It is one of the few materials, classified as a bioactive biomaterial that supports bone in growth and osseointegration when used in orthopedic, dental and maxillofacial applications. Fish bone and scale is a rich source of hydroxyapatite. The hydroxyapatite content of fish skeleton may vary between 40-60%. Generally, very high heat treatment is used for extraction of HAp from bone and this temperature gives a higher strength to HAp structure. The high temperature also burns away any organic molecules such as collagen protein. Hydroxyapatite, found in fish is chemically similar to mineral components of bone and hard tissues in mammals. Approximately, 65-70% of the fish bone is composed of inorganic substances. Almost all these inorganic substances are hydroxyapatite composed of calcium, phosphorous, oxygen and hydrogen.



Hydroxyapatite derived from fish scale

Squalene:

Squalene is a highly unsaturated hydrocarbon present in the liver oil of certain species of deep-sea sharks mainly Centrophorus and Squalidae spp. The liver oil of these species contains high percentage of squalene (90%) which can be isolated and purified and can be used as a dietary supplement. It belongs to a class of antioxidant molecules called isoprenoids. Squalene is found

to be a proficient chemo preventive agent against lung metastasis in mice bearing lung carcinoma. Squalene revives damaged body cells and aids to revitalize cell generation. Its chief attribute is the protection of cells from oxidation reactions. Squalene assists to clean, purify, and detoxify the blood from toxins, facilitating systemic circulation. It purifies the gastrointestinal tract and kidneys, causes better bowel movement and urination. Squlalene helps in regulating the female menstrual cycle and also improves irregular and abnormal cycles.

Taurine:

Taurine is a sulfur-containing non-protein amino acid (2-aminoethanesulfonic acid), with multiple functions like neurotransmission, cell volume regulation, stabilization of cell membranes and in the transport of ions such as calcium, sodium, potassium and magnesium. Taurine is one of the most abundant amino acids in the brain, retina, muscle tissue, and organs throughout the body, and taurine deficiency is associated with cardiomyopathy, retinal and tapetum degeneration, renal dysfunction, immune deficiency, muscle atrophy, developmental abnormalities, premature aging, and impaired reproduction. It can be synthesized from methionine and cysteine with the help of vit B6. The importance of taurine in biological system has only been recognized in the recent past and is now considered as a 'conditionally essential amino acid' having key functions in the visual pathways, the brain and nervous system, cardiac function, and cholesterol metabolism. The osmoregulatory role of taurine in facilitating the passage of sodium, potassium, calcium and magnesium ions into and out of cells, thereby stabilizing the structural and functional integrity of cell membranes was well discussed in earlier reports. It is involved in detoxification of xenobiotics and also essentially required for efficient fat absorption and solubilization. Taurine has a protective effect on the tissue damage that results from oxygen free radicals in mercury induced toxicity. It plays a crucial role in prenatal and infant development. Epidemiological studies have shown that increased taurine intake is associated with diminished risk of hypertension. The deficiency of taurine does not impose immediate health issues, however long-term deprivation can affect a multitude of metabolic pathways. It is a key ingredient of bile and has a major role in the maintenance of normal gastrointestinal development and functions. Taurine is found in greater concentrations in all animal products. Meat, breast milk, dairy products, and fish are good sources of taurine. Shell fish contain higher concentration of taurine compared to that of fin fish. Zhao et al. (1998) determined the taurine concentration of a variety of common marine fish species and reported

the highest content in crustacean and molluscs, ranging from 300-800 mg per 100 g meat. Apart from that red algae are considered as a good edible source of taurine. A possible beneficial action of taurine against Parkinson's and Huntington's disease by attenuating oxidative stress and apoptosis is proposed. Even though, the cellular and biochemical mechanisms mediating the actions of taurine are not fully revealed, mounting evidences suggest that taurine might be a key functional ingredient for use as a nutritional supplement to protect against oxidative stress, neurodegenerative diseases, atherosclerosis and hypertension.

Glucosaminoglycans:

Glucosaminoglycans (GAGs) are linear polysaccharides with repeating sequences of disaccharides consisting of an amino sugar (*N*-acetylglucosamine, or *N*-acetylgalactosamine) and uronic acid (glucuronic acid or iduronic acid) or galactose. The major members of GAGs are hyaluronic acid or hyaluronan (HA), keratin sulfate (KS), chondroitin (CS), dermatan sulfate (DS), heparin and heparin sulfate (HS). HA is a high molecular weight molecule, typically with 2 -10 x 10^7 Da and 2–25 μm chain length, whereas, other GAGs are short-chain molecules with of less than 50 kDa, more commonly 15–20 kDa. Hyaluronan lacks sulfate groups and is not covalently linked to protein, but the rest of the glycosaminoglycans are covalently linked to a protein core and contain sulfates at various positions. Dermatan sulphate is distinguished from chondroitin sulfate by the presence of iduronic acid. Keratan sulfates contains sulfated galactose and *N*-acetylglucosamine in place of uronic acids. GAGs are primarily considered as the components of various structural and connective tissues. Apart from the structural role, GAGs have been found to be associated with the regulation of a number of proteins, including chemokines, cytokines, defensins, growth factors, enzymes, proteins of the complement system and adhesion molecules. Apart from that, a few members like heparin possess anticoagulant, and anti-inflammatory properties. Dermatan sulfate (chondroitin sulfate B), also has a range of biological properties, although it has not yet been considered for therapeutic purposes. Marine heparin extracted from shrimp and sea squirt has proven anti-inflammatory properties.

Pigments*:*

Astaxanthin, fucaxanthin, melanin etc. from different fish resources are found to have a variety of bioactive properties. The filleting discards of salmonids and the shell wastes of crustaceans contain significant amounts of carotenoid pigments such as astaxanthin and canthaxanthin. The protective role of carotenoids against the oxidative modification of LDL cholesterol could be explored by incorporating in health drinks. Carotenoids are also highly sought after as natural food colours. Cephalopod ink is

another less tapped reservoir of a range of bioactives having therapeutic and curative values. It is an intermixture of black pigment melanin, glycosaminoglycans, proteins, lipids, and various minerals. Cephalopod ink has been reported to have anti-radiation activity, antitumor activity, immunomodulatory activity, procoagulant function and so on. The pigment melanin can be used both as a natural colorant as well as antioxidant, in addition to a number of other therapeutic and prophylactic properties including anticancer, antihypertensive, Anti IDA etc.

Melanin:

Cephalopods comprising mainly squids and cuttlefishes form an important resource of world oceans and their economic importance is growing exponentially. Consequently, cephalopods have emerged in recent years as an important component of the marine products, and are considered as a major delicacy in export markets. While several products (fillets, tubes, rings etc.) are made from cuttlefish, squid and octopus, considerable quantity, including the ink sac is disposed as waste. Interestingly, the cephalopod ink was identified as the most useful resource for the commercially important pigment melanin. Basically, squid ink is an intermixture of melanin, proteins, lipids, carbohydrates, glycosaminoglycans, various minerals etc. The predominant components are melanin and protein-polysaccharides complex. Each ink sac of sepia has ~1 g of melanin, and melanin constitutes ~15 % of the total wet weight of ink with other proteins.

The basic structure of melanin comprises of covalently linked indole structure (Takaya and others 1994). Melanin performs a number of biological functions in the body, the main function being to protect the organism from harmful agents such as ultraviolet (UV) radiation; melanin is capable of dissipating over 99% of absorbed UV light. Besides, in the biological system, melanin plays a vital role in providing mechanical strength and protecting proteins from degradation. Numerous reports published in last thirty years reveal the therapeutic, prophylactic and curative value of cephalopod ink. The anti-ulcerogenic properties and anti-inflammatory activity of squid melanoprotein against paw edema was demonstrated in 80’s by Mimura et al. through a series of rat model studies. Later on, several researchers confirmed the effect of squid melanin on both phenylbutazone induced ulceration in gastric mucosa and secretion of gastric juice in rats. Apart from that, melanin has been reported to have radio-protective activity, antitumor activity, immunomodulatory activity, procoagulant function and so on. Natural melanin has been reported to have defense activity, protection function and metal chelating ability. It could participate in physiological and pathological activities in human body and even

in the treatment of Acquired Immune Deficiency Syndrome (AIDS). A new generation photo-thermal dopamine-melanin colloidal nanospheres was developed by Liu et al. (2012) which could efficiently damage tumour cells at low power density and short duration, without damaging healthy tissues. Melanin also functions as photoprotective and chemoprotective pigment, protecting the body from damaging radiations, as observed at an effective dose of 50 mg/kg body weight in mice model. Similarly, oral administration of melanin for protection against radiation was reported by Dadachova et al (2016). The protective activity of melanin is primarily attributed to the inhibition of radiation-induced hematopoietic damages. Several other physiological studies conducted on squid ink also revealed significant effects on granulopoiesis of hemopoiesis impaired mice induced by ^{60}Co γ irradiating or cyclophosphamide, but has no effect on erythropoiesis. Melanin has been widely and conventionally used as an antioxidant and natural colorant in food formulation. The most interesting thing is that melanin can be used as food additives to prevent the rancidity caused by the presence of bacteria by quenching the bacterial quorum sensing. Squid melanin was reported to have hemopoietic function in Iron Deficiency Anaemic rats, which might be exploited as a safe, efficient new iron tonic. Deficiency of melanin is associated with disorders such as vitiligo and oculocutaneous albinism. Interestingly, melanin is thought to play a protective role against the age-associated and notice-induced hearing loss. Recently, the anti-ageing property of melanin was demonstrated in mice model, suggesting its use in neutracosmetic formulations. Even though melanin is a part of normal human diet, research on dietary intake of melanin is not much explored.

Melanin from cuttlefish ink

Marine algae

Algae, in particular, are virtually fat and calorie-free, making them increasingly sought for commercial purposes. Macroalgae, generally referred as seaweeds, have been found to be good sources of dietary fiber and carotenoids with antioxidant activity and play important roles in the prevention of neurodegenerative diseases. Several bioactive compounds have been isolated from brown algae with different pharmacological activities such as cytotoxic, antitumor, nematocidal, antifungal, anti-inflammatory and antioxidant. Algins, carrageenans and agar are examples of polysaccharides derived from algae that are widely used as thickeners and stabilizers in foods as well as for gels. Sulphated fucans, carrageenans and ulvans, have been known to act as modulators of coagulation as well as reveal antithrombotic, anti-inflammatory, antioxidant, anticancer and antidiabetic activities, among. Soluble polysaccharides from algae have tremendous potential as dietary fiber for human nutrition and are being evaluated as new possible prebiotic compounds. Microalgae are considered important producers of some highly bioactive compounds found in marine resources; they can be used to improve food nutritional profile due to their richness in PUFAs and pigments such as carotenoids and chlorophylls.

Chapter 14

Role of statistics in research

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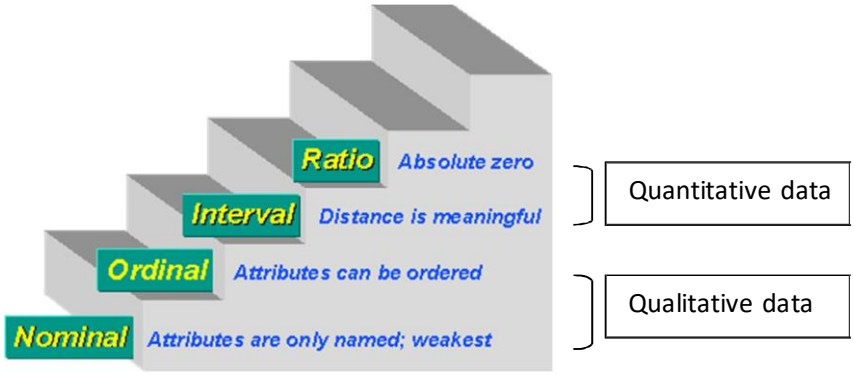
Descriptive Statistics

Statistics is a set of procedures for gathering, measuring, classifying, computing, describing, synthesizing, analyzing, and interpreting systematically acquired data. The data can be collected either in qualitative or quantitative in nature.



Descriptive Statistics gives numerical and graphical procedures to summarize a collection of data in a clear and understandable way. Inferential statistics provides procedures to draw inferences about a population from a sample.

Types of Descriptive Statistics

1. Graphs & Frequency Distribution: It summarizes the distribution of individual observations or range of values in a given set of observations.
2. Measures of Central Tendency: It computes the indices enabling the researcher to determine the average score of a given set of data.
3. Measures of Variability: It computes indices enabling the researcher to indicate how a given set of data spread out.

Frequency Distribution

Frequency distribution organizes raw data or observations that have been collected. Frequency distribution can be computed for grouped as well ungrouped set of data.

Ungrouped Data

Listing all possible scores that occur in a distribution and then indicating how often each score occurs

Grouped Data

Combining all possible scores into classes and then indicating how often each score occurs within each class. It is easier to see patterns in the data, but lose information about individual scores.

For making a frequency table following Guidelines should be followed

- Intervals should not overlap, so no score can belong to more than one interval
- Make all intervals of the same width
- Make the intervals continuous throughout the distribution (even if an interval is empty)
- Use optimum class intervals
- Choose a convenient interval width

Graphical Display

Graphical display is used to depict certain characteristics and trends in a given set of data

Graphs for quantitative data

- Histogram
- Frequency Polygon
- Graphs for qualitative data
- Bar Chart
- Pie Chart

Histogram and Frequency Polygon

Histogram consists of a number of bars placed side by side

- The width of each bar indicates the interval size
- The height of each bar indicates the frequency of the interval
- There are no gaps between adjacent bars
- Continuous nature of quantitative data

A frequency polygon represents the shape of the data. It can be conceptualized by connecting the midpoints of the classes at the height specified by the frequency.

Bar Graph

- The qualitative data is summarized in a frequency, relative frequency, or percent frequency distribution
- On the horizontal axis, the labels used for each of the classes are specified
- On the vertical axis, frequency is specified
- The bars are separated to show that each class is a separate category

Pie Chart

- Commonly used graphical device for presenting relative frequency distributions for qualitative data

- Use the relative frequencies to subdivide a circle (360^0) into sectors that correspond to the relative frequency for each class
- A class with a relative frequency of 0.25 would take $0.25(360) = 90^0$ of the circle

Measures of Central Tendency

The central tendency of a distribution is an estimate of the 'centre' of a distribution of values of a given set of distribution. The major measures of central tendencies are

1. Mean
2. Median
3. Mode
4. Harmonic mean
5. Geometric mean

The mean is the arithmetic average of data values. It computes by adding up the observations and divide by total number of observations. It is the most commonly used measure of central tendency and it is affected by extreme values (outliers).

The median is the "middle most observation" in a given set of observations. If n is odd, the median is the middle number and if n is even, the median is the average of the 2 middle numbers. Median is not affected by extreme values.

The mode is the most frequently observation in a given set of observations. Mode is not affected by extreme values.

The harmonic mean is the average of the reciprocal of the observations

The geometric mean is the n^{th} root of the products of the observations

Averages or measure of central tendency are representatives of a frequency distribution, but they fail to give a complete picture of the distribution. Measures of central tendency do not tell anything about the scatterness of observations within the distribution.

Measures of Dispersion

Measures of Dispersion quantify the scatterness or variation of observations from their average or measures of central tendencies. It describes the spread, or dispersion, of scores in a distribution. The three most commonly used measures are

a) Range
b) Variance
c) Standard Deviation

Range is the simplest measure of variability and it is the difference between the highest and the lowest observation in a given set of data. It is very unstable and unreliable indicator.

Range= H-L

Variance measures the variability of observations from its mean. It computes the sum of squared difference between observations and mean. Standard Deviation is the square root of variance.

$$\sigma^2 = \frac{\sum (X - \mu)^2}{N}$$

Measures of Relative Dispersion

Suppose that the two distributions to be compared are expressed in the same units and their means are equal or nearly equal, then their variability can be compared directly by using their S.Ds. However, if their means are widely different or if they are expressed in different units of measurement, SDs cannot be used as such for comparing their variability. In such situations, the relative measures of dispersions can be used.

The coefficient of variation (C.V) is a commonly used measure of relative dispersion and it is ratio of SD to the Mean multiplied by 100.

C.V. = (S.D / Mean) x 100

The C.V. is a unit-free measure and it is always expressed as percentage. The C.V. will be small if the variation is small. Of the two groups, the one with less C.V. is said to be more consistent.

Graphical Representation of the data

In a graphical representation the data is represented by symbols, such as bars in a bar chart, lines in a line chart, or slices in a pie chart. A chart can represent tabular numeric data, functions or some kinds of qualitative structures. Graphs make it easier to see certain characteristics and trends in a set of data

The Graphs for quantitative data are

- Histogram
- Frequency Polygon

The Graphs for qualitative data are

Bar Chart

Pie Chart

Histogram and Frequency Polygon

A histogram is a graphical representation showing a visual impression of the distribution of data. It is an estimate of the probability distribution of a continuous variable. A Histogram consists of a number of bars placed side by side

- The width of each bar indicates the interval size
- The height of each bar indicates the frequency of the interval

- There are no gaps between adjacent bars
- Continuous nature of quantitative data

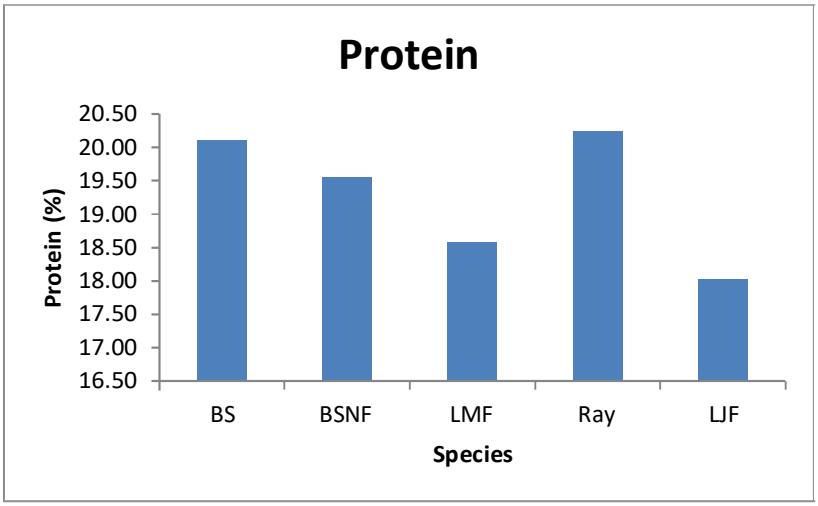
A frequency polygon represents the shape of the data. It can be conceptualized by connecting the midpoints of the classes at the height specified by the frequency.

Example of histogram

Bar Graph

A bar graph is a chart with rectangular bars with lengths proportional to the values that they represent. The bars can be plotted vertically or horizontally. A vertical bar chart is sometimes called a column bar chart.

- The qualitative data is summarized in a frequency, relative frequency, or percent frequency distribution
- On the horizontal axis, the labels used for each of the classes are specified
- On the vertical axis, frequency is specified
- The bars are separated to show that each class is a separate category

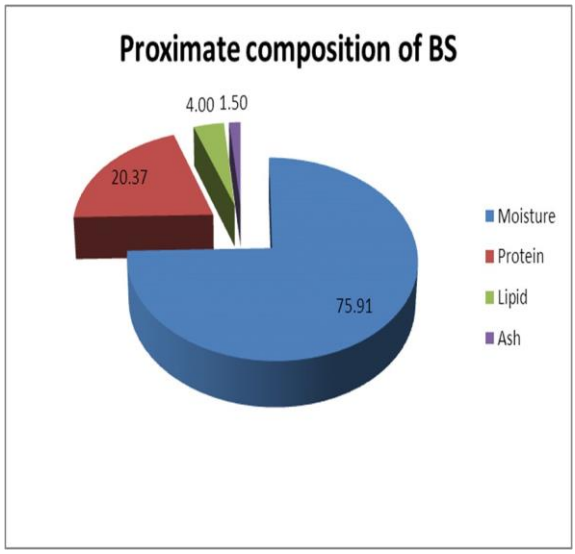


Pie Chart

A pie chart (or a circle graph) is a circular chart divided into sectors, illustrating proportion. In a pie chart, the arc length of each sector (and consequently its central angle and area), is proportional to the quantity it represents.

- Commonly used graphical device for presenting relative frequency distributions for qualitative data
- Use the relative frequencies to subdivide a circle (360^0) into sectors that correspond to the relative frequency for each class

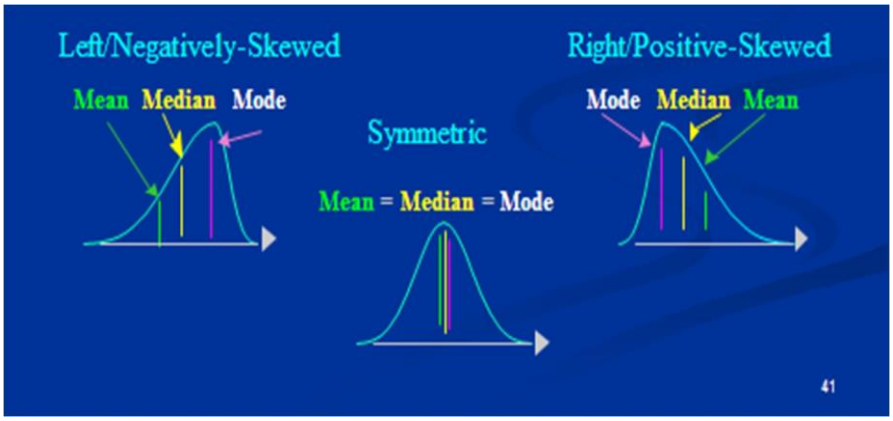
- A class with a relative frequency of 0.25 would take $0.25(360) = 90^{0}$ of the circle

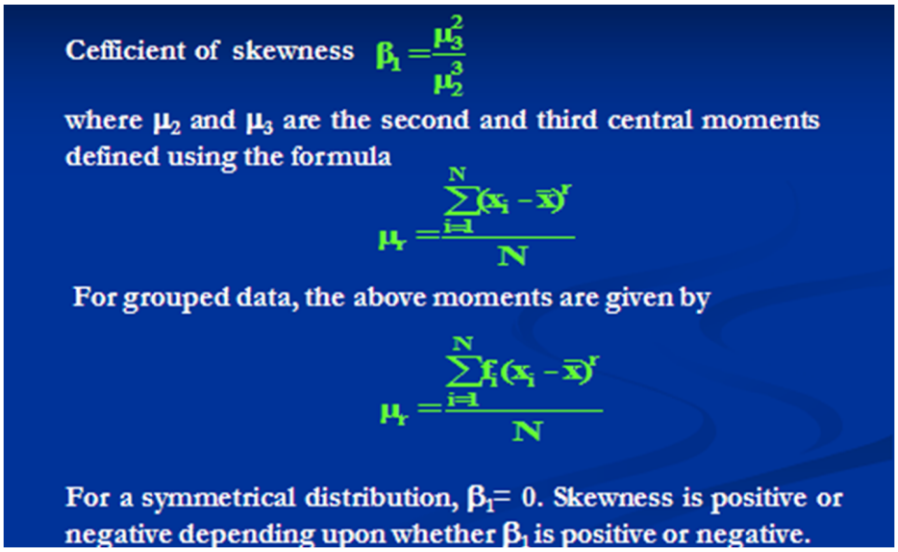


Distribution of a given data

Skewness and Kurtosis are the main statistics used to measure the shape or distribution of a given set of data.

Skewness is a measure of the asymmetry of the probability distribution of a real-valued random variable. The skewness value can be positive or negative, or even undefined. Qualitatively, a negative skew indicates that the *tail* on the left side of the probability density function is *longer* than the right side and the bulk of the values (possibly including the median) lie to the right of the mean. A positive skew indicates that the *tail* on the right side is *longer* than the left side and the bulk of the values lie to the left of the mean. A zero value indicates that the values are relatively evenly distributed on both sides of the mean, typically implying a symmetric distribution. Kurtosis measures the peakedness of shape distribution of a given set of data. The distribution is called normal if $\beta_2 = 3$; β_2 is more than 3, the distribution is said to be leptokurtic β_2 is less than 3, the distribution is said to be platykurtic (where $p_2 = \frac{}{\frac{2}{2}}$)





Exploratory Data Analysis

Exploratory data analysis employs a variety of techniques (mostly graphical)

- Scatter Plot
- Stem and Leaf
- Boxplot

Five Number System gives a good identification of center and spread of the data

- Maximum
- Minimum
- Median = 50^{th} percentile
- Lower quartile $Q_1 = 25^{th}$ percentile
- Upper quartile $Q_3 = 75^{th}$ percentile

Scatter Diagram

- A graphical presentation of the relationship between two quantitative variables.
- One variable is shown on the horizontal axis and the other variable is shown on the vertical axis.
- The general pattern of the plotted points suggests the overall relationship between the variables.

Stem-and-Leaf Display

- Shows both the rank order and shape of the distribution of the data.
- It is similar to a histogram on its side, but it has the advantage of showing the actual data values.
- The first digits of each data item are arranged to the left of a vertical line.
- To the right of the vertical line we record the last digit for each item in rank order.
- Each line in the display is referred to as a stem.
- Each digit on a stem is a leaf.

Box Plot

- A boxplot is a graph of the five – number summary
- A central box spans the quartiles
- A line in the box marks the median
- Lines extend from the box out to the smallest and largest observations
- Boxplots can be drawn either horizontally or vertically

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Chapter 15

Application dryers in fishery sector

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In India, Fish production has increased manifold since independence. About 16 million people are involved in the fishing industry in India. Fishermen in India catch fish as major aquatic products and are intended mainly for domestic consumption and sale in the local market. However, in the case of over catch, tremendous losses occur because the fishermen have neither access to markets in big cities nor to the international market due to poor product quality and the absence of good marketing and distribution system. As an alternative, fishermen can convert the catch into value-added products *viz.*, dried fishery products, smoked fish, etc, with enhanced shelf life and market value.

Traditional drying method and its drawbacks

Drying preserves fish from decay by removal of moisture from fish, thereby arresting the growth of bacteria, the action of enzymes, and chemical oxidation of the fat. Out of total catch, 30-40 % of fish is dried or processed for export and local consumption. Open-air sun drying is the traditional method employed in India to dry fish and fishery products, known for higher microbial load and lower product quality. It denotes exposure of the commodity to direct solar radiation and the convective power of the natural wind for the removal of moisture. But it often results in inferior quality of product due to its dependence on weather conditions and vulnerability to the attack of dust, rains, insects, pests, and microorganisms. Also, it requires a longer drying time.

Solar dryers for high-quality products

Solar drying is an alternative that offers numerous advantages over the traditional method, apart from being environmentally friendly and economically viable. In solar drying, a structure, often of very simple construction, is used to enhance the heating effect of solar radiation. Compared to sun drying, solar dryers can generate higher air temperatures and consequential lower relative humidity, which are conducive to improved drying rates and hence lower moisture content of the final products.

Major parts of Solar dryers and its advantages

The essential parts of the solar dryer (Fig. 1) include the solar collector, drying chamber, and airflow system (Inlet & Exhaust). The solar collector consists of a glass cover and an absorber plate. The drying chamber consists of trays stacked one above another at an equal distance in which the material to be dried is placed. The ambient air enters into the solar collector in which air gets heated up and moves to the drying chamber and flows across the trays. The heated air after removing moisture from the material moves out through the exhaust system. The advantages of solar drying are,

- Uniform and hygienic drying
- Eco-friendly / No GHG emissions
- Low cost
- Energy efficient
- Quality and food safety
- Reduced drying time

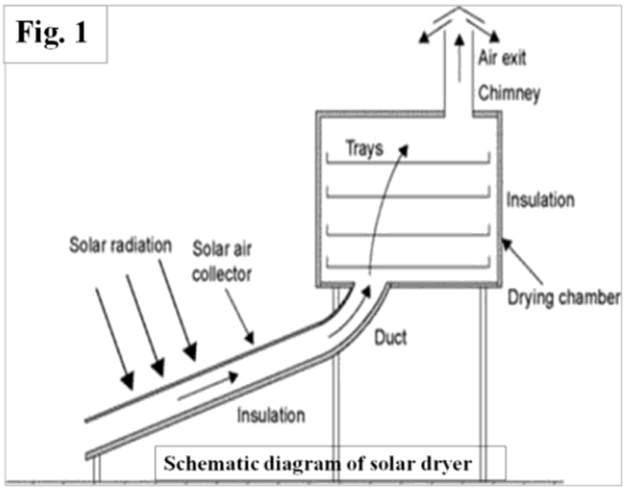

Fig 1. Schematic diagram of basic solar dryer

Different types of CIFT dryers

ICAR-Central Institute of Fisheries Technology (CIFT), Cochin, has been in the service of the nation since 1957 under the Indian Council of Agricultural Research (ICAR), New Delhi. CIFT

has developed low-cost, energy-efficient, and eco-friendly dryers like Solar tray dryer, Solar cabinet dryer, Solar tunnel dryer, etc based on solar energy for quality drying of fish. Apart from fish, this dryer is also suitable for drying other agricultural products like fruits, vegetables, spices, and condiments. All of these dryers are provided with alternative heating sources to continue the drying process during off sunshine hours especially during night time, cloudy and rainy days.

In the CIFT Solar dryers, the labor requirement is considerably reduced compared to open sun drying in beaches/coir mats because of the elimination of the cleaning process due to sand and dust contamination. The re-handling process like spreading, sorting, and storing because of non-drying or partial drying due to unfavorable weather conditions and spoilage due to rain is also not required. The drying time is reduced considerably with improved product quality. Improved shelf life and value addition of the product fetches higher income for the fisherfolk. The eco-friendly solar drying system reduces fuel consumption and can have a significant impact on energy conservation.

The design of solar dryers varies from simple direct dryers to more complex hybrid designs. Hybrid model solar dryers are having LPG, biogas, biomass, or electricity as alternate backup heating sources for continuous drying of fish even under unfavorable weather conditions. ICAR-CIFT has developed different models and capacities of solar dryers for the hygienic drying of fish. The capacity of these hybrid solar dryers varies from 6 to 110 m^2 of tray spreading area for drying various quantities of fish varying from 10 kg to 500 kg.

Solar dryer with LPG backup (50-60 kg)

ICAR-CIFT designed and developed a novel system for drying fish using solar energy supported by environment-friendly LPG backup (Fig. 2). In this dryer during sunny days fish will be dried using solar energy and when solar radiation is not sufficient during cloudy/ rainy days, LPG backup heating system will be automatically actuated to supplement the heat requirement. Water is heated with the help of solar vacuum tube collectors installed on the roof of the dryer and circulated through heat exchangers placed in the PUF insulated stainless steel drying chamber. Thus, continuous drying is possible in this system without spoilage of the highly perishable commodity to obtain a good quality dried product.

This dryer is ideal for drying fish, fruits, vegetables, spices, and agro products. It helps to dry the products faster than open drying in the sun, by keeping the physicochemical qualities like color, taste, and aroma of the dried food intact and with higher conservation of nutritional value. A programmable logical controller (PLC) system can be incorporated for automatic control of temperature, humidity, and drying time. Solar drying reduces fuel consumption and can have a significant impact on energy conservation (Murali et al. 2020; Murali et al. 2021).

Fig.2. ICAR-CIFT Solar-LPG hybrid dryer

Solar dryer with electrical backup (20 kg)

Effective solar drying can be achieved by harnessing solar energy by specially designed solar air heating panels and proper circulation of the hot air across the SS trays loaded with fish (Fig. 3). Food grade stainless steel is used for the fabrication of chamber and perforated trays which enable drying of fish hygienically. Since the drying chamber is closed, there is less chance of material spoilage by external factors. An alternate electrical backup heating system under controlled temperature conditions enables the drying to continue even under unfavorable weather conditions like rain, cloud, non-sunny days, and in night hours so that the bacterial spoilage due to partial drying will not occur. Improved shelf life and value addition of the product fetches higher income for the fisherfolk. The eco-friendly solar drying system reduces fuel consumption and can have a significant impact on energy conservation.

Fig. 3. ICAR-CIFT Solar-electrical hybrid dryer

Solar dryer with electrical backup (40 kg)

The dryer consists of four drying chambers with nine trays in each chamber (Fig. 4). The trays made of food-grade stainless steel are stacked one over the other with a spacing of 10 cm. The perforated trays accomplish a through-flow drying pattern within the dryer which enhances drying rates. Solar flat plate collectors with an area of 7 m^2 transmit solar energy to the air flowing through the collector which is then directed to the drying chamber. The capacity of the dryer is 40 kg. Electrical backup comes into a role once the desired temperature is not attained for the drying process, particularly during rainy or cloudy days.

Fig. 4. ICAR-CIFT Solar- electrical hybrid dryer

Solar tunnel dryer

ICAR-CIFT developed a low-cost, energy-efficient solar tunnel dryer for bulk drying of fish and fishery products. This dryer can be used by fishermen or small-scale fish processing units for

bulk drying during seasonal higher catch/excess landing of fish. The capacity of the solar tunnel dryer is 50 kg with a floor area of 12 m^2 (Fig. 5). The materials of construction are UV stabilized transparent polythene sheet for roof cover, black absorber sheet for the floor, supporting frames of CPVC, and GI rod. Three ventilator fans of 0.5 hp were provided for air inlet and moisture removal. The trays with tray holders were placed inside the dryer for spreading and hooking the fish for drying. This tent dryer was designed as a stand-alone system as it does not require any external power source/electricity. The fans were operated through a solar PV panel fitted on the rooftop of the dryer and associated battery setup. It is also affordable and suitable for Indian fisherfolks.

Fig. 5. ICAR-CIFT Solar-tunnel dryer

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Chapter 16

Advanced fish drying techniques

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Introduction

Fish is a highly nutritious food than meat and egg and it is highly perishable because of its high moisture content which is about 80%. Fish preservation is essential immediately after the catch to increase the shelf life of fish. Preservation methods help to maintain the quality of fish for a longer period of time, prevent spoilage and decomposition, retain its original nutritional contents, and make transportation and storage of fish easier. Fish preservation techniques vary with the type, nature, size, and condition of fish. Improper handling and processing of fish lead to immediate spoilage of fish resulting in poor quality.

Conventional preservation techniques such as chilling, freezing, drying, and chemical preservation are widely used for fish preservation throughout the world. Among the various preservation techniques drying of fish is the oldest preservation technique and drying means the preservation of fish by removing water from it through heating. Drying removes the moisture content up to a certain level to prevent microbial growth thereby providing greater shelf life, and reduction in weight, volume, transportation, and storage space. Two commonly used drying methods are natural and artificial drying. Natural drying includes sun drying, and solar drying, whereas artificial drying includes a microwave, fluidized bed, spouted bed, infrared, convective drying, desiccant drying, freeze drying, osmotic, vacuum drying, pulsed electric field, high hydrostatic pressure, superheated steam drying, heat pump and spray drying *etc*.

Natural drying methods are associated with disadvantages like contamination and damage by dirt, insects, rodents, birds, and animals. Sun drying of fish often results in low-quality products since drying is slow normally it takes five to seven days. Therefore, it is necessary to choose an advanced method of drying to obtain good quality products (Curran and Trim, 1985). Artificial drying methods have advantages like less drying time, good quality drying, better process control, operational safety, and higher capacity.

1.1 Advanced drying methods

1.1.1. Solar drying

Solar energy has been used all around the world to dry food products. Solar drying is the use of equipment to collect the sun's radiation in order to harness the radioactive energy for drying applications. Good product quality can be retained with the control of radioactive heat. It is mainly used to dry products like grains, fruits, vegetables, meat, and fish. A solar food dryer improves the open air sun drying in the following ways:

1. Solar dryers enhance the drying time because it directly traps heat inside the dryer using translucent, glazing over the collection area and raising the temperature of the air.
2. It is a more efficient method of drying than open sun drying. Food materials can be dried more quickly so less will be lost to spoilage.
3. Drying is being done in a hygienic environment and is less likely to be contaminated.
4. Drying foods at optimum temperatures and in less time enables solar dryers to retain more nutritional values such as vitamin C.
5. Using freely available solar energy instead of conventional fuels to dry products or using a cheap supplementary supply of solar heat, so reducing conventional fuel demand can result in significant cost savings.

1.1.2. Fluidized bed drying

In fluidized bed drying (FBD) system, the air is allowed to pass through the bed of solid material in the upward direction with a velocity greater than the settling rate of solid particles. It is mainly working on the fluidization of solid materials. Since hot air is introduced from the bottom of the system at high pressure the solid particles which have to be dried will be in a suspended state in a stream of air. Heat transfer is accomplished by direct contact between the solid material and hot air. Vaporized liquid is carried away by the hot air.

For most of food applications, a batch-type fluid bed dryer is a better choice since small quantity of wet material to be processed. Recent developments in FBD include mechanically agitated FBD, use of pulsating flow, and use of immersed tubes for efficient heat transfer *etc*. The advantages of FBD systems are as follows: 1. Drying temperature is low thus minimizing the quality degradation by thermal effects. 2. Uniform drying results in particles having even dryness. 3. The effectiveness of heat and mass transfer is high since there is direct contact between wet material and hot air.

1.1.3. Infrared drying

In recent years, infrared drying has gained popularity as an alternative drying method for foods. IR is electromagnetic radiation that is in the region of 0.78 – 1000μm. It is transmitted and absorbed by the food surface and gets changed into heat. Generally, the far-IR region (3 – 1000μm) is used for food processing since most of the food materials are having the ability to absorb IR in this region. IR radiation impinges on the surface of the material which has to be dried and penetrated into it. Absorption of radiation increases the molecular vibration inside the material and resulted in heat generation on both the inside and surface of the material concurrently (Sakai and Hanzawa, 1994). Faster heat generation inside the material increases the movement of moisture towards the outer surface. External hot air movement over the surface of the material can remove the moisture from the surface and influence the further mass transfer from the material. IR drying provides less drying time, is highly energy efficient, uniform in drying, and has good quality dried products. Infrared offers faster drying of products with minimum energy consumption and nutrient losses than conventional dryers. Also, IR heating provides high heat transfer with less drying time and energy cost. Drying using IR radiation will result in better quality products than another drying process since the heating is fast and uniform. It can be used for various food materials like grains, flour, vegetables, pasta, meat, and fish.

1.1.4. Vacuum drying

Vacuum drying is a process in which materials are dried in a reduced-pressure environment, which lowers the temperature required for rapid drying. Major advantages of vacuum drying are as follows: less energy is needed for drying, it is highly suitable for heat-sensitive food materials, faster method than other drying methods, it retains the integrity of materials *etc*. In general, vacuum drying is performed in combination with other drying techniques.

1.1.5. Superheated steam drying

In superheated steam drying, the drying gas in a convective dryer is replaced with superheated steam. Superheated steam at certain pressure enters in drying chamber and removes the moisture from wet foods and the exhaust from the dryer is also superheated steam with a lower specific enthalpy. A part of the steam can be recycled back after compression and the excess can be either used directly or removed from the system. Any conventional convection and conduction dryer can be easily adapted to a superheated steam dryer. It is an attractive drying medium for some products which gives better quality products in absence of oxygen.

1.1.6. Freeze drying

Freeze drying or lyophilization is a dehydration process used to preserve material and make it into more convenient for transport. It is a method of water removal from material by sublimation. This drying process is divided into three stages: pre-freezing of wet material, primary drying (sublimation of frozen water under vacuum), and secondary drying stage (desorption of residual bound water from the material). Freeze drying is initially freezing the material and then reducing the surrounding pressure to allow the frozen water in the material to sublimate directly from the solid phase to the gas phase. It is one of the best methods of water removal and results in a final product of much higher quality compared to any other drying technique. A comparative review of drying technologies showed that freeze drying, vacuum drying, and osmotic dehydration are considered too costly for large-scale production of dried products (Khin *et al.*, 2005).

1.1.7. Heat pump drying

A heat pump is a device that transports energy from a low-temperature source to a higher-temperature sink. This transfer requires an input of work which may be supplied mechanically as in a vapor-compression cycle. The most common type of heat pump operates on the vapor-compression cycle and a basic unit consists of the evaporator, compressor, condenser, and expansion valve. Heat transport is achieved through phase change of the working fluid (refrigerant). The refrigerant in the evaporator absorbs heat and vaporizes at low pressure and temperature. As the vapor condenses at a higher pressure in the condenser, it rejects heat at a higher temperature. When used in a drying system, the heat pump dryer cools the process air first to saturation, and then further for condensation of water (dehumidification), thus increasing the drying potential of air. In the process, it also recovers low-grade heat (sensible and latent) from the air, which is made available at the condenser as sensible heat of higher quality. A heat pump dryer consists of a heat pump system and a dryer, the performance of the dryer is greatly affected by the performance of the heat pump system. Heat pump drying is a technology by which materials can be dried at low temperatures and in an oxygen-free atmosphere using less energy than common drying methods. This drying recorded less drying time than other drying methods and it is simple to design.

1.1.8. Dielectric drying:

Electromagnetic energy of microwave and radio frequency (RF) can directly interact with foods to quickly raise center temperature since most food materials are dielectric materials and can

store electric energy and convert it into heat. It is volumetric heating and quick raise of temperature is possible.

In microwave drying, microwaves can penetrate materials and heat resulting in water removal during drying. Microwave energy at 915 and 2450 MHz can be absorbed by water-containing materials and can be converted into heat. Food materials are dried by the interactions between the electromagnetic energy and polar molecules within the material. Polar molecules rotate in response to the applied oscillating electromagnetic waves. The reorientation of molecules in a high-frequency electric field occurs frequently and rapidly, resulting in molecular friction that generates heat. It is an energy-efficient technology and can maintain the quality of food materials upon drying. Some disadvantages of microwave drying are non-uniformity in drying, limited penetration depth, lack of equipment for large-scale production *etc*.

Radiofrequency energy generates heat volumetrically within wet material based on combined mechanisms of dipole rotation and conduction effects. The free space wavelength in the RF range is 20-360 times longer than that of commonly used microwave frequencies, allowing RF energy to penetrate foods more deeply and provide better heating uniformity in food materials than microwave energy. Therefore, radio frequency (13.56, 27.12, and 40.68 MHz for industrial applications) thermal processes have the potential to reduce thermal quality degradation in the drying of foods. Major challenges for using RF heating in the food industry are non-uniform heating which leads to overheating in corners, edges, and center parts of intermediate and high moisture food.

1.1.9 Hybrid drying/Combined drying

Hybrid drying techniques are becoming common because the combined technology receives the benefits of individual processes. Combined drying is considered as the best technique to reduce energy consumption and improve quality (Raghavan *et al*., 2005). Combined drying technologies involve the implementation of different modes of heat transfer and two or more stages of the same or different types of dryer. Currently, some new techniques such as microwave, infrared, and radio frequency assisted drying have been used to reduce drying time and improve the final quality of dried products. Many various combinations of drying methods can be used to avoid the disadvantages of a single drying method such as long drying time, high power consumption, and low product quality. Combined drying methods include parallel and

tandem drying. Parallel drying uses two or more drying methods simultaneously. Tandem drying involves the use of one drying method followed by one or more other drying methods.

Conclusion

Drying is an important process to preserve food materials and to extend the shelf life. Different drying methods are available for drying of foods and each has its own advantages and disadvantages. Traditional drying methods (sun, solar, hot air oven drying) are simple to use but have low energy efficiency and longer drying time. Thus it negatively affects the colour, flavor and nutrient content of dried products. Some advanced drying methods (freeze drying, microwave, heat pump, and vacuum drying) offer a wide scope for the production of best quality dried products. But usage of these methods for drying is restricted due to its high cost. Therefore, cost-effective alternative systems such as combined/hybrid drying can be used for the drying of products with minimum cost and simple technologies. Combination drying with an initial conventional drying process followed by microwave/vacuum or simultaneously two methods hot air with infrared/microwave/vacuum has proven to reduce drying time with improved product quality and minimizing energy requirements.

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Chapter 17

Novel Engineering Solutions for Postharvest fisheries sector

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Introduction

Fisheries and aquaculture are a significant source of food production, nutritional security, employment, and income generation in India. The fisheries sector offers a major contribution of livelihoods for more than 20 million fishers and fish farmers; pays INR 1.75 trillion annually to India's economy; and is a key export earner, with fish being one of the most important agricultural commodities to be exported from India.

Fishermen in India catch fish as major aquatic products and are intended mainly for domestic consumption and sale in the local market. Fish is being an important source of animal protein, and because of its highly perishable nature, fish has to be processed and stored appropriately to avoid any postharvest losses. But, in the case of over-catch, tremendous losses occur because the fishermen have neither access to domestic markets nor the international market due to low product quality and the absence of good marketing and distribution system. As an alternative, fishermen can convert the catch into value-added products *viz.*, dried fishery products, smoked fish, etc., while extending its shelf life and adding to its market value.

Drying techniques

Drying is one of the oldest methods of food preservation. It is one of the unit operations in the postharvest phase. Drying is the process of removing moisture from food by circulating hot air through it. Drying reduces the water activity of food which in turn helps to arrest or delay the microbial activity and chemical reactions thus dried food can be stored for longer time. The predominant food drying methods are open sun drying. It is one of the cheapest methods drying and energy is freely available, renewable and abundant. But it takes several days to dry agricultural commodities in out-doors. As the weather is uncontrollable, sun drying can be risky. And also, direct exposure of the food material to unhygienic open conditions may cause dust, excreta, pests, insects and microbial infestations and yield inferior quality product. To overcome the disadvantages of open sun drying, mechanical dryers with electric heating system are generally used. But this involve running costs due to high electricity consumption and are not

recommended due to exploitation of non-renewable sources of energy. Solar drying is most effective for fish drying as it is using renewable energy for drying. And also, numerous investigations have shown that solar drying can be a means of food preservation since the product is dried under controlled conditions and completely protected during drying from rain, dust, insects and animals.

Solar drying is an alternative that offers numerous advantages over the traditional method, apart from being environmentally friendly and economically viable. In solar drying, a structure, often of very simple construction, is used to enhance the heating effect of solar radiation. Compared to sun drying, solar dryers can generate higher air temperatures and consequential lower relative humidity, which are favourable to better drying rates and hence lower moisture content of the final products.

Solar dryer can perform drying for food preservation only during sunny days, and hence the drying efficiency depends largely on climatic conditions and the season (Nukulwar., 2022). Hybrid solar dryers are more reliable as there is a back-up system to provide heating in it. Solar-electrical hybrid dryer is more trustworthy as auxiliary system is electrical heating coil (Alfiya et al., 2019). Based on the aforesaid facts, CIFT had designed and developed, energy efficient and cost-effective solar hybrid dryers of different capacities for drying of fish/shrimps.

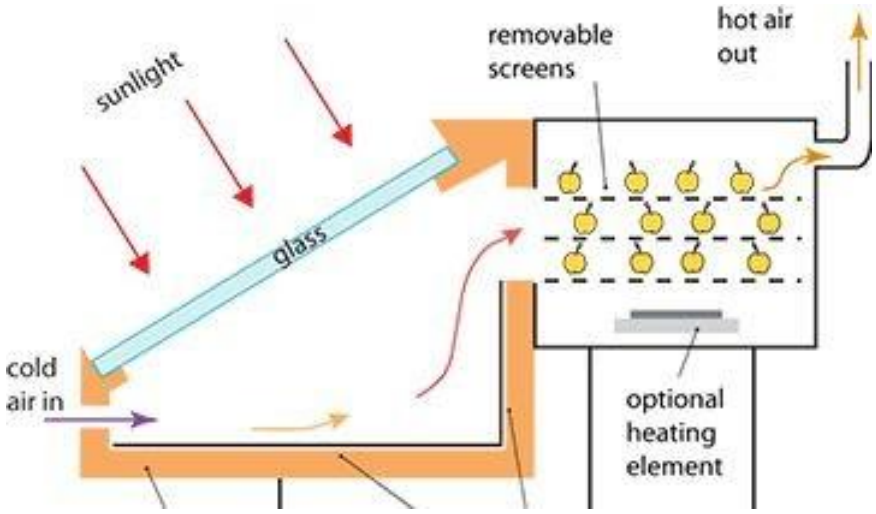

Fig.1. Solar drying technique

Different types of CIFT dryers

ICAR-Central Institute of Fisheries Technology (CIFT), Cochin, has been in the service of the nation since 1957 under the Indian Council of Agricultural Research (ICAR), New Delhi. CIFT has developed low-cost, energy-efficient, and eco-friendly dryers like Solar tray dryers, Solar cabinet dryers, Solar tunnel dryers, etc. based on solar energy for quality drying of fish. Apart from fish, this dryer is also suitable for drying other agricultural products like fruits, vegetables, spices, and condiments. All of these dryers are provided with alternative heating sources to continue the drying process during off-sunshine hours, especially during nighttime, cloudy and rainy days.

The design of solar dryers varies from simple direct dryers to more complex hybrid designs. Hybrid model solar dryers are having LPG, biogas, biomass, or electricity as alternate backup heating sources for the continuous drying of fish even under unfavorable weather conditions. ICAR-CIFT has developed different models and capacities of solar dryers for the hygienic drying of fish. The capacity of these hybrid solar dryers varies from 6 to 110 m^2 of tray spreading area for drying various quantities of fish varying from 10 kg to 500 kg.

The solar dryer is ideal for drying fish, fruits, vegetables, spices, and agricultural products. It helps to dry the products faster than open drying in the sun, by keeping the physicochemical qualities like color, taste, and aroma of the dried food intact and with higher conservation of nutritional value. A programmable logical controller (PLC) system can be incorporated for automatic control of temperature, humidity, and drying time. Solar drying reduces fuel consumption and can have a significant impact on energy conservation.

Fig.2. ICAR-CIFT Solar-LPG hybrid dryer

Solar dryer with LPG backup (50 kg)

ICAR-CIFT designed and developed a novel system for drying fish using solar energy supported by environment-friendly LPG backup (Fig. 2). In this dryer during sunny days fish will be dried using solar energy and when solar radiation is not sufficient during cloudy/ rainy days, LPG backup heating system will be automatically actuated to supplement the heat requirement. Water is heated with the help of solar vacuum tube collectors installed on the roof of the dryer and circulated through heat exchangers placed in the PUF-insulated stainless steel drying chamber. Thus, continuous drying is possible in this system without spoilage of the highly perishable commodity to obtain a good quality dried product.

Fig. 3. ICAR-CIFT Solar-electrical hybrid dryer

Solar dryer with electrical backup (20 kg)

Effective solar drying can be achieved by harnessing solar energy from specially designed solar air heating panels and proper circulation of the hot air across the SS trays loaded with fish (Fig. 3). Food-grade stainless steel is used for the fabrication of chamber and perforated trays which enable drying of fish hygienically. Since the drying chamber is closed, there is less chance of material spoilage by external factors. An alternate electrical backup heating system under controlled temperature conditions enables the drying to continue even under unfavorable weather conditions like rain, cloud, non-sunny days, and night hours so that bacterial spoilage due to partial drying will not occur. Improved shelf life and value addition of the product fetch higher income for the fisherfolk. The eco-friendly solar drying system reduces fuel consumption and can have a significant impact on energy conservation.

Solar dryer with electrical backup (40 kg)

The dryer consists of four drying chambers with nine trays in each chamber (Fig. 4). The trays made of food-grade stainless steel are stacked one over the other with a spacing of 10 cm. The perforated trays accomplish a through-flow drying pattern within the dryer which enhances drying rates. Solar flat plate collectors with an area of 7 m^2 transmit solar energy to the air flowing through the collector which is then directed to the drying chamber. The capacity of the dryer is 40 kg. Electrical backup comes into a role once the desired temperature is not attained for the drying process, particularly during rainy or cloudy days.

Fig. 4. ICAR-CIFT Solar-electrical hybrid dryer

Solar tunnel dryer (50 kg)

ICAR-CIFT developed a low-cost, energy-efficient solar tunnel dryer for bulk drying fish and fishery products. This dryer can be used by fishermen or small-scale fish processing units for bulk drying during seasonal higher catch/excess landing of fish. The capacity of the solar -

Fig. 5. ICAR-CIFT Solar-tunnel dryer

dryer is 50 kg with a floor area of 12 m^2 (Fig. 5). The materials of construction are UV-stabilized transparent polythene sheet for the roof cover, black absorber sheet for the floor, supporting frames of CPVC, and GI rod. Three ventilator fans of 0.5 hp were provided for air inlet and moisture removal. The trays with tray holders were placed inside the dryer for spreading and hooking the fish for drying. This tent dryer was designed as a stand-alone system as it does not require any external power source/electricity. The fans were operated through a solar PV panel fitted on the rooftop of the dryer and associated battery setup. It is also affordable and suitable for Indian fisherfolk.

Multi-purpose emission less biomass dryer

Biomass dryer consists of drying chamber, blower, biomass furnace and a hot air recirculatory system (Fig. 6). The capacity of this dryer is 30-40 kg with 10 trays. Biomass furnace capacity is 25 kg (wood) with the dimension 0.77 m x 1.76 m x 1.42 m. Biomass dryer is provided with a blower of 0.5 hp and axial fan of 0.25 hp. This dryer is suitable for drying fruits, vegetables, spices, condiments and fish. Biomass dryer is highly economical to operate where the biomass availability is abundant and free of cost.

Fig. 6. ICAR-CIFT Biomass dryer

Household electrical dryer

Electrical dryer consists of drying chamber, blower, exhaust fans and a heating coil (Fig. 6). The capacity of this dryer is 10 kg with 10 trays with dimension of 0.54 m × 0.55 m × 0.25 m. The trays are made of aluminium frame and stainless-steel wire mesh. Drying chamber is having the dimension of 0.56 m x 0.61 m x 1.65 m. The heat source is the electrical coil of 1500 W (2 Nos).

This dryer is suitable for drying agricultural commodities. The cost of electrical dryer is low, but the operational costs is high due to the sole electricity consumption.

Fig.7. Household Electrical dryer

Advanced drying techniques

Advanced drying methods have advantages like less drying time, quality drying, better process control, operational safety, and higher capacity. Infrared (IR) drying can be considered to be an artificial drying method and it can sustain throughout the day. In recent years, infrared drying has gained popularity as an alternative drying method for foods. IR is electromagnetic radiation that is in the region of 0.78 – 1000 μm. It is transmitted and absorbed by the food surface and gets changed into heat. Generally, the far-IR region (3 – 1000 μm) is used for food processing since most of the food materials are having the ability to absorb IR in this region. IR radiation impinges on the surface of the material which has to be dried and penetrated into it. Absorption of radiation increases the molecular vibration inside the material and resulted in heat generation on both the inside and surface of the material concurrently (Sakai and Hanzawa, 1994). Faster heat generation inside the material increases the movement of moisture towards the outer surface. External hot air movement over the surface of the material can remove the moisture from the surface and influence the further mass transfer from the material. IR drying provides less drying time, is highly energy efficient, uniform in drying, and has good quality dried products. Infrared offers faster drying of products with minimum energy consumption and

nutrient losses than conventional dryers. Also, IR heating provides high heat transfer with less drying time and energy cost. Drying using IR radiation will result in better quality products than another drying process since the heating is fast and uniform.

Advantages of using IR for drying include flexibility of operation, simplicity of the equipment, fast response of heating and drying, easy installation to any drying chamber, and low capital cost (Sandu, 1986). It can be used for various food materials like grains, flour, vegetables, biscuits, pasta, meat, and fish. A simple IR dryer consists of an inlet and outlet hopper, manual conveyor system, IR lamp arrangements, voltage regulator, and timer relay. Food product enters from the inlet hopper to the manual conveyor and it moves parallel to the IR lamps and dried. The IR radiation intensity can be adjusted via the voltage regulator and intermittent IR drying can be implemented by a timer relay.

Fig. 8. Batch type Infrared dryer

Batch type infrared dryer

A batch type infrared dryer was developed by ICAR-CIFT, which is having a loading capacity of 4 kg fish. The dryer consists of a chamber with a dimension of 1.22 m × 0.92 m × 0.38 m. The material of construction of this dryer is marine plywood coated with aluminium foil inside the chamber. The drying material can be kept in a single tray, which is made of food grade stainless-steel (SS304) material having a dimension of 1.0 m × 0.9 m. The heating source is infrared lamps of 150 W (8 Nos). The drying time of this dryer varies between 2-4 hours based on the type of the product. Fishery products or any agri products can be dried using this dryer.

Hot air assisted infrared dryer prototype

Fig. 9. Hot air assisted infrared dryer prototype

Pilot scale infrared dryer

A pilot-scale hot air-assisted continuous infrared dryer was designed and developed by ICAR-CIFT and it is presented in Fig. 10. The major components assembly of the pilot scale dryer comprised of belt conveyor, infrared radiation heating system, hot air generation and circulation, power transmission, feed hopper, discharge chute, and control panel. The drying chamber of 2.22 x 1.19 x 1.30 m was made from stainless steel sheets with 25 mm thick glass wool insulation and a folding door opening at the front. Both the outer and inner sides of the drying chamber were covered with 1 mm thick stainless-steel sheet. The conveyor dryer has a four-layer conveying system with a loading area of 2 m^2 on each layer. The conveying system was composed of end rollers and conveyor belts (2 x 1 m), both were made of stainless steel (SS 304) material. The size of the dryer and loading area was selected based on the calculations obtained from the assumed dryer capacity and bulk density of the product to be dried. Stainless-steel (SS 304) feed hopper (0.98 × 0.10 × 0.19 m) was designed in such a way to feed the sample throughout the width of the top layer conveyor belt as a single layer. Sample discharged from the feed hopper to the top layer conveyor belt was conveyed along and transferred first to the second layer, then to the third and at last to the fourth layer using stainless steel discharge fixed at the end of each layer. From the fourth layer, the dried sample will be discharged through the discharge chute. The drying chamber was fitted with a ceramic infrared heater of 250 W which emits radiation at a wavelength of 2.5 – 10 µm. A total of ninety-six IR heaters (twenty-four numbers in each layer) were fixed over each layer of the conveyor belt at a distance of 10 cm from the belt surface. The provision to cut-off IR intensity of each layer to its half load was

provided using a PLC (Siemens LOGO X50) and HMI (Siemens LOGO TDI) for situations where full power is not required. Switching on and off the IR heaters of each layer was also controlled by the PLC and HMI automatically. The drying chamber has six air inlets (d = 0.20 m) and two exhaust (rectangular mesh opening) ducts for hot air circulation and to remove humid air. A temperature sensor (J-type thermocouple) was fixed inside the chamber to measure the air temperature during drying. A discharge chute was placed to collect the dried samples.

Fig. 10. Pilot-scale hot air-assisted continuous infrared dryer

Fish descaling machineries

Descaling is the process of removal of scales from fishes. Removing the scales of fishes is a laborious and time-consuming activity which also requires skilled man power. Mechanization of descaling process could significantly reduce the handling time thereby shortening the pre-processing period. Moreover, it reduces the drudgery of labour involving in manual descaling of fishes. Use of descaling machine reduces the overhead costs and enhances the quality of the final product. In this context, ICAR-Central Institute of Fisheries Technology (CIFT) has designed and developed three types of fish descaling machines. These machines can be used to remove the scales of fishes from all types/sizes/species of fishes.

Hand operated fish descaling machine

The descaling capacity of the machine is 3 kg, and made of Stainless-steel (SS 304). The major parts of the machine are a base frame made of 1inch square shaped SS tube and a rotating drum. The drum is made of perforated SS sheet fitted in a strong SS frame and having diameter and length of 255.5 mm and 270 mm, respectively. Descaling is done inside the closed chamber by rotational action of cylinder. Friction between the fishes and projections of perforated SS

cylinder during the rotation of drum removes the scale. Leak proof door with lock is provided for loading and unloading purposes. A hand pedal is fitted on the side to rotate the drum manually.

Fig. 11. Hand operated fish descaling machine

Table top motorized fish descaling machine

It is made of SS 304 and has 5 kg capacity. It contains a 0.5 HP AC motor with belt reduction mechanism to achieve the required drum speed of 20-30 rpm. Frame is fabricated using 1-inch square shape SS tube with suitable covering in electrical parts. The drum is made of perforated SS sheet and having suitable internal projections to remove the scales. Drum is also provided with a leak proof door with suitable lock.

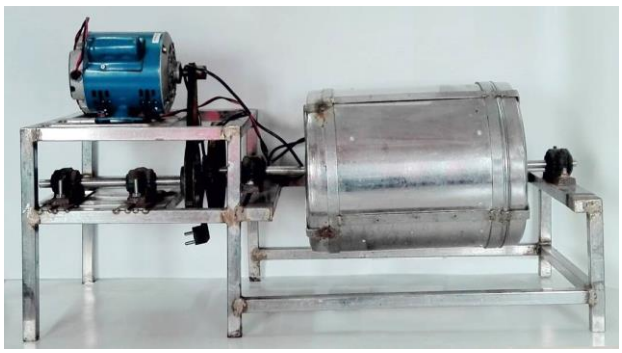

Fig. 12. Motorized fish descaling machine

Fish descaling machine with variable drum speed

It is made of SS 304 and has 10 kg capacity. It contains a 1.5 HP induction motor and a variable frequency drive (VFD) to vary the speed of the drum depending on the variety of the fish loaded. The drum is made of perforated SS 304 sheet fitted on a strong SS Frame. Water input facility is provided in the drum for easy removal of the scales from the drum. The outlet pipe also

provided to remove scales and water from the machine. Since speed of the drum is influencing descaling efficiency, an electronic RPM meter is attached with the descaling machine to set the required RPM. The machine takes 3-5 minutes to clean 10 kg fish depending on the size.

Mini fish descaling machine

To cater the household needs related to fish descaling, a motorized version of 1 kg capacity fish descaling machine was developed at ICAR-CIFT. It can be used in home kitchens and hotels for easy removal of fish scales. The equipment consists of a rotating drum, nylon brush, motor and frame to support the assembly. The diameter and length of the drum are 190 mm and 225 mm respectively. Inside of the drum is riveted with perforated stainless-steel mesh. The fish can be fed to the drum and motor switched on for descaling action. The machine can be loaded with 1 kg of fish in single batch for effective removal of scales. Cleaning of the machine can be done easily by detaching the drum with perforations inside. The system is ergonomically designed in such a way that even women can work on it without any drudgery. The drum speed of descaling machine is optimized with respect to the efficiency level and it was found that maximum efficiency can be attained at 22 rpm drum speed at the loading capacity of 1 kg of fish. Descaling of Sardine and Tilapia required 5 minutes to attain efficiencies of 84.60% and 79.59% respectively.

Fig. 13. Variable speed fish descaling machine

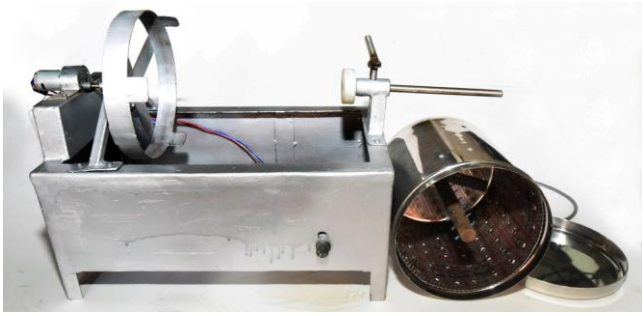

Fig. 14. Mini fish descaling machine

Refrigerated mobile fish vending Kiosk

Exposure of fishes to the atmosphere often leads to contamination by means of dust, insects and flies apart from deterioration of quality in terms of freshness and taste. ICAR-CIFT, Cochin, has designed and developed a low-cost energy efficient refrigerated hygienic mobile fish vending kiosk to sell fish at consumer's door step under hygienic conditions in village/urban/municipality areas with proper waste disposal system. The unit was fabricated mainly using food grade material stainless-steel (SS 304) with transparent poly carbonate/toughened glass sheets for the display. The kiosk can carry 20-30 kg fish under refrigerated storage. It was designed considering the maximum weight that a man pulls on a rickshaw. The main components of the kiosk model are chilled fish storage cum display unit with three chambers, a hand operated de-scaling machine (3 kg) and fish dressing deck with wash basin, water tank, cutting tool, waste collection chamber and working space. The main feature of the prototype is that consumer can see the fishes directly through transparent cover and select according to their choice of purchase. A digital sign board is attached in the front of kiosk to display the available fishes and their rates.

Fig. 15. Refrigerated mobile fish vending Kiosk

Under ideal operating conditions, the unit can extend the shelf life of fish for 4 to 5 days and increases marginal benefit to fish vendors/sellers. The kiosk is affordable to small scale and retail fish vendors/sellers. The technology also helps to change the unhygienic handling and marketing practices of fish by the vendors/sellers/fisherfolks. The traditional fish vending systems are soon going to be replaced with refrigerated mobile fish vending units developed by ICAR-CIFT.

Parameters	**Specifications**
Full body material	SS 304 Food grade material
Fish display unit top	Poly carbonate/toughed glass material
Loading capacity	20kg under chilled storage and 80 kg under Ice box
Refrigeration capacity	400 L
Insulation	Puff insulation (3-inch thickness)
Electric rating	220-240V (AC 50 - 60 Hz.)
Power consumption	300 watts
Temperature of chilled storage	2-3°C
Design variation	It can be customized according to the requirement (U shape, L shape, straight line etc.)

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Chapter 18

An introduction to biochemical aspects of fish preservation

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Biochemistry of foods emphasizes the importance of biochemistry in the rapidly developing field of food science, and to provide a deeper understanding of those chemical changes occurring in foods. The development of acceptable fruits and vegetables on postharvest storage is dependent on critical biochemical transformations taking place within the plant. Meat and fish similarly undergo post-mortem chemical changes which affect their consumer acceptability. In addition to natural changes, those induced by processing or mechanical injury affect the quality of foods. Such changes can be controlled through an understanding of the chemical reactions involved. Increased sophistication in food production has resulted in the widespread use of enzymes in food-processing operations. Biodeterioration of foods by various microorganisms involved in the degradation of proteins, carbohydrates, oils, and fat with special reference to the individual biochemical reactions responsible for food deterioration are important. Meat is basically defined as the flesh of animals used as food. In fish, however, it is the muscle which provides the main nutritional source. While meat is a major source of high- grade protein, fish flesh also provides man with high quality protein; the amount consumed of the latter is increasing annually. Unfortunately, both are expensive foods. The greatest per capita consumption of meat and fish is found in the advanced areas of the world, generally speaking Europe, North America, and Australia. In the developing continents, Africa, Asia, and Latin America, where there is already a deficiency of high-grade proteins, consumption of meat and fish is low, resulting in a high incidence of malnutrition. Such a deficiency of essential amino acids, particularly lysine, methionine, and tryptophan and micronutrients, can now be considered the world's most urgent problem, rather than a shortage of a total quantity of food. With respect to fish muscle, changes occur after death accompanying the decline of pH, including loss of water-holding capacity. This is due to the lactic acid produced, which might be a possible index of freshness in fish. Since lactic acid is known to increase in the post-mortem muscle, at those animals which undergo violent death struggle not only exhibit a reduction of glycogen levels but also ATP and creatine phosphate, which is certainly true for fish muscle.

The development of rigor mortis is highly dependent on temperature. The length of time which elapses between the death of fish and the onset of rigor mortis is ultimately determined by the relative activities of the enzymic systems involved in the synthesis and breakdown of ATP. This, in turn, is controlled by the levels of creatine phosphate, ATP, and glycogen within the muscle tissue at the moment of death. In well-fed, well-rested animals these levels are all high, so that a longer delay period is observed prior to rigor development, producing a meat of low pH and high quality. Any subjection of the animal to starvation or struggling would result inevitably in a much shorter delay period, producing an inferior meat product. The fall of pH to an acidic state accompanied by the various exothermic reactions, such as glycolysis, have a profound effect on the properties of the muscle proteins for fish. The sensitivity of proteins to increase in temperature or marked changes of pH is well established. The sarcoplasmic proteins of fish are generally more stable than the myofibrillar proteins, being unaffected by dehydration or prolonged cold storage. Actin and myosin, the major myofibrillar proteins of fish muscle undergo important changes closely linked with the development of rigor mortis. For instance, in the pre-rigor stages, meat actin and myosin are dissociated, myosin being extractable in solutions of high ionic strengths. Fish actin and myosin are also dissociated during the pre-rigor phase, but are far more labile, associating together at the slightest injury. This has tended to render the isolation of pure fish myosin an extremely difficult operation. As the ATP level decreases, actin and myosin gradually associate to form inextensible actomyosin, an essential criterion for the establishment of rigor. Meat that is cooked during this period is extremely tough in texture. During the development of post-rigor tenderness, however, actomyosin does not dissociate back to actin and myosin, but other subtle changes proceed. A number of studies have been carried out with respect to the denaturation and precipitation of the sarcoplasmic proteins. Fish sarcoplasmic proteins are far more stable than the corresponding myofibrillar proteins. They possess a far greater thermolability and solubility than their counterparts in meat, and do not appear to be involved in fish texture. Comparatively little autolytic enzyme activity appears during the onset of rigor, although some small changes in amino acids are reported in sterile cod muscle. Any deterioration occurring during cold storage is generally attributed to bacterial activity as a result of contamination in the fish samples. A prominent post-mortem change in fish muscle is the loss of fluid or exudation, which is related to the ability of the respective muscle proteins to bind water. In the pre-rigor state, fish muscle possesses a high water-holding

capacity that falls within the first few hours following death to a minimal level coincident with the development of rigor mortis. This minimal level corresponds to the final pH of 5.3-5.5, which coincides with the isoelectric point of the principal muscle proteins. These changes are also associated with a decrease in the ATP level. The significance of pH on the water-holding capacity of fish muscle has tended to be overlooked, since the pH is higher than that for meat, hardly ever falling below 6.0 even in full rigor. However, it has since been established that considerable losses of water occurred from excised fish muscle similar to those reported earlier for mammalian skeletal muscle. A rapid rise in expressible fluid is found in cod which increased after storage in ice for a 168-hour period. Although, as mentioned previously, the post-mortem pH in fish muscle hardly ever falls below 6.0, certain species, including halibut and mackerel, have been found to exhibit a post-rigor pH approaching that of meat. A decrease of pH in halibut which resulted in protein insolubility on approaching the isoelectric zone, producing a pale, soft, exudative condition closely resembling a condition found in pork. This condition in halibut is known as chalkiness and has been a particular problem in the fishing industry of the Pacific Northwest, since fish found in this state are generally rejected by the consumer. However, this condition can be alleviated by allowing the fish to remain alive following capture, thus permitting dissolution of the excess lactic acid, resulting in a normal post-mortem pH following death. On dissolution of rigor mortis, a gradual tenderization of fish muscle occurs. Post-rigor fish muscle provides less of a problem in toughness when cooked compared with that cooked in rigor. Meat will generally reach an optimum acceptable tenderness after an aging period of around 10-18 days storage at 0°-5°C.

Besides the influence of the change in water-holding capacity on post-mortem tenderness for meat, insoluble nonprotein nitrogen, namely peptides and amino acids presumably derived from muscle protein by the activity of proteolytic enzymes also are responsible for tenderness. The sarcoplasm within the muscle fibres contains lysozomes, cellular organelles which can be removed by differential centrifugation. These contain hydrolytic enzymes, including cathepsins, proteolytic enzymes active at an acid pH. These enzymes are liberated when the lipoprotein membranes of the lysozomes rupture at pH levels lower than that normally found in vivo, presumably during post-mortem aging. Proteolysis by cathepsins is the most likely theory to account for the increase in tenderness developed during the post-mortem aging of fish muscle. Several theories have been postulated to account for post-rigor tenderness; however, the

evidence supporting the cathepsin theory still remains inconclusive. Since the sarcoplasmic proteins are more readily denatured under post-mortem conditions, they would consequently be more susceptible to protease attack. The cathepsins appear to have an optimum pH of around 5.5 and are active at a fairly high temperature, i.e., 37°C. The majority of studies on fish quality have been studied during frozen storage, since fish sold in shops as fresh are actually frozen, but thawed prior to sale. Under such conditions microbial spoilage is arrested, although other changes, chemical and physical, can develop. It is generally accepted that a minimum storage temperature of — 18°C is necessary in order to retain the desired fish quality, although these conditions are not always adhered to during the commercial distribution of the frozen fish products. Solubility changes of the total extractable proteins is an index of the changes taking place in the frozen muscle proteins due to denaturation. The decrease in tenderness is proportional to the decrease in "actomyosin" extractability in whitefish muscle. These changes in protein are probably due to a decrease in the water-holding capacity of the thawed muscle, resulting in an aggregation of the proteins. In fish muscle the lipids also appear to be linked with the decrease in protein solubility, as well as the production of off-flavours. The lipids located in fish muscle are highly unsaturated. Free fatty acids are found to increase in saltwater fish during frozen storage; the rate is shown to be both temperature and species dependent. In frozen white-fish, free fatty acids are thought to be liberated as a result of enzymic cleavage of phospholipids and triglycerides. The significance of the free fatty acids on the insolubilization of the fish protein is established through interaction studies of lipids with myofibrillar proteins. Either linoleic or linolenic acid is responsible to reduce the solubility of cod actomyosin. In addition to this reaction other reactions such as protein-protein interactions have been demonstrated, which also effect protein insolubilization. Most of the studies concerning fish texture have been related to protein solubility changes, cell fragility, and free fatty acid liberation under specified conditions of temperature and time. With regard to the autolytic processes taking place during the post-mortem changes in fish muscle, these have not been studied to any great extent. It has however, been reported that the cathepsin activity of fish muscle is considerably greater than that of mammalian muscle, but the significance of this with respect to fish tenderization is not known. Changes produced in fish by the naturally occurring microflora inherent to the living animal, as opposed to the post-mortem contamination of the carcass by bacteria from external sources need to be studied. Although fish muscle is generally regarded as sterile, observation of

bacteria in muscle have been reported in some seawater fish, as well as in several freshwater fish. However, the latter observation leads to the conclusion that the bacteria isolated from fresh-water fish did not belong to the family of water bacteria and, therefore, might have come from the feed. However, freshly caught marine fish have considerable numbers of bacteria located on their skin as well as on their gill surfaces. Following death, the mechanisms involved in their control are no longer functional, as bacterial growth presumably occurs with movement into the various tissues throughout the vascular system. A particular problem in marine fish is the presence of trimethylamine oxide (TMAO) which is reduced by bacterial and enzymatic action to trimethylamine (TMA), a spoilage product of marine fish. The estimation of TMAO, TMA, and total volatile basic nitrogen might provide a useful index of freshness for marine fish. In short, a number of chemical reactions happen in fish muscle after death and during storage, which directly or indirectly determine the quality of fish. A thorough knowledge in this aspect is absolutely necessary to supply good quality fish to consumers and to address the problem of malnutrition in developing countries. As fish is a highly perishable item, special attention is needed, to keep its quality. However, the presence of essential nutrients and micronutrients in large quantities in fish makes it very special globally in nutrition point of view, hence the preservation of its quality and prevention of post-harvest loss is the need of the hour.

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Chapter 19

An overview of seafood borne bacterial pathogens

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Seafood is generally considered microbiologically safe when cooked and offers several health benefits including reduction of cardiovascular diseases, contribution to improving bone strength and congenital developments in infants, reduction of joint pains and inflammations etc. However, when the seafood is consumed in raw form such as fresh, live, partially cooked, etc. despite having these advantages, are associated with foodborne illness. Rapid industrialization has resulted in the release of sewage and other industrial effluents into natural water bodies, increasing the chances of seafood borne diseases.

The seafood-borne outbreaks are mainly caused by bacteria, viruses, and parasites. The major risk recognized for the contamination of seafood by pathogenic bacteria is by the exposure of food chain to contaminated water. The water runoff from polluted areas such as waste waters from agricultural, industrial and sewage will significantly change the microbial flora of the harvesting water bodies and culture ponds which will result in the contamination of seafood with pathogens like, pathogenic *E. coli*, *Salmonella*, *Campylobacter* etc. or viruses such as Hepatitis A, Norwalk etc. The consumption of raw or partially cooked seafood especially bivalve molluscs can be one of the major contributing factors for the spread of seafood borne pathogens. Another reason for the spread of contaminating pathogens in seafood is the poor personal hygiene of workers and food handlers. Inadequate storage temperature and use of poor-quality raw material in the preparation of seafood etc. will increase the risk of illness due to bacteria. Many of the pathogens grow rapidly at room temperature. Fish or fishery product left at ambient temperature is easily spoiled and contaminated with pathogens. This chapter covers the details of major seafood borne bacterial pathogens including emerging pathogens that are causing serious threat to food safety measures.

Salmonella

Infection caused by *Salmonella* continues to be the major cause of seafood borne outbreaks globally. The main source of contamination is associated with raw oyster, salmon, tuna, value

added products of tuna, sole etc. Infection due to *Salmonella* causes gastrointestinal disease and typhoid fever in human. *Salmonella* induced seafood borne outbreaks are reported from several countries worldwide. Non typhoidal serovars are generally associated with seafood borne outbreaks. It was reported that USA alone contributes about 1 million cases of food borne non-typhoidal disease globally. In India, the prevalence of *Salmonella* is ranged between 30.5% in fish to 34.1%. The prevalence rates were low in cold temperate regions such US, Spain and Mexico, ranging from 1.5% to 16.4%. The major serovars of *Salmonella* reported from seafood samples of fishing harbours and fish markets in Cochin (India) were *S. weltevreden, S. rissen, S. typhimurium* and *S. derby*. *Salmonella* infection occurs either through the contact with infected animals, or through the consumption of contaminated seafoods.

Pathogenic *Escherichia coli*

Escherichia coli is a commensal bacterium commonly in the intestinal tracts of warm-blooded animals including humans. Hence, the presence of this bacterium in food products indicates faecal contamination. There are around 700 serotypes of *E. coli* that are generally non-pathogenic in nature, however, there are certain pathotypes that are pathogenic to human being; enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC) enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and Shiga toxin-producing *E. coli* (STEC). This classification is based on their O:H antigen types, virulence characteristics and clinical syndromes. ETEC causes gastroenteritis in humans and low dose of toxin production is sufficient for the excessive fluid secretion and diarrhoea in humans as well as in infants. EPEC causes infantile diarrhoea and the outbreak is mostly seen in least developed countries due to the poor sanitation and hygiene habits. The STEC is highly virulent and is grouped under enterohemorrhagic *E. coli* (EHEC). *E. coli* O157:H7 of EHEC category cause diarrhoea and hemolytic uremic syndrome (HUS) in humans and several infections have been reported in many parts of the world. Virulence in STEC is due to the presence of virulence genes such as either stx1, or stx 2, and both, ehxA and eae genes. The minimal dose of less than 100 cells are able to cause food poisoning in humans.

Staphylococcus aureus

Staphylococcal food borne illness is due to the consumption of food contaminated with membrane-damaging, invasive, one or more staphylococcal toxins. The presence of

Enterotoxigenic S. aureus in fishery products and fish processing environments have been reported from India (Murugadas, 2017). Infection due to Methicillin Resistant *Staphylococcus* aureus (MRSA) is mostly hospital acquired and the high prevalence of this bacterium in health care sector is reported from all over the world. There are only two incidences of food borne outbreaks due to MRSA. MRSA outbreak that resulted in mortalities were reported from Netherlands where banana was implicated as the source of infection. The ingestion of contaminated shredded pork barbeque and coleslaw resulted in food poisoning outbreak due to MRSA in United States. The prevalence of S. aureus in Indian seafood ranged from 9 % to 23 % during the period from 1985 to 2016.

Vibrio parahaemolyticus

The food borne outbreaks caused by *Vibrio parahaemolyticus* are associated with consumption of raw, partially cooked seafood especially bivalve mollusc. This bacterium was first reported as an entero-pathogen in a food borne diseases in Japan in 1950 due to the consumption of partially cooked sardine. It has been considered as the one of the leading causes of food poisoning agent globally. Food borne illness due to the presence of these bacteria have been frequently. It has been detected in many seafood samples including eel, octopus, squid, shrimp, oyster, sardine, tuna, mackerel, perch, pompano, etc. Most of the environmental strains are non-pathogenic and does not cause any infections. Pathogenic strains are characterized by the presence of haemolysin genes such as tdh and/ or trh gene (Okuda, 1997). Most of the pathogenic environmental strains carry trh gene whereas presence of tdh gene is more in clinical strains that cause infection. Main symptoms of infection include gastroenteritis, wound infection and in rare cases, septicemia can occur. No dominant serovars were involved in food poisoning until the appearance of O3:K6 pandemic serotype in India in 1996.

Vibrio cholerae

The transmission route of *V. cholerae* to human occurs mainly through aquatic environments particularly water. There are reports of this pathogen in fish and fishery products from several parts of the world. Several cases of rejections of consignments of seafood in international trade due to the presence of *V. cholerae* has been reported. Generally environmental strains are non-pathogenic and do not possess any virulence related genes such as ctx, zot, ace, and tcpA. The survival and evolutionary dynamics of *V. cholerae* in water causes the emergence of diverse sero

and biovariants of *V. cholerae* due to the gene transfer mechanisms. The horizontal and lateral gene transfer mechanism causes the acquisition of virulence genes, antigenic types such as O1 and O139 etc. Toxigenic *V. cholerae* of classical biotype, had been responsible for infections previously and many epidemic outbreaks were reported in the 19th century which was gradually replaced with an emerging strain of the El Tor biotype in 20th century. Re-emergence of classical biotype together with El Tor strains were reported in Bangladesh during 1982 and these strains were frequently reported in gastroenteritis and diarrhoea from this area until 1993. Another epidemic strain of *V. cholerae* carrying O139 antigen was first reported in 1992 in Southern Asia. The incidence of cholera due to O139 and O1 Biotype El Tor strains gradually increased thereafter in India and Bangladesh. Subsequently, the variant of O1 El Tor (hybrid) which carry tcpA classical genes or classical ctx A or ctx B genes have been reported from clinical cases of cholera from Bangladesh. The non-toxigenic strains of O1 are different in terms of its biochemical and serological properties. Clinical and environmental origin of non-toxigenic strains of O1 have been reported from several countries. However, the non-toxigenic strains lacking toxigenic genes also has the potential of causing diarrhoea in human. The mechanism of virulence and pathogenicity of this strain remains unknown.

Listeria monocytogenes

Listeria monocytogenes is major concern in lightly preserved food products and the prevalence of this bacterium in considerably increased in ready to eat fishery products. Seafood has the highest risk among the minimally processed products. *L. monocytogenes* enters into seafood by cross-contamination and the presence of this pathogen in seafood have been reported from different seafood products. Prevalence rate of this pathogen in seafood products varies from 0 to 17 %. However, the prevalence in seafood is relatively low compared to other food products such as dairy and other animal products. The mortality rate due to *L. monocytogenes* infection is very high ranging from 20% to 30% in immunocompromised patients and hence an important public health concern. The symptoms of infection include septicemia, meningitis, gastroenteritis, pneumonia, and spontaneous abortion. Regulatory agencies such as FDA, ISO, WHO, etc. have included this pathogen in zero tolerant category in processed food products due to its survivability in wide environmental conditions. This pathogen is able to withstand high NaCl concentration of upto 20%, pH range of 4.3 to 9.8, temperature range of 0.5 to 45oC, and

low water activity of 0.91. This pathogen is very well adapted to grow in refrigerated condition, and pose serious risk to the chilled and frozen products once it is contaminated.

***Yersinia* spp.**

The genus *Yersinia* belongs to Enterobacteriaceae family. Presently, it comprises of 16 species and two species (*Y. enterocolitica* and Y. pseudotuberculosis) are pathogenic to human. *Y. enterocolitica* is widely distributed in aquatic and animal reservoirs with swine serving as a major reservoir. Yersiniosis is caused by *Y. enterocolitica* of which virulence biotypes associated with infections are biotypes 1B, 2, 3, 4, and 5. The spectrum of disease ranges from mild diarrhoea to acute gastroenteritis, enterocolitis and pseudo appendicitis in humans. *Y. enterocolitica* is able to withstand freezing for long period of time and remain viable after extended frozen storage which raises public health concerns in the low temperature preservation and processing of seafood.

Clostridium botulinum

C. botulinum are grouped under Gram positive bacteria, and are anaerobic spore producing bacilli of important public health concern in seafood industry. This bacterium is autochthonous to the aquatic environment and aquatic sediments and forms major reservoir of this pathogen. The toxigenic types of *C. botulinum* belong to type A, B, E and F. The major risk factors in seafood are due to the presence of these toxigenic types. Botulinum food poisoning is due to the consumption food contaminated with preformed toxins of *C. botulinum* and low oral dose of 70 µg is sufficient to causes illness in human. Its prevalence in seafood depends upon several factors such as topographical location, culture practices, detection methods etc. The fish poses serious risk due to its direct contact with sediment and the ingestion of spores through contaminated feed/sediment. This bacterium is a major concern in packaged seafood products where cold chain is not maintained during storage, transport and distribution chain. The favourable condition for the growth of *C. botulinum* in preserved products such MAP or vacuum-packed products include, pH of about 4.6, water activity of 0.93%, low salt upto 3%, temperature range of 3oC to 50° C.

Emerging pathogens in seafoods

Apart from the well reported seafood borne pathogens, several other pathogens are also emerging throughout the world irrespective of the geographical conditions, and able to cause infectious diseases in the current century. It is not always true that emerging pathogens are a new category of microorganisms, instead it can be already established pathogens in which the virulence or resistance to disease characteristics is high as a result of stressful conditions such as changes in the habitat, climate, overdose of antibiotics etc. It is important to study the time of emergence of particular bacteria of infectious category to the food chain via source tracking and establishment of national network of surveillance system, so that the epidemic spread can be controlled by effective implementation of the mitigation measures and re-emergence can be prevented.

Vibrio vulnificus

Vibrio vulnificus a halophilic bacterium belonging to Vibrionaceae and widely distributed in brackish water and marine environments. High concentration of these bacteria can be seen in filter feeding bivalves that inhabits coastal polluted waters. So, the major risk factor for the food borne outbreak is the consumption of contaminated raw or partially cooked shellfishes. Infection can also occur through open wounds and may lead to septicaemia in fatal cases. The fatality rate of *V. vulnificus* infection ranges from 20 to 60%. Recently, this bacterium has emerged as public health significant bacteria due to its high fatality rate all over the world.

***Campylobacter* spp.**

Campylobacter spp. causes gastrointestinal disease termed camplyobacteriosis and one of the leading causes of food borne outbreaks in developed countries. Since 2005 to 2019, this bacterium has been implicated in gastrointestinal disease of more than 2,20,000 people in EU and ranks first in foodborne outbreak followed by *Salmonella* and *Yersinia*. The USA reports 8.45 lakh cases of *Campylobacter* infection per year. The outbreak is mainly due to ingestion of contaminated food products, where the chicken alone contributes to about 25% of the infections. The incidence of *Campylobacter* spp. have been reported in other types of food animals such as cattle, pig, cows, sheep etc. *Camplyobacter pleridis* and *C. lari* subsp. concheus were isolated from shell fish. The *Campylobacter* spp. is a commensal bacterium to poultry and the intestinal tract carry huge amount of this bacterium. The rupture of intestinal tract while processing can

disseminate the content to skin. Cross contamination with shellfish harvesting area and handlers can result in seafood borne outbreak. Shellfish associated *Campylobacter*osis was first reported during 1980s where 28 persons were infected after eating raw clams.

***Cronobacter* spp.**

Cronobacter species belongs to the family Enterobacteriaceae and is considered as an opportunistic pathogen in neonates. Among 7 species of *Cronobacter,* three species are pathogenic to human, namely *C. sakazakii, C. malonaticus and C. turicensis*. Out of these, C. sakazakii causes high mortality rate of about 40-80% in neonates. This bacterium has been isolated from wide range of food sources such as dairy products, plant-based products, dried fish, shrimp, seaweeds and minimally proceeds products. This bacterium is considered as an emerging pathogen of seafood recently due to its survivability in low moisture foods such dried fish product. However, the seafood borne outbreak due to this bacterium was not reported so far.

***Arcobacter* spp.**

Arcobacter is an emerging zoonotic pathogen, belongs to Campylobacteraceae and is closely related to the Genus *Campylobacter*. They are able to survive in low oxygen condition, and well adapted to temperature of less than 30°C. Arcobacter causes bacteraemia, gastroenteritis and diarrhoea. Out of 27 species, three species are major pathogenic strains causing disease, namely *A. butzleri, A. cryaerophilus* and *A. skirrowii*. Food borne infection associated with chicken and vegetables have been reported. Seafood borne outbreak due to *Arcobacter* was not reported so far, however reports of isolation of *Arcobacter* from fish, shellfish, and seawater are available.

Vibrio mimicus

Vibrio mimicus is an important emerging zoonotic pathogen in seafood that causes disease in aquaculture fishes as well as gastroenteritis in human. Major reservoir of this pathogen are raw oysters, fish, turtle eggs, shrimps, cray fish. Davis et al. (1981) studied the biochemical characteristics of atypical *V. cholerae* by biochemical tests revealed new species of sucrose negative strain for which the name *Vibrio mimicus* sp. nov. strain was proposed. *V. mimicus* carrying ctx gene is reported as pathogenic strain that can cause severe watery diarrhoea and gastrointestinal disorders. In India, there were only few reports of this organism from seafoods.

Food safety with respect to seafood pathogens is an important in terms of public health perspectives as over 200 types of diseases are due to the consumption of contaminated foods. (To ensure food safety, routine microbiological screening tests should be validated in real time so that the contaminated food products get detected. National regulations shall be enforced for ensuring food safety that includes the strict implementation of food hygiene and sanitation programme through Hazard analysis and critical control point (HACCP), together with Good management practices (GMP), standard operating Procedures (SOPs), Sanitation standard operating procedures (SSOPs) practices from production to consumption stages, there by the product becomes safe at all stages of production, processing and distribution levels. The harmonization of these practices in international trade ensures the safety of seafood products, globally.

Chapter 20

Environmental impacts of fishing

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General ecological impacts due to fishing

Fishing can have a significant impact on ecological processes on a large scale, and badly managed fisheries develop excessive fishing capacity, leading to overfishing with social and economic consequences. An ecosystem that was originally stable, mature, and efficient becomes stressed and immature as a result of overfishing. By targeting and reducing the abundance of high-value predators, fisheries deeply modify the trophic chain and the flows of energy across the ecosystem. Fishing also alters habitats by destroying and disturbing bottom topography and the associated habitats including seagrass, seaweed, mangrove, algal beds, coral reefs, and benthic communities. The alteration of the habitat by various fishing activities may be physical like the introduction of artificial structures or mechanical such as the use of bottom trawls, or chemical such as the leaching of pesticides, heavy metals, drugs, hormones, etc to the marine environment result in changes in productivity. Some aspects of fishing can have significant and long-lasting effects, e.g. destructive fishing techniques or inadequate fishing practices; pollution, use of ozone-depleting refrigerants, dumping at sea of plastic debris that can entangle marine animals or be swallowed by turtles; loss of fishing gear, possibly leading to ghost fishing; lack of selectivity, affecting associated and dependent species, resulting in wasteful discarding practices, juvenile mortality, added threat to endangered species, etc. Poorly-managed fishing practices can damage coastal ecosystems and contribute to ecosystem contamination with food residues, waste, antibiotics, hormones, diseases, and alien species.

Fishing involves the construction of the fishing vessel, gear & other accessories which channelled the harvest process. Both of these processes cause many environmental impacts. During the last 50 years, the introduction of synthetics in construction/fabrication gradually replaced natural materials such as wood for fishing boat construction and natural fibres such as cotton, manila, sisal, jute, coir, etc in the fabrication of fishing gears due to their high breaking strength, high resistance to weathering, low maintenance cost, long service life and better uniformity in characteristics also affect the marine ecosystems.

Environmental impacts of major boat building materials in aquatic system

Several technologies evolved over the years in the fishing industry which have improved the fish catch as well as the effort and the related inadequate practices leading to damage to the ecosystem and these ecological impacts were well explained in much of the literature. Hence, this chapter mainly dealt with the environmental impacts of boatbuilding materials and emissions from fishing.

In fishing boat construction, the common materials used in India include wood, glass/fiber reinforced plastic (FRP/GRP), aluminium, steel, plywood, ferrocement, etc. While selecting a material for boat construction some basic factors to be considered are type, size, speed, the shape of the vessel, availability and suitability of the material, and economic and environmental viability. The performance and efficiency of a boat are directly dependent on the choice of the boat-building material which also has a direct impact on the environment. By taking these facts into account, a boat designer can select the best possible alternative for building a boat of high efficiency and durability. A fishing boat is made up of different components and their construction is a complex process. Certain quantities of greenhouse gases (GHGs) are produced in the process of manufacture, transportation, and utilization of these components, which can be converted in terms of equivalent CO2. Every ocean has marine debris, and more than 60% of it is plastic that comes from the fishing industry, offshore platforms, recreational shipping, etc (Cheshire et al., 2009; Eriksen et al., 2014; Pham et al., 2014., Richardson et al., 2019).

At present, the larger class of fishing vessels are made of steel while vessels belonging to the medium and lower categories mostly use wood for construction. Fiberglass, ferrocement, and aluminium are the new substitutes for conventional boat building materials as these can improve the lifespan of the boats. However, traditional fishing boats still play a vital role in this era. Despite its obvious advantages, all boat-building materials are susceptible to the effects of the marine environment, for example, glass fibres are the most selected material for boat construction, which are vulnerable to the effects of sunlight in marine conditions. Fiberglass-reinforced plastic (FRP) is a polyester resin-based composite, reinforced with fine strands of glass filaments. Glass fiber is prone to osmosis, and gelcoat gets faded in sunlight resulting in the attack of UV radiation. FRP fragments have a higher density than seawater and will tend to concentrate nearshore. The polyester resins or epoxy resins in the FRP undergoing physical &

chemical degradation lead to the release of microplastics which affects the environment. Marine organisms consume these plastic particles and end up in the human food chain causing severe health issues. Additionally, the deteriorating and peeling paint with high concentrations of tributyltin and lead from the abandoned boats may provide a long-term environmental issue

Aluminium alloys are prone to corrosion if untreated or damaged. When new alloys are exposed, an oxide layer is formed on their surface but this oxide layer does not protect the alloy in the long term when exposed to marine environments. Periodically the paint system will need to be removed in areas of stress and the corrosion treated. Careful inspection on an annual basis of all weld seams helps in early identification of the occurrence of this problem. Aluminium reacts with some copper-based antifouling paints causing serious corrosion in environmental conditions. Therefore, antifouling containing metallic copper or cuprous oxide should never be used on aluminium, whilst copper thiocyanate-based antifouling can be used if the aluminium is primed properly.

The most common form of corrosion in steel is rust. Such a reaction will take place only in the presence of water. A marine environment is therefore an ideal place for rust to occur. Due to the high flexibility and strength of steel, it is hard to break, but impact damage may well result in a dent owing to the metal stretching and deforming locally. This can present problems for a protective coating, which may not be so flexible.

The fibrous nature of timber means that it has a tendency to absorb moisture from the atmosphere, and swell and contract to varying degrees depending on the type of construction. For a varnish or paint coating to stay intact it has to be quite flexible in nature. Moisture content in wood allows the growth of fungal spores, which leads to rotting and decay. Wood can also be subjected to the attack of marine borers, which eat the wood fibers. Therefore, it needs to be protected by good-quality preservatives and coatings. Many different kinds of wood can be used, which can differ immensely.

A comparative table giving the carbon consumption in the production of these materials

Material	Net carbon emissions (kg C/metric) [1]	Net Carbon emissions including Carbon storage within material (kg C/ metric ton)[2]
Framing Lumber	33	-457
Medium density fibre board	60	-382
Steel	694	694
Aluminium	4532	4532
Plastics	2502	2502

Net carbon emissions in producing 01 ton of material (OECD, 2010)

While considering the environmental and economic sustainability of different boat building material, wood is an ideal material still preferred for marine boat construction. Wood is a functionally efficient material which reduce carbon footprint thereby reducing the environmental impact and simultaneously balance the cost objective. Environmental impact of any material can be evaluated through Life cycle assessment procedure or LCA. The environmental impact of wood from the very first state of harvesting to the end of the product was studied and compared with other materials and found that wood as a material for boat construction contributes less pollution to the environment compared to concrete, steel, aluminium, etc.

Studies have found that wood products have less embodied energy and are more environmentally friendly as they are involved in less carbon footprint as well as air and water pollution. Furthermore, residues of wood industries are utilized in either by-product manufacturing or fuel and clean bio-energy. As forests act as a carbon sink and prevent climate change and greenhouse gas, increasing wood use ensures sustainable development by reducing emissions, increasing renewable wood use, and thus helping the national economy.

Fishing Vs Energy use

Commercial fishing operation mainly utilizes fossil fuels which result in the emission of greenhouse gases. The active cost of fishing is less understood and consequently receives less attention to GHG emissions than the direct impact on fishery stock and marine ecosystem. Similarly, in the harvest process, several reoccurring inputs are required for every fishing operation, viz. fuel, lubricant, ice, freshwater, etc. These inputs have their own carbon footprint value for construction/extraction/process, especially fuel contributes more than 95% out of all

the components. Despite the fact that the prevailing pre-harvest phase of marine capture fisheries lacks general detail and standardization about LCA/carbon footprint studies; such studies and their findings can be useful in formulating constructional/operational recommendations to improve the environmental performance of fisheries, under the context of an ecosystem approach to fisheries along with future certification and different eco-labeling of fisheries. Studies related to pre-harvest, harvest, and post-harvest fisheries LCA/carbon footprint analysis would be more appreciated by policymakers for the regulation of fishing boat yards and other related fishing ventures.

Based on behaviour and habitat, there are different methods of fish harvest and on the basis of their operation, the quantum of fuel and energy requirement also varies. As per the study by Parker et al., 2018, the world fishing fleet burned about 40 billion liters of fuel and emitted 179 million tonnes of CO2 equivalent and other GHGs to the atmosphere. Overcapacity and irresponsible use of fossil fuels leads to increased levels of fuel consumption in fishing contributing to climate change in the long run. India contributes 134 million metric tonnes (2.7%) of CO2 emission due to total marine capture fisheries, against 90 million metric tonnes (3.9% of global production) of fish production. The emissions due to fishing were not given importance as compared to other sectors for emission in India, however, the contribution of the fisheries sector is negligible which roughly may be <1% of global GHG emission. The other associated important environmental parameters by which the health of the environment, humans, and resources can be evaluated due to the fishing process are; terrestrial acidification, formation of fine particulate matter, Water consumption, Ionizing radiation, ozone formation, human carcinogenic toxicity, fossil resource scarcity, mineral resource scarcity environment deterioration, human health, resource depletion, and stratospheric ozone depletion, etc.

Different types of vessel and gear combinations are used for fishing to exploit various fish stocks. The important fishing practices are trawling, gillnetting, longlining, dol netting, purse seining, etc. One major reason for the substantial increase in eq. CO2 emission by the construction process is the increase in the number and efficiency of fishing boats otherwise called overcapacity, which need more inputs and equipment, resulting in more eq. CO2 emission.

In modern fisheries, the major direct and indirect energy inputs can be systematically analyzed using process analysis and input-output techniques. Mostly direct fuel inputs are used primarily for vessel propulsion. On average direct fuel energy inputs account for between 75 and 90% of the total energy inputs, irrespective of the fishing gear used or the species targeted. The remaining 10 to 25% generally depends on vessel construction and maintenance, and the provision of labour, fishing gear, bait, and ice if used which depends on the character of the fishery and the scope of the analysis conducted. The secondary energy-consuming activities, which include onboard processing and storage are negligible compared to primary energy consumption in terms of fuel burned. The study of environmental burden is important in relative resource-use analysis and greenhouse gas (GHG) impacts in climate change mitigation. It has got emphasis due to the high instability in fossil fuel costs which has potentially lasting impacts on the economic performance of various fishing systems.

The effects of fishing and its implications on ecosystems, especially from the boat-building sector or the usage of energy, fuel, and emissions, were not particularly addressed and are anticipated to have significant effects on ecological sustainability and food security globally.

Chapter 21

Pathogenic *Vibrios* of public health and aquatic animal health

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Introduction

Classification of Vibrios

Domain - Bacteria

Phylum - Proteo bacteria

Class - Gamma proteobacteria

Order - Vibrionales

Family - Vibrionacea

Genus - Vibrio

Vibrios are the diverged group of organism and mostly had the history of pandemics. They are inhabitants of natural aquatic ecosystem like ocean, River, wells and ponds. They are gram negative facultative anaerobes motile by using single polar flagella. Vibrios do not form spores and capsules. Most of the Vibrios are not fastidious and they tolerate high alkaline pH. Vibrios can do both Oxidative and fermentative utilization. They are distributed throughout the world with more occurrence in the tropical region. Vibrio occurrence in the temperate regions are more in hotter months. They have the peculiar ability to go to viable but nonculturable state in adverse environmental conditions. Vibrio can be classified in to cholera causing and non-cholera and they are Vibrio cholera and Vibrio mimicus. The important non-cholera pathogenic vibrios are *Vibrio parahaemolyticus*, Vibrio vulnificus, Vibrio harveyi and Vibrio alginolyticus.

Vibrios are zoonotic in nature and with the fish they can affect the higher vertebrates also. Aquaculture is the food production sectors from water. So any inhabitant in water can affect the production adversely. the sudden onset of diseases, especially by Vibrio spp. is becoming a great concern in larval and juvenile penaeids, and fishes. Hence, the monitoring of aquaculture environments for pathogenic Vibrios is essential to control the spread of Vibrio infections. The members of the genus Vibrio are the most important food-borne and aquatic pathogens which are responsible for illness in humans and cause large-scale mortality in the aquaculture sector. Nowadays in the international trade of marine fishes, testing of Vibrio species has become a criterion of microbiological testing. Even though Vibrio species are a common inhabitant of the

aquatic environment, some species are emerging as pathogens which can cause up to more than 50% of deaths of all clinical cases. Major Vibrio sp. viz. V. harveyi, *V. parahaemolyticus*, V. alginolyticus, V. anguillarum, *V. vulnificus*, V. mimicus, and V. splendidus are usually associated with shrimp diseases. V. harveyi is associated with luminescent vibriosis in shrimps e.g., Litopenaeus vannamei and Penaeus monodon and it is the most important etiological agent for mass mortality in P. monodon. The mode of infection in fish mainly consists of penetration of bacterium to the host tissue mainly by the chemotactic activity, followed by deployment of the iron sequestering system and eventually damages the fish through extracellular products i.e., hemolysin and protease.Gram negative, curved, comma shaped bacilli

Traditional method of detection of pathogenic Vibrio species

There are well-established isolation and biochemical confirmation procedures for pathogenic Vibrio spp. Which were described in ISO and BAM protocol for Vibrios. First stage in traditional detection methods exploits the ability of Vibrio species to grow rapidly at relatively high pH values. Media containing sodium chloride and with a pH of about 8.6, such as alkaline saline peptone water (ASPW), are used for enrichment. Typically, a 6-hour preliminary enrichment (at 41.5oC for fresh products, or 37oC for frozen or salted products) is followed by a second enrichment in ASPW at 41.5oC (for *V. cholerae* and *V. parahaemolyticus*) or 37oC (for other species) for 18 hours. Preliminary identification based on colony appearance on TCBS agar is traditionally confirmed using classical biochemical tests. The second enrichment culture is inoculated onto thiosulphate citrate bile salts sucrose (TCBS) agar and one other optional selective medium and incubated at 37oC for 24 hours. On TCBS agar, V. mimicus colonies are green, *V. parahaemolyticus* colonies appear blue-green and V. harveyi colonies are green in color. Selective chromogenic agar media specifically designed for the differentiation of pathogenic Vibrio species are also available.

Vibrio choleraas a human pathogen and aquatic pathogen

Vibrio cholera is the organism responsible for the disease cholera, an acute illness. The diarrhea cause by cholera is specific with rice water stool. The body will become dehydrate and mortality can occur in hours. This can be cultured with alkaline peptone water enrichment and Thiosulphate citrate bile salt sucrose agar streaking. After 24 h the TCBS will have yellow round flat colonies of 2-3 mm size. Vibrio cholerae has more than 200 serotypes with O antigens. Only

serogroup O1 and O139 are found to cause cholera epidemics. The 01 serogroup is divided into two biotypes, Classical and El tor, both of which can cause epidemics. The classical bio-types susceptible to polymixin, VP negative and do not produce hemolysin to lyse heamocytes. Whereas El-tor biotype insusceptible to polymixin, VP positive and produce hemolysin to lyse heamocytes. So far 6 pandemics are caused by Cholera bacteria classical biotype now the cholera occurrences are by 7th pandemic are from Eltor biotype. But this is relatively less fatal and it will survive in human body for more days. Human cholera infection starts with injestion of the cholera bacterium through food or water. It colonizes the small intestine and produce cholera-toxin in to the host cells. This cause rapid efflux of chloride ions and water to the intestinal lumen. This causes the diarrhea and dehydration. Vibrio cholera is not causing any apparent cholera disease to fish and shrimp. According to Koch postulate it is not causing any disease. But it can be isolated from aquaculture environment and fish gut. Aquatic environment is the major reservoir of Vibrio cholerae before and after the outbreak. Recent evidences support the theory of the fish and water birds can be vectors of cholera outbreak. Most of the Vibrio cholera outbreak are caused by under cooked fish consumption. The Eltor biotype infection in Bengal was brought by Hilsa which acted as a reservoir.

***Vibrio parahaemolyticus* as a human pathogen and aquatic pathogen**

The first reported occurrence of *Vibrio parahaemolyticus* is in Japan in 1950, where the under-cooked bacteria affected 272 patients and killed more than 20 people. Until then the *Vibrio parahaemolyticus* was not much considered as a pathogen. *Vibrio parahaemolyticus* is a non-cholera Vibrio which cause gastro-enteritis. This is a halophilic Vibrio which can live in water of 0.5-8% salt. The infections are caused by consumption of under-cooked or raw shellfish. It can cause extra intestinal infections also. It can also cause infection to the cooked product from the uncooked product. The occurrence is there in almost all water bodies with necessary sodium requirement. The major virulence factor is hemolysin (TDH, TRH) and cytolysins. The TDH is the major toxin present in 95% of the *Vibrio parahaemolyticus* and it can be seen as haemolysin in wagatsuma agar. Thermolablile haemolysin also reported from *Vibrio parahaemolyticus*. This also cause similar result in heme supplemented blood agar. The toxins are having cardio-toxicity, cell toxicity and center toxicity. The toxins are releases as monomers to extra-bacteria space and they become oligomer to make pore in the host cells. This can also spread through open wounds and cause septicaemia. The toxin production is correlated with Urease production in the *Vibrio*

parahaemolyticus. The disease propagation in cells needs ammonia which can be produced by the Urease positive *Vibrio parahaemolyticus*. More than 800 food-borne disease outbreaks were reported in china, 40 % are from *Vibrio parahaemolyticus* alone.

The *Vibrio parahaemolyticus* is a deadly pathogen for shrimp which cause early mortality syndrome. It causes heapatopancreatic necrosis and sloughing of intestinal epithelium. The *Vibrio parahaemolyticus* infections have caused major losses in aquaculture industry.

Food poisoning due to *Vibrio parahaemolyticus* occurs in warmer months. It is associated with Fish, crab, shrimp, lobsters and oysters. If consumers eat the under cooked seafood contaminated with *Vibrio parahaemolyticus* the disease occurrence is confirmed. The feces of patients are contaminated with this bacterium and it mostly follow the fecal oral route. It causes fever, chills. Nausea and water like stools. The shock from the toxin sometime gives death.

***Vibrio vulnificus* as human and aquatic pathogen**

Vibrio vulnificus is a halophilic aquatic Vibrio which has relatively low occurrence compared to Vibrio cholera and *Vibrio parahaemolyticus*. It can occur worldwide from temperature ranging from 9-35℃ and salinities ranging from 0.5- 35. It causes diseases such as necrotozing fasciitis, Gastero enteritis and wound infections. This mostly infects person with underlying medical conditions such as liver diseases, immune-compromisation and iron storage disorders. The bacteria possess cytolysins, hemolysin and specialized siderophores (Vulnibactin) as immune factors. This can produce amine putrescene and cadavarine from ornithine and Lysine. They can neutralize the gut acid and can cause gastro-enteritis. Vibrio vulnificus produces superoxide dismutase and nullify the peroxide present in the neutrophils. So the infection can also travel through the neutophils. The bacteria have 3 bio types. Biotype one is arginine negative, ornithine Indole and lysine positive. The biotype two is Indole and ornithine negative. the first biotype is known to cause disease to the human. And second biotype is known to cause fish diseases. Third biotype has the mixed characteristics and its geographical distribution restricted to Israel.

The contamination of Vibrio vulnificus will not cause any odour or appearance change. It is present in warm waters and can be accumulated in filter feeding bivalves. The fatality is very high compared to the bio-safety level 3 and 4 pathogens such as plague, anthrax and Ebola. In immuno compromised persons the consumption can cause gastro-enteritis which if untreated

can enter bloodstream and can be fatal. The wound infections could start after the handling of infected fish and seafood, especially shellfish and after the practice of aquatic activities such as swimming. More than 50% of primary septicaemia due to Vibrio vulnificus result in death within the first 72 h of hospitalization. If there is infection diagnosed due to Vibrio vulnificus, immediate and appropriate antibiotic treatment with surgical intervention if necessary.

The vulnificus is known to cause Gastroenteritis, primary sepsis, and wound infection. Rare cases of spontaneous bacterial peritonitis, Pneumonia, Endometritis, Meningitis, Septic arthritis, Osteomyelitis, Endophthalmitis, Keratitis to human beings.

An aquatic Vibrio Disease - Early mortality syndrome

The AHPND Acute Hepatopancreatic Necrosis Disease is caused by *Vibrio parahaemolyticus*, Vibrio punensis, V. harveyi, and shewanella sp with the disease causing plasmid pVA1. The plasmid code for the Pir toxin A and Pir toxin B (Photorhabdus luminous insect related) This is one of the reason behind shrimp aquaculture collapsed in South-Asian countries. Develops quickly, starting approximately 8 days post stocking and severe mortality (up to 100%) occur within 20–30 days. The toxins can cause opaqueness, organ liquefaction and death.

Control method for zoonotic Vibrio diseases in aquatic food production sectors

- The handlers should not be immune compromised
- Should wear gloves while handling diseased fishes
- Fish source should be disease free.
- Farm should have bio-security measures
- The disease farm water should be treated with bleaching powder before release
- Water quality parameters should be optimum
- For human beings it should be personal hygiene and washing hands before and after handling fish.

Chapter 22

Introduction to Bioinformatics

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Introduction

Micro-organism weighs about 60 % of living beings despite being invisible in natural ecosystem. Most of them are either sub-microscopic or microscopic in nature. They are nature friendly for the sustenance of our ecology. However, a few species are of pathogenic in nature to the flora and fauna of our ecosystem. Fisheries, a highly dynamic ecosystem are however, no exception on this count as fish pathogens play havoc in the present food security regime. In this context, it is also noteworthy that in India, fisheries is a bio economy that contributes over 1% of GDP and over 5% of agricultural GDP that itself account for about 10% of global fish production.

Intensive fish farming, by and large leads to disease problems resulting in serious economic losses. Bacterial pathogens are primarily the root cause of fish diseases. Some of the important pathogens among them include *Salmonella, E.Coli, Campylobacter, Staphylococus spp., Enterococus spp. Aeromonas hydrophila, Vibrio spp. and Edwardsiella tarda***.** It also includes other pathogenic bacteria such as *Streptococcu*s spp. and *Pseudomonas spp.* These pathogens cause many foodborne illnesses such as typhoid fever, diphtheria to mankind too. Microorganisms express their pathogenicity by means of their virulence. The determinants of virulence of a pathogen with its genetic or biochemical or structural features attribute to produce disease in a host. In bacterial host mediated pathogenesis, (e.g., tuberculosis), tissue damage results from the toxic mediators released by lymphoid cells rather than from bacterial toxins. This underscores the need to understand the pathogenesis and pathology of diseases in fishery environment by identifying the pathogens.

Microbiology

Microbiology is the study of microscopic organisms. They are unicellular (single cell) or multicellular (cell colony). Microbiology includes sub-disciplines like virology, mycology, parasitology and bacteriology. Microbiologists carry out biochemical test for checking, understanding and identifying of microorganisms. However, in the modern age Microbiologists do carry out tests on molecular approaches by extraction or detection of nucleic

acid, either DNA or RNA sequences to ensure and validate the test results generated through conventional diagnostic methods.

Antonie van Leeuwenhoek is considered a father of microbiology as he observed the microscopic organisms in 1676, using simple microscopes. However, microbiology has been evolved as a scientific discipline in the 19th century through the systematic but scientific microbial studies carried out by **Louis Pasteur**. The main challenge faced by microbiologist is the size and numerals of microbes present in a unit space. It is difficult to assess microbes quantitatively owing to its infinitesimally small size in dimension and infinitely large in numbers, present in the host. However high-end computing and software facilities coupled with computer aided instrumentations have facilitated the data generation process and data analytics and thus mitigated the problems faced by microbiology per se, as a scientific discipline to a greater extent.

Biotechnology

Biotechnology is an applied science to use living systems to develop or make products. In other words, it is the technological application that uses living beings, largely microbial living organisms, or derivatives thereof, to make or modify products or processes for specific use. Exploitation of biological processes for industrial and other purposes through genetic manipulation of microorganisms is also well established in this field of science. For thousands of years now, mankind has been using biotechnology in the field such as agriculture, fisheries, food science and medicine. The term Biotechnology is believed to have been coined in 1919 by Hungarian agricultural engineer **Károly Ereky**. In the late 20th and early 21st centuries, Biotechnology has been expanded to include new and diverse scientific disciplines like genomics, recombinant gene techniques, applied immunology, and pharmaceutical therapy.

While some microorganisms do have associated with human illnesses, major chunk of microbes are positively instrumental for numerous beneficial processes such as industrial fermentation (e.g. the production of alcohol, vinegar and dairy products), antibiotic production and as vehicles for cloning in more complex organisms such as plants. Scientists have also benefitted their knowledge of microbes to produce biotechnologically important enzymes such as T*aq* polymerase, reporter genes for use in other genetic systems and novel molecular biological techniques such as the yeast two-hybrid system.

A variety of biopolymers, such as polysaccharides, polyesters, and polyamides, are produced by microorganisms. Microorganisms are used for the biotechnological production of biopolymers with tailored properties suitable for high-value medical application such as tissue engineering and drug delivery. Some polyester materials are used for fabrication of fishing gears either.

Microorganisms are beneficial for microbial biodegradation or bioremediation of domestic, agricultural and industrial wastes and subsurface pollution in soils, sediments and marine environments. The ability of each microorganism to degrade toxic waste depends on the nature of each contaminant. Since sites typically have multiple pollutant types, the most effective approach to microbial biodegradation is to use a mixture of bacterial and fungal species and strains, each specific to the biodegradation of one or more types of contaminants.

The monstrous genera *Thiomargarita* and *Epulopiscium* in which some species of bacteria that measure over 600 to 700 μm in length or diameter and are visible to the naked eye. However, large bacteria are rare in nature, Most of the bacteria size around 0.4 and 2 μm in diameter and 0.5 and 5 μm in length. It is all the more important to see that bacteria are boring, at least in morphological sense. Table1 gives the perception size of some of the bacteria.

Size, Shapes and arrangements of bacteria

Three basic shapes of bacteria are coccus (spherical shaped), bacillus (rod shaped) and spiral**.** An average coccus is about 0.5-1.0 micrometer (μm) in diameter. An average bacillus is 0.5-1.0 μm wide by 1.0-4.0 μm long. Spirals come in one of three **forms**, a vibrio, a spirillum, or a spirochete. Some typical bacteria and the size are given below.

Table 1: Size and dimension and of some important bacteria

SL. No.	Name	Dimension of size (μm=micron)
1.	*E.coli*	1-3 x 0.4-0.7 μm
2.	*Salmonella*	0.7-1.5 x 2-5 μm
3.	*Vibrio spp.*	0.5 x 1.5-3.0 μm
4.	*Clostridium spp*	0.3-2 x 1.5-20 microns
5.	*Aeromonas hydrophila*	0.3-1.0 x1.0-3.5 μm

Bacteria usually are microns in diameter (10^{-6} meters). Only general shape and major morphological features are visible in light microscope. In general, bacteria range from 0.2-10 microns. A scanning elecronmicroscopic (SE) micogram of *Vibrio parahaemolyticus* is given in fig.1

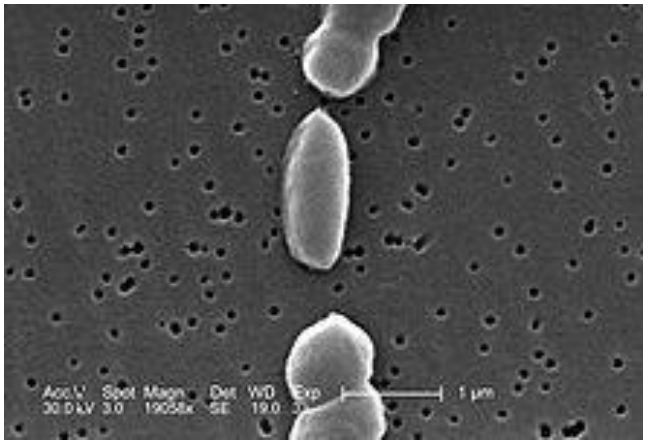

Figure 1: SE Micogram of *Vibrio parahaemolyticus*

(Image source https://en.wikipedia.org/wiki/Vibrio_parahaemolyticus#/media/ dt.06/06/2018)

The excessively large number of microorganism present in a very small unit space and the infinitesimally small dimension of the microorganisms by nature are the challenges faced by microbiology as a scientific discipline. The digital era of computational technology mitigates this problem to a considerable extent by facilitating data collection from laboratory using high-end computer aided instruments, data processing and analysis for generation of information with the help of high speed and high precision computing facilities. Huge volume of genomic data is by far a challenging task for development, processing and management of databases either. Thanks to the development in the field of informatics and computing facilities with sophisticated programming codes in the modern era, available with us to cope up with the situation largely.

Important pathogens and likely problems with fish/fishery products

Bacteria in food may cause illness in humans by infection or intoxication. Examples of some of the types of bacteria that may be found in seafood that cause foodborne illness by infection are *Vibrio, Salmonella, Shigella, and Listeria*. From 1973 to 2006, *Vibrio* species accounted for 38% of the outbreaks associated with seafood and 54% of the illnesses. *Salmonella* and *Shigella* each were associated with about 10% of the reported illnesses, and *Listeria monocytogenes* approximately 1% too. Foodborne intoxications occur when patients consume pre-formed toxins that are produced by certain types of bacteria when they grow and multiply in the food. *Clostridium botulinum* can produce a potent neurotoxin during growth under anaerobic conditions (absence of oxygen) usually associated with vacuum packed, improperly canned, or fermented products. *C. botulinum* toxin was associated with

almost one fourth of the seafood related outbreaks from 1973 to 2006 and caused 152 illnesses and 38% of all hospitalizations. Bacteria such as *Staphylococcus aureus* can produce enterotoxins that cause foodborne illness, but less than 5% of the seafood associated outbreaks and illnesses were associated with this pathogen over the past three decades. Preventing the growth of these bacterial pathogens is important to prevent infection or intoxication when seafood is eaten and is all the more relevant in this Antimicrobial resistance regime.

Fish diseases

Many diseases are caused in fishes due to pathogens which cause havocs to fisherman community as well as fishery industries. Some of the fish pathogens, causing diseases *are Aeromonas hydrophyla, A. salmonicida, Pseudomonas fluorescens, P. putrefaciens, Flexibacter columnaris, Edwardsiella tarda, Vibrio alginolyticus* and *V.parahaemolyticus*. It is highly imperative to identify the pathogens either by biochemical method or by molecular method through the study of genomic sequence to tackle the causative reasons for the diseases prevailing in fish and fishery environment including fish processing industry. Though biochemical methods are proven methods for identifying the micro-organisms, molecular methods through the study of genomic sequences of the pathogenic cells is all the more paramount in the present data analytic environment of high precision computing facility. At times it could be used as a mode of corroboration for conventional microbial detection techniques.

Genomics

Genomics is an interdisciplinary field of science focusing on the structure, function, evolution, of genomes. A genome is the complete set of DNA of an organism, including all of its genes. Unlike in genetics, where the study of individual genes and its roles in inheritance is emphasized, genomics aims at the collective characterization and quantification of genes, which drive and direct the production of proteins with the assistance of enzymes and messenger molecules. In turn, proteins make up body structures such as organs and tissues as well as control chemical reactions carry signals between cells. This has a bearing on our response to different stimuli either, which account for the unique emotional characteristics of all living being. Genomics also involves the sequencing and analysis of genome through uses of high throughput DNA sequencing and sequence analysis tools to study the evolutionary relationships through phylogenetic tree. Identification of organism is also possible by incorporating the logics of the phylogenetic tree in the algorithms of the software module. It also assembles and analyzes the

function and structure of all genes of the entire genome to advance the genomic and proteomic study. Advances in genomics have triggered a revolution in microbiological research and systems biology to facilitate the understanding all the organ systems including muscular system and nervous system. This sort of generation of genomic data, its warehousing, its analysis through different analytic tools for generation of information on biological system, have emerged as a new scientific discipline viz., Bioinformatics.

Bioinformatics

Bioinformatics is an interdisciplinary field of science that develops methods and software tools for understanding biological data. As an interdisciplinary science, bioinformatics combines computer science, statistics, mathematics to analyze and interpret biological data. Bioinformatic tools have been used for *in-silico* analysis (Computer aided analysis) of biological data. These tools are developed based on principles and concepts of mathematical and statistical theory applied in Data Analytics. Bioinformatics is used as an umbrella term for the biological studies based on database management system and data analytic tools developed on mathematical and statistical techniques befitting to the software algorithm tailored for the module. Common uses of bioinformatics include the identification of candidate genes whereby identify a species under microbiological wet lab study. Often, such identification is made with the aim of better understanding the genetic basis of diseases, unique adaptations, desirable properties (esp. in microorganism species), or differences between populations. In a less formal way, bioinformatics also tries to understand the organizational principles within nucleic acid and protein sequences. This will supplement and corroborate biochemical and other conventional techniques to strengthen microbiology as a science.

The genomic study, analysis and interpretation of genomic data is based on the concept of **Central Dogma of Molecular Biology**. This dogma elucidates that “The gene region of DNA in the nucleus of the cell is copied (transcribed) in to the RNA and RNA travels to protein production sites and is translated in to protein. This underlines the fact that DNA and the embedded genes are responsible for morphological characteristics and manifestation of response to every stimulus of living organisms. Genomic approaches have opened up new vistas for increasing the quality and their by productivity of biological systems. During the last decade, omics (field of study in biology ending in -omics, such as genomics, proteomics or metabolomics) has witnessed an information explosion. Omics

databases contain huge amount of information that are not amenable to traditional analytical approaches. In a multi-disciplinary area with a blend of biology, mathematics and computing science that can be used to derive biological insights from various omics data. It is an application of computing technology along with informatics for the management of biological information. A diagrammatic representation of genome is shown in fig.2.

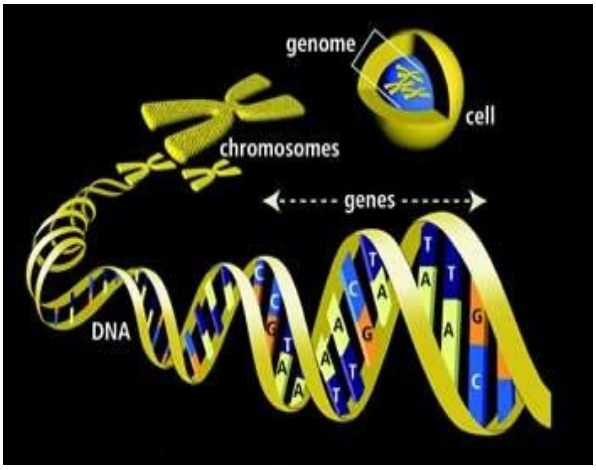

Figure 2: Genomics diagram

(Image source.http://www.differencebetween.info/difference-between-gene-and-genome dt.30-07-2017)

Bioinformatics has thus been emerged as a new scientific discipline which involves the analysis and interpretation of various types of data that includes nucleotide and amino acids sequences, protein domains and protein structures.

Deoxyribonucleic acid (DNA)

Deoxyribonucleic acid (DNA) is a thread-like chain of nucleotides carrying the genetic instructions used in the growth, development, functioning and reproduction of all known living organisms . DNA and ribonucleic acid (RNA) are nucleic acids; alongside proteins, lipids and complex carbohydrates (polysaccharides), they are one of the four major types of macromolecules that are essential for all known forms of life. Most DNA molecules consist of two biopolymer strands coiled around each other to form a double helix. The two DNA strands are called polynucleotides since they are composed of simpler monomer units called nucleotides. Each nucleotide is composed of one of four nitogen-containing nucleobases (cytosine [C], guanine [G], adenine [A]or thymine [T]),
a sugar called deoxyribose, and a phosphate group. The nucleotides are joined to one another in

a chain by covalent bonds between the sugar of one nucleotide and the phosphate of the next, resulting in an alternating sugar-phosphate backbone. The nitrogenous bases of the two separate polynucleotide strands are bound together, according to base pairing rules (A with T and C with G), with hydrogen bonds to make double-stranded DNA. The complementary nitrogenous bases are divided into two groups, pyrimidines and purines. In a DNA molecule, the pyrimidines are thymine and cytosine; the purines are adenine and guanine.

DNA stores biological information. The DNA backbone is resistant to cleavage, and both strands of the double-stranded structure store the same biological information. This information is replicated as and when the two strands separate. A large part of DNA (more than 98% for humans) is non-coding, meaning that these sections do not serve as patterns for protein sequences. The two strands of DNA run in opposite directions to each other and are thus antiparallel. Attached to each sugar is one of four types of nucleobase. It is the sequence of these four nucleobase along the backbone that encodes biological information. RNA strands are created using DNA strands as a template in a process called transcription. Under the genetic code, these RNA strands are translated to specify the sequence of amino acids within proteins in a process called translation.

Within eukaryotic cells, DNA is organized into long structures called chromosomes. During cell division these chromosomes are duplicated in the process of DNA replication, providing each cell its own complete set of chromosomes. Eukaryotic organisms store most of their DNA inside the cell nucleus and some of their DNA in organelles, such as mitochondria or chloroplasts. In contrast prokaryotes (bacteria and archaea) store their DNA only in the cytoplasm. Within the eukaryotic chromosomes, chromatin proteins such as histones compact and organize DNA. These compact structures guide the interactions between DNA and other proteins, helping control which parts of the DNA are transcribed. DNA was first isolated by Friedrich Miescher in 1869. Its molecular structure was first identified by **James Watson and Francis Crick** at the Cavendish Laboratory within the University of Cambridge in 1953, whose model-building efforts were guided by X-ray diffraction data acquired by Raymond Gosling, who was a post-graduate student of Rosalind Franklin. Anything a cell could possibly want is stored in its DNA. When a cell wants to build a protein, it finds the appropriate piece of DNA, makes a copy of it (called RNA), and uses the instructions in the copy to make the protein. In living organisms, DNA does not usually exist as a single molecule, but instead as a pair of molecules that are held

tightly together. These two long strands entwine like vines, in the shape of a double helix. The nucleotide contains both a segment of the backbone of the molecule (which holds the chain together) and a nucleobase (which interacts with the other DNA strand in the helix). A nucleobase linked to a sugar is called a nucleoside and a base linked to a sugar and one or more phosphate groups is called a nucleotide. A polymer comprising multiple linked nucleotides (as in DNA) is called a polynucleotide.

The backbone of the DNA strand is made from alternating phosphate and sugar residues. The sugar in DNA is 2-deoxyribose, which is a pentose (five-carbon) sugar. The sugars are joined together by phosphate groups that form phosphodiester bonds between the third and fifth carbon atoms of adjacent sugar rings, which are known as the 3′ and 5′ carbons, the prime symbol being used to distinguish these carbon atoms from those of the base to which the deoxyribose forms a glycosidic bond. When imagining DNA, each phosphoryl is normally considered to "belong" to the nucleotide whose 5′ carbon forms a bond therewith. Any DNA strand therefore normally has one end at which there is a phosphoryl attached to the 5′ carbon of a ribose (the 5′ phosphoryl) and another end at which there is a free hydroxyl attached to the 3′ carbon of a ribose (the 3′ hydroxyl). The orientation of the 3′ and 5′ carbons along the sugar-phosphate backbone confers directionality (sometimes called polarity) to each DNA strand. In a double helix, the direction of the nucleotides in one strand is opposite to their direction in the other strand: the strands are antiparallel.

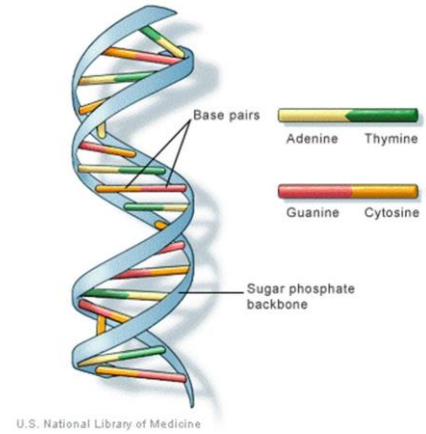

Figure 3: DNA double helix structure

The four bases found in DNA are adenine (A), cytosine(C), guanine (G) and thymine (T). These four bases are attached to the sugar-phosphate to form the complete nucleotide, as shown for adenosine monophosphate. Adenine pairs with thymine and guanine pairs with cytosine. It

was represented by A-T base pairs and G-C base pair. Proteins are the 'machinery' of a cell. They can perform many functions like transportation, structural support, movement and metabolism. Proteins are made from amino acids. There are twenty different amino acids that are used to build millions of different protein molecules. The principle of bioinformatics is that these molecules can be studied by using computers to analyze the DNA, RNA, and amino acid sequences from which they are created. Because there are so many different molecules, the best way we have of understanding how the entire system works is to use bioinformatics.

Base pairing

In a DNA double helix, each type of nucleobase on one strand bonds with just one type of nucleobase on the other strand. This is called complementary base pairing. Here, purines form hydrogen bonds to pyrimidines, with adenine bonding only to thymine in two hydrogen bonds, and cytosine bonding only to guanine in three hydrogen bonds. This arrangement of two nucleotides binding together across the double helix is called a **Watson-Crick base pair.**

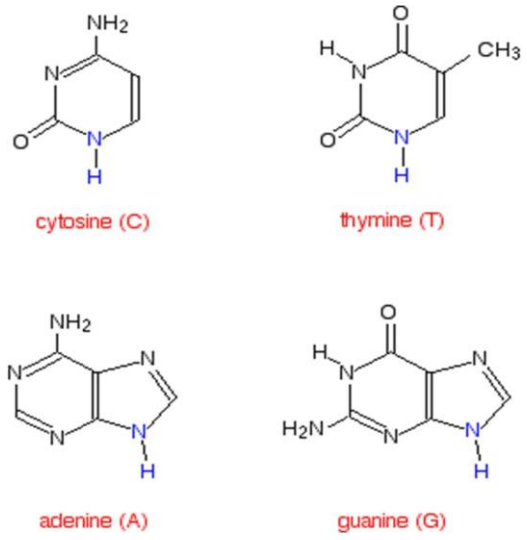

Figure 4: Chemical Structure of nucleobase

Traditionally molecular biology research was carried out entirely at the experimental lab but the huge increase in the scale of data being produced in this genomic era has necessitated incorporating computer and computing science in to research process. Sequence generation and its subsequent storage, interpretation and analysis are solely computer dependent. However, the molecular biology of an organism is a very complex issue with research being carried out at

molecular level. The first challenge facing the bioinformatics community today is the intelligent and efficient storage of this massive data by providing reliable access to this data.

Computer Systems

A computer system allows users to input, manipulate and store data. It includes hardware's like processor, monitor, keyboard, mouse and other peripheral components along with software like operating system and other system and application programmes. All of these components also can be integrated into all-in-one units, such as desktop or laptop computers. Very high speed and repetition of processing of data, high precision accuracy of result derived after data processing and its capacity storage area are the forte of computer systems which could be harnessed for advancement of Science and research. Though the numerical data collected are however large or small it be, the processing with very high speed and generation of result with desired accuracy can be achieved through suitable instruction given to the computer system called computer programming. Logical flow of computer system is given in Figure 5.

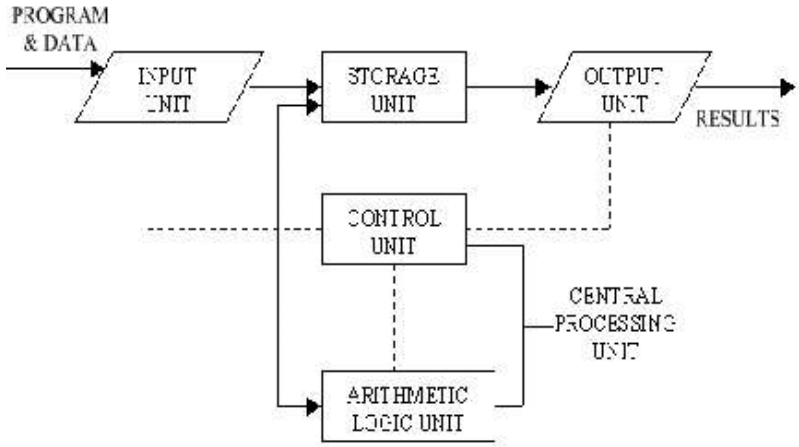

Figure 5: Logical flow of computer system

On these count, data processing of voluminous microbiological data with generation of result with high accuracy is the order of the day with high-end computing system armed with versatile system and bioinformatics software and 4GL programming languages like Java and Perl.

Bioinformatics and available web based genomic databases

Bioinformatics is the study of information processing and management in biotic systems. National Centre for Biotechnology Information (NCBI) defines "bioinformatics as the field of science, in which biology, computer science and information technology merge in to a single discipline. There are three sub discipline within bioinformatics: the development of new algorithms and statistics with which to assess relationships among members of large data sets; the analysis and interpretation of various types of data including nucleotides and amino acid sequences, protein domain and protein structures and the development and implementation of the tools that enable efficient access and management of different types of information. At the beginning of genomic revolution, the main concern was creation and maintenance of databases to store biological information such as nucleotide and amino acid sequences which are produced tremendously due to revolutionary developments in the fields of informatics and microbiology. This databases could be used to access existing data and to submit new and revised data to NCBI, (http://www.ncbi.nlm.nih.gov/)

Biological databases

Biological databases are huge databases mostly sequence data generating from major genome sequencing projects all over the world. The information about DNA, protein and functions of protein must be stored in an intelligent fashion so as to solve problems quickly by the available information stored in the data bank in the clouds, many of which are accessible by concerned user on internet. Some of the web based databases are available in table2

Table 2: Standard web based genomic databases

Sl. no.	Name of the database	Description
1.	PDB (Protein Data Bank)	Databank contains Protein Structures
2.	Swiss-Prot	Databank containing protein sequence and their functions
3.	ENZYME	Databank containing enzymes and their functions
4.	EMBL	Databank containing all nucleotide sequences of all genes sequenced till date.
5.	DDBJ	DNA Databank of Japan
6.	IMG	Integrated Microbial Genome System- A genome browsing and annotating platform of complete microbial genome.

Using data banks one can perform all kinds of comparisons and search queries. With this known information we can perform all kinds of comparisons with sequences generated from our wet lab studies for species identification. If you know a protein which causes a disease in human, you might look in to a databank to see if a similar protein has previously been described and what this protein does in human body. This known information has wide pharmaceutical application in Health Science. The NCBI site is one of the world's premier web site for biomedical and bioinformatics research (http://www.ncbi.nlm.nih.gov/). Based within the National Library of Medicine at National Institute of Health , USA, the NCBI hosts many databases used by medical and research professionals. The service includes PubMed (the bibliographic database), GenBank (the nucleotide sequence database) and the BLAST algorithm for sequence comparison. It is established in 1988 as a national resource for molecular biology information. NCBI creates public databases, conduct research in computational biology, develop software tools for analyzing genome data and disseminate biomedical information all for a better understanding of molecular processes affecting human health and diseases.

On-line software (Bioinformatics tools) for genomic data analysis for species identification and management has revolutionized genomic data analysis of nucleotide and amino acid sequences. In bioinformatics, BLAST for Basic Local Alignment Search Tool is an algorithm for comparing primary biological sequence information, such as the amino acid sequences of proteins or the nucleotides of DNA sequences. A BLAST search enables a researcher to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold. Thanks to the genomic databases available in the databank for comparison with the data under study and the automation of the searching based on the bioinformatics software tools to get the insight of the study.

Digital and computer aided equipments available for micro biological lab

High precision computer aided equipments are the order of the day for collection and analysis of microbiological data in modern science. This could facilitate computer aided data collection process for development of proper database management thereon. These equipments do generate accurate data with high precision accuracy. This could also help us to generate bias- free information with high degree of accuracy, which is a must in any scientific discovery or invention. Some of the important and must-have equipments are listed in the **table3** fit microbial study.

Table 3: List of high precision computer aided equipments of microbiology lab

Sl. No.	Name of the equipment	Use
1.	Shaking Water Bath	Heating at precision temperature
2.	Automated Colony Counter	Estimate No. of bacteria in a sample
3.	Electronic Colony Counter	Counting the colonies of bacteria in a petri-dish
4.	Magnetic Stirrer	Dissolving chemical substance effectively
5.	Sonicator	Rupture cells using high frequency waves
6.	Vortex Mixer	Used for thorough mixing of liquids in test tubes
7.	Laminar Flow Chamber	Used for aseptic transfer of sterilized materials, as well as for inoculation of microbes
8.	Electronic Cell Counter	Used to directly count the number of bacteria in a given liquid sample
9.	Microscopes	Used for visual observation of morphology, motility, staining and fluorescent reactions of bacteria
10.	Spectrophotometer:	Measuring the differences in colour intensities of solutions.
11.	Automatic Bacteria Identification System	Used for automatic computer-assisted identification of bacteria
12.	PCR Thermo cycler	Used to amplify segments of DNA via the polymerase chain reaction (PCR)
13.	Ultra-centrifuge	Precipitating large biological molecules from solution or separate them by their different rates of sedimentation.
14.	Gas Chromatography (GC)	Used for separating and analyzing compounds that can be vaporized without decomposition
15.	High Performance Liquid Chromatography (HPLC)	Technique used to separate, identify, and quantify each component in a mixture.
16.	Thin Layer Chromatography (TLC)	Technique used to separate non-volatile mixtures.
17	Paper Chromatography	A simple technique of separating constituents in a sample solution using a chromatography paper

Genomic software

Online soft wares and genomic databases as mentioned above has revolutionized genomic analysis and made genomics a separate branch of science in the age of digital technology. Identification of microorganism especially fish pathogens using analysis of genomic sequences using bioinformatics tools available with NCBI give more teeth for analysis of genomic data. We can customize software kits to identify the fish pathogens using NCBI freeware available. Now many software tools are available in the market, both free software and paid software.

Conclusion

Most critical task of bioinformatics involves the finding of genes in the DNA sequences of various organisms, developing methods to predict the structure and functions of the newly discovered proteins and structural RNA sequences, clustering protein sequences in to families of related sequences, development of protein models, aligning similar proteins and generating phylogenetic trees to examine the evolutionary relationships. The sequencing of the genomes of microbes should have enormous benefits for the biological systems including human health in general and fishery eco-system in particular. Computational analysis of this sequence data generated by genome sequencing is critically important. Bioinformatics tools can be used to search for the gene within this genome to understand their functions with the help if high-end computing facilities. Microorganism enjoys key position in the sustenance of fish farming eco system. Though most of the microorganism is environment friendly there is some pathogenic microbe in aquatic system which affects fish health and in turn human health. Microbes play an important role in the degradation of fish products, thus better knowledge of the microbiological conditions throughout the supply chain of fisheries and fish processing industry; thereby optimize fish product quality and fishery resource utilization. Under these circumstances, a regular monitoring of fish health and the quality of fish products in perspective of microbiology with bioinformatics analytic tools is of paramount for better management of the prevailing food safety and security regime.

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Chapter 23

Introduction to Fisheries Economics

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Introduction

Economics is derived from the Latin words 'eikos' and 'nemein' which means 'to manage household' and is a social science concerned with the production, distribution, and consumption of goods and services. It dealt with how people allocate scarce resources for production, distribution, and consumption, both individually and collectively. There have been various streams of thought that have influenced the growth of this field of enquiry and are termed 'classical', 'neo classical', 'modern' etc. and the stream of knowledge and enquiry evolves.

Adam Smith is considered the father of Classical economics who propounded theories regarding the study of the nature and causes of national wealth and considered wealth getting and spending behaviour as the primary objective of economics. Alfred Marshall a Neo classical economist was the first to shift the focus of economics from 'wealth' to 'welfare', and called it "the study of mankind in the ordinary business of life; it examines that part of individual and social action which is most closely connected with the attainment and with the use of the material requisites of well-being".

Professor Lionel Robbins defines "Economics is the science that studies human behavior as a relationship between ends and scarce resources which have alternative uses". It was not confined to the industry and market and it related to the basic levels of social business interactions, barters, and loans.

Prof. Paul. A. Samuelson defined it as:

"Economics is the study of how people and society end-up choosing, with or without, the use of money, to employ scarce productive resources that could have alternative uses to produce various commodities and distribute them for consumption, now or in the future among various persons or groups". Costs and benefits of improving patterns of resource allocation were analyzed.

In Indian history, Arthashastra by Kautilya has chapters on politics, governance, welfare, economics, protecting key officials and king, gathering intelligence about hostile states, forming strategic alliances, and conduct of war and includes ancient economic and cultural details on agriculture, mineralogy, mining and metals, animal husbandry, medicine, forests and wildlife.

Significance, Scope and Areas

It is understood that resources are scarce but wants tend to be unlimited which calls for optimal allocation of resources. This leads to a choice to be made and how best to make them looking at the impacts on various aspects like income, savings, wages, employment, money, banking, inflation, which has both individual and societal dimensions. Various theories, methods and analytical techniques are used to optimize the utilization of the available resources and reduce wastages. For the stability of an economy it is essential for any country or society to have sound economic practices to survive and prosper in the long run.

The scope of the subject is wide and varied and has application in business, environment, natural resource management (including agriculture and fisheries), human resource management, marketing, etc.

The broad areas under which it is studies is as follows:

- Microeconomics, which focuses on the behaviour of individual consumers and producers
- deals with individuals, households, firms and industries or individual prices, wages or incomes.
- economic motives and behaviour patterns of individual consumers and produces involved in organizing and operating individual business firms or industries.
- Macroeconomics, which examine overall economies on a regional, national, or international scale.
- economic system as a whole, of the aggregate consumption and demand and of the aggregate saving, investment and employment in the economy. It deals with aggregates and averages of the system rather than individual items in it

Economic systems and processes

An economic system is a means by which societies or governments organize and distribute available resources, services, and goods across a geographic region or country. Economic systems regulate the factors of production, including land, capital, labor.

The two major economic systems: capitalism and socialism but most countries use some combination of the two known as a mixed economy. In pure capitalism, there is private ownership, and markets and prices coordinate and direct economic activity. At the other end of the spectrum is complete control by the state. In a mixed economy, there are elements of both these systems at play, e.g. India where both government controlled and private economic activities function side by side.

The basic questions of economics system are:

• What to Produce?

Should a society direct most of its resources to the production of military equipment or to other items such as food, clothing, or housing?

• How to Produce?

Should a society direct most of its resources in machinery or use less machinery to employ more workers and lower unemployment?

• For Whom to Produce?

Once a society decides what to produce and how to produce it they must decide who is going to get it and who is going to share in what is produced.

Economic processes include actions, and operations that involve the production and sale of goods and services. Activities can be the following:

• Primary: uses natural resources directly to provide the basic raw materials for industry

• Secondary: uses raw materials to produce or manufacture something new.

• Tertiary: provides services to people and businesses

• Quaternary: knowledge-based part of the economy, which typically includes knowledge-oriented economic sectors such as information technology; media; R&D; information-based and knowledge-based services - consultation, education, financial planning, blogging, and designing

Basic Concepts

Presented below are a few basic concepts:

Demand and supply

It is now clear that wants are unlimited and the resources to satisfy these wants are limited and have alternative uses. The want satisfying power of a good or service is called its utility. By production we actually generate utility which on consumption is destroyed or met. Demand and supply analysis helps to understand production and consumption of goods and services. Demand is the desire for a commodity or service supported with necessary purchasing power and willingness to pay a price for it. It is always with reference to price and time. When the demand schedule, that is the demand for a particular product at a particular price, is plotted in a graph, the demand curve obtained. Demand curve slopes downwards indicating that other things remaining constant, a fall in price will cause an increase in demand and a rise in price will lead to a fall in demand.

The law of demand reveals the relationship between the price of a commodity and the quantity demanded of it in a market. The law of demand states that other things being equal, the quantity demanded of good increases with a fall in price and vice versa. Thus, quantity demanded varies inversely with price.

The term supply refers to the quantity of a good offered for sale at a given price. Like demand, supply is always with reference to a price and a point of time. In case of supply and price, a direct, relationship exists. The law of supply states that other things remaining equal, as the price of a commodity increases, its supply also tends to increase. The supply curve has a positive slope and it mores upwards to the right.

There are several determinants of both demand and supply. Demand is dependent on (i) Price of the good, (ii) Price of the substitute goods, (iii) Income of the consumer and (iv) Tastes and preferences of the consumer. The determinants of supply are (i) Price of the good which is to be

supplied, (ii) Price of other goods, (iii) Prices of factors of production, (iv) Number of producers or sellers, (v) Entry of new firms, (vi) Technology, (vii) Government policy etc.

Costs

Costs are the expenditure or money spent on an item or for a specific purpose or cause. Costs are of various types. Capital cost are expenditure incurred on capital items like land, buildings, vehicles, machineries and equipment, farm or firm infrastructure, etc.

Costs can be fixed or variable. Variable costs are costs incurred on an item or input which varies with the level of output. This could include costs of inputs. In aquaculture it could be manure, Lime, fertilizer, feed, cost of fingerlings, fuel, power, wages, etc. Fixed costs incurred on an item does not vary with the level of output and includes depreciation, interest on capital and variable costs, repairs and maintenance costs, taxes etc.

• Total cost: Variable + Fixed costs

• Average cost: Total cost divided by total output. Mean cost of producing one unit of the output.

• Marginal cost: It is the cost incurred on one more or additional unit of the product.

• Opportunity cost: It is the next best alternative foregone

Income/ benefit refers to the sale proceeds of an individual or firm.

• Total Income = Total Yield (kg) x Unit price (Rs/per unit of output)

• Net Income = Total Income-Total Cost

• Average Income = Total income/ Yield

• Marginal income is the income obtained from one additional unit of the output.

Benefit-Cost ratio (BCR)

It indicates the returns on a rupee of investment or expenditure

Cost - benefit ratio (Variable cost basis) = Total income/ Total variable cost

Cost - benefit ratio (Total cost basis) = Total income/ Total cost

BCR over 1 indicates that the activity is profitable.

Application in Fisheries

Fisheries play a significant role in the economy and in supporting the livelihood of an estimated 14 million people in the country. It contributes to national income, to food fish production, provides livelihood support and employment, is a foreign exchange earner, and contributes to incomes in other ways like eco-tourism. The fisheries sector as a system has a macro level dimension in its contribution to the national economy etc. At the process level, we can look at it from the production, distribution, consumption angle. Fisheries Economics thus can be very basically defined as the production, distribution, and consumption of fish and seafood and all financial aspects of the fishing and seafood industry (including aquatic life in fresh water).

• Production – how fish/ or any other product is produced

• Distribution – how fish/ or any other product is marketed/ traded

• Consumption – how fish/ or any other product is consumed

Production is the transformation of one or more inputs into one or more outputs by creation of utilities (satisfying goods and services; and need not necessarily be matter). Utilities are

(i) form utility - changing its physical form

(ii) place utility - transporting it to another place

(iii) time utility – stored to be used or traded later

(iv) possession utility - value customers have while buying a product.

Factors of production include basic ‘inputs’ like land, labour, capital and organization and these factors have a price which can be rent, wage, interest etc. While asking the basic question on what, how and how much to produce, we also need to understand the premises like wants being unlimited and resources being scarce and a choice to be made. The factors of production or the inputs have alternative uses and through judicious decision making the best use of these resources need to be made. The relation emanating from the basic questions are as follows:

What to Produce? (Product-Product relationship)

How to produce? (Factor-Factor relationship)

How much to produce? (Factor-product relationship)

Factor-product relationship

Production results in transformation of one or more inputs into one or more outputs and the production function explains the functional relationship between the inputs used and output obtained. A very simple production function is given below:

• If Y refers to the fish produced and x1, x2, x3 and x4 refer to the inputs used, then the production function is specified as follows:

Y = f (x1, x2, x3, x4,….) [Cobb-Douglas production function]

Production functions vary with the type of production and associated factors and assumptions. Appropriate functional forms need to be used to draw meaningful conclusions. Several functional forms like linear, log linear, quadratic, polynomial, parabolic, etc. are used and a suitable one can be identified based on the analytical requirements. Functional form could be decided on the basis of scatter plots, reveals how the input and output data are distributed and indicates the overall trend

A simple production function in aquaculture (say, carp culture) could look like the following:

In carp culture, the quantity of fish farmed represents the output for which various inputs like seed, feed, fertilisers etc. are used.

The Cobb-Douglas production function in carp culture could be specified as follows:

Y = f (m, u, s, f, r, g, 1) where,
Y = output of farmed carps in kg/ha.
m = cattle manure in kg/ha
u = urea in kg/ha
s = super phosphate in kg/ha
f= stocking density in numbers/ha
r = rice bran in kg/ha
g = groundnut oil cake in kg/ha
l = labour in man days

Marketing

Market is the very basic foundation on which the capitalist economy rests and operates. The price for a commodity is determined by the nature of the market. The price can be regulated by state or determined in open market. The buyer and seller can meet, negotiate and transact over an agreed price. Marketing is another dynamic area of study in the sector, bith the domestic marketing and international trade

Chapter 24

Input and service delivery system in fisheries

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India is endowed with a broad range of marine and aquatic resources, which support a thriving fish economy. Bounded by the Indian Ocean along its southern, eastern and western borders, India's exclusive economic zone (EEZ) extends over a distance of 8 129 km and encompasses an area of 2.02 million km^2. As well as the ocean, a variety of inland water bodies – rivers and canals, reservoirs, lakes, lagoons, floodplain wetlands, and brackish water ponds – all add to the diversity of aquatic resources in the country. India is the fourth-largest capture (marine and inland) fisheries and second-largest aquaculture nation in the world (FAO, 2020). India is the second largest fish producer in the world accounting for 7.58 percent of the global production. India's fish production reached an all-time high of 14.16 million metric tonnes in 2019-20. This sector contributes 1.24 percent to GVA in the economy and 7.28 percent to GVA from agriculture. Export of marine products in 2019-20 was 12.9 lakh metric tonnes and Rs 46,662 crore. Several initiatives of the central government, such as the Blue Revolution and the Pradhan Mantri Matsya Sampath Yojana (PMMSY), have attempted to tap the potential of the sector (Economic review,2021).

The entire fisheries system is divided in to capture fishery and culture fishery. India is the 2nd largest producer of fish in the world and about 68% of India's fish comes from the aquaculture sector. In terms of employment, the sector supports the livelihood of over 28 mn people in India especially the marginalized and vulnerable communities. The Government of India estimates that the fisheries sector supports the livelihood of nearly 16 million people in India at the primary level, and almost twice that number along the value chain (Van Anrooy et al.,2 022). Therefore, an efficient delivery system for fishery inputs and services can play a crucial role in the growth of farm income. The most of the fishers and input dealers are experiencing challenges and constraints in accessing and supplying the fisheries inputs respectively. The most notable constraint faced by farmers is access to farm inputs due mainly to poor delivery system in country.

Major inputs required for the fishery development are given below

1. Labour

Labour is the important element of any production system. Major labour market in the fishery sector constitutes by the fishermen community. The Government of India estimates that the fisheries sector supports the livelihood of nearly 16 million people in India at the primary level (Table 1), and almost twice that number along the value chain (Van Anrooy et al.,2022). In terms of employment, the aquaculture supports the livelihood of over 28 mn people in India especially the marginalized and vulnerable communities. However, the sector witnessing a downward mobility or migration of labours from fishery to other sectors due to economically not viable and unprofitable especially after the modernisation.

Table 1: Number of fishermen engaged in fishery activities

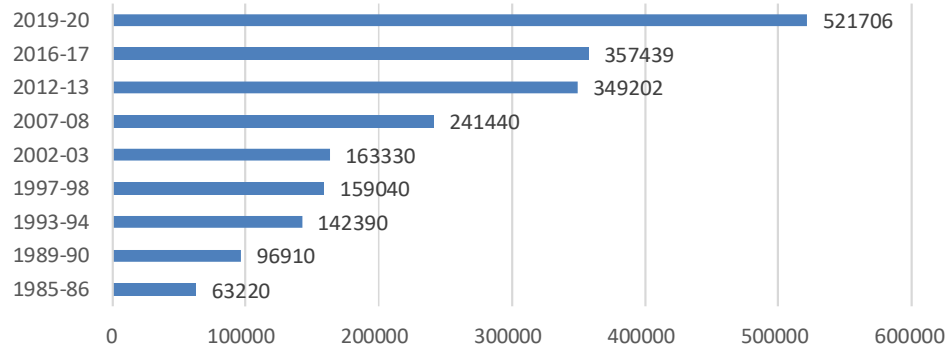
	Male	Female	total
Inland	1,3,0,13,978	10103842	2,31,17,820
Marine	26,51,652	22,99,065	49,45,717
	1,56,65,630	1,23,97,907	2,80,63,537

Source: Fishery statistics 2020

2. Fish seed and feeds

Fish seed means fish egg, larva or post-larva of fish or the spawn, fry or fingerling of fish. Fish seed production means all the operations leading up to and including final harvesting of the seed from the seed crop field. The freshwater aquaculture system in the country is primarily confined to the major Indian carp, Katla, Rohu, and Mrigala, while exotic carp, gorge carp, silver carp, and common carp become the second major group (Shukla et al.,2021). An adequate supply of carp seeds of the required species at the appropriate time is essential for the success of aquaculture activities (Katiha et al.,2003). Major inputs for the aquaculture system are feed and seeds of fishes. In past decades, the major seed source was wild catches from natural water bodies such as rivers, streams, estuaries, and the sea. In recent years technologies have been developed for high and quality production of fish seeds, such as selective breeding, hypophysation, induced breeding by hormonal injection (ovaprim, ovatide),and intensive breeding(Katiha et al.,2003).The development of indigenous technology of hypophysis revolutionized the spawning of major carp.

Figure 3. Fish seed Production (lakh fry)



Studies show that one of the biggest limitations of aquaculture development is the chronic shortage of quality fish seeds and feed, which has been overcome by technological advances in fish feed and seed production. Moreover, the availability, quality, and quantity of fish seeds have a significant impact on the aquaculture industry (Nyimbili&Musuka, 2017).

3. Craft and gear

Vessel and gear are the major fishing equipment's. Fishing gears are defined as tools used to capture marine/aquatic resources, whereas how the gear is used is the fishing method. Additionally, a single type of gear may also be used in multiple ways. Different target species require different fishing gear to effectively catch the target species. Trawlnet , Gillnet , Driftnet , Ringseine , Purseseine , Boatseine ,Bagnet ,Shoreseine , Castnet ,Hooks & line are the important gears used in India for fishing. Technological advances in introducing new equipment for fishing gears, the mechanization of fishing crafts, and the introduction of modern methods for navigation and fish location have led to a significant increase in fish production in India over the years. Based on the technology used in the vessel it is further divided into three, mechanised, motorised and non-motorise.

Mechanized craft: Any fishing craft with engine permanently fitted to the hull, which uses machine power for both propulsion as well as fishing operation like casting and pulling the net, operating lines, etc., is identified as mechanized craft. It includes Trawler, Gillnetter, Purseseiner, Dolnetter, Ringseine.

Inboard craft: Any fishing craft that has an engine permanently fitted to the hull or central portion of the craft, which is used only for propulsion and not for fishing operation, is identified

as Inboard craft. It includes Wooden Built, Iron Built, Wood Fibre etc. ***Motorized (Outboard)*** craft: Any fishing craft that has an engine fitted temporarily outside the craft, which is used only for propulsion and not for fishing operation, is identified as motorized craft. Dugout canoe, Plank built boat, Plywood boat, Fibre glass boat.

Non-motorized craft: Any fishing craft that does not use any kind of machine power for propulsion as well as fishing operation. Dugout canoe , Catamaran , Plank built , Ferro cement , Thermocol , Outrigger canoe, Masula boat.

4. Ice and cold storage facility

Safety and quality issue is a major concerned that affect the efficiency of the supply chain of fish. Since fish is a highly perishable commodity, it starts spoilage within a short period of period time. Ice is the major material used for chilling purpose. Ice plants play major role in fish quality management during transportation and processing. Available ice plants and cold storage facility in different marine state of India is given in table 2.

Table 2: Ice plants and other cold storage facilities sanctioned under blue revolution scheme from 2015-16 to 2019-20 in India

Items	No
Ice plants	221
Cold storage facility	8
Ice plant cum cold storage unit	104
Refrigerator and insulation trucks	206
Insulator truck 6t capacity	112

Source: fishery statistics, 2020

Development in ice plants and cold storage units facilitate to improve the countries fish export. One major issue is the lack of awareness about need to use ice, non-availability of good quality ice and affordable prices. The institutional mechanism to assure quality and safety of fish is limited to occasional inspection by the authorities, but is quite inadequate and doesn't serve as a deterrent. One immediate necessity is to provide infrastructure and facilities for cold storage across the supply chain, including the retail markets.

Service delivery system in fishery sector

2.1. Credit delivery

Availability and access to adequate, timely and low-cost credit from institutional sources is particularly important for small and marginal farmers. Along with other inputs, credit is essential for establishing sustainable and profitable farming systems.While examining the credit delivery system in the fisheries sector, which mainly involves informal players such as auctioneers-middlemen, third-party shareholders and private moneylenders; and formal sources such as fish fed societies, cooperative banks, commercial banks and non-banking financial institutions.

2.1.1. Informal credit financers

a. Auctioneers / Commission agents

This is usually a feature of inter-linked deals, in which the commission agent/auctioneer enters into an output-tying contract with the vessel-owner, and the fisherman in need of a loan. The contract is purely an unwritten and on mutual trust between payee and payer. Under the commission agent system, fishermen get credit under the condition that the future catches from their vessels are marketed through the commission agent/auctioneer at an agreed-upon rate of commission. Commissions are based only on the quantity of fish catch up and uncorrelated to the amount of outstanding debt. As long as a debtor fisherman has an outstanding loan, he is bound by the contract not only to continue selling their catches through the creditor-auctioneer but also to pay the due commission per catch.

b. Third party

Third-party share is another way to raise funds for capital expenses or unforeseen expenses such as repairs and maintenance. These shares are usually issued to people outside the fishing community or to businessmen outside the locality those who wish to invest in the fishing business. Interest is paid as a share of the harvest income from fishing. The value of a share in a fishing vessel is generally determined unilaterally by the primary shareholders, but it is strongly related to the financial performance of the vessel in question, the experience of the captain, and the general reputation of the shareholders and crew.

c. Money lenders

Money lenders played an important role in the credit financing among the fishermen community. Factors such as the urgency of funding requirements and faster access with less procedure have made them more acceptable. Interest rate charged by the money lenders are predetermined rate at regular intervals. Volumes of catch up, type or condition of vessel are not a considerable condition for availing loans.

d. Fisherman to fisherman

In addition to the above informal loans, the fishermen also resort to mutual loans, which are interest-free financial transactions based on the trilateral relationship between the fishermen. The triadic relationship between the debtor, the creditor, and the community ensures that the parties involved are insured against each other at any time through a severe financial crisis through forced transaction systems (baiju et al., 2019). It reveals the culture and unity of the fisherman community.

2.1.2. Formal credit institution

The formal agencies in delivery of credit for fisheries include scheduled commercial banks (CBs), regional rural banks (RRBs), cooperative societies, and private sector banks. These agencies lend credit for several activities in fisheries sector. In case of traditional fishers (artisanal fishers), the Kerala State Co-operative Federation for Fisheries Development Ltd. (Matsyafed) provides credit to a diverse set of activities.Some of non-banking financial institutions are also rendered credit services to the fisherman. These are the financial companies registered under companies act 1956 and are providing loans and advances, acquisition of shares, stocks, bonds, hire-purchase, insurance business under the RBI rule of law (Baiju et al.,2019). Easy access to loans without sufficient guarantees is the main advantage of this institution and this is the winning card of these forms of institutions however interest rates are higher than banking institutions. Muthoot fin crop, Bajaj finance are some of the leading creditors in this sector.

Micro finance is another major bank of beach. It provides loans to poor fishermen for the financial needs of their families and small businesses. For example, in Kerala, Society for Assistance to Fisherwomen (SAF), an agency functioning under the Department of Fisheries,

GoK provides micro credit to fisherwomen to initiate micro enterprises, and cultivate thrift among fisherwomen. There are several other agencies in India that disburse credit to fisherfolk though SHG platforms.

2.2. Market system in fishery sector

A market is a place where the exchange of goods and services takes place as a result of the interaction of buyers and sellers either directly or through intermediary agents and institutions. Marketing is the series of human activities by which a product is exchanged between the producer and the consumer during which the place, time, form and possession desires of the consumers are satisfied. To make fish available to consumers at the right time and in the right place requires an effective marketing system. Fishermen who catch fish by labouring overnight (from common-property water bodies) do not usually sell fish in retail markets. At the break of day, they take their catches to places where traders meet them and bargain by the lot (FAO, 2022). The domestic fish marketing system in India is neither efficient nor modern and is mainly carried out by private traders with a large number of intermediaries between producer and consumer, thereby reducing the fisherman's share in consumer's rupee. Fish marketing system of the state can be broadly classified into two such as traditional and modern system of fish marketing. The traditional fish marketing system is more common in the state, even though modern and digital marketing models have recently emerged. In the case of marine fishes, marketing starts from the fish landing centres whereas, in the case of inland fishes, marketing starts at farm gate.

2.2.1. Traditional fish marketing system

Traditionally fish marketing and distribution systems have involved collecting, processing and transporting fish from fishermen in remote landing areas to major consumption centers. Fresh fish is sold from the landing site to intermediate processors who smoke the fish (sometimes the smoking is done by family processors) and sell to wholesalers or middlemen at a distance, who pass through some middlemen and are finally sold to customers.Fish landing centres are the primary fish markets from where fishes are transported to the wholesale or retail markets and these centres had the maximum number of intermediaries like auctioneers, commission agents, retail traders and export agents (Aswathy*et al*, 2014). In the traditional marketing system, a large number of intermediaries are involved. Various marketing channels involved in the marine fish

marketing system is given below, almost similar marketing channel exist in inland fisheries (CMFRI, 2020). **Marketing channel** is defined as a path traced in the direct or indirect transfer of title of a product as it moves from a producer to an ultimate consumer or industrial user. Thus, a channel of distribution of a product is the route taken by the ownership of goods as they move from the producer to the consumer or industrial user. Kohls and Uhl have defined marketing channel as alternative routes of product flows from producers to consumers. The number of intermediaries between the fishermen and the final consumers varies in different marketing channels, based on the quantum of landings and the effort required to perform various marketing functions such as assembling, cleaning, grading, processing, storing and transportation (Sathiyadhas*et al*, 2011). Different market channels in the traditional marketing system given below.

Channel 1: Primary market/landing centre →Auctioneer → Agents of freezing plants → Freezing plants → Fish stalls/ Exporters → Consumers

Channel 2: Primary market/landing centre → Auctioneer → Processors (curing) → Wholesalers (dry fish) → Retailers/ Exporters → Consumers

Channel 3: Primary market/landing centre → Auctioneer → Wholesalers (primary market) → Wholesalers (retail market) → Retailers → Consumers

Channel 5: Primary market/landing centre → Auctioneer → Commission agent → Wholesalers (interior market) → Retailers → Consumers

Channel 6: Primary market/landing centre → Auctioneer → Retailers/On-line retailers/Bulk purchase → Consumers

Fish from the distant landing centres were able to reach, wholesale and retail markets due to the technological advancements in marine fish transport and processing. The perishable nature of fish, on the other hand, necessitated its prompt disposal at each point of transaction, resulting in the involvement of many intermediaries in the marketing channel, leading to high marketing costs and margins (Aswathy et al., 2014). Besides auctioneers, market intermediaries in the traditional marketing system includes wholesalers, retailers, vendors, marine/ inland fishermen cooperatives, contractors. They were involved in the supply chain and undertake various activities such as cleaning, grading, sorting, processing, icing, packaging and transporting at

various levels of marketing. For instance, in Kerala fishermen welfare society (Matsyafed) performing the auctioneer's duty to avoid the exploitation of auctioneers. They also provide credit to the needy.

Market functionaries or institutions move the commodities from the producers to consumers. Every function or service involves cost. The intermediaries or middlemen make some profit to remain in the trade after meeting the cost of the function performed. In the marketing of agricultural commodities, the difference between the price paid by consumer and the price received by the producer for an equivalent quantity of farm produce is often known as farm-retail spread or **price spread**. Sometimes, this is termed as **marketing margin**. The total margin includes: (i) The cost involved in moving the product from the point of production to the point of consumption, i.e., the cost of performing the various marketing functions and of operating various agencies; and (ii) Profits of the various market functionaries involved in moving the produce from the initial point of production till it reaches the ultimate consumer. The absolute value of the marketing margin varies from channel to channel, market to market and time to time. Marketing costs and margins for major marine fish species in Kerala is depicted in table 3.

Table 3: Marketing costs and margins for major marine fish species in Kerala

Particulars	Seer fish	Tunnas	Pomfrets	Mullets	Mackerels	Oil sardines
Marketing channel I: Fishermen (Kerala)-Auctioneer-Commission agent-retailer-consumer (Kerala)						
Marketing costs as share of landing price (%)	2.9	16.7	4.4	10.0	5.1	11.4
Marketing margins as share of landing price (%)	33.7	31.7	37.3	34.0	38.5	45.7
Fishermen's share in consumers' rupee (%)	70.0	63.8	67.1	65.9	66.3	59.5
Marketing channel II: Fishermen (Kerala)-Auctioneer-Women vendors-consumer (Kerala)						

Marketing costs as share of landing price (%)		1.0	2.4	3.0	5.8
Marketing margins as share of landing price (%)		41.5	49.4	48.5	27.5
Fishermen's share in consumers' rupee (%)		70.2	65.9	66.0	75.0
Marketing channel III: Fishermen (Karwar)-Auctioneer-Commission agent (Wholesaler)-wholesaler-auctioneer-retailer-consumer (Kerala)					
Marketing costs as share of landing price (%)	8.1	9.3	20.9	26.9	77.9
Marketing margins as share of landing price (%)	69.7	29.5	66.6	60.7	108.1
Fishermen's share in consumers' rupee	56.8	53.2	42.6	29.1	15.0 (%)

Source: Aswathy, 2014

2.2.2. Modern marketing system

Online fish marketing is an innovative approach in the fish marketing system, trying to meet the increasing demand and delivery of high-quality fresh fish at an affordable rate within shortest time period (Salim, 2018). The rise of e-groceries and latest cost-effective freezing technologies had increased online fish retailing (Vishal ,2015).Online marketing of fish is also a growing business, especially after the Covid pandemic. Digital marketing/e-marketing, often called online marketing, internet marketing or web marketing, has gained popularity over the past decade. With the advent of social networks, e-marketing also now boasts of a new branch of social media marketing. People prefer to shop at home rather than crowd purchase. Online

platforms like WhatsApp and Facebook can be useful for this. Web marketing, blog marketing, you tube marketing are different form of online marketing. Example 'Fishwaale' in assam, India's first e-fish market platform (Singh, 2021), 'LIVE to FISH' and 'Pachameen' in Kerala are some successful ventures in this area. Elimination of intermediaries is the prime feature of online markets.

In an efficient marketing system, the share of fishermen is higher due to the lesser involvement of the middlemen.A market can be graded as efficient, only when the price spread is minimum (Narayanakumar and Sathiadhas, 2006).Price spread is the difference between the price received by the producer and the price paid by the consumer for any given commodity at a point of time in a market. Marketing efficiency is the ratio of market output (satisfaction) to marketing input (cost of resource). An increase in this ratio represents improved efficiency and a decrease denotes reduced efficiency. A reduction in the cost for the same level of satisfaction or an increase in the satisfaction at a given cost results in the improvement of efficiency. Some of the problems in fish marketing include high perishability and weight of materials, high diversity in size and weight among species, high cost of storage and transportation, lack of assurance of quality and quantity of the commodity, low demand elasticity and high price spread (Kumar et al.,2008).

Insurance system

Insurance is one of the widely adopted means for risk management and is used the world over as an effective instrument for containing and mitigating a wide variety of risks such as asset risks, production and management risks, market risks, personal and health risks (Parappurathuet al.,2017).In the case of fisheries, insurance covers risk factors such as loss or damage to fishing vessels, gear and equipment, loss of fish and human life at sea, stock failure due to disease, climate change, and for subsequent natural calamities likes cyclone, flood and droughts etc. The institutional mechanism available to cover the risk in the fisheries sector is very less and the main policy schemes in the sector are accident insurance, vessel insurance and insurance cover for selected stock in aquaculture.

Accident insurance: It is the most promising insurance product in the capture fishery sector and covers active fishermen's risk of life or disability while engaged in fishing activities. Among the

insurance schemes, 'Group Accident Insurance Scheme for Active Fishermen' is the major scheme currently in operation, which covers the life and disability risks of the boat crew.

Vessel insurance: vessel insurance covers risk of loss and damage to the craft's hull and body while engaged in fishing at sea. Due to high premium, the number of vessel insurance subscription quite low among boat owners. And also available vessel insurance policies are quite low in fishery sector.

Concerns over input -service delivery system for the fisheries development

a. Lack of formal institutional credit mechanism

In the absence of the formal sector financing, the credit requirement is met through informal means, which possess the fishermen in the circle of debt trap and poverty. the biggest drawback of the output-tying credit system is that it leaves the fisherman permanently indebted, unable to get rid of his outstanding debts, and forced into a permanent bond of commission payments. Formal credit institutions are not accessible to the fishermen. Lack collateral security and low debt repaying capacity are the major barriers to accessing formal credit services. Special attention should be paid to this.

b. Lack of market information

The actors in the whole supply chain needs information on various dimension- arrival of fish (inland and marine, in various markets), varieties of fish available in various markets and fish prices. However, market intelligence system on fish is highly under-developed, which hinders policy development and best-informed consumer decision making.

c. Lack of quality fish seeds

The non-availability of quality fish seed was the major constraint in culture fisheries. which has been overcome by technological advances in fish feed and seed production. Moreover, the availability, quality, and quantity of fish seeds have a significant impact on the aquaculture industry.

d. Inadequate infrastructure developments

The fisheries sector remains vulnerable to losses, despite a fair amount of share in the national exports, due to multiple reasons. The main reason for the same is poor post-harvest infrastructure

facilities. In India demand for fish and fishery product has been increasing at the same time the loss in the post-harvest fisheries has been massive, estimated at around 15 percent due to inadequate post-harvest infrastructure in the country.For instance, hook and line kind of fishing, dumping of the catch and poor container facilities make the harvest vulnerable to losses. Further, the type of vessel and facilities such as availability of ice, drainage facilities and access to the markets are other key components that influence the post-harvest loss on-shore. Similarly, the nature of retail and wholesale markets for the catch including processing of the catch is crucial in determining the loss off-shore (Sivagnanam, Priya and Pulikkamath, 2019). The fisheries sector has specific characteristics with reference to its harvesting and post-harvest handling. Hence, it needs infrastructure that takes care of its quality from harvest to final consumption.

e. Inadequate risk covering mechanism

One of constraints of risk financing mechanisms in the marine fisheries sector is lack of adequate, and affordable insurance policy schemes in the country. Not only marine but also inland fisheries such as fish farming face the same. For instance, the number of independent insurance policies in India is very few in vessel insurance. Currently, four public insurance companies hold less than 1,000 active policies. According to the latest maritime census (2016), the number of fishing vessels operating in the country is 164302, of which 42656 are mechanized, 95957 are motorized, and 25689 are conventional. The number of insured craft in India is estimated to be 5000-7000. In other words, only 3-4 percent of the country's fleet is insured (Van Anrooy et al. 2022). In addition, available risk covering policies are not affordable to the poor fishermen due to high premium rate.

f. Market intermediaries and inefficiency

About three-fourths of total marine fish landed in Kerala is marketed domestically. . The fish marketing system in the state is highly complex, involves multiple stakeholders, intermediaries and benefactors with high level of diversity in market structure and conduct. Though modern and innovative marketing models are emerging in recent years, marketing practices followed are predominantly old and traditional in many areas with inefficiencies pervasive across the value chain. The major market imperfection in fish supply chain emerges in the stage of auctioning. Fish auctioning is highly unorganized and is rooted in traditions. The market charges and operations are unregulated, and is characterized by monopoly elements. There is barriers to entry

as a fish auctioneer (Kumar *et al*, 2008).Other than performing the function of auctioning, their activities are both horizontally and vertically integrated: they serve as a major agents for informal credit to the fishing sector, financing both capital requirements for acquiring fishing vessels and daily fishing operations, supplying of axillary inputs like ice, providing fuel (diesel, kerosene) on credit etc. The credit offered to the fishermen is tied with output marketing operations. The real interest rate charged by the auctioneers is much higher than the market interest rate. However, one useful function is that the auctioneers shoulder the risks in financing fishing operations as fish catch depends on an element of probability, and therefore the repayment is a risky affair. Further, there are several irregularities persist in the structure, conduct and performance of the marketing system, as is observed in case of price determination, weighing and quality checking, payment, large element of reduction in quantity of fish on several pretexts etc. In that sense the fish auctioning system has large element of imperfections and exploitative elements. On the other end, consumers are charged high for their fish purchase. Over a period of time, the retail price of fish has increased at a higher rate compared to several other food commodities, resulting in large price spread. Further, this renders several consumers inaccessible to fish.

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Chapter 25

Fish consumption in India: Patterns, determinants and consumer perspective

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Fish and seafood offer a much healthier diet than any other terrestrial meat products (Bogard et al., 2015). Being a great source of unsaturated fatty acids, amino acids, vitamins and minerals, coupled with its low-fat content (Yaktine and Nesheim, 2007) fish always tops the list as an important cuisine for people all around the world (Burger et al., 1999; Turan et al., 2006) making any diet sustainable, safe and nutritious. On a global basis, fish is considered as the third major source of dietary protein after cereals and milk (FAO, 2020). In major studies (Brunso, 2003; Gross, 2003), consumers have regarded fish as healthier compared to other non-vegetarian foods. Significant contribution of fisheries sector is evident in the fight to end global hunger, achieve food security, and improve nutrition (Bennet et al., 2021). 20 per cent of the total animal protein intake of 3.1 billion people is met by fish with per capita food fish consumption rising from a mere 9.0 kg in 1961 to 20.5 kg in 2018 (FAO, 2020).

According to National Sample Survey Organization (NSSO) report, the monthly per capita fish consumption of urban and rural India is 0.27 kg and 0.25 kg. The ICMR recommendation of fish consumption is 12 kg/year, which is yet to be achieved in India with a predicted per capita fish consumption of 6.6 kg in 2030 by World Bank (Msangi et al., 2013). Government of India has also set a target of 20 MT fish production by the year 2022-23 by laying renewed focus on the sector through a flagship scheme “Blue Revolution” (Shasani et al., 2020). But an entirely different situation exists in Kerala state with a per capita fish consumption of 2.26 kg in rural and 2.21 kg in urban areas (NSSO, 2012). Being a coastal state and leading fish producer of the country, both fresh and dried fish are important items of Kerala diet. Identifying the factors influencing consumption of fish and studying consumption behaviour aids government in alleviating hunger and malnutrition among deprived sections (Sajeev et al; 2021).

Most Indians have a positive attitude towards seafood and consider it as an important part of healthy and balanced diet. The annual per capita consumption of fish for the entire Indian population is estimated at 5-6 kg whereas for the fish-eating population it is found to be 8-9 kg.

Average annual per capita fish consumption is highest in Kerala state at 30 kg which is very high compared to that of other states of India (Shyam, *et al.* 2015). Issues of fish adulteration have been widely discussed by media and have created an increased health, safety and quality consciousness among consumers. These issues have created new drivers and barriers to fish consumption with fish consumers changing their fish purchase behaviour and market choice. The article discusses the emerging drivers and barriers to fish consumption wherein, the factors identified as influencing fish consumption were consolidated into a framework of fish consumption.

Drivers and barriers to fish consumption: important factors

Empirical evidence shows differences in the use of information sources by consumers depending on the food product, the communicated information about the food product and the potential health or safety risk of the food product (Gutteling and Wiegman, 1996; Jungermann *et al.*, 1996). With respect to fish, consumers mostly use personal sources of information, such as fishmongers and family and friends (Pieniak *et al.*, 2007). Pieniak *et al.* (2010 a,b) identified knowledge as a relevant determinant of fish consumption. Consumers with a higher level of knowledge about fish were found to eat fish more frequently. Knowledge studies focused mainly on production aspects, whereas consumer information and education campaigns have mainly been focused on the health and nutritional benefits of fish, as well as on convenience issues acting as barriers to consumption (Olsen, 2003; Verbeke and Vackier, 2005). Olsen (2004) identified four salient beliefs reasonable in forming seafood / food consumption attitude as: taste, distaste (negative affect), nutrition (Steptoe *et al.*, 1995) and quality / freshness. After the taste issues the nutritional aspects are the second prominent factor that affect consumer's food attitude, it is directly related to health and healthy eating behaviour (Olsen, 2001). The quality of the fish/seafood freshness is another prime determinate. In this regards, frozen fish are treated as "non-fresh" "bad quality" "tasteless" "watery" "boring" (Olsen, 1998). Olsen in 2004, found price, value for money and household income are not barrier in seafood consumption, while Verbeke & Vackier, in 2005, reported that price negatively affect the fish consumption attitude.

Fish consumption: feedback from consumer behaviour studies

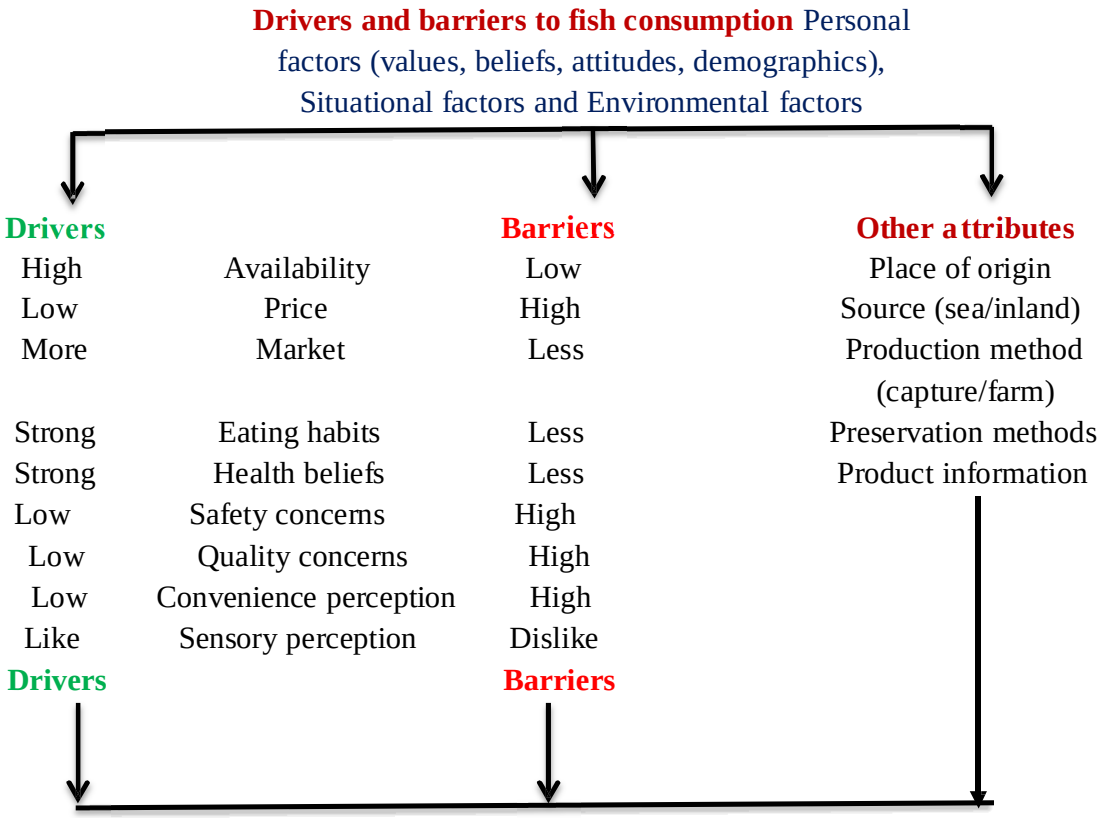
A study on knowledge and perception of fish consumers with respect to health benefits of fish consumption, safety and quality of fish and major drivers and barriers to consumption was done

among consumers in Kerala State, India. The state was identified for the study due to its predominantly high fish consuming population having annual per capita fish consumption rates higher than global average. **'**Transreg' procedure revealed that for 'price of fish' was the most important driver or barrier in Kerala. When the coastal and non-coastal districts were compared, there was marked difference in the drivers and barriers with 'Source of fish (marine/inland)' being the most important driver in coastal districts while 'Safety of fish' emerged as the most important driver for consumers of non-coastal districts. For consumers in Ernakulam; 'Source of fish (marine/inland)'was the most important driver while in Kozhikkode 'health benefits from eating fish' acted as the biggest driver. In Palakkad 'place of origin' of fish was the most important driver while 'market accessibility' was the most important driver in Kottayam.

A study on six major tribes of Wayanad, Kerala; in which data were gathered from 200 tribal households covering different socioeconomic backgrounds, identified that Adiyan followed by Vettakuruman tribes had highest per capita fish consumption. While Sardine is the most consumed and preferred fish among Wayanad tribes, the percapita consumption (1.03kg/month) was estimated far below the Kerala average. Price of fish ranked as the most important barrier of tribal fish purchase and consumption while the 12 determinants of fish consumption analyzed were found highly associated with the health values of tribes.

In another study conducted among urban consumers of Kerala, Conjoint analysis revealed that the factors like 'place of origin of fish', '24x7 accessibility' and 'sensory perception' were the most contributing drivers while 'price of fish' and 'availability of favourite fish' were the most important barriers to online fish purchase.

The review of the drivers and barriers to fish consumption using 'Theory of Planned Behaviour' as a base provided a framework for quantity, frequency and characteristics of fish consumed (Sajeev *et. al*., 2018). Personal factors like values, beliefs, attitudes and demographics had huge influence on fish consumption. Factors like availability, price, market, eating habits, health beliefs, safety and quality concerns and sensory and convenience perception acted as both driver as well as barrier in varying degrees.



Sajeev *et al.*, 2018

Fish consumers mostly use personal sources of information such as fishmongers and family and friends to arrive at a purchase decision. Consumer knowledge is an important determinant of fish consumption. Consumer information and education campaigns have mainly been focused on the health and nutritional benefits of fish. However, convenience issues (such as fish preparation, quality evaluation and fish species) have been found as an important barrier to fish consumption. Other attributes like place of origin (local/outside), source of the fish (marine/inland), production method of fish (capture/farm), preservation methods (frozen/chilled) and product information (information available/not available). All the above factors in combination decide the quantity, frequency and characteristic of fish consumed. Hence the most important drivers and barriers to fish purchase identified among the above studies has to be considered by existing and upcoming entrepreneurs.

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Chapter 26

Overview: Safety and quality of fish

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Safety is an essential pre-requisite for quality and represents the minimum standard that guarantees seafoods on the hygienic-sanitary point of view. A further safety tool is represented by the product traceability and labelling that supply detailed and precise information to the chain operator, only some of them reaching consumers: identification number of each lot, identification number and name of the fishing vessel or name of the aquaculture production unit, date of catches or the date of production, quantities of each species (kg or no of individuals), name and address of suppliers, scientific name and commercial designation of species, relevant geographical area, production method (farmed or captured) and whether the fisheries products have been previously frozen or not (Reg. CE 1224/2009). Ethical aspects too are assuming an increasing importance for consumers, when they are sensitive to the fact that seafood they will use were obtained with sustainable fishery/aquaculture systems and with respect for animal welfare.

Product quality evaluation starts from a careful examination of the external aspect of the species under interest, on the basis of the distinctive features such as skin or carapace or valve colour, and morphological traits of commercial interest. Proper morphology and merchantable traits are evaluated through a series of length and weight measures. Length measures also have an important role at commercial level for the main species. A minimum size, below which fishing and marketing are not allowed, was fixed for each main species (Reg. EC 1967/2006, Annex III). This was decided because the smaller specimens, still juveniles, must be protected to assure a sustainable exploitation of resources. For commercial size fish the measures to be pointed out are gutted weight/ fillet yields, and condition factor. Condition factor (body weight/length ratio) indicates the fish corpulence within species, often related to body and meat adiposity. Apart from the feeding history, some quality aspects can also differ according to size because with increase in fish body weight and age, muscle and mesenteric fat incidences increase, while the one of bony tissue decreases. The fish reared in floating cages generally show less fat, both in viscera and in fillet, and better sensorial quality, in comparison to the ones reared in tanks. The

different nutritional state, the higher energetic consumption/swimming activity and the streamlined flow in the cage are at the basis of the main differences, making them similar to the product captured in the wild. The chemical, nutritional and dietetic characteristics, peculiar of the species but markedly influenced by extrinsic parameters, such as quantity, quality and feeding modality.

The physical and organoleptic characteristics can be evaluated through the behaviour of rigor mortis phases (pre-rigor, full rigor, rigor release), the changes of dielectric properties (indices of fish integrity loss), pH, colour, texture and freshness/quality state. Freshness state evaluated by sensorial methods through examination of the general aspect of eyes, skin, gills, odour of gills, flesh texture, resilience and colour on the raw product, and flesh texture, colour, taste, flavour and juiciness on the cooked product is able, even alone, to be a reliable index of seafood quality. Evaluation methods more frequently used are the ones officially accepted in Europe (Reg. EU 2406/1996) distinguishing three freshness classes, very fresh (Extra), fresh (A), bad quality (B), below B fish being discarded for human consumption, or the Quality Index Method, a specific demerit index that assumes 0 value in very fresh fish, increasing value with quality worsening. In the case of reared product, freshness state of each species could even be estimated from the harvesting date, when a correct and uninterrupted cold chain has been assured. The time period in which seafood is marketable (shelf life) could also be evaluated both by sensorial methods, and as total viable count (TVC) or charge of individual specific spoilage organism (SSO), the latter are the ones better developing at the selected conservation conditions (for example Pseudomonas in refrigerated product, Photobacterium in Modified Atmosphere Packed, MAP product). Raw product is considered unfit for human consumption according to the sensorial parameters days before in comparison to the edibility threshold indication as TVC (107 cfu/g), the later resulting more fit as spoilage index in cooked product. Among the physical traits instrumentally determined, some of them are to be mentioned: skin and fillet colour, important for fish with pigmented flesh- evaluated (CIELab system) through the colorimetric parameters lightness (L*), redness (a*) and yellowness (b*), chroma and hue - and texture, important both as product sensorial aspect and for processing attitude. Texture increases with the muscular fibre density and diameter, and with the quantity and ageing of collagen structure. At the same weight, wild fish generally have flesh more consistent than those of cultured ones, also for their lower fat quantity and the greater muscular tissue activity for swimming. Texture decreases as fish

freshness declines, and can be considered as a non-destructive index of freshness. Other useful aspects for the instrumental evaluation of quality changes in the final phases of shelf life are the levels of biogenic amine (histamine, putrescine, cadaverine) of malonaldehyde, secondary lipid oxidation compounds and odour volatile compounds.

Major developments in the field of fish safety and quality have had a significant impact on international trade during the last few decades. Technological developments in fish handling and processing coupled with increasing consumer food safety and quality awareness have resulted in the adoption of HACCP-based systems and scientifically-based risk assessment methodologies. This is reflected in international regulatory framework of the SPS and TBT Agreements of the WTO and the normative work of CODEX Alimentarius. Based on available food borne illness reports, the decrease in food borne diseases has coincided with the implementation of HACCP-based food safety assurance measures. However, fish safety and quality issues related to indigenous microorganisms, chemical or veterinary drugs are increasingly of concern. This reflects the need for a food chain approach in the analysis of hazards and risks to develop integrated risk management strategies. However, a food chain approach also requires substantial multidisciplinary scientific information given the need of science-based risk analysis.

FAO provides direct assistance to member countries via the CODEX Committees and other expert groups with a focus on training and capacity building in developing countries. In response to increasing demands for pertinent and succinct scientific and technical information upon which to conduct adequate hazard and risk analysis, the FAO has launched the Aquatic Food Programme in collaboration with the Canadian Food Inspection Agency with the expectation of creating a peer reviewed comprehensive knowledge base of integrated aquatic food to safety and quality information from a food chain approach. Safety and quality concepts are incorporated in the FAO Code of Conduct for Responsible Fisheries, particularly Articles 6 and 11.

ISO 8402: 1995 standard defines general quality as: “the totality of features and characteristics of a product or service that bears its ability to satisfy stated or implied needs.” The ISO 9000 standard quality system modified the quality concept, shifting the attention from the final product to all the processes contributing to its production. This integrated management system approach, in which planning, personnel involvement, documentation of activities and the attitude

towards a continuous enhancement became the basis of the new management model. This is the background of the total quality concept.

In a fishery chain, total quality could be defined as the complex of the characteristics able to satisfy the organoleptic, health, use/price convenience requirements of the purchaser/consumer, constantly found and obtained through a correct management of the production chain, with respect of welfare and environment sustainability, and made known in full transparency through traceability and labelling.

Practical implementation of international regulatory frameworks commenced in the early 1980s when many countries engaged in reforming their fish inspection systems to implement preventive HACCP-based safety including quality systems and supporting hygiene and sanitary requirements. While there is growing and strong evidence that the implementation of HACCP based systems has contributed to improve fish safety and quality significantly, there has been recent growing awareness of the importance and need of an integrated, multidisciplinary approach to safety and quality, considering the entire fish food chain. FAO defines the food chain approach as recognition that the responsibility for the supply of food that is safe, healthy and nutritious is shared along the entire food chain by all involved with the production, processing, trade and not the least the consumption of food. Stakeholders include farmers, fishermen, food processors, transport operators, distributors, consumers, as well as governments obliged to protect public health. This holistic approach to food safety along the food chain differs from previous models in which responsibility for food safety concentrated mainly on the food-processing sector and government control services. The implementation of a food chain approach requires an enabling policy and regulatory environment at national and international levels with clearly defined rules and standards, establishment of appropriate food control systems and programmes at national and local levels, and provision of appropriate training and capacity building (FAO, 2003). Efforts to integrate these developments into fish safety and quality policies are ongoing at national, regional (e.g., European Union, EU) and international (e.g., Codex Alimentarius Commission) levels. Fish safety regulators have been applying a host of control measures, from mandating the use of HACCP to increasing testing, with varying degrees of success. However, the various scientific tools available to support the development of a food chain approach present limitations, which needs to be recognized and considered, including gaps in research data. Indeed, much of the data needed to develop science-based

strategies are often incomplete, non-existent or require extensive resources to generate. In addition, the link between the food safety criteria and public health objectives is not always present in current safety regulations. Consequently, improved scientific tools must be adopted and novel approaches must be sought so that the need for regulatory control can be balanced with the need for regulatory flexibility and with the expectation that a regulatory agency's actions reflect the most current and effective scientific methods available to protect the public health.

In the fish industry, there are five broadly defined needs on which a strategy in support of a food chain approach to food safety should be based:

- Fish safety and quality from a food chain perspective should incorporate the three fundamental components of risk analysis–assessment, management and communication–and, within this analysis process; there should be an institutional separation of science-based risk assessment from risk management–which is the regulation and control of risk.
- Traceability from the primary producer, through post-harvest treatment, processing and distribution to the consumer must be improved.
- Harmonization of fish quality and safety standards, implying increased development and wider use of internationally agreed, scientifically based standards is necessary.
- Equivalence in food safety systems–achieving similar levels of protection against fish-borne hazards and quality defects whatever means of control are used–must be further developed.
- Increased emphasis on risk avoidance or prevention at source within the whole food chain–from farm or sea to plate–, including development and dissemination of good aquaculture practices, good manufacturing practices and safety and quality assurance systems (i.e., Hazard Analysis and Critical Control Point [HACCP]), are necessary to complement the traditional approach to fish safety and quality management based on regulation and control.

The principles of achieving harmonization of standards and equivalency in food control systems and the use of scientifically based standards are embodied in the two binding agreements of the WTO (the Agreement on the application of sanitary and phytosanitary [SPS] measures and the Agreement on technical barriers to trade [TBT]). The SPS Agreement confirms the right of WTO member countries to apply measures necessary to protect human, animal and plant life and

health. The purpose of the SPS Agreement is to ensure that measures established by governments to protect human, animal and plant life and health, in the agricultural sector, including fisheries, are consistent with obligations prohibiting arbitrary or unjustifiable discrimination on trade between countries where the same conditions prevail and are not disguised restrictions on international trade. It requires that, with regard to food safety measures, WTO members base their national measures on international standards, guidelines and other recommendations adopted by the Codex Alimentarius Commission (CAC) where they exist. This does not prevent a member country from adopting stricter measures if there is a scientific justification for doing so or if the level of protection afforded by the Codex standard is inconsistent with the level of protection generally applied and deemed appropriate by the country concerned. The SPS Agreement states that any measures taken that conform to international Codex standards, guidelines or recommendations are deemed to be appropriate, necessary and not discriminatory. Finally, the SPS Agreement requires that SPS measures are to be based on an assessment of the risks to humans, animal and plant life using internationally accepted risk assessment techniques. The objective of the TBT Agreement is to prevent the use of national or regional technical requirements, or standards in general, as unjustified technical barriers to trade. The agreement covers standards relating to all types of products including industrial products and quality requirements for foods (except requirements related to SPS measures). It includes numerous measures designed to protect the consumer against deception and economic fraud. The TBT Agreement basically provides that all technical standards and regulations must have a legitimate purpose and that the impact or cost of implementing the standard must be proportional to the purpose of the standard. It also states that, if there are two or more ways of achieving the same objective, the least trade restrictive alternative should be followed. The agreement also places emphasis on international standards, WTO members being obliged to use international standards or parts of them except where the international standard would be ineffective or inappropriate in the national situation. The aspects of food standards that TBT requirements cover specifically are quality provisions, nutritional requirements, labeling, packaging and product content regulations and methods of analysis. Unlike the SPS Agreement, the TBT Agreement does not specifically name international standard setting bodies, whose standards are to be used as benchmarks for judging compliance with the provisions of the Agreement. Risk analysis is widely recognized today as the fundamental methodology underlying the development of food

safety standard that provides adequate health protection and facilitates trade in food (FAO, 2001). There is a fundamental difference between a hazard and a risk. A hazard is a biological, chemical or physical agent in, or condition of food, with the potential to cause an adverse health effect. In contrast, risk is an estimate of the probability and severity in exposed populations of the adverse health effects resulting from hazard(s) in food. Risk analysis is a process consisting of three components: risk assessment, risk management and risk communication. Risk assessment is the scientific evaluation of known or potential adverse health effects resulting from human exposure to food-borne hazards. Risk management is the process of weighing policy alternatives to accept, minimize or reduce assessed risks and to select and implement appropriate options. Risk communication is an interactive process of exchange of information and opinion on risk among risk assessors, risk managers and other interested parties. The responsibility for the supply of fish that is safe, healthy and nutritious should be shared along the entire chain from primary production to consumption. Development and implementation of Good Aquaculture Practices (GAP), Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP) are required in the food chain step(s). Government institutions should develop an enabling policy and a regulatory environment, organize the control services, train personnel, upgrade the control facilities and laboratories and develop national surveillance programs for relevant hazards. The support institutions (academia, trade associations, private sector, etc.) should also train personnel involved in the food chain, conduct research on quality, safety and risk assessments and provide technical support to stakeholders. Finally, consumers and consumer advocacy groups have a counter balancing role to ensure that safety and quality are not undermined by political considerations solely when drafting legislation or implementing safety and quality policies. They also have a major role in educating and informing the consumer about the major safety and quality issues. The general principles of GHP/HACCP were adopted by the Codex Alimentarius Commission (CAC) in 1997 and 1999 (FAO, 2001). They include requirements for the design and facilities, control of operations (including temperature, raw materials, water supply, documentation and recall procedures), maintenance and sanitation, personal hygiene and training of personnel. Similarly, the Codex Committee on Fish and Fishery products is working on a draft code of practice for fish and fishery products, including aquaculture products, which integrates these general principles and adapts them to aquaculture. However, this Code is not intended to cover extensive fish farming systems or integrated

livestock and fish culture systems that dominate production in many developing countries. Control and prevention of chemical pollutants and biotoxins require the implementation of appropriate monitoring and surveillance programs. This is particularly important for mollusc culture, filter feeders that can concentrate pollutants, biological agents and biotoxins. The Codex Code of Practice describes the requirements for surveys and monitoring of the harvesting and growing areas to determine sources of domestic and industrial pollution, classification of the areas into suitable for harvesting, relaying or non-suitable for growing or harvesting, and the frequency and methods of monitoring.

Fish Safety and Quality Knowledge Base Fish and seafood are produced from a great variety of plant and animal aquatic species. A risk analysis focused on a specific hazard such as a pathogen or a contaminant requires a substantial amount of scientific and technical information. Each species has different safety and quality attributes related to local conditions and production methods in addition to the type of food commodity, which also has specific processing and preservation requirements. Better utilization of aquatic resources and the harmonization of fish safety and quality systems require access to updated scientific and technical information not the least in light of the SPS Agreement of the WTO that require science-based risk analysis of food hazards. Over the years, FAO has experienced this first hand in risk assessment exercises such as *Listeria monocytogenes* or Vibrio spp. These also proved to be cost and resource intensive given the often-lengthy time frames and the number of experts involved. Since the late 1990s, FAO has been aware of the need for integrated and succinct technical information. The current thinking of a food chain approach to safety and quality simply exacerbates this need. In addition to potential food hazards that may be introduced via handling and processing, fish and seafood production methods include the fisheries of wild populations and aquaculture where safety and quality also depends on the local conditions of the environment and habitat. Given FAO's normative work and capacity building mandate in developing countries, FAO is launching the Aquatic Food Programme in collaboration with the Canadian Food Inspection Agency and other international organizations. Although the Internet offers a wide range of scientific information, finding adequate and pertinent information can be perplexing for a novice user of the Internet. In addition, today's electronic information dissemination capabilities are less of a challenge than the work involved in updating information. The understanding and integrated management of

risks along the entire food chain requires substantial integration of technical information based on the latest available scientific literature and knowledge.

Fishery products are the most traded food in the world. The globalization and further liberalization of world fish trade presents new safety and quality challenges. Upgraded scientific tools must be implemented and novel flexible methods to safety must be sought so that regulatory actions can reflect the most current scientific evidence, and this in turn helps to share the responsibility for safety among the stakeholders of food chain. Fish safety and quality assurance will require enhanced levels of international co-operation in promoting harmonization, equivalency schemes and standards setting mechanisms based on science. The SPS/ TBT agreements of the WTO and the benchmarking role of the Codex provide an international platform in this respect. Important reforms to tackle these issues have been initiated in the USA (NAS, 2003), the EU (2000) and many other countries. Unfortunately, developing countries are at a disadvantage because of insufficient/inadequate national capacities and resources. International organizations such as FAO must revamp their programmes and seek the necessary resources to assist in this endeavour.

Chapter 27

Spoilage indices for fish and shellfish

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

1. Microbial
2. Physical
3. Chemical

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

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

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

Total Volatile Bases:

Volatile bases are produced by spoilage bacteria in fish. They are basic nitrogenous compounds such as ammonia, trimethylamine (TMA), Trimethylamine oxide (TMAO), and Dimethylamine *etc*., The most commonly used index of quality for the freshness of fish is the Total Volatile Base Nitrogen value (TVBN) along with Trimethylamine. Fish with a TVBN value of 20mg/100g is considered very fresh. The limit of acceptability of TVBN is 35-40 mg/100g beyond which the fish is considered as spoiled.

Trimethylamine (TMA):

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

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

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

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

Ammonia:

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

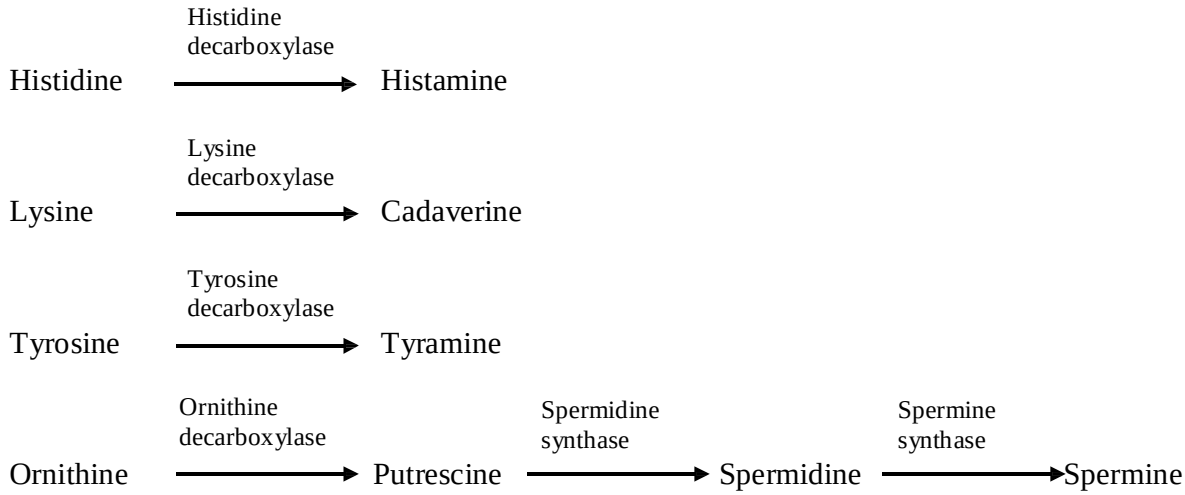
Biogenic amines:

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

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

These fishes are found to be having high free histidine content which gets converted into histamine during spoilage. The biogenic amines formed during the spoilage of fish are found to be thermally stable and thus can be used as an indicator of poor quality of raw material in

preserved/processed fishery products. Cadaverine and putrescine are found to be potentiators of histamine. The direct precursors of histamine, cadaverine, and putrescine are histidine, lysine, and ornithine respectively. Putrescine is also an intermediate of a metabolic pathway that leads to the formation of spermidine and spermine.



Although biogenic amines have been associated with fish spoilage, the legal limit has been established for histamine only. As per Food Safety and Standards Regulation (FSSR, 2011), the maximum permissible level of histamine content in fish and fishery products is 200mg/Kg. Fishes with histamine content up to 20mg/kg are considered to be safe for consumption, 20-100mg/Kg is probably safe while ≥100mg/kg is toxic and unsafe for consumption. Studies also indicated that cadaverine and putrescine can also be used as freshness indices for fish and shellfish respectively. Fish and fishery products containing cadaverine below 15mg/100g are considered as good for consumption, 15-20mg/100g indicates potential decomposition, and over 20mg/100g advanced decomposition.

The Quality Index (QI) and Biogenic Amine Index (BAI) are also used to indicate the freshness of fish.

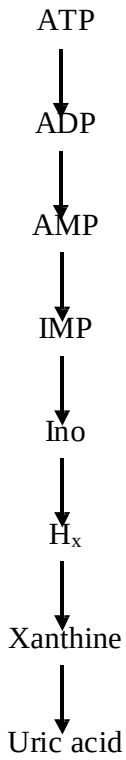
$$\mathrm{QI} = \frac{\text{Histamine} + \text{Putrescine} + \text{Cadaverine}}{[1 + (\text{Spermidine} + \text{Spermine})]}$$

$$\mathrm{BAI} = (\text{Histamine} + \text{Putrescine} + \text{Cadaverine} + \text{Tyramine})$$

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

Nucleotide degradation products:

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



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

$$K\ (\%) = \left[\frac{\text{Ino} + \text{H:x}}{\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{Ino} + \text{H:x}}\right] \times 100$$

Lipid oxidation products:

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

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

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

taste rancid. During prolonged storage of fish, PV, AV and TBA values may increase reaching a peak and decline.

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Chapter 28

Chemical contaminants 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

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.

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 hours to a few days. Histamine concentration required to produce poisoning varies with respect to the susceptibility of each individual. In case of susceptible individual 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 analyzed by High Performance Liquid Chromatography (HPLC) methods with pre and post column derivatization and UV–visible or fluorescence detection. LC with tandem mass spectrometry (MS/MS) can also be a useful approach for an unequivocal confirmation of the studied analytes.

Antibiotics

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

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

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

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

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

Pesticides

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

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

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

Food additives

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

• To maintain product consistency – E.g: emulsifiers, stabilizers, thickeners, etc.

• To improve nutritional quality – E.g: vitamins, minerals

• To improve product safety and quality – E.g: preservatives, antioxidants

• To aid in process or preparations – E.g: leavening agents

• To enhance sensory characteristics of the product

Classification of food additives

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

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

Chapter 29

Biological hazards in fish and fishery products

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Fish and fishery products consumption trend has increased in recent times because of globalization and healthy food awareness. Changes in life style of human has also contributed for pace in consumption. Foodbome illness (commonly known as food poisoning) is often caused by consuming food contaminated by bacteria and/or their toxins, parasites, viruses, chemicals, or other agents. Fish and fishery products may get contaminated with various pathogenic bacteria due to unhygienic handling practices which may results in food poisoning. Biological contaminants of food are harmful and hazardous substances of biological origin in the food that can cause foodborne illness when they are consumed. Each year worldwide, unsafe food causes 600 million cases of foodborne diseases and 4,20,000 deaths. 30% of foodborne deaths occur among children under 5 years of age. WHO estimated that 33 million years of healthy lives are lost due to eating unsafe food globally each year, and this number is likely an underestimation.

Contributing to this underestimation is that many foodborne illnesses lack the severity, duration, and specific diagnosis required for definitive identification and intervention. Biological contaminants could be microorganisms, so small that only can be seen by a microscope such as bacteria and versus, or could be large such as some parasites. Bacteria can grow and multiply rapidly if food is not taken care for temperature. Bacterial and viral Pathogens are the primary food safety concern with regard to fish and fishery products. Some types of fish may also contain naturally occurring parasites. Poor handling practices, such as failure to prevent raw foods from coming in contact with cooked or ready-to eat foods (cross contamination), and lack of proper temperature control are significant factors that can lead to pathogen growth and foodborne illness. To prevent the outbreak of foodborne illnesses, it is crucial for food service professionals to understand all aspects of biological contaminants from how they grow and reproduce to how they contaminate food and infect humans. These hazards can come from raw materials or from food processing steps.

Bacterial Pathogens

Bacterial Pathogens are very common in fish and fishery products including viral pathogens and parasites. Bacterial Pathogens Pathogen contamination and growth is often an important factor in food-borne illness. Pathogenic bacteria can cause illness in human, either by infection or intoxication. Food borne infections are caused by swallowing live pathogens that grow within the body, usually in the intestinal tract. Intoxication is a condition caused by swallowing preformed toxins means toxins produced by microorganisms in the food before it is eaten. Most of the pathogenic bacteria are not present in fish caught from off-shore waters, but contamination occurs during handling of the material. If the time and temperature conditions are favourable, these organisms get an opportunity to grow and multiply at a faster rate. Consumption of such fish is dangerous and it will lead to food poisoning.

Bacterial Pathogens:

1. *Aeromonas spp.*
2. *Bacillus cereus*
3. *Campylobacter jejuni*
4. *Clostridium botulinum*
5. *Clostridium perfringens*
6. *Pathogenic Escherichia coli*
7. *Listeria monocytogenes*
8. *Salmonella spp.*
9. *Shigella spp.*
10. *Pathogenic Staphylococcus aureus*
11. *Faecal Streptococci*
12. *Plesiomonas shigelloides*
13. *Pathogenic Vibrio spp.*
    a. *Vibrio cholerae*
    b. *Vibrio parahaemolyticus*
    c. *Vibrio vulnificus*
14. *Yersinia enterocolitica*

***Aeromonas* spp.**

The genera *Aeromonas* comprise Gram-negative, facultatively anaerobic, oxidasepositive, glucose-fermenting rod-shaped bacteria, generally motile. *Aeromonas* species viz. *A. hydrophila*, *A. sorbia* and *A. caviae* has been described as emerging food-borne pathogens. Besides gastroenteritis *A. hydrophila* may cause cholera like infections. *Aeromonas spp.* are natural members of aquatic environments and is commonly found in fish and fish products of all aquatic environments. *A. hydrophila* is very resistant organism and it can survive in food items stored in cold for long period. Oysters have been implicated in food-borne disease. *Aeromonas* associated diarrhoea has been reported from different parts of India. Some *Aeromonas* spp. are psychrotrophs and some others are enteropathogenic. Studies have shown that very high percentage of the isolates from fish and fishery products produced hemolysin (79.2%) and cytotoxin (91.7%). Psychrotrophic *Aeromonas* strains are able to grow at 4-5°C and produce toxin in oysters at 5°C. Combination of chilling, salting and/or acidification is effective means of preventing the growth of *Aeromonas*.

Bacillus cereus

Bacillus cereus is a facultative anaerobic, catalase-positive, toxin-producing gram-positive bacterium found in soil, vegetation, and food. It commonly causes intestinal illnesses with nausea, vomiting, and diarrhea. However, it has been associated with serious infections in immuno-compromised hosts and can cause septicemia as well as endophthalmitis, which can lead to vision loss. Bacillus cereus is a well-known cause of food-borne illness, but infection with this organism is not commonly reported because of its usually mild symptoms. A fatal case due to liver failure Food poisoning caused by B. cereus may occur when foods are prepared and held without proper refrigeration for several hours before being served. B. cereus is an aerobic spore-forming bacterium. It is commonly found in soil, on vegetables, and in many raw and processed foods. Two types of illnesses have been attributed to B. cereus. The first is characterized by abdominal pain and diarrhea. It has an incubation period of 4-16 hours and symptoms that last for 12-24 hours. The second is characterized by an accute attack of nausea and vomiting. It has an incubation period of 1-5 hours. Diarrhea is not common with the second type of illness. Colonies of B. cereus have an irregular perimeter and are opaque on sheep blood

agar. When grown on an egg yolk agar, a zone of opacification will be noted due to lecithinase production.

B. cereus is a common food contaminant. Effective control measures depend on Notes: destruction by a heat process and temperature control to prevent spore germination and multiplication of vegetative cells in cooked, ready-to-eat foods. Measures to reduce or eliminate the threat of food poisoning by B. cereus include: 1) Avoid preparing food too far in advance of planned service, 2) Avoid holding cooked foods at room temperature, 3) Use quick chill methods to cool foods below 45ºF (7.2ºC) within 4 hours of preparation; store in shallow pans/ small quantities with the food less than 4 inches deep; if food is especially thick (e.g., refried beans), store no more than 3 inches deep, 4) Hold/store hot foods above 140ºF (60ºC) until served, and 5) Reheat foods rapidly to 165ºF (74ºC) or above.

Campylobacter jejuni

They are very small, Gram-negative, microaerophilic, curved thin rods with corkscrew motility. *C. jejuni* is widely distributed in the intestinal tract of poultry, livestock, and warm-blooded domestic animals. It is a very common and important cause of diarrheal illness in humans. Symptoms include profuse diarrhea (sometimes bloody), abdominal pain (intensity and duration can be somewhat severe), headache, weakness, and fever. Many infections occur without symptoms. *C. jejuni* is transmitted through: contaminated foods, including raw clams, mussels and oysters; person-to-person contact; and contaminated water. Cross-contamination of foods by dirty food-contact surfaces, including cutting boards and hands, may be the most frequent route of transmission. Since the infective dose of *C. jejuni* is thought to be small, time/temperature abuse of food products is not necessary to result in this illness. *Campylobacter jejuni* is widely distributed in the intestinal tract of poultry, live-stock and warm-blooded domestic animals.

Contaminated food including raw clams, mussels and oysters, person to person contact, cross contamination of food by dirty food contact surface etc. Incubation period is 3-5 days. Profuse diarrhoea, abdominal pain, headache and fever and meningitis in neonates. Infective dose ranges from 500 to 10,000 cells. This organism survives refrigeration and freezing.

C. jejuni can be controlled by thoroughly cooking fish and fishery products and by stressing the importance of proper (and frequent) hand and equipment washing and sanitary food-handling practices.

Clostridium botulinum

Clostridium botulinum is a dangerous food poisoning organism and it produce a very deadly, exotoxin when grows in food. The food poisoning is known as 'botulism''. It is an anaerobic, Gram-positive, spore-forming rod. The spores are highly heat resistant. Eight different toxins i.e. A, B, C1, C2, D, E, F & G known to exist. Type- E is present in sea mud and is mostly involved in botulism food poisoning in fish and fishery products. Food poisoning is due to the ingestion of toxin.

C. botulinum is found throughout the environment and has been isolated from soil, water, vegetables, meats, dairy products, ocean sediments, the intestinal tracts of fish, and the gills and viscera of crabs and other shellfish. *C. botulinum* is a spore-forming bacteria that grows in the absence of air. These characteristics allow it to survive normal cooking temperatures and to grow in a vacuum packaged and modified-atmosphere environment. *C. botulinum* produces a powerful neurotoxin that causes botulism. Growth is necessary for *C. botulinum* to produce toxin. Symptoms include diarrhea, vomiting, abdominal pain, nausea and weakness. These are followed by double, blurred vision and dilated, fixed pupils. In severe cases, paralysis of the muscles responsible for breathing can cause death. The type of *C. botulinum* Type E that is most common in fish and fishery products is of particular concern because it grows at temperatures as low as 38 F and produces little noticeable evidence of spoilage. *C. botulinum* Type A is the form of this bacteria that is most common in land-based products. It is a common contaminant on processing equipment. It will grow at temperatures no colder than 50 F and produces a putrid odor in products in which it grows. However, its spores are much more heat-resistant than the Type E form of the bacteria.

Because *C. botulinum* produces heat-resistant spores and requires the absence of oxygen for growth, botulism has been most commonly associated with improperly canned food (usually home canned). Semi-preserved fish and fishery products, including smoked, salted and fermented fish, have also been identified as causes of botulism.

C. botulinum can be controlled by inhibiting growth of the bacteria or by destroying it in fish and fishery products. Proper thermal processes for canned fish and fishery products destroy the bacteria. Heavy salting or drying to reduce the water activity below 0.93 and fermentation or acidification to below pH 4.6 are effective means of preventing *C. botulinum* growth. Maintaining proper storage temperatures alone is not considered an adequate control measure for *C. botulinum* Type E because of its ability to grow at low temperatures and because of the severity of the illness. Nonetheless, in many products, it is an important second barrier to growth.

Clostridium perfringens

C. perfringens is commonly found in soil, dust, and the intestinal tract of animals. It is a spore forming, anaerobic (oxygen-free growth conditions) bacterium. Food poisoning caused by *C. perfringens* may occur when foods are cooked and held without maintaining adequate heat or refrigeration before serving. The illness is a self-limiting gastroenteritis with an incubation period of 8-15 hours and a duration of 12-24 hours. The symptoms, which include intense abdominal cramps, gas, and diarrhea, have been attributed to a protein enterotoxin produced during sporulation of the organism in the intestine. The presence of small numbers of *C. perfringens* is not uncommon in raw meats, poultry, dehydrated soups and sauces, raw vegetables, and spices. Because the spores of some strains are resistant to temperatures as high as 100ºC for more than 1 hour, their presence in foods may be unavoidable. Furthermore, the oxygen level may be sufficiently reduced during cooking to permit growth of the clostridia. Spores that survive cooking may germinate and grow rapidly in foods that are inadequately refrigerated after cooking. Thus, when clinical and epidemiological evidence suggests that *C. perfringens* is the cause of a food poisoning outbreak, the presence of hundreds of thousands or more of these organisms per gram of food substantiates the diagnosis.

Control measures emphasize proper food preparation and storage techniques, especially temperature control. Control measures include: Rapid, uniform cooling of cooked foods of cooked foods to < 10ºC (50ºF) within 2-3 hours; Hot holding of cooked foods at or above 60ºC (140ºF); Reheating cooled or chilled foods to a minimum internal temperature of 75ºC (167ºF) immediately before serving; Not leaving foods at room temperature or thawing frozen foods at room temperature; Preventing cross-contamination of cooked foods with bacteria from raw foods by using separate food-contact surfaces for preparing raw and cooked foods items, or by

thoroughly cleaning and sanitizing food contact surfaces after being used for raw products; Maintaining food preparation areas so that they are free of soil and dust; Cleaning and sanitizing meat slicers, meat-cutting equipment, food contact surfaces, and other equipment after use; and Using good personal hygiene methods, and thoroughly washing hands frequently when handling food products, especially after handling raw products and before handling cooked products.

Escherichia coli

E. coli are Gram-negative, rod-shaped, non-spore forming facultative anaerobic bacteria. *E. coli* are naturally found in the intestinal tracts of all animals, including humans. Most forms of the bacteria are not pathogenic and serve useful functions in the intestine. Pathogenic strains of *E. coli* are transferred to fish and fishery products through sewage pollution of the coastal environment or by contamination after harvest. *E. coli* food infection causes abdominal cramping, water or bloody diarrhea, fever, nausea, and vomiting.

Generally this organism is harmless: Pathogenic strains of E.coli are considered to be harmful.

- Enterotoxigenic *E. coli* (ETEC) - Gastroenteritis
- Enteropathogenic *E. coli* (EPEC) - Infant diarrhoea
- Enteroinvasive *E. coli* (EIEC) - Bacillary dysentery
- Enterohemorrhagic *E. coli* (EHEC) - Newly added category
- Enteroadherent *E. coli* (EAEC) - Hemorrhagic colitis (E.coli 0157:H7)

E. coli can be prevented by heating fish and fishery products sufficiently to kill the bacteria, holding chilled fish and fishery products below 40^0F, preventing post cooking cross-contamination, and prohibiting people who are ill from working in food operations. The infective dose of *E. coli* is dependent upon the particular strain from only a few organisms to millions. For this reason, time/temperature abuse of food products may or may not be necessary to result in illness.

Listeria monocytogenes

It is generally accepted as a food borne pathogen. Outbreak of disease is very rare but considered to be very serious due to high rate of mortality. *L. monocytogenes* is widely distributed in nature. A variety of animals can serve as hosts for this organism. The bacterium is often associated with the intestinal tract of domestic animals, birds and humans. About 1% of human population is

known to carry *L. monocytogenes.* This organism is Gram-positive, micro- aerophilic, non-spore forming, motile rods. It can survive freezing and thawing; if the load is more than 5x104 /ml. in milk it can withstand pasteurization. *L. monocytogenes* grows in refrigerated temperatures (even 1° C) and it can survive both acidic and alkaline pH. This is the most heat resistant pathogenic bacteria among non-spore formers.

L. monocytogenes is widespread in nature and has been isolated from soil, vegetation, marine sediments and water. In the early 1900s, *L. monocytogenes* was recognized as a bacterium that caused illness in farm animals. More recently, it has been identified as the cause of listeriosis in humans. Most healthy individuals are either unaffected by *L. monocytogenes* or experience only mild flulike symptoms. Victims of severe listeriosis are usually immunocompromised. Those at highest risk include: cancer patients, individuals taking drugs that affect the body's immune system, alcoholics, pregnant women, persons with low stomach acidity and individuals with AIDS. Severe listeriosis can cause meningitis, abortions, septicemia and a number of other maladies, some of which may lead to death.

The greatest threat of listeriosis is from ready-to-eat products that do not require further cooking at home. *L. monocytogenes* in raw food that will be cooked before consumption is less of a concern to the food industry since the bacteria are killed during cooking. L monocytogenes has been isolated from raw fish, cooked crabs, raw and cooked shrimp, raw lobster, surimi and smoked fish. One of its most significant characteristics is its ability to grow at temperatures as low as 31ºF. *L. monocytogenes* can be prevented by thoroughly cooking fish and fishery products and by preventing cross-contamination once the fish and fishery products is cooked. Since the infective dose of *L. monocytogenes* is thought to be small, time/temperature abuse of food products may not be necessary to result in illness.

***Salmonella* spp.**

Salmonella are enteric organisms producing enteric fever and food borne gastroenteritis. More than 2500 serotypes of this organism are known to exist at present and more are added to the list every year. Food poisoning due to *Salmonella* is known as "Salmonellosis" infants, elderly and the under nourished are more susceptible to the disease and in such individual salmonellosis is known to occur even from one single cell of *Salmonella*. *Salmonella* are non-spore forming,

mostly motile (exception *S. pullorum* and *S. gallinarum*) facultative — anaerobic, Gram-negative rods.

Salmonella is naturally found in the intestinal tracts of mammals, birds, amphibians and reptiles but not in fish, crustaceans or mollusks. *Salmonella* is transferred to fish and fishery products through sewage pollution of the harvest environment or by contamination after harvest. Freshly caught marine fish are usually free from *Salmonella*. However, fish from polluted coastal waters are usually contaminated with this organism. *Salmonella* food infection causes nausea, vomiting, abdominal cramps and fever. Outbreaks of *Salmonella* food infection have been associated with raw oysters, salmon, tuna salad, shrimp cocktail, stuffed sole and gefilte fish.

Salmonella can be prevented by: heating fish and fishery products sufficiently to kill the bacteria, holding chilled fish and fishery products below 40 F, preventing post-cooking cross-contamination and prohibiting people who are ill or are carriers of *Salmonella* from working in food operations. The infective dose of *Salmonella* is thought to be extremely variable, relatively high for healthy individuals and very low for at-risk individuals, such as the elderly or medically compromised. For this reason, illness could result even without time/temperature abuse, but abuse has been a contributing factor in many outbreaks.

Shigella spp.

The disease caused by *Shigella* is generally known as `shigellosis' which is not indigenous in foods, transmitted through food or water contaminated with human excreta. *Shigella* are Gram-negative, facultative anaerobic, non-sporulating, non-motile, rod-shaped bacteria. They are the most difficult enteric pathogens to isolate. Man is the only known natural host for *Shigella*. The organisms pass the acid barrier of the intestine, multiply in the gut and produce ulceration of large intestine followed by dysentery. Four serological groups i.e. A, B, C, and D. The major species in *Shigella* comprises *Shigella dysenteriae; S. flexneri; S. boydi* and *S. sonnei. S. dysenteriae* causes the most severe illness They survive longest when food holding temperatures are 25°C or lower.

Shigella is naturally found in the intestinal tract of humans. *Shigella* is transferred to fish and fishery products through sewage pollution of the coastal environment or by contamination after harvest. *Shigella* produces an illness called Shigellosis, which causes mild diarrhea, fever, abdominal cramps and severe fluid loss. Hazards from *Shigella* can be prevented by eliminating

human waste contamination of water supplies and by improved personal hygiene for people who are ill or are carriers of *Shigella* and work in food operations.

Staphylococcus aureus

Since 1930, it is known that contamination of food with coagulase — positive staphylococci could cause food poisoning, as the organism growing in food materials in considerable numbers, secretes exotoxin. Staphylococcal food poisoning is caused only by certain well defined strains of S. aureus; such strains are known as enterotoxigenic strains. Food-borne out breaks due to coagulase-negative strains of Staphylococci are seldom reported. S. aureus are known to produce 9 different types of enterotoxins designated as enterotoxin A, B, C1, C2, D, E, F, G and H. This is the most drought resistant pathogenic bacteria and they cannot compete with general bacterial flora.

Humans and animals are the primary reservoirs for S. aureus. S. aureus can be found in the nose and throat and on the hair and skin of 50 percent of healthy individuals. However, the bacteria can be found in air, dust, sewage and surfaces of food-processing equipment. S. aureus can produce a toxin if allowed to grow in food. The toxin is not destroyed by the cooking or canning processes. S. aureus has the ability to grow and produce toxins in food with very little available water (.85 aw, 10 percent salt), which would prevent the growth of other pathogens.

S. aureus food poisoning causes nausea, vomiting, abdominal cramping, watery or bloody diarrhea, and fever.

Hazards from *S. aureus* can be prevented by: minimizing time/temperature abuse of fish and fishery products, especially after cooking, and requiring that food handlers engage in proper hygiene.

Faecal *Streptococci*

Faecal streptococci are Gram-positive, facultative anaerobic, non-spore forming non-motile and catalase negative cocci. Faecal streptococci are comparatively resistant to many adverse conditions. About 30% reduction of faecal streptococci takes place during freezing at -40°C, during subsequent storage at -18°C not much of reduction in count takes place even after 2 years of storage.

Primary habitat and source of contamination are same as in the case of *E. coli*. One gram of faeces contains 10^6 to 1^{08} faecal streptococci, therefore their presence in food product is generally regarded as an indication of faecal contamination. Just like *E. coli*, faecal streptococci are absent in off-shore water but are present in considerable numbers in coastal waters. Unclean boat deck, utensils, water and ice are the major source of contamination.

Vibrio cholerae

It is the causative agent of cholera. The current definition of *V. cholerae* consists of the classical (non-hemolytic) and El Tor (hemolytic) biovars. The El Tor *vibrios* are generally more infectious than the classical *V. choleraeserotypes* and it can survive longer in the environment. The only natural habitat of *V. cholerae* is man. *V. cholerae* is found in estuaries, bays, and brackish waters. It is naturally occurring and is not necessarily related to sewage contamination. *V. cholerae* tends to be more numerous in the environment during warmer months.

There are a number of types of *V. cholerae*, and these produce very different symptoms. One type, Vibrio cholerae 01, initially causes abdominal discomfort and mild diarrhea. As the illness progresses, the symptoms may include: watery diarrhea, abdominal cramps, vomiting and dehydration. Death can occur. Susceptibility to cholera is enhanced in people who have had gastric surgery, take antacids or have type O blood. Outbreaks of this type of cholera have been associated with oysters, crabs and shrimp from the Gulf of Mexico. *V. cholerae* 01 has also been recovered from Chesapeake Bay waters, although no illness has been reported from that area. Another type of *V. cholerae*, non-01, causes diarrhea, abdominal cramps and fever. Nausea, vomiting and bloody diarrhea have also been reported. The severity of the symptoms is dependant, in part, upon the specific strain. In its most severe form, *V. cholerae* non-01 has resulted in septicemia (blood poisoning) in individuals with medical conditions that weaken their immune systems. The illness has been associated with consumption of raw oysters, but the bacterium has also been found in crabs. Hazards from *V. cholerae* can be prevented by cooking fish and fishery products thoroughly and by preventing cross-contamination once the fish and fishery products is cooked.

Vibrio parahaemolyticus

V. parahaemolyticus is a marine pathogen present in marine and brackish-water. They are Gram-negative, rod shaped bacteria which are non-sporulating, halophilic, motile, and oxidase-

positive. *V. parahaemolyticus* is naturally occurring in estuaries and other coastal areas throughout most of the world. In most areas, *V. parahaemolyticus* is more numerous in the environment during the warmer months and, as a result, most outbreaks occur during the summer. The most commonly experienced symptoms of *V. parahaemolyticus* illness include: diarrhea, abdominal cramps, nausea, vomiting and headache. Fever and chills are less frequently reported. The illness has been associated with consuming contaminated crabs, oysters, shrimp and lobster. Hazards from *V. parahaemolyticus* can be controlled by thoroughly cooking fish and fishery products and preventing cross-contamination after cooking. Control of time/temperature abuse is also an important preventative measure.

It can cause food poisoning when it is consumed in large numbers (more than 10^5/g of Kanagawa-positive strains), along with food materials. This type of food poisoning is more in countries like Japan, where there is a habit of eating un-cooked fish and fishery products. In recent years, the incidence of *V. parahaemolyticus* infection has been increasing in many parts of the world, and this has been attributed to the emergence of a new clone of the 03: K6 serotype carrying only the *tdh* gene. The onset of symptoms is within 12 h of eating infected food. Icing the material immediately after catch, washing with potable water and improvement of hygiene are considered as remedial measures.

Vibrio vulnificus

V. vulnificus is a naturally occurring marine bacterium. It is an emerging pathogen, phenotypically similar to *V. parahaemolyticus.* Mortality is up to 60%. It is the part of the normal bacterial flora of estuarine and marine waters. *V. vulnificus* is Gram-negative, halophilic, lactose-positive, rod shaped bacteria. All strains are pathogenic; infection dose is not known! Infection is associated with the consumption of raw fish and fishery products particularly oysters. *Vibrio vulnificus* requires salt for survival and is commonly isolated at salinities of 7 ppt to 16 ppt. It is primarily found in the Gulf of Mexico, but it has also been isolated from the Atlantic and Pacific oceans. The numbers of the bacterium in the environment are highest during the warmer months of April through October.

The most common symptoms include: skin lesions, septic shock, fever, chills and nausea. Abdominal pain, vomiting and diarrhea are less frequently reported. Death occurs in about 50 percent of the cases. A number of medical conditions make individuals more susceptible to the

life threatening effects of this bacterium, including: liver disease, alcohol abuse, cancer, diabetes, chronic kidney disease, immunosuppressive drug or steroid usage, low stomach acidity and AIDS. *V. vulnificus* sepsis has been associated with the consumption of certain molluscan shellstock.

Hazards from *V. vulnificus* can be controlled by thorough cooking of shellfish and by preventing cross-contamination once the fish and fishery products is cooked. The risk of *V. vulnificus* infection may also be reduced by rapidly refrigerating oysters from the Gulf Coast during warm-weather months. Individuals in the "high risk" groups should not consume raw molluscan shellfish. Icing is very effective to reduce the load of the organism. This organism is closely associated with oyster tissues and is not removed fully by controlled purification methods such as UV light assisted depuration. No effective means commercially exist for elimination of the health hazard in oyster intended for raw consumption and so, it is advised to avoid raw fish and fishery products completely.

Yersinia enterocolitica

Y. enterocolitica is naturally found in soil, water and domesticated and wild animals. Yersiniosis causes diarrhea, vomiting, abdominal pain and fever, often mimicking appendicitis. Outbreaks have been associated with oysters and fish. Hazards from Y. enterocolitica can be prevented by: heating fish and fishery products sufficiently to kill the bacteria, holding chilled fish and fishery products below 40° F and preventing post-cooking cross-contamination.

Plesiomonas shigelloides

The genera *Plesiomonas* comprise Gram-negative, facultatively anaerobic, oxidase positive, glucose fermenting, rod shaped bacteria, generally motile. It is an emerging pathogen, mostly associated with fresh water and seawater in warm months. This organism is predominantly associated with fish and fishery products. *P. shigelloides* was implicated as the causative agent for diarrhoea after consumption of fish and fishery products in Hong Kong and USA. It cannot grow at chilled condition, but can survive. Growth can be prevented by chilling, moderate salting/acidification.

Fungal hazards

The fungi associated with foods are generally yeasts and moulds. The greatest concern for food safety are mycotoxins eg. aflatoxin, fusarin, patulin, etc. which are produced by moulds and may be associated with chronic illness, such as cancer. Fungi needs lesser moisture for growth compared to bacteria. If the water activity (aw) is less than 0.60 there will not be any growth of fungi or other microorganisms. Water activity of biscuits is 0.30 and sugar is 0.10.

Viral Pathogens

Viruses contaminate the foods same way as bacteria. It reproduces only within susceptible living cells. A ready to eat food containing a pathogenic virus is a health hazard. Viruses don't reproduce in food; it exists in foods without growing, so they need no food, water or air to survive. Viruses don't cause spoilage but may cause illness. It can survive in human intestine, water, frozen foods etc. for months. Viruses can be found in people who were previously ill. Adequate cooking can destroy it.

Major Viral Pathogens in fish and fishery products includes:

• Hepatitis A Virus

• Norwalk Virus

Hepatitis A

This virus survives better at low temperatures and are killed at high temperatures. As a result, most outbreaks of hepatitis occur during winter and early spring. Viruses can remain alive for long periods of time in seawater and have been shown to survive over one year in marine sediments. Both raw and steamed clams, oysters, and mussels have been implicated in outbreaks of hepatitis A. Symptoms of hepatitis A include weakness, fever and abdominal pain. As the illness progresses, the individual usually becomes jaundiced. The severity of the illness ranges from very mild (young children often experience no symptoms) to severe, requiring hospitalization. The fatality rate is low, and deaths primarily occur among the elderly and individuals with underlying diseases.

Hepatitis A can be prevented by thoroughly cooking fish and fishery products and by preventing cross-contamination of cooked fish and fishery products. But hepatitis A appears to be more resistant to heat than other viruses. A laboratory study showed that hepatitis A virus in infected

oysters were inactivated after heating at 140 F for 19 minutes. Therefore, mollusks steamed only until the shells open (a common cooking practice) are not exposed to heat long enough to inactivate hepatitis A virus.

Norwalk Virus

Norwalk virus is considered a major cause of nonbacterial intestinal illness (gastroenteritis). Illness from Norwalk virus has been associated with eating clams (raw and steamed), oysters and cockles. Norwalk virus causes nausea, vomiting, diarrhea, abdominal cramps, and occasionally fever in humans. Hazards from Norwalk virus can be prevented by thoroughly cooking fish and fishery products and by preventing cross-contamination of cooked fish and fishery products. Additionally, a recent outbreak has demonstrated that controlling overboard discharge of untreated sewage from shellfish harvesting vessels would reduce the incidence of illness attributable to Norwalk virus. Viruses can be prevented by thorough cooking and preventing cross contamination of cooked foods.

Parasites in fish and fishery products:

Major parasites significant for human health includes:

• *Anisakis simplex*

• *Pseudoterranova decipiens*

• *Diphyllobothrium latum*

Anisakis simplex

Anisakis simplex, commonly called herring worm, is a parasitic nematode or roundworm. Its final hosts are dolphins, porpoises and sperm whales. The larval (wormlike) stage in fish and squid is usually 18 to 36 millimeters in length, 0.24 to 0.69 millimetres in width and pinkish to whitish in colour.

Anisakiasis, the human illness caused by *Anisakis simplex*, is associated with eating raw fish (sushi, sashimi, lomi lomi, ceviche, sunomono, Dutch green herring, marinated fish and cold-smoked fish) or undercooked fish.

Parasites in fish are considered a hazard only in fish that the processor knows or has reason to believe will be served raw or undercooked. In other products, parasites are considered filth but

not hazardous. The FDA has established three freezing processes to kill parasites. Freezing and storing at -4ºF (-20ºC) or below for 7 days (total time), or freezing at -31ºF (-35ºC) or below for 15 hours, or freezing at -31ºF (-35ºC) or below until solid and storing at -4ºF (-20ºC) or below for 24 hours is sufficient to kill parasites. FDA's Food Code recommends these freezing conditions to retailers who provide fish intended for raw consumption. Note: these conditions may not be suitable for freezing particularly large fish (e.g. thicker than six inches).

Pseudoterranova decipiens

Pseudoterranova decipiens, commonly called "codworm" or "sealworm," is another parasitic nematode or roundworm. The usual final hosts of Pseudoterranova are gray seals, harbor seals, sea lions and walruses. The larval stage in fish are 5 to 58 millimeters in length, 0.3 to 1.2 millimeters in width and yellowish, brownish or reddish in color.

These nematodes are related to *Anisakis simplex* and the disease associated with infections is also termed anisakiasis. These nematodes are also transmitted to humans through raw or undercooked fish. Control of Pseudoterranova is the same as for *Anisakis simplex*.

Diphyllobothrium latum

Diphyllobothrium latum is a cestode, or tapeworm, that parasitizes a variety of fish-eating mammals of the northern latitudes. A similar species is found in the southern latitudes and is associated with seal hosts. Cestodes have a structure that allows them to attach to the intestinal wall of their host and have segmented bodies. Cestode larvae found in fish range from a few millimeters to several centimeters in length and are white or gray in colour.

Diphyllobothrium tapeworms primarily infect freshwater fish. But salmon and related fish can also carry the parasites. *Diphyllobothrium* tapeworms are usually found unencysted and coiled in musculature or encysted in viscera. These tapeworms can mature and cause disease in humans. These cestodes are also transmitted to humans through raw or undercooked fish. Control of *Diphyllobothrium* is the same as for *Anisakis simplex*.

Conclusion:

Proper food handling can prevent most foodborne illness and diseases. Consumers must follow WHO's five keys to safer food -

1. Keep clean:
    - Thoroughly wash raw fruits and vegetables with tap water.
    - Keep clean hands, kitchen and chopping board all the time.
2. Separate raw from cooked:
    - Do not mix raw food and ready-to-eat food.
    - Do not mix raw meat, fish and raw vegetables.
3. Cook thoroughly:
    - Thoroughly cook all meat, poultry and fish and fishery products, especially shellfish.
    - Reheat all leftovers until they are steaming hot.
4. Keep food at safe temperatures:
    - Refrigerate cooked food within two hours of preparation
    - Never defrost food at room temperature. Defrost frozen food in the refrigerator, cold water or in the microwave.
5. Use safe water and raw materials.
    - Use safe drinking water for food preparation.
    - Check use-by dates and labels while buying packed food.

Chapter 30

PRPs for Fish Processing Establishments

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Pre-requisite programs (PRPS)

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

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

Employee training and training records

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

processing, packing, or holding activities for food. Processors must maintain records of this training for at least 2 years.

Good Manufacturing Practices (GMP)

Good Manufacturing Practices (GMPs) provides the basis for determining whether the facility, processing methods, practices and controls used to process food products are suitable to allow for the production of safe and wholesome food and whether the products have been processed under sanitary conditions. GMPs outline the minimum standards that a food processing facility needs to meet including, but not limited to, personnel, buildings and facilities, equipment, production and process controls, raw materials, and manufacturing operations. GMPs were first released in 1969 as 21 CFR Part 110, and revised in 1986 and again in 2015 (21 CFR Part 117). The 2015 updated version of GMPs explicitly address the allergen cross contact. "Cross-contact" differs from "cross-contamination". Allergen cross-contact is the unintentional incorporation of undeclared food allergens into food while cross-contamination is the contamination of food with bacterial, chemical or physical hazards.

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

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

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

Sanitation Control Procedures (SCPs)

Sanitation Control Procedures are the necessary procedures to meet specified GMPs requirements which, in the absence of control, could impact food safety. When SCPs are in place, HACCP plans can more effectively focus on the hazards associated with the product or process and rather than the processing plant environment or employee practices. The Seafood HACCP Regulation SCPs (21 CFR part 123.11) include one recommendation and three requirements. It is recommended that processors create a written sanitation standard operating procedure (SSOP) that describes how sanitation procedures will be performed. Written SSOPs would outline the goals, methods and activities that are needed to be performed in order to meet the SCP requirements. Well-designed, written SSOPs that are properly implemented are an effective means to prevent insanitary conditions associated with the processing environment and employee practices that may contribute to food safety hazards.

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

Eight Key Sanitation Areas

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

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

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

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

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

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

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

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

These eight key areas of sanitation should be monitored at a frequency sufficient to ensure conformance. In addition to that the monitoring results and corrections made for any deficiencies must be recorded. The frequency or time for monitoring will vary according to various types of products and the schedule of operations. The SCP monitoring forms or records must include the name and location of the processor, the date and time the monitoring was performed, corrections made and the signature or initials of person conducting the monitoring. The sanitation monitoring, corrections and sanitation controls recordkeeping may be performed as part of a firm’s HACCP Plan controls, or separately.

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

Chapter 31

HACCP Principles 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:***

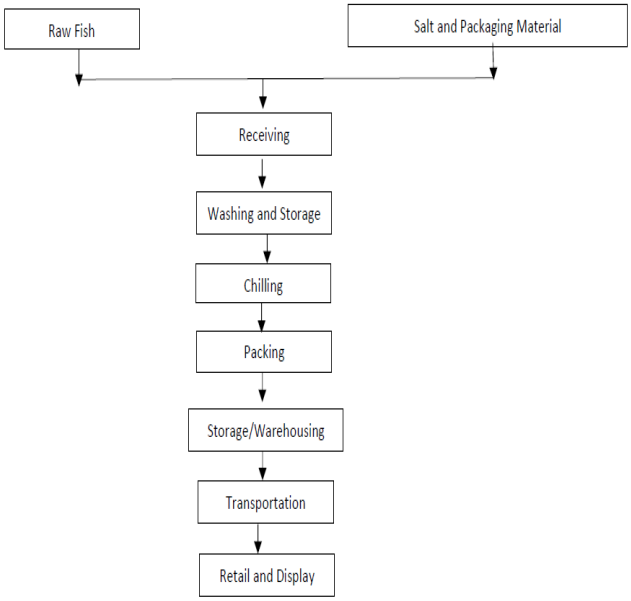
A full description of the product should be drawn up, including relevant safety information such as: harvesting area and technique; raw materials and ingredients used including commercial and Latin name of the fish; factors that influence safety such as composition, physical/chemical parameters, such 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.



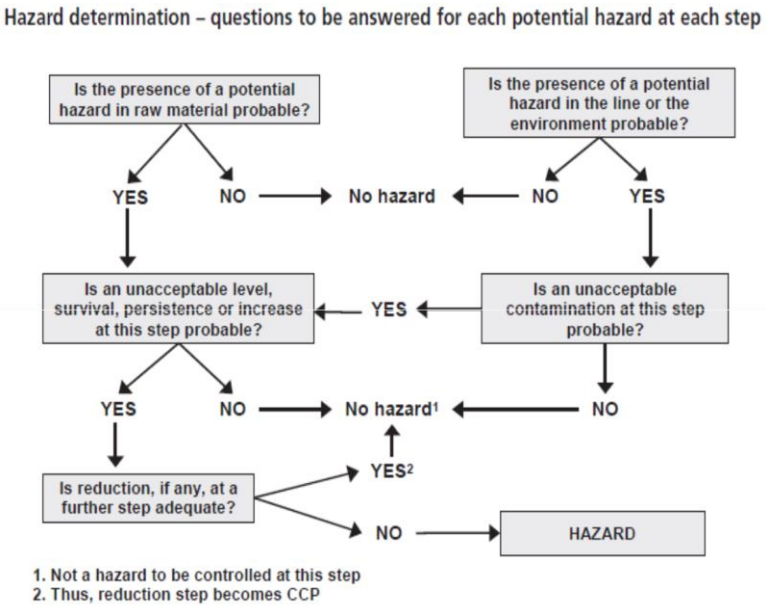
5. On site verification of the process flow diagram:

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:



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)

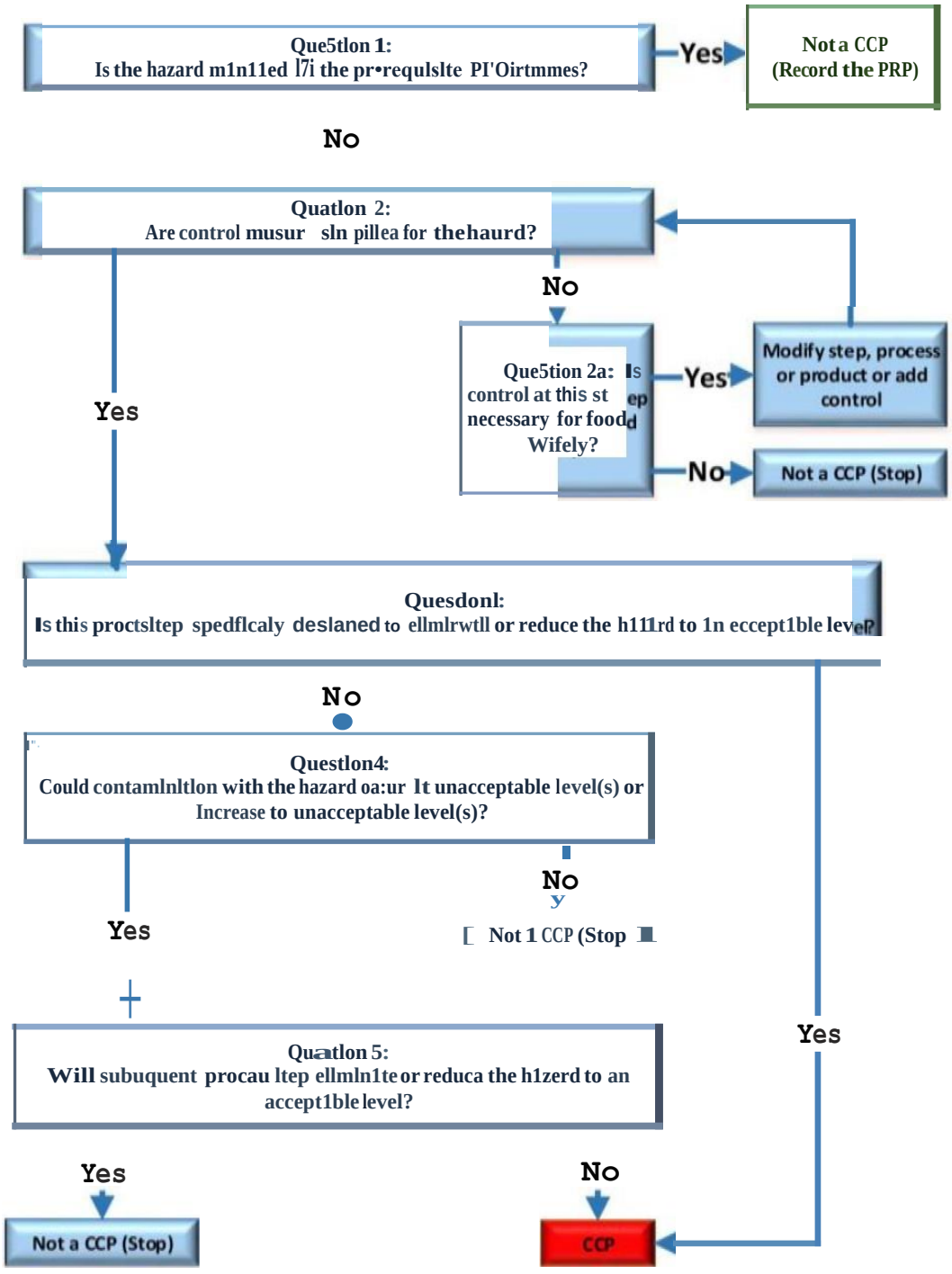
Physical hazard:

- 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.



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.

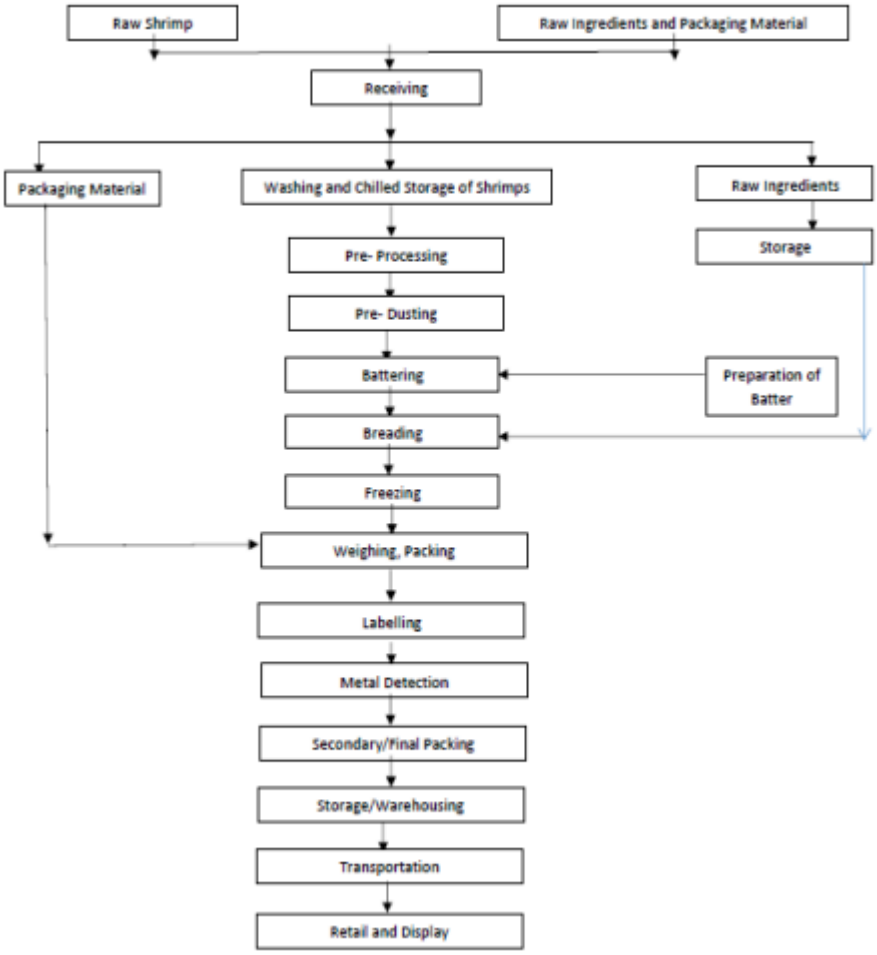
7. Establish documentation concerning all procedures and records appropriate to these principles and their application

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



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 Stop	Hnrd Typo	Potential homd	Iikolihood	Severity	Risk	Preventive Measure	Q■	Q2	Q2A	Q3	Q4	QS	CCP Y/N	Rmison for decision
1.	Receiving Of Shrimp	Biological	Microbial pathogens	M	L	ML	Controlled Infurther processing steps	V						N	Reduced to acceptable level in the subsequent freezing step.
		Chemical	Sulphite Pesticide Antibiotidin case of Aquaculture	M	L	ML	Adherence to raw material specifications Supplier's guarantee that suphiting agents are not used and the raw product is free from pesticide residues. Supplier's gurantee taking into account withdrawal period	V						N	Supplier's declaration Adherence to spedfications.
		Physical	None												
	Receiiing of other raw material	Biological	None												
		Chemical	None												
		Physical	Presence of foreign material	M	L	ML	Takencare by PRPs a	V						N	Visual Inspection to detect presence of fore gn material
I.c.	Receiving and storage of Packaging materiaI	Biological	Contaminatio n due to poor storage conditions	L	M	LM	Takencare by PRP's	V						N	Maintain good air quiity, cleanliness and humidity of the storage room
		Chemical	None												
		Physical	Low quality packaging material	L	M	LM	Takencare by PRp's	V						N	Purchase specifications and visual inspection of all lots of packaging material Packaging materiaI used must be

															food grade.
2.	Washing	Biological	Microbial Pathogens	M	■	ML	Taken care by PRPs and eliminated during retorting stage Use only potable water for washing	V				-		N	Microbialpathogens are reduced or eliminated in the subsequent pre--cooldng and retorting stage. Testing of potable water done against 1510500 standard requirements.
		Chemical	None												
		Physcal	None												
3.	Storage	Biological	Microbial pathogens	M	■	ML	Time-Temperature control	V						N	Adherence to PRP's control microbialmultiplication.
		Chemical	None												
		Physical	None												
4.	Pre-processng	Biological	Microbial pathogens	M	■	M■	Taken care by GHP	V						N	Adherence to GHP prevents microbialcontamination
		Chemical	None												
		Physcal	Metal Fragments	M	■	M■	Controlled in the foUowing steps	N	V		N	V	V	N	Controlled during the metal detection step.
5	Pre-dusting	Biological	Microbial pathogens	M	L	MI	Controlled by GHP	V						N	Adherence to GHP
		Chemical	None												
		Physcal	Metall fragments	M	L	ML	FinalProduct is passed through metal detector	V						N	There are chances of metal contamination from the conveyor belts and equipment. Metal detection step elininate the hazard.
6.	Battering	Biological	Microbial Pathogens	M	L	ML	Taken care by PRPs and GHP	V						N	Adherence to GHP controls bactertalmuiplcation.
		Chemical	None												
		Physcal	Metall fragments	M	■	ML	FinalProduct is passed through metal detector	V						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		MH	Taken care by PRPs							N	Adherence to GHP controls bacterial multiplication
		Chemical	None												
		Physical	Metal fragments	M		MH	Final Product Is passed through metal detector							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					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							Metal fragments entering into the product from the processing

														V' 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		MH	Temperature to be maintained	N						N	Finished Product Storage done makes hazard unlikely to occur.
		Chemical													
		Physical	None												
14.	Transportation	Biological	Microbial pathogens	M		MH	Cleaning of vehicles Time-temperature control							N	Controlled by sanitation programmes and PRP's
		Chemical	None												
		Physical	None												
15.	Retail& Display	Biological	Microbial pathogens	M		MH	Adherence to GHP							N	SOP for finished product storage during retail and display makes hazard unlikely to occur
		Chemical	None												
		Physical	None												

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

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

Conclusion

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

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Chapter 32

Overview of chromatography and mass spectrometry

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Introduction

A Mass Spectrometer is an analytical instrument that measures the masses of individual molecules which have been converted into gas-phase ions. Molecules in a liquid-phase need to be converted into a gas-phase for the mass spectrometer to be able to measure them. Ions are separated, detected and measured by their mass-to-charge ratios (m/z). Mass spectrometers hyphenated with liquid chromatograph (LC-MS/MS) are widely used in chemical and biological research now a-days, to identify and elucidate structures of unknown metabolites, protein etc. in biological tissue, plant material, microbial broth etc. It is also used for targeted analysis of known compounds such as pesticides, antibiotics, vitamins, amino acids, phospholipids etc. In the field of marine bioactive compounds, LC-MS/MS are widely used to determine metabolite profile of marine plants, fish, crustaceans, micro algae, and microbes. Structure elucidation of marine bioactive peptides is another possible application of LC-MS/MS. It is also used for targeted analysis of phenolic acids, flavonoids, carotenoids, vitamins, phospholipids etc. in the marine plants and animals.

Liquid Chromatography

A high-performance liquid chromatograph (HPLC) or ultra-high performance liquid chromatograph is a common front end of a LC-MS/MS system. HPLC/UHPLC separates mixture of compounds based on the principle of adsorption chromatography where the mobile phase is liquid solvent and the stationary phase is solid sorbent particles tightly packed inside a metal column. When the stationary phase is polar in nature, the type of chromatography is called normal phase chromatography; while in case of reverse phase chromatography the stationary phase is non polar. Reverse phase chromatography is most commonly used with mass spectrometry because of its repeatability, relatively lower maintenance, and chromatographic resolution for wide range of mid-polar to non-polar compounds. Most common type reverse phase stationary phase material is C18, where the silica particle surface is modified with 18 carbon chain length hydrocarbons. Similarly, C30 and C8 columns are used for separation of

highly nonpolar and relatively polar compounds respectively. Normal phase chromatography commonly uses unmodified silica as stationary phase and used for chromatographic separation of polar compound mixture such as fatty acids and tocopherol isomers. In reverse phase chromatography water in combination with acetonitrile or methanol is most common type of mobile phase, where the solvent elution programme starts with high aqueous content and gradually ramped to high organic content. In case of normal phase chromatography, water cannot be used as mobile phase because of its interaction with silica particles. A combination of nonpolar and relatively polar solvents is used as mobile phase, where the elution programme starts with high content of nonpolar solvent and the content of polar solvent is gradually increased. The following figure presents different parts of the HPLC/UHPLC.

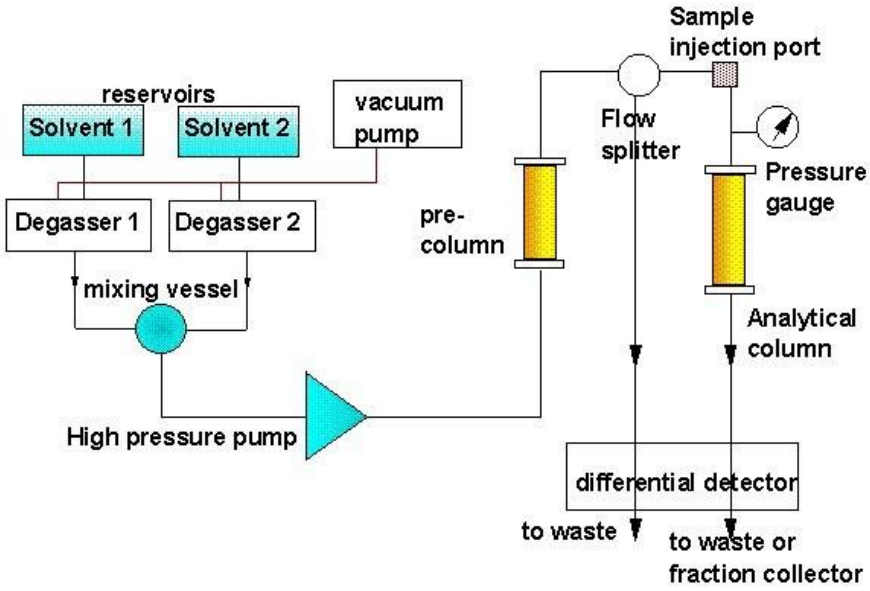


Figure 1. Different parts of a liquid chromatograph

Chromatographic resolution is directly proportional to the length of the chromatographic column, and inversely proportional with the particle size, inner diameter, and pore size. Hence, a short length column with finer particle size, shorter inner diameter, and smaller pore size can achieve the same chromatographic resolution in less time which will take longer in a column of higher length, with bigger particle size, longer inner dia and bigger pore size. However, the solvent back pressure is extremely high in such short columns and can be used only with UHPLC where the pump is equipped to handle back pressure up to 18000 psi. UHPLC is a popular front

end of mass spectrometer due to short analysis time, sharp peak shape, and less consumption of organic solvents.

Mass spectrometer

In a mass spectrometer the compounds introduced in liquid phase form gas phase ions in the ion source. Next the ions are separated in a mass analyzer and finally they reach the detector. The detector shows the output in the data system as a mass spectrum, total ion chromatogram (TIC), base peak ion (BPI) chromatogram, or extracted ion chromatogram (XIC). There are different possible ion sources and mass analyzer combinations in different mass spectrometers which are used for different application needs. The following figure shows a schematic of major parts of a mass spectrometer and lists different possible ion sources and mass analyzers.

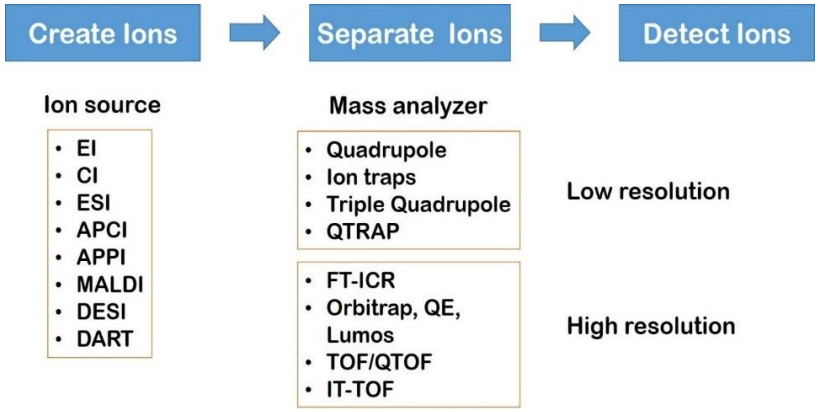


Figure 2. Schematics of different parts of mass spectrometer

The electron impact (EI) and chemical ionization (CI) ion sources are found in gas chromatograph hyphenated mass spectrometer (GC-MS) and the ionization happens under complete vacuum. EI is a hard ionisation technique, where the molecular weight ion is almost completely broken down into fragments. Hence, for molecular weight determination, CI ion source is preferred in GC-MS; where a pseudo molecular ion with reagent gas (most commonly methane or ammonia) is formed through a soft ionisation technique. Electron spray ionisation (ESI), atmospheric pressure chemical ionisation (APCI), atmospheric pressure photo ionisation (APPI), fast atom bombardment (FAB), matrix assisted laser desorption ionisation (MALDI), desorption electron spray ionisation (DESI), direct analysis in real time (DART) are prominent ion sources in different LC-MS. These ion sources are used based on the polarity and molecular weight range of the target analytes or analyte classes, as shown in the following schematics.

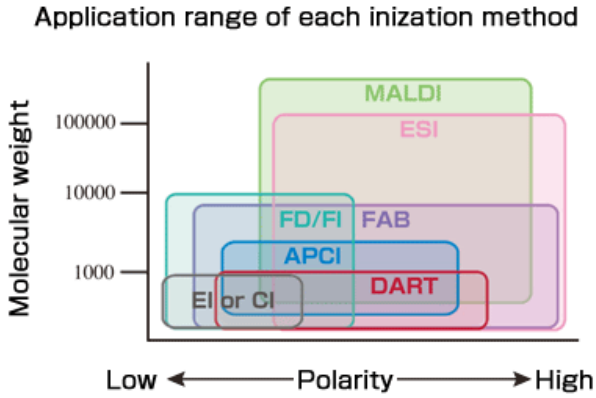


Figure 3. Application range of different ion sources

ESI is most commonly used ion source with LC-MS as a wide range of compounds with medium polarity to high polarity, and low to high molecular weight can be analyzed. APCI is suitable for compounds with low polarity which do not ionize sufficiently in ESI. APPI is suitable for highly non polar compounds such as persistent organic pollutants. MALDI is prominently used for intact mass determination of proteins.

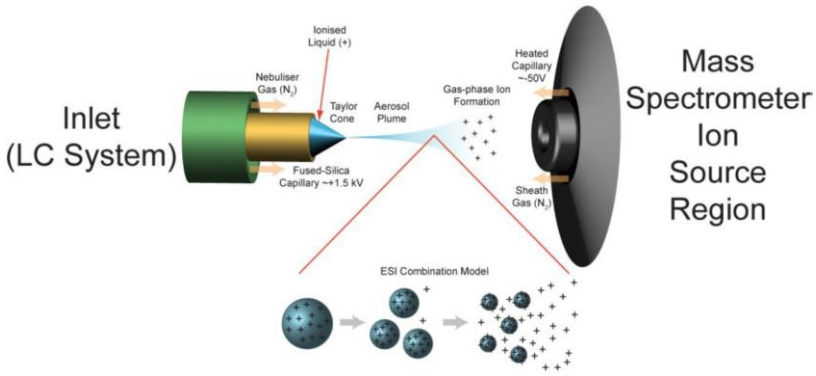
In ESI ion source, the compound in liquid phase is nebulized through a charged capillary. In positive ionisation mode a positive charge is applied, where in negative ionization mode a negative charge is applied. The solvent around the droplets containing charged ions is rapidly evaporated by the heater gas and ion source temperature. Hence, the droplets become smaller and smaller, finally releasing only gas phase ions. The ESI is a soft ionization technique, where the most commonly formed ions are $[M + H]^+$, and $[M - H]^-$, depending on the ionisation operating mode. These ions of a compound are called adduct/pseudo molecular weight ion/parent ion/precursor ion. Some other common adducts found in ESI ion source are listed below.

Positive polarity adduct	Mass difference*	Negative polarity adduct	Mass difference*
$[M + H]^+$	+1.0078	$[M - H]^-$	-1.0078
$[M + NH_4]^+$	+18.0344	$[2M - H]^-$	-
$[M + Na]^+$	+22.9898	$[M - H + H_2O]^-$	+18.0106
$[M + K]^+$	+38.9637	$[M - H + CH_3OH]^-$	+32.0262
$[M + H_2O + H]^+$	+18.0106	$[M - H + CH_3CN]^-$	+41.0265
$[M - H_2O + H]^+$	-17.0027	$[M + Cl]^-$	+36.4609
$[M - 2H_2O + H]^+$	-35.0133	$[M + Br]^-$	+79.9040

A cone voltage or declustering potential is applied on the ion source cone to further push the generated gas phase ions towards the mass analyzer. Hence, the flow rate of nebulizer gas, heater gas, ion source temperature, cone voltage/declustering potential are important parameters that

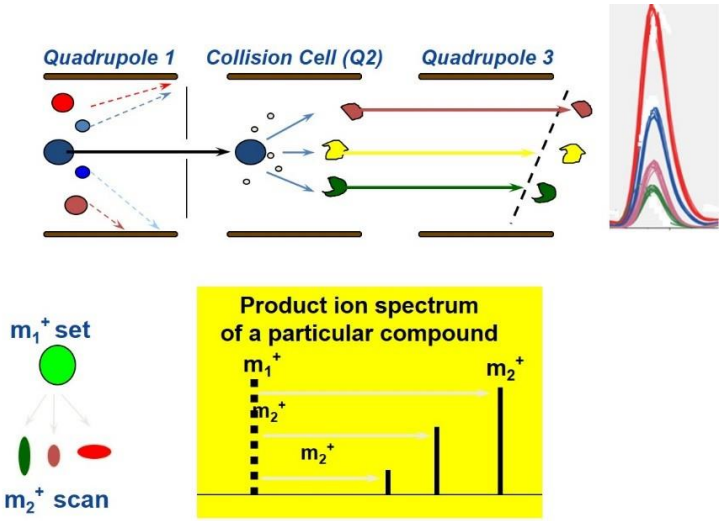
need to be optimized in analyses using ESI ion source. Following is a schematic of the operation of ESI ion source.

Figure. Schematic of ESI ion source.



A quadrupole system uses four cylindrical magnets that are set parallel to each other and function to filter ions based on their mass-to-charge ratio (m/z). The analyzer consists of two pairs of like charged magnets that oppose each other and keep the ions within the ion path of the quadrupole under vacuum. Ions are filtered based on their masses as they traverse the linear ion path. When a linear series of three quadrupoles is used, the resulting triple stage quadrupole analyzer is able to both filter and fragment the ion stream. In most cases, the first (Q1) and third (Q3) quadrupoles act as mass filters, while the second (Q2) quadrupole dissociates ions by having them collide with argon, helium or nitrogen gas. Quadrupole-based mass analysers excel at tracking single ions or reactions for extended periods of time. This is why they are preferentially used in the targeted analysis of compounds, especially known compounds such as drugs and pollutants. This is also why quadrupole mass analyzers are often used in the fields of food safety, environmental analysis, clinical and forensic toxicology studies. The triple quadrupole (QQQ) mass spectrometer (MS) consists of a series of three quadrupoles and selects ions of specific mass-to-charge ratios (m/z) when a specific DC/RF voltage combination is applied. The first and third quadrupoles (Q1) act as mass filters, while the Q2 acts as a collision cell. Triple quadrupole MS systems can be operated in a tandem MS/MS assay called Selected Reaction Monitoring (SRM) (sometimes also called Multiple Reaction Monitoring (MRM)) mode. SRM is a highly selective mode whereby a fixed set of DC and RF voltages is applied to the quadrupole, permitting only one precursor ion, which is measured by its m/z, to pass. After the Q1 filters that specific precursor ion, the Q2 produces product ions via collision of the precursor ion with a neutral gas

(e.g., nitrogen) in a process called collision-induced dissociation (CID). Product ions progress to the Q3, where only a specific m/z is permitted to pass. By breaking the ion apart into its component fragments, a given molecular species can be identified not only by its mass but by product identity. In this way, SRM reduces noise and increases selectivity. Following schematic presents the working of a triple quadrupole mass analyzer in MRM mode.



LC-MS/MS is a versatile technology with wide range of application in marine bioactive compound analysis. LC-MS/MS can be used for free amino acid analysis in serum, tissue or plant material extracts. The instrument with ESI and MALDI ion source has prominent application in the field of proteomics and peptide sequencing of bioactive peptides. The technique is also used for structural elucidation of bioactive compounds through molecular weight determination, and tandem mass spectra fragmentation pattern. High resolution mass spectrometer can be used for high throughput metabolomics profile of biological materials and can derive important insights in biological experiments.

Chapter 33

Advanced Microbiological techniques for human health significant pathogens

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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 is 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 different 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℃ and 65℃ for 60 min. In LAMP, four primers comprising two inner primers and two outer primers are used to target six specific regions of target DNA. Cauliflower-like DNA structures bearing multiple loops as well as stem-loop DNAs of different sizes are the final products of LAMP. Large amount of amplicons can be produced by LAMP within 60 min which is usually 103-fold or higher as compared to simple PCR. The LAMP amplicons can be detected by agarose gel electrophoresis or SYBR Green I dye.

Problems/draw-backs with automated systems

Several factors have contributed to the current dearth of automation in microbiology labs. These include the ideas that microbiology is too complex to automate, no machine can replace a human in the microbiology laboratory, automation is too expensive for microbiology laboratories, and microbiology laboratories are too small to automate. Microbiology samples are more complex

for analysis by conventional methods. Humans are generally considered capable of performing tasks faster than machines and that machines cannot think. The perception that machines cannot exercise the critical decision-making skills required to process microbiology specimens has persisted. Specifically, human observation of organism growth on agar plates is still considered essential by many. Automation has historically been considered too expensive for microbiology. It simply has not been viewed as cost-effective. Although automation is justified for chemistry, the relative test volumes for microbiology are much smaller, making automation seemingly less attractive. Most microbiology laboratories have been considered to be too small for automation. Automation may have a place in the very largest microbiology labs, it does not have a place in the average-sized laboratory as these labs are small, automation would be underutilized. At last shortage of well trained personnel for operation of automated instruments also play an important role in automation of microbiology laboratory.

Chapter 34

Regulatory requirements for fish and fishery products

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Introduction

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 un-eviscerated 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 help 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 Finfi!;h, Eviscerated or Unevi cerated**	CODEX SJAN 36-1981
3.	**Standard for Canned Shrim»s or Prawns**	CODEX STAN 37-1981
4.	**Standard for Canned Tuna and Bonito**	CODEX STAN 70-1981
.	Standord for Canned Crob Mtot	CODEX STAN 90-1981
6.	**Standerd for Quick Frozen Shrimps or Prawns**	CODEX STAN 92-1981
7.	**StandtVd for Sardines and SArdine-Type Product**	CODEX STAN 94-1981
8.	**Standard for Ouiek Frozen Lob!;ters**	CODEX STAN 95-1981
9.	**Standard for Canned Finftsh**	CODEXSTAN 119-1981
10	**Standard for Quick Frozen Block!; of Fi!;h Fillet!;, Minced Fi!;h Fle!;h and Mixtures of Fillet!; and Minced Fish Flesh**	CODEX STAN 165-1989
11	**Standard for Quick Frozen Fish Sticks (fish Fine;ers), 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	Q12 6[1 §7·1282
13	Standord for Dried Shork fins	· DEX STAN 189-199 3
14	Gtnerol Sto.ndord for Quick frootn fish Fillets	CODEX STAN 190-1995
IS	**Standard for Quick Frozen Raw Squid**	CODEXSTAN 191-1995
16	**Standard for Crackers from Marine and Freshwater Fish, Crustaceans and Mollusee.n** Shellfish	CODEX STAN 222-2001
17	**Standard for Boiled Dried Salted Anchovies**	CODEX STAN 236-2003
18	**Standard for Salted Atlantic Herrin,e and** Salted Sprat	CODEX STAN 244-2004
19	**Standard for Sturc;eon Caviar**	DE TAN29 -201
20	**Standard for Live and Raw Bivalve Molluscs**	':ODEX S AN 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 pro duction, 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 fishe!)' pro ducts 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	**Prawnr./Shrimp Caned in Brine**
IS 2237	1997	**Prawn$ (Shrimps) - Frozen**
IS 3336	1965	**Shark Liver Oil for Veterinary U$e**
IS 3892	1975	**Frozen Lobster Tails**
IS 4304	1976	**Tuna Canned in Oil**
IS 4780	1978	**Pomfret, Fresh**
IS 4793	1997	**V..'hole Pomfret - Frozen**
IS 5734	1970	**Sardine Oil**
IS 6121	1985	***Lactarius* sp Canned in Oil**
IS 6122	1997	**Seer Fish (*Scomberomon{s* Sp.) - Frozen**
IS 6123	1971	**Seer Fish (*Scomberomon{s* 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.

Chapter 35

Nano technology and its applications in fisheries

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Introduction

Materials size below 100 nm size usually considered as nano materials and it is considered as an emerging area of science and technology last 20 years. The nano materials as powders, nanotubes or nano 2D sheets were extensively employed for different applications. Nano materials were synthesised either top to bottom or bottom up methods. These materials were characterized by SEM, TEM, FT Raman and XRDs. Nano materials used mainly in fisheries to develop antifouling strategies, slow release nutraceuticals, material protection from degradation and sensors.

The term nanotechnology was coined by Prof Taniguchi, Japan in 1974 conference of the Japanese Society of Precision Engineering [1,2]. Nano technology is a domain of scientific activity oriented on synthesis, characterization, application of devices and materials and technical systems which functions at nano structures having 1 to 100 nm size [1]. Prof R. Feynman [3] American Physicist and Nobel Prize winner was the first person pointed out the importance and promising outlook for nano particles during his lecture entitled "There's Plenty of Room at the Bottom. An Invitation to Enter a New Field of Physics," delivered on December 29th 1959 at the California Institute of Technology. He pointed out that "... when we have some *control* of the arrangement of things on a small scale we will get an enormously greater range of possible properties that substances can have, and of different things that we can do ... The problems of chemistry and biology can be greatly helped if our ability to see what we are doing, and to do things on an atomic level, is ultimately developed." Later scientists realized the potential of nano particulate materials during the last decade has tremendous advancement in nano research. Governments and private sectors of the world invested huge sums to reap the benefits from novel applications of nano materials.

Nanotechnology

The principle of nano technology is that the material with known properties and functions at normal size exhibit different behaviour and functions at nano scale. By decreasing the size of

the material the surface area per unit material will increase enormously and this helps greater interactions with reactive sites. Nano technology implied that the process of fabricating and/ or controlling the material sized between 1 to 100nm.

Classification of nano materials

The 7th International Conference on Nanostructured materials recommended the following classification of nano materials

- Nano particles
- Nano porous structures
- Nano tubes and nano fibers
- Nano dispersions
- Nano structured surfaces and films
- Nano crystals and clusters.

Among the different types of nanomaterials, nanoparticles, nano tubes and nano fibres are the most economically important items and they are extensively used.

Carbon nano materials

The fullerene was discovered in 1985 by Robert Curl, Harold Kroto and Richard Smalley [3,4]. It is shaped like a footballs with an empty core. The number of carbon atom in fullerene was ranged from 20 to several hundreds. Simio Lijima [5-7] and it has quasi one dimensional tube structures, which are formed by wrapping basic planes of graphite hexagonal lattice into seamless cylinders. CNT are single or multi layered and they can be opened and closed. These CNTs have an array of interesting magnetic, electronic and mechanical characteristics. It is light weight with higher strength and can conduct electricity better than copper. CNTs are extensively used in packaging material and added as additive to prepare anti-static packaging material. CNTs are considered as unique since it has stronger bonding between the carbon atoms and the tubes can have extreme aspect ratios. The characteristics of CNTs different and it depends on how graphene sheets rolled up to form the tube causing it to act either metallic or as a semiconductor. carbon nanotubes do not have free chemical bonds, therefore despite their small sizes, they do

not display *surface* effects. CNTs are studied thoroughly and the countries like Japan commercially manufacturing hundreds of tons of CNTs.

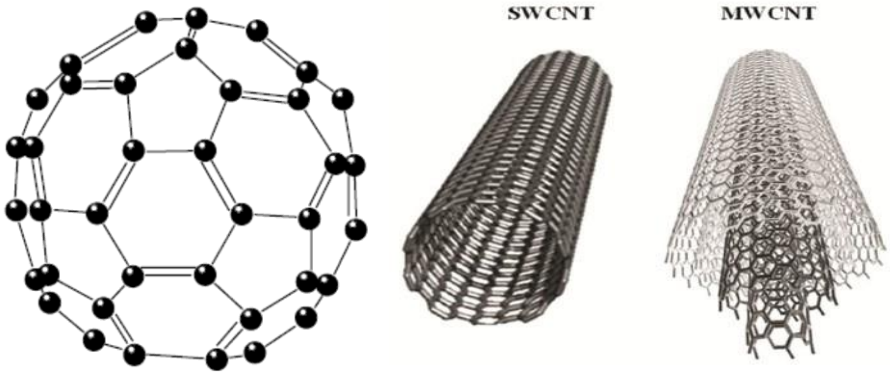

Fig 1. A) Fullerene C60 molecule B) SWCNT and C) MWCNT.

There are different types of carbon nanotubes viz single walled (SWCNTs) and multiwalled carbon nano tubes (MWCNTs). SWCNT has one layer whereas MWCNTs are having a collection of nested tubes of continuously increasing diameters. There may two or higher number of tubes or walls. Each wall is separated at a certain distance between the inner and outer tubes through interatomic forces. Carbon nanotubes are extensively applied for strengthening the rebar to concrete.

Synthesis of nano materials

There are two approaches used for the synthesis of nanomaterials, viz., top-down principle and bottom-up approach [5,6]. The bottom up technology is based the development of nanomaterials of desired structure directly from "lowest level" elements (atoms, molecules, structure blocks etc). Here we have to identify the desired material in advance. The carbon nanotubes are synthesised by passing simple carbohydrates (eg acetylene) through a volume containing catalysts at a temperature of 600 – 800^{o}C. CNTs are formed on the catalysts [7]. Development of nanomaterials from larger size particles to lower sizes is termed as top-down approach. Eg. Synthesis of nano cerium oxide from cerium chloride. Dilute solutions of cerium nitrate were oxidized using ammonia under controlled environment and then calcined at 400 oC will give nano cerium oxide.

Equipments for testing nanomaterials

The instruments used for characterization of nanomaterials are

- Transmission Electron Microscopes
- Scanning Electron Microscopes and its variants like Scanning Tunneling Microscope,
- Near field Scanning Optical Microscope etc.
- X – Ray Diffraction,
- Atomic Force Microscopes
- FT Raman spectroscope,
- UV- Vis Spectrophotometers
- Particle size analyzer with zeta potential etc.

Characterization of nano materials

Nanostructures have interesting features and physico-chemical characteristics and successful use of nanotechnology is possible only after a careful study of their properties. Some of the properties to be studied generally are mechanical, thermo physical, electrical, magnetic, optical and chemical properties. The details are available in different text books of nanotechnology [9].

Applications of nano technology

Material science: the major application in material science is the development of new materials. CIFT is doing research on development of new aluminium metal matrix composites by incorporating nano cerium oxide, nano samarium oxide, nano titanium oxide etc.

Antifouling strategies:

Fig 1. A) PE cage net b) PE cagenet after 3 months c) PE cagenet treated with PANI+nano CuO after three months exposure in the estuary.

Biofouling is a major problem in the aquaculture cage nettings and its management measures are very expensive. CIFT carried out research on nano material coated aquaculture cage nets and tests revealed that the coatings were efficient in preventing the biofouling in cage nets. Polyethylene cage nettings surface was modified with polyaniline and the nano copper oxide coating prevented the attachment of foulers.

Medicine and bio nanotechnology: Nano materials can be used for precise drug delivery, to the targeted organs or body parts or tissues.

Nano sensors: Design of nano sensors and nano devices of autonomous or as administered inside the human body. This will help the recognition of molecules of specific types like cancer and its treatment [13-16]. Nano materials like gold and other organo polymeric composites were successfully employed for the development of thermochromics sensors, colorimetric sensors and electrochemical sensors for detection of contaminant in the human body or food products or adulterants. Nano engineered biodegradable material incorporated with insulin used for slow release insulin to control blood glucose concentrations [18]. Applications of nano materials in medicine are like mucosal lining treatment [19,20] and inflammatory bowel treatment using nano pharmaceuticals [21].

Food science:

Nano materials were potential to apply as food supplements For example, antioxidant nutrients may be included in nanocomposites, nanoemulsions, nanofibers, nanolaminates and nanofilms, or nanotubes etc.

Research in CIFT

Nano application in aquaculture cage nets

Nano copper oxide coated HDPE cage nets

Polyethylene fibres are extensively used to prepare the aquaculture cage nets. Polyethylene is non polar polymeric molecule and difficult to introduce the biocide over the molecule. Generally biocide coatings were made over the cage nets using adhesives. The major disadvantages of biocides like copper oxide coating over the cage net is leaching to the aquatic environment and disposal of nets after use. The major advantage of nano materials as biocide very less quantity used, increased surface area of exposure and exhibit higher efficiency. Since polyethylene is non

polar we have undertaken different methodology to make the polyethylene surface polar. The surface was coated with in situ synthesized polyaniline, a conducting polymer. Over this surface nano copper coated and their characteristics were studied. Uniform coating of polyaniline and copper was showed by Scanning electron micrograph and Atomic force micrographs. The formation of the biocide was verified by analyzing FTIR spectra [24]. Polyaniline coated polyethylene showed IR absorption was shifted from 1362 to 1396 cm-1 indicating the attachment of polyaniline over PE. Quinanoid peak of NH4+/NH+ in polyaniline was exhibited at 1047/1161 cm-1 and the same was shifted further to 1070 / 1179 cm-1 due to nano copper coating over polyaniline.

To study the biofouling resistance of the treated net can be evaluated by different methods. The field evaluation of the cage net showed the excellent biofouling resistance after 90 days exposure in the estuarine environment. The experiment was repeated by constructing a cage with treated and control panels and exposed in the Vizhinjam coast for 7 months (fig 1). The fishes grown in the cages and controlled environments were compared and exhibited significant difference in growth was shown.

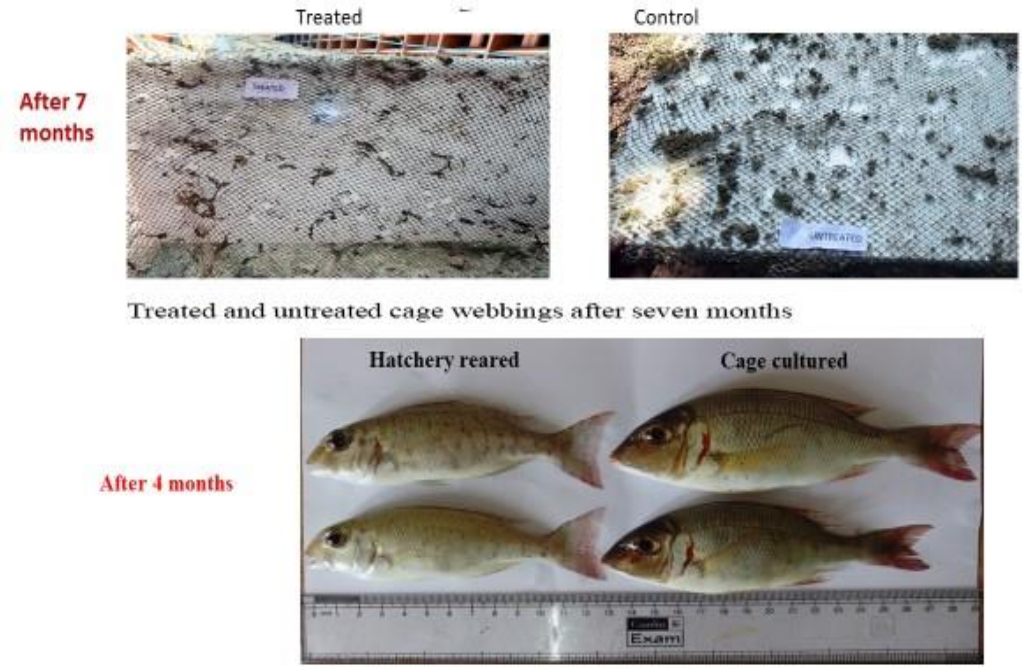

Fig 2. Control and treated net after 7 months exposure in the marine environments.

Different tests to verify the biofouling resistance are mentioned in detail by Ekbalad et al 2008. Deterrence of biofouling organisms to the treated surface was tested by cyprid assays. The treated surfaces were exposed to the testing organisms in natural or artificial seawater at controlled environments. Callow et al 1997 described assays using microorganisms like Ulva zoospore over the treated surface. The exposed surface in controlled environment were evaluated based on the attachement of spores. Callow et al and Schultz et al [25, 26] described about the determination of adhesive strength using a calibrated flow channel. Diatom assays were generally carried out using Navicula perminuta [27] by suspending the treated surface in artificial seawater containing chlorophyll a 0.30 ug ml-1. After 2 h exposure the surface were evaluated for the adherence and deterrence of organisms. Antibacterial property of the biocide treated surfaces were evaluated using two marine bacteria viz Cobetia marina and Marinobacter hydrocarbonoclasticus [28, 29]. The former bacteria is considered first settled microbes over marine exposed surfaces. The measurement were carried as per the protocols described by Akesso et al [28].

Societal Issues

As with any emerging technology, the full consequences of pervasive incorporation into society are currently unknown. For example, what are the outcomes if the byproducts of nanoshells or nanoparticles, or the nanoparticles themselves, used in cancer treatment enter circulation and healthy tissues and cells?. Other issues like free radical formation during sun exposure [22], health environment and safety issued [23]. The ethical and legal ramifications of nanotechnology are primed for public consideration. The greater the awareness and understanding of nanotechnology among the society is essential for safe application and reaping the benefits. The society must be more informed about advantages and disadvantages of nanotechnology through public deliberations, discussions and suitable decisions by the public and government for brighter tomorrow

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Chapter 36

Techniques in molecular detection of seafood borne pathogens

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The conventional procedures for the detection of pathogens include selective enrichment and plating procedures for the initial screening of the pathogens that can be further identified by series of biochemical and phenotypical tests. The conventional detection and typing methods have been used for many years as a preliminary screening of pathogens surveillance and outbreaks. However, the advent in food safety practices and the increased awareness among consumers together with relatively higher occurrence of foodborne outbreaks resulted intensive investigation of the quality and safety of the products via several advanced, rapid techniques. Traditional identification of microorganisms relies on the growth of bacteria on media that are often time consuming and un-reliable whereas molecular detection assays are clearly rapid and highly specific for detection of a number of pathogens. Molecular detection methods are based on the analysis of nucleic acid so that the specificity, sensitivity and robustness of the testing protocol is much superior than the culture-based methods.

Molecular detection methods

There have been number of molecular methods have been developed for the detection of pathogens in seafood such as colony hybridization, Polymerase chain reaction methods, loop mediated amplification assays etc.

Polymerase chain reaction and its types

Polymerase chain reaction (PCR) is one of the fundamental techniques in various molecular microbiology experiments and refers to a set of procedures for the in vitro enzymatic amplification of a desired DNA fragment or gene from the whole genome of an organism. PCR offers the synthesis of several million copies of a target DNA sequence from a one or few copies of the sequence. PCR techniques is used widely in various diagnostics and forensic investigations, and becomes essential for many common procedures such as cloning, sequencing, microaarays etc. PCR has three main stages in which, the double stranded DNA is denatured by heat (denaturation stage) and then the temperature is lowered to allow annealing

of two specific primers by complementary base pairing on the opposite strands of the DNA (annealing stage). Taq polymerase directs the synthesis of the new strand from the primed sites in both directions that results in double stranded DNA (extension stage) and the procedure is repeated for 25-40 times in a thermocycler. In each cycle, the target DNA is replicated by a factor of 2 so that, after the completion of PCR, millions of copies of DNA are available for downstream applications. In addition to the amplification of a target DNA sequence by the typical PCR procedures there are several specialized types of PCR have been developed for specific applications. They are

Quantitative (Real Time) PCR

Real Time PCR is one of the PCR based assays to monitor the amplification of a particular gene /gene product in real time basis without any need for the post amplification process for visualizing DNA such as agarose gel electrophoresis, capillary electrophoresis etc. The fluorescent dye added to the reaction mixture allows the monitoring of the amplification staring from the first cycle of the PCR run and concomitantly the fluorescence is increased to 2 to 1000-fold as amplification progresses. Thus, based on the fluorescence, the DNA can be quantified over wide range of concentrations with the help of standard curves. Further, the data generated from the amplification process can easily be analyzed. The sensitivity and reliability of the result is significantly higher compared to conventional PCR. Real time PCR can be used for viral/bacterial quantification, gene/allele copy number, allelic discrimination assays (SNPs) gene expression, Methylation studies etc. The real time detection of the nucleic acid amplification is achieved by nonspecific or sequence specific strategies. The nonspecific method uses intercalating dyes which can able to produce fluorescence while binding with double stranded DNA (ds DNA). The commonly used nonspecific dye in real-time PCR is asymmetric cyanine dye called SYBR Green I. This dye has higher affinity to ds DNA compared to that of ethidium bromide and the intensity of the bound dye is higher (magnitude of 1000 folds) than the free form of syber green. This enables an increase in fluorescence during amplification. However, once the melting of the double stranded DNA after polymerisation causes the denaturation of DNA and signal strength falls off due to the detachment of fluorescent dye. Other dyes of this category include, O-PRO-1, BEBO, YOYO -1. The major advantage of nonspecific dyes are less expensive, and can be used with any pair of primers/target. The disadvantage is that it binds non-specifically to any ds DNA yielding signal from nonspecific products.

However, this can be verified at the end with the help of melt curve analysis by subjecting the amplicon to a temperature range beyond its melting temperature.

The sequence specific strategies employ the use of either hydrolysis probes or hybridization probes. These probes are synthesized based on the sequences of the internal fragments of the two primers. The quantification of the PCR product is done by measuring the fluorescence signal strength based on either quenching or FRET mechanism. Hydrolysis probes are the probes which are hydrolyzed due to 5'-3' exonuclease activity of DNA polymerase during the elongation stage of the PCR cycle. TaqMan Probe is widely known hydrolysis probe for RT PCR application. It is nothing but a oligo sequence labelled with reporter dye in one end (5'end) and quencher dye at the other end. In intact the fluorescence emitted from the reporter dye is banned due to the presence of quencher dye in its close proximity. During PCR run, DNA is denatured and both primer and the probe annealed to the target DNA. However, the Taq polymerase has exonuclease activity will cleave the probe and the reporter and quencher dye get separated, thus allowing the fluorescence emission from the reporter dye when it excited with a suitable light source. As amplification progress, the signal strength gets increased enabling the quantification of DNA. The melting point of the probe should be 10 degrees higher than primer Tm (melting point) as cleavage of the probe take place only during the elongation step of the PCR. In addition to TaqMan Probe, TaqMan MGB probes are also used. The Minor groove binder increase the melting temperature of the probe and it increase the duplex stability particularly for shorter probes. In case of hybridization probes the fluorescent signal is obtained due to the structure changes in the secondary structure of the probe during hybridization phases. The changes in the structure causes increase the distance between reporter and quencher dye preventing the fluorescence resonance energy transfer (FRET) from a reporter dye to quencher dye. The probe in its intact form is a hair pin like structure and behaving non-fluorescence chromophore due to close proximity of both quencher and reporter dye. However, the conformation changes during hybridization demands separation of both dyes and the far distance among the dyes prevent the energy transfer through FRET mechanism. Thus, the increased fluorescent signal from the reporter dye enables the quantitative estimation of the DNA. With both types of assays, the exponential increase in fluorescence is used to determine the cycle threshold (Ct) which is the number of PCR cycles at which significant exponential increase in fluorescence is detected.

Using a standard curve for Ct values at different DNA concentrations, quantitation of target DNA in any sample can be made.

Reverse Transcription (RT-PCR)

RT-PCR (or Reverse Transcription PCR) is used when the target nucleic acid is RNA. The central dogma in molecular biology explains about the direction or flow of information in which the DNA of the organism encodes the genetic information, intern transfer to RNA by the process of transcription and then to protein via translation process. As RNA is highly unstable and enzymatic amplification is difficult and need to reverse transcribed to cDNA for amplification. The reverse transcriptase, an enzyme that converts RNA into cDNA. This cDNA can be used for PCR and reverse transcription process may be combined in a tube, as the initial heating step of PCR being used will inactivate the transcriptase enzyme. The Tth polymerase is used for the enzymatic amplification due to its inherent RT activity, and can carry out the entire reaction. As the phenotype of an organism is explained by the RNA or protein fractions. So, RT-PCR is used in expression profiling of specific gene or gene products. It can also be used in RNA transcript analysis where in transcription start and termination sites are determined. Also, it enables the mapping of exons and introns of the gene sequence.

Nested PCR

Nested sets of primers can be used to improve PCR yield of the target DNA sequence. In nested PCR, two primer sets are used in which the first round of PCR is performed with one primer set for 15-30 cycles, then second set of primer is used for second round PCR, for an internal region of the first amplified DNA for an additional 15 to 30 cycles. The PCR product of the first round of PCR is used as DNA template for the second PCR. Thus, the nested PCR method increases the sensitivity and specificity of DNA amplification. The specificity is particularly enhanced because this technique almost always eliminates any spurious non-specific amplification products. This is because after the first round of PCR any non-specific products are unlikely to be sufficiently complementary to the nested primers to be able to serve as a template for further amplification, thus the desired target sequence is preferentially amplified. However, the increased risk of contamination is a drawback of this extreme sensitivity, and great care must be taken when performing such PCRs, particularly in a diagnostic laboratory.

Multiplex PCR

Multiplex PCR enables simultaneous amplification of many sequences or gene using two or more set of primers in one PCR. The presence of many PCR primers in a single tube could cause many problems, such as the increased formation of misprimed PCR products, "primer dimers", and the amplification discrimination of longer DNA fragments. For this type of PCR amplification, primers are chosen with similar annealing temperatures. The lengths of amplified products should be similar; large differences in the lengths of the target DNAs will favour the amplification of the shorter target over the longer one, resulting in differential yields of amplified products. In addition, Multiplex PCR buffers contain Taq polymerase additive, which decreases the competition among amplicon and the discrimination of longer DNA fragments during Multiplex PCR. Multiplex PCR products can be further hybridised with a gene-specific probe for verification.

Colony PCR

Colony PCR is used mainly in cloning procedure to screen the correct DNA vector constructs. Here, bacterial colonies are directly taken from the culture plate by touching a single colony using a sterile loop or tip and transferred into a PCR mix. DNA extraction from the cell is not carried out here. The denaturation step of the PCR cycle releases the DNA. In order to achieve the release of DNA from the cell, either the time period or the temperature may be extended to get an optimum amplification condition.

Loop-mediated Isothermal Amplification Assays

Loop-mediated isothermal amplification (LAMP) has been widely used to detect pathogenic bacteria in food (Zhao et al., 2011). In contrast to conventional PCR, LAMP is carried out in isothermal conditions of temperature 60-65oC with the use of specific primers. It has high DNA strand displacement activity which is mediated by Bst polymerase enzyme from Geobacillus stearothermophilus. The optimum temperature of this enzyme is 60-65oC.The DNA strand displacement is achieved by the use of 2 sets each inner and outer primers which are specific to the target DNA. The amplification initiates with the hybridization of forward primer with the target DNA and starts the synthesis of new strand. Then, the forward outer primer hybridizes again with the same original reverse target sequence and the synthesis of this new forward strand continues until the enzyme finds the 5′ end of the first strand created with the use of the inner

primer. Then owing to the property of BSt polymerase, the strand displacement of the first forward strand further forms a loop at one end due to the hybridization of the inner primer with target DNA. This will again serve as the template for the reverse inner and reverse outer primers and subsequently dumbbell like structure forms due to the strand displacement activity. Owing to the high displacement activity of the Bst DNA polymerase, a huge amount of DNA with a high molecular weight is rapidly generated. This allows target DNA amplification until 109 copies in less than one hour.

Molecular typing methods

Several molecular typing methods have been developed which examine the relatedness of isolates by studying their molecular composition, homology and presence or absence of specific genes etc.

Randomly amplified polymorphic DNA (RAPD)

RAPD is a typing technique based on PCR reaction in which very short nonspecific primers are used for the amplification of targeted gene (Williams et al., 1990). As the primers are short, they should be able to bind many genomic sites throughout the bacterial genome. This analysis requires relatively low annealing temperature. The resulting multiple PCR products are then separated in agar gel electrophoresis. This method is simple and independent of phenotypic characters but its reproducibility from the random priming units is very low. The multiple band pattern generated by the RAPD-PCR is followed by dendrogram analysis to generate fingerprint profiles for the test organism. This method can be used to determine the clonal variations in bacterial strains. This method has been used in food borne bacterial pathogens including *V. parahaemolytcus,* Escherichia coli, *Salmonella*, *Shigella* etc.

Ribotyping

Ribotyping is rapid and specific techniques which uses the information from rRNA for the identification of bacteria. It involves the digestion of bacterial genomic DNA with specific restriction enzymes and the resulting fragments are separated in a gel matrix. The separated fragments are transferred to nylon membrane and hybridization will carry out with a labeled 16S or 23S rRNA probe. Analysis of such hybridized fragments can able to identify the bacteria of interest.

Restriction Fragment Length Polymorphism (RFLP)

In PCR-RFLP, the amplified DNA is cut into short specific sequence by restriction enzymes and the resulting fragments are then separated by size using agarose gel electrophoresis. The restriction fragment profiles are very efficient in comparison of different strains. Important advantages of PCR-RFLP include inexpensiveness and lack of requirement of advanced instruments. Disadvantages include the requirements of specific restriction enzymes and difficulty to identify the variation in the nucleic acid sequence analyzed. RFLP analysis has been widely used for the identification of bacterial species and biotypes.

Direct genome restriction enzyme analysis

Direct genome restriction enzyme analysis is method used for genetic diversity analysis where the DNA is cut using an endonuclease enzyme and produces a small discrete DNA fragments of 30-40 number and sizes ranging from 500-2500bp. These fragments are separated in non-denaturing polyacrylamide gel electrophoresis. Visualization of banding patterns is carried out by silver staining.

Pulsed field gel electrophoresis (PFGE)

Pulsed field gel electrophoresis (PFGE) is a typing technique widely used in epidemiological studies. It is currently recognized as a golden fingerprinting method due to the highly discriminating power as compared to other typing methods. The method involves the separation of large DNA molecules by cutting the DNA with restriction enzymes. The fragmented DNA pieces can be separated based on size using an electric field. PFGE is different from conventional DNA electrophoresis because PFGE can separate very large fragments to generate a fingerprint by constantly changing the direction of the electric field. Analysis of fingerprint pattern is carried out by software program (BioNumerics) and that can be compared with national data base (Pulse net). PFGE is a time consuming and labor intensive method. This typing method has been used in several food poisoning outbreaks to pin point the relatedness of the strains through the space or time. This has been used in several foodborne pathogens of which the most popular is methicillin resistant S. aureus.

Multi locus sequence typing (MLST)

Multi locus sequence typing (MLST) is proposed in 1998 for the characterization of human pathogen Neisseria meningitides. Since then, it has been widely used in epidemiological and population analysis of different bacteria. This technique uses the sequences of internal fragments of usually 6 to 8 house-keeping genes or loci (Urwin and Maiden, 2003). In MLST, approximately 450-500bp internal fragments of each gene are sequenced and variations within the house keeping genes are utilized to study the genetic relatedness of the bacterial strain. An arbitrary allele number is used to denote each unique sequence of a given locus. Similarly, an arbitrary sequence type (ST) number is assigned to each unique combination of alleles (or allelic profile). Thus, it enables to identify the DNA sequence variations in a set of housekeeping genes and characterizes the strains by their unique allelic profiles and assigned sequence types.

DNA sequencing techniques

Sequencing technique provides all information about the biochemical properties, hereditary etc by analyzing the order of nucleic acid in polynucleotide chain of the whole DNA molecules or specific fragment or gene after amplification of the same. It has high discriminatory power, 100 % typeability and good reproducibility compared to other detection or typing techniques. The first-generation sequencing technology includes Sanger's sequencing (Chain termination method) and chemical degradation methods. In sanger sequencing, the radioactive/fluorescent labelled deoxyribonucleotides lacking 3'hydroxy group which are unable to bind with DNA polymerase were used so that halt in the progression of extension reaction occurs thereby the resulting ddNTP bases were further run in polyacrylamide gel to yield the nucleotide sequences in the given gene fragment. In chemical degradation method, chemical cleavage of an end labelled DNA to fragments were done using specific chemicals, such a dimethyl sulphate, hydrazine etc. followed by high resolution gel electrophoresis and detection by audioradiography. Short gun sequencing is one of the improvements in first generation sequencing techniques in which, the overlapping DNA fragments were cloned and sequenced separately and then assembled together to long contiguous sequence.

The second-generation sequencing includes pyrosequencing, and next generation sequencing approaches (sequence by synthesis or sequence by ligation). In pyrosequencing, the liberated pyrophosphates (two molecules of phosphate group) from each nucleotide while adding to the

DNA strands during extension reactions were measured with the help of ATP sulfurylase and luciferase enzyme. The pyrophosphate and adenosine phosphosulphate reacted together in the presence of ATP sulfurylase yielded ATP and luciferin which in turn converted to fluorescent oxyluciferin compound in the presence of luciferase. Pyrosequencing approach uses this measured fluorescence from pyrophosphate synthesis. Another two approaches for the next generation sequencing include clonal amplification by bead-based emulsion PCR and bridge PCR. Bead based PCR for sequencing is done by sequencing by ligation process in which the adapter is attached to the bead via ligation followed by water in oil emulsion PCR in which the DNA fragments gets amplified inside the droplet into millions copies. After PCR, the magnetic separation of amplified DNA beads from non-amplified DNA beads were done and sequenced by placing the beads in the sequencing slide. In bridge-based PCR, the DNA attaches to the flow cell mounted with numerous nucleotides where the DNA attaches to the complementary sequences and bend over and attached to next oilgo forming a bridge. The polymerase enzyme synthesis the reverse strand so that the two strands releases and straighten. The result is a cluster of DNA forward and reverse strand clones. Here, the sequencing is done with help of polymerase enzyme.

The third-generation sequencing techniques include single molecule real time Platform and nanopore sequencing. The PacBio Sequencing is done by passing DNA (sequence with adapter) molecule through the illuminated volume in a nano well and raw fluorescent signals from each fluorescent nucleotide when its attached to the strand during extension reaction were captured. The nucleotide sequences were determined based on the fluorescent intensities specific to each nucleotide incorporation. Nanopore sequencing technology involves the passing of DNA molecule through nanoscale pore, then the changes in electrical field surrounding the pore is measured.

Chapter 37

Novel extension approaches for technology dissemination in fisheries

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Trends in aquaculture and fisheries

Global fisheries have made rapid strides in recent years by establishing its strong hold over increasing food supply, generating job opportunities, raising nutritional level and earning foreign exchanges. These benefits become more important when placed in the context of current challenges in food production, nutritional security, social transitions and growing climate uncertainties. Fish and fishery products are the most traded food commodities in the world accounting for 1% of world merchandise trade in value terms representing more than 9% of total agricultural exports all over world (FAO, 2014). About 38% of the global fish production enters international trade in various forms and shapes, generating an export earning of nearly US$148.1billion with a record import at US$140.6 billion during 2014. Mostly the developing countries that account for over 60% of global fish catch, which has continued to expand at an average annual rate of 8.8% (FAO, 2009 & 2012) and play a major role in the global trade of fish and fish products contributing around 50% of fishery exports in value terms and more than 60% in quantity terms supplied by them (World Bank, 2011). At the same time, demand for fish products are likely to rise as a result of rising populations that are expected to reach 9.3 billion by 2050. Developing countries have a positive trade balance due to their increasing involvement in global fisheries trade. Developing country like India may have higher proportion of population growth but its impressive economic growth over the past two decades has resulted in steady increase in per capita income in real terms that in turn increases the purchasing power of people resulting in increasing demand for food to feed & ensure nutritional security of the population. As a result of which it brought inconsistency in fish consumption pattern across the coastal, marine and hill region.

It is estimated that fish production generally contributes 0.5 – 2.5 % of GDP globally (Allison 2011). In spite of that globally an estimated population of more than 1.3 billion people are in extreme poverty (2016), 795 million people (2015-16) are estimated to be in chronic hunger and an estimated one third of children in the developing world under five years of age are stunted

(Conway 2012). Fish is considered as the most affordable and frequently consumed animal-source food in low income food deficit countries in sub-Saharan Africa, Latin America and Asia (World Bank,2006). It is an important source of a wide range of intrinsic micronutrients, minerals and fatty acids. It accounts for about 17 % of most affordable, easily digestible, high-quality animal protein and 6.7 % of all protein, all essential amino acids, essential fats (e.g. omega-3 fatty acids), vitamins and minerals thus contributing to a great extent to food and nutrition security in many Asian and African countries where large proportion of population are still in hunger and under nourished (Kent,1987). Besides small-sized fish species are excellent source of many essential minerals such as iodine, selenium, zinc, iron, calcium, phosphorus, potassium, and vitamins such as A, D and B. About 150 g of fish provides about 50–60 % of daily protein requirements for an adult. On an average, fish provides about 20–30 kilocalories per person per day. In addition, dietary diversity of the region is mainly influenced by different quantitative and qualitative attributes viz., income, price, preference, market, type and quality of products, cultural traditions, beliefs as well as various geographical, environmental, social and economic factors that influences the fish consumption pattern.

Despite the significant contributions by the sunrise sector, global debates on fisheries issues and policies appear to be dominated by concerns over environmental sustainability, overfishing and overcapacity. In this context, it is alarming to note that the sector has not received adequate attention from the social scientists to understand its various socio-economic dynamics to prove the sunrise sector as a potential driver of local and national economic development.

Major concerns in fisheries

Food security has become the prime concern with the increasing trend of population growth in a country. Over the last fifty years, the food grain production in India has increased considerably, but the advantage of this increase in food grain production has not been reflected in the per capita availability of food grains. As per estimate, the human population and food grain production in India has grown up by 2.09% and 2.36%, respectively from 1961 to 2011, whereas the annual per capita availability of food grains has come down from 171.1 kg in 1961 to a level of 169 kg in 2011 showing a decreasing trend of 1.17 %. In case of fish, Asia accounts for almost two-thirds of global fish consumption i.e. 21.4 kg per capita per year in 2011 – a level similar to Europe (22.0 kg/cap/yr) and North America (21.7 kg/cap/yr), and close to the levels of Oceania

(25.1 kg/cap/yr), whereas Africa, Latin America and Near-East have lowest per-capita consumption (10.4, 9.9 and 9.3 kg/cap/yr in 2011, respectively). Although annual per capita apparent consumption of fish products has grown steadily in developing regions (from 5.2 kg in 1961 to 17.9 kg in 2011) and in Low Income Food Deficit Countries (LIFDCs) that increases from 4.4 kg in 1961 to 8.6 kg in 2011, it is still considerably lower than in developed regions (from 17.1 kg in 1961 to 23.0 kg in 2011). It is clearly evident that rising population is nullifying the effect of growth in food grain production, keeping aside several other factors which determine the access to food grains. In this context, increasing fish production to meet the challenges of nutritional security has drawn the attention of the planners and policy makers. Hence, aquaculture is considered as a promising food production sector for high quality protein food and providing livelihood to the rural populace, which needs to be more efficient and cost-effective. However, there is multitude of challenges associated with the growth of this industry.

The fishery sector is a major foreign exchange earner for any developing countries. In India, its foreign exchange earnings were estimated to increase by 16 to 20 per cent in 2005 and 26 to 42 per cent by 2015. Nearly 85 per cent of the export benefits are projected from shrimp export alone. Because of its potential and rich source of animal protein, fish demand has been rising in both the developed and developing world at more than 2.5 percent per year (Peterson and Fronc, 2007) and demand levels were raised in proportion to increase in income in highly populated countries like China and India, (Garcia and Rosenberg, 2010). In view of higher production in fisheries, producers may lose from price fall in the domestic market; where prices were estimated to fall by 15 to 20 per cent by 2005 and 27 to 54 per cent by 2015. In spite of the phenomenal success of the sector, still there are some major issues related to the economic and nutritional conditions of fisher folk in addition to some important concerns in the context of rising environmental hazards, depressing prices world over, emerging new economic challenges following establishment of WTO, IPR & SPS issues, compliance of several multilateral agreements, etc.

In the post- harvest front, the processing industries face multifarious problems like complicated exporting procedures, high shipping costs, cut-throat competition in the industry, changing quality standards of importing countries, irregularity in supply of raw materials, hygiene problems and non-availability of quick transportation facilities from the fishing port to the processing units, etc. As a result of which trade-driven commercial fish farming is suffered that

reduces the livelihood opportunities of small scale dry fish processors, petty traders within the communities of poor fishermen.

Environmental degradation poses a challenge to the phenomenal success of the fishery sector in promoting food security and adversely creates impact on nutritional rights and livelihood status of the fishermen communities for whom fish and fishery products are critical for their health benefit and wellbeing. As per directives of international conventions like Kyoto Declaration and Code of Conduct of Responsible Fisheries, this trade-driven, resource depletion sector can be sustained through by-catch reduction and juvenile fishing ban. The benefit of this may be accrued through policy level intervention by institutions within the legal framework.

Small-scale fisheries are normally characterized by low capital input activities, low capital investments, lack of equipment and labor-intensive operations followed by traditional fishers. They also usually operate as semi-subsistence, family-based enterprises, where a share of the production is kept for self-consumption (Garcia *et al*., 2008). Traditional fishers dominate the marine sector and they are socially deprived, educationally weak with very high occupational rigidity. There is inequity in the distribution of yield and effort in marine fishing in case of traditional fishing communities. They are unorganized with least social security. The informal social security system in the form of sharing of earnings among the community prevailing in the traditional fishing is hardly seen in the mechanized fishing. There are also huge regional variations in productivity among them.

Technologies are the main drivers of growth. Hence, systematic technological interventions backed by appropriate policy and institutional support are vital for making the aquaculture operations sustainable and economical. Generally, the technologies and trade interventions reinforce each other which can be characterized as skill-based, cost effective, capital intensive which can bring a change in the performance of the sector. Keeping eye upon this, following strategies have been suggested for an accelerated fishery development with focus on poverty alleviation of poor fishers:

- Commodity-centered approach
- System approach
- Prioritize technology on the basis of needs and problems at micro and macro levels

- Skill development/upgradation of the fishers
- Monitoring the technology demonstrations programs and assess the impacts.
- Innovate and strengthen institutions and policies
- Enhance investment and reorient policies to facilitate percolation of benefits to all sections of the society.
- Follow ecological principles
- Emphasize on domestic market demand and consumers' preferences
- Strengthen database and share it for a better planning and policy making in the sector.

Extension systems for sustainable development

Unlike India, the economy of developing and underdeveloped countries in sub Saharan Africa, Latin America, Asia inclusive of 22 Low Income Food Deficit Countries (LIFDCs) is predominantly agrarian economy, where agriculture inclusive of fisheries provides employment and livelihood to majority of the rural households, but the condition of both farmers/fishers and farming is in alarming state.

Hence, there is an urgent need to reform that agriculture allied sectors in holistic, scientific and systematic approach to meet the recent challenges due to climate change and global competitiveness so as to achieve sustainable production and growth under different agro-climatic conditions.

As per the report of world commission on Environment and Development (1987), sustainable development meets the needs of the present generation without compromising the ability of future generation to meet their requirements. The FAO committee on Fisheries (1991) defines sustainable development more elaborately as the management and conservation of national resource base and the orientation of technological and institutional intervention to ensure the attainment of human needs for present and future generation including fulfilment of social and economic demands and conserving the natural resource base. In response to that FAO developed a code of conduct for Responsible Fisheries (FAO,1995) that provides principles and guidelines for ensuring sustainable exploitation of marine resources. Sustainable fisheries can be possible through responsible fishery, which envisages rational fishery management that address a range

of issues dealing with resource status, environmental health, post-harvest technology, trade and export, socio-economic benefits, legal and administrative support. Sustainable agricultural systems must be resource-conserving, socially supportive, commercially competitive, and environmentally sound. Hence, the agriculture research system must place emphasis on generation of resource conservation technology (RCT) along with strong forward-backward linkage between research-extension system. It involves design and management procedures that work with natural processes to conserve all resources, promote ecosystem resilience and self-regulation, minimize waste and environmental damage, while maintaining or improving farm productivity and profitability (MacRae et al., 1990).

The role of extension in fisheries cannot be ignored. Strong extension system is the key to bring the desired changes to meet the present day challenges related to sustainable fisheries. Basically, the end product of the fisheries extension system is to work with fisheries within an agro-climate and economic environment by providing suitable technologies to enrich knowledge and upgrade skills to improve better handling of natural fish resources and applying the cutting-edge technologies to achieve desired production level. Extension system plays a pivotal role in empowering fishers and other stakeholders to make fish farming more participatory, demand-driven, knowledge intensive and skill supportive for disseminating most appropriate technical, management and marketing skill to improve profitability in fisheries that can overcome the emerging challenges and concern, thus developing a synergistic pathway for enhancing productivity along with quality produce in order to sustain production base and ensure ecological and livelihood security. The extension system needs to disseminate a broad array of information starting from farm to fork in an integrated manner for safe delivery from field to the consumer considering all the aspects of conservation and production technologies, post-harvest management, processing and value addition. Such knowledge based decision should be incorporated in reshaping of extension approaches. In present scenario, the extension system envisages a transformation from technology driven to market driven extension, where fishers would give emphasis on commercialization of fish and fish based products, maintenance of quality, fulfilling consumers' demands, etc., in the program planning process for the effectiveness of any extension programme.

Further, with the advent of global competitiveness and market liberalization, our prevailing extension system has to be strengthened with innovative extension approaches to tackle the

recent challenges in fisheries *viz.*, climate change, weather aberrations, dwindling resources and quality and safety of products; so that fishers can adjust their production portfolio keeping eye upon the emerging trends in food consumerism in domestic as well as global markets. Grooming fishers with proper information support for taking right decision related to fish production essentially requires a strong network of extension systems, supported with government initiatives and strong linkage among extension scientists and functionaries working for fishery sector development. This would ensure the livelihood security of millions of fisher communities by improving the quality production and creating better job opportunities, which intends to bring out planned changes to meet the needs of the present generation without compromising the future generation's requirements.

Innovative extension approaches for technology dissemination in fisheries

Earlier in developing countries, the extension personnel were involved in diffusion of farm technologies generated by public research organizations, mostly disseminated through appropriate mechanism, viz., On Farm Trials (OFT), frontline demonstrations (FLD), field visits, fishers' meetings, media use, etc. This process had the conceptual backup from the 'diffusion of innovation' model. But in the last two decades, the paradigm shifts in development pivots to the enhanced concern for future generations to meet their basic needs, accordingly the nature, design and integration of fisheries technologies are drawing attention of the extension professionals and practitioners across the globe. In India, different models for transfer of technology have been tested and some robust extension approaches have been validated. Furthermore, the frontline extension system of the country has been revisited and sharpened through fishers oriented approaches for technology adaptation and dissemination. The extension system in India has been designed to move beyond technology and beyond commodity through reciprocal fishers-research-extension linkages. Fish farmers still suffer from lack of access to appropriate services like credit, inputs, market, extension, technologies etc. Keeping eye upon this, the World Development Report has focused on need to restructure and revamp agricultural extension system as a tool for realizing the growth potential of farm sector against the widening demand–supply pressures for ensuring sustainable fisheries, inclusive, pro-poor socio-economic development. Therefore, participatory technology development and participatory extension approaches emerged as a part of integration of the '*interdependence model*' and the '*innovation systems framework*' that offered more inclusive ways of involving the institution in technology

generation, customization and diffusion. Extension approaches have to be redefined depending upon the components involved for sustainable growth and livelihood security of the farmers for which a conceptual framework has to be developed in response to recognizing and considering different livelihood assets viz., *human, social, physical, natural and financial resources*. Some of the following innovative extension approaches originating from multiple sources must be adopted on trial basis to make fisheries more lucrative and sustainable which can be replicated in the fishery sector interwoven with numerous challenges like increased production with sustained natural resources, growing market demand for processed products having entrepreneurial opportunities, protection and conservation of environment, and promoting international trade.

An analysis of national extension systems in the Asia and Pacific region by Qamar (2006) observes that agricultural extension is undergoing a major transformation as a result of failure of public extension systems perceived to be outdated in the context of globalization, decentralization, and information technology revolution. Extension systems in many developing countries are undergoing a paradigm shift to more fishers -oriented approaches based on rural innovation that emphasize the importance of interactive, integrated and multidisciplinary oriented mutual learning between formal and informal knowledge systems (Friederichsen, 2009).

a. Asset Based Community Development (ABCD) approach

As per the traditional approach to development, poor people see themselves as people with special needs that can only be met by outside supporting agencies. But Asset Based Community Development (ABCD) approach intends for the development of community based on the principle of identifying and mobilizing individual and community 'assets', rather than focusing on problems and needs. It is an extension approach in which a community's micro-assets are linked with its macro environment. It believes that communities can initiate and sustain the process of growth and development themselves by recognizing and harnessing the existing, but often unrecognized assets, and thereby promoting local economic potential to drive its development process (Rans & Green, 2005). The approach is optimistic in nature, because the focus is on *'what is possessed by the community, rather than the problems of the community."*

The focal point in this approach is asset and not the need of the community. Assets of individuals, associations and institutions are identified after an extensive survey and assets are then matched with the need of the people to empower communities to control their futures and create tangible resources such as services, funds and infrastructures etc. *(Foot and Hopkins, 2010*. In fishery, ABCD approach gives greater emphasis on reducing the use of external inputs and on a high degree of social mobilization in which the assets of the poor (*social, physical, financial as well as human*) can be utilized to bring sustainable livelihoods in fisheries through number of different fishery related activities.

Five Key Assets in ABCD

As per ABCD approach there are 5 categories of asset inventories such as individuals, associations, institutions, physical assets and connections

1. **Individuals**: Every individual has got certain assets, gifts and qualities; such individual is at the center of ABCD approach.
2. **Associations**: Groups of people working with a common interest are critical to community mobilization.
3. **Institutions**: The assets of institutions help the community capture valuable resources and establish a sense of civic responsibility.
4. **Physical Assets**: Physical assets such as land, buildings, space, and funds are other assets that can be used.
5. **Connections:** These are the exchange between people sharing their assets by various methods.

b. Rural advisory services (RAS)

Rural Advisory Services (RAS) refer to all the different activities that provide the information and services needed and demanded by farmers and other actors in rural settings, to assist them in providing their livelihoods by developing their technical, organizational and management skills and practices (GFRAS, 2011; FAO, 2010). RAS designers and implementers must recognize the diversity of actors in extension and advisory fields (public, private, civil society); the need for extending support to farmers' producer organizations (FPO) and rural communities (beyond technology and information sharing) including advice related to farm, organizational

and business management; and explaining the role of facilitation and brokerage in rural development and value chains. In the case of aquaculture, large-, medium- and small-scale fishers need different types of RAS support. The large aquaculture farms are mostly self-reliant and need only regulatory support, while medium-sized farms need mobilization and facilitation support in addition to regulatory support. Small aquaculture farms need more education and input provision alongside facilitation (Kumaran, 2014). Timely sharing of research recommendations can address the problem of disseminating information to fishers. In this direction, innovative strategies are being formulated keeping the fishers' needs and capacities in mind to pass on appropriate technologies by combining Internet, telecommunications, video, and print technologies that may bridge the information gap and empower fishers to make better production and marketing decisions (McLaren et al. 2009).

In fishery sector, RAS helps in

- Providing management and business development support appropriate to the scale, resources and capacities of each fisherman.
- Better understanding markets (prices, products, seasonality, standards, value addition etc.) related to fish and fish products.
- Linking fishers to other stakeholders involved in provision of varied support and services.
- Creating platforms to facilitate interaction and sharing among the various stakeholders including FPOs to ensure coordinated support to fishers.
- Exploiting information communication technologies (ICTs) to provide fishers with a range of information related to weather, prices, extension programmes and generic information regarding fisheries.
- Facilitating the formation of FPOs and also collaborate with FPOs to strengthen the demand and supply side of RAS.
- Promoting institutional and policy change to enable and support small-scale fishery.

RAS encourages the formation/ organisation of groups by involving individual fishers, who have little influence over the social, economic and political processes affecting them, but as a group/ organizations and networks they can deal with their specific challenges and make their voice

heard. Such groupings can act as platforms to articulate concerns, exchange knowledge, influence policies and engage in collective action so that their agriculture remains sustainable and profitable. Effective formation of Rural Resource Centres (RRCs), Fishermen Cooperative Society, Farmers producers Organisations (FPOs) can be instrumental by galvanizing collective action in order to ensure better access to markets and to support innovation by their members in related activities (Sundaram, 2014).

c. Model Village System of Extension (MVSE) approach

MVSE is an integrated and holistic extension approach where *community participation* is prioritized for suitable technological interventions in the fisheries to bring all-round development in fisheries sector in terms of *socio-economic upliftment, technological empowerment, self-governance* thereby enhancing the futuristic knowledge base and skills through *participatory framework*. MVSE emphasizes on involvement of all stakeholders in the process to converge their activities with a stake in the food value chain *linking producer to consumer*. Nevertheless, MVSE is an action research taken up in fishers' farm based on the principle of leveraging the activities, investments and resources from outside agencies/ externally aided projects resulting higher productivity, ensuring food security and sustainable improvement in overall quality of life by promoting leadership, self-dependency of the community in food chain. Economically viable, ecologically compatible and socially acceptable suitable technologies are successfully intervened in a cluster approach through participatory mode by integrating the multi-disciplinary research. The cluster of villages is adopted as model village, the success of which is later replicated to other villages. The village is developed as a commodity village branding for a particular commodity in the market.

MVSE approach works on the following principles:

- Promotes self -governance among the fishers
- Skill improvement and leadership development among the fishing community.
- Establishing linkage through pluralistic convergence of various stakeholders associated in the sector.
- Encouraging the market opportunities through commodity based village development (CBVD).

d. Farmers Field School (FFS) approach

The FFS extension approach is an alternative to the top down extension approach which was evolved as a method to solve complex field level issues in fisheries sectors. FFS aims to build fishers' capacity to analyze their production systems, identify problems, test possible solutions, and eventually encourage the participant member to adopt the practices most suitable to their farming systems (FAO, 2003 c). This is a learning-by-doing approach which emphasizes group observation, discussion, dissection, modification, and promotes field-based experimentation, analysis for collective decision making followed by actions. The FFS approach is an innovative, participatory and interactive learning approach that emphasizes problem solving and discovery based learning. FFS also provides an opportunity to fishers to practice and evaluate sustainable resource use technologies, and adoption of new technologies by comparing with their conventional technologies developed in congruent with their own tradition, culture and resource use pattern. The goal of FFS approach is such that, after observing and comparing the results of field level experimentation fishers will eventually "own" and adopt improved practices by themselves sidelining the conventional ones without any external compulsion. Field day is being organized at the end of the season to give visibility to the entire activities to convince the non-adopters. Exchange visits with other FFS is also encouraged to learn by association and comparison A group of 20-25 fishers can form a Farm School under the guidance of a FFS facilitator. Extension workers, NGO workers, fishermen co-op members or previously trained fishers can become Farmer Field School (FFS) facilitators. The facilitators are trained by master trainers, who have expertise in the particular subject matter. FFS is a time bound activity usually covering one production cycle or a year.

It is also significant to note that irrespective of the merits of the technology, the acceptance to technologies is influenced by the extension method. Farmer Field School (FFS) model has been accepted as a good methodology because it is exclusively participatory. A special feature of this extension approach was that it reached poor and female-headed households and lower-caste households much better than the regular extension services (Tiwari et al. 2010). FFS was also found to be effective in avoiding barriers like socio- economic constraints, infrastructure problem and incompatibility of technology for the adoption of sustainable fishery practices.

The basic component of FFS is setting up of a Participatory Comparative Experiment (PCE), commonly referred to as Participatory Technology Development (PTD), whereby the fishers put the FFS concept into practice under close monitoring and supervision by the FFS members. A PCE can be developed in the field of agriculture, livestock, fishery, forestry, agro-forestry, livelihood system and others.

Principles of Farmer Field School(FFS)are as follows: -

- Field is the learning place.
- Emphasizes hands on and discovery based learning.
- Farmers become experts.
- Integrated and learner defined curriculum.
- Doing is better than learning/ seeing.
- Experiences are the start of all learning.
- Link to actual field situations and should be relevant to local needs and problems.
- Participatory monitoring and evaluation.
- Fishermen are decision makers.

e. Market Led Extension (MLE) approach

In order to make farming more enterprising, extension professionals need to be pro-active beyond the regular objective of maximizing the productivity of the fishers by transferring improved technologies rather fishers should be sensitized on various aspects of farming like culture, harvest, quality, processing and value addition, consumer's preference and market intelligence. This will help the fishing community to realize high returns for the produce, minimize the production costs, and improve the product value and marketability that may lead to realize the concept of doubling farmers' income (DFI). With the globalization of agriculture, emphasis on productivity and profitability to the farm enterprises has been increased and, therefore the demand- driven agriculture (and allied sectors) has led to the paradigm shift from production-led extension to market- led extension. There are many challenges in the agricultural marketing system, which can be resolved through the efforts of market- led extension models.

In this approach, fishers are viewed as 'Fish-entrepreneurs' who expects high returns 'Rupee to Rupee' from his produce by adopting a diverse baskets of package of practices suitable to local situations/ farming systems with optimum cost benefit ratio (C:B ratio) ensuring maximum share of profit by exploring the market demand. Goal of market led extension is to facilitate fishers to get better price. Market led extension focuses on harnessing the ICT tools to access market intelligence including likely price trends, demand position, current prices, market practices, communication network, etc. besides production technologies.

For farmers, as the extension system is more credible source of farm technologies, the extension personnel ought to be knowledge- and skill-oriented in relation to production and marketing of agricultural goods. Thus, revamping the extension system will have a catalytic role for ushering in farmer-led and market-led extension; which can subsequently alleviate poverty and ensure livelihood security. In the light of this, the challenge remains to motivate the extension personnel to learn the new knowledge and skills of marketing before assigning them marketing extension jobs to establish their credibility and facilitate significant profits for the fishing community. SWOT analysis of the market, Organization of Farmers' Interest Groups (FIGs), capacity development, establishing linkage and synergy, harnessing ICTs, digital marketing etc are the competencies required by the extension personnel in order to effectively implement market led extension.

f. Digital Extension approach

Extension reforms brought a transformation in fishery extension system through introduction of Information and Communication Technologies (ICTs). The ICT-enabled extension system referred to as Digital Extension has the potential for enabling the empowerment of fishing communities by improving their access to information and sharing knowledge with innovative e-agriculture initiatives (Saravanan, 2010a).

With the phenomenal growth in information and communication technology, use of IT application in agriculture will bring remarkable change in the attitude and knowledge level of user. Basic requirement is to provide most appropriate information in such a capsule that can be easily understood and used by them. This approach will strengthen the extension system for better dissemination of technology. As a case study the contribution of Digital Green, a NGO that uses an innovative digital platform for community engagement to improve lives of rural

communities across South Asia and Sub-Saharan Africa is remarkable. Digital Green associate with local public, private and civil society organizations to share knowledge on improved farm practices, livelihoods, health, and nutrition, using locally produced videos and human mediated dissemination. As per the study, the Digital Green project (participatory digital video for agricultural extension) increased the adoption of certain farm practices seven times higher compared to traditional extension services and the approach was found to be 10 times more cost-effective per dollar spent. Hence, along with ICT-based advisory services, input supply and technology testing need to be integrated for greater impact and content aggregation from different sources require to be sorted in granular format and customized in local language for rapid adoption of technologies (Balaji et al., 2007&Glendenning and Ficarelli, 2011).

The effectiveness of this innovative extension approach depends on capacity building, people's participation along with government initiative to provide strong infrastructure to be worked with the cutting edge technologies. The farmer friendly technology dissemination process needs to be handled with careful planning by the incorporation of information communication technology. The use of ICT application can enhance opportunities to touch the remote farmers to live in close proximity of the scientific input. The computer based web portals namely aAQUA, KISSAN Kerala, TNAU AGRITECH Portal, AGRISNET, DACNET, e-Krishi, ASHA, India Development Gateway (InDG) portal, Rice Knowledge Management Portal (RKMP), Agropedia, KIRAN, AGMARKNET, ITC-e-Choupal, Indiancommodities.com, Mahindra Kisan Mitra, IFFCO Agri-Portal, Agrowatch Portal, iKissan, etc. along with some mobile based Apps like mKRISHI® Fisheries, riceXpert, Pusa Krishi, Krishikosh, m4agriNEI, CIFTFISHPRO, CIFT Lab Test, CIFTraining etc. launched in India are some of the successful digital intervention for technology dissemination.

The use of internet, mobile and video- conferencing assists the IT enabled farmers to utilize the facilities for their favors for which the most suitable permanent infrastructure is the basic requirement. Strong linkages need to be established between direct ICT interventions and it should be part of the national level program on holistic agricultural development.

g. Disruptive Extension approach:

Recently, a new extension approach christened as 'disruptive extension' comes into limelight which is considered as an innovative extension approach that creates a new paradigm of

extension that eventually disrupts an existing approach followed by extension professionals in the field of agriculture and allied sectors. It is an entrepreneurial oriented sustainable extension system that can able to transform every link in the food chain, from farm to fork. It is a cost-recovery extension approach the fulcrum of which lies between resource exploitation on one side and resource conservation on another side that influence the livelihood security and technology sustainability for small scale farm holders. It deals with the following principles:

- Importance of good governance in agriculture (and allied fields) that considers the resource rights of the farmers.
- Emphasis on growing interest among the stakeholders by explicit analysis of field level issues for technology adoption.
- Potential to resolve the social conflicts for equal access to community resources through Memorandum of Understanding (MOU).
- Based on cost recovery mechanism.
- Ensure commitment to optimum resource management and maximum economic benefit to improve food security.
- Provision of community based social insurance.
- Maintaining the sustenance of the technology supports through custom hiring approach.
- Focus on pluralistic convergence of different partners to build a network of linkage with various entities around the farm households.
- Encouraging the farmers-scientist interaction for technology development, assessment and application through Farmers' FIRST approach.

Global agriculture embraces diverse actors in its endeavour to feed about 10 billion people in the planet by the end of 2050. The small, marginal & landless farmers are extremely vital for food security due to shrinking of resource day by day. The contribution of women fishers also cannot be ignored particularly in on-farm operations, harvesting, post-harvest management, processing etc., especially in fishery and animal husbandry sector. Hence, in today's scenario innovation in agriculture extension is the key to address the growing challenges, which need to be validated, integrated and scaled up and further recommended for large scale implementation

by the policy makers. The innovative extension approach should be based on capacity building, skill development, people's participation along with government initiative to provide policy support to be worked with the cutting-edge technologies. Much effort has been initiated in going beyond the farm and the fishers and focus on beyond the technology to a wider innovation system.

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Chapter 38

Microbial toxins in seafood

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Introduction

According to the Food and Agriculture Organization (FAO, 2020), global fish production has reached to 179 million tonnes in 2018 with a total value of USD 401 billion. Out of that, 156 million tonnes were used for direct human consumption and remaining 22 million tonnes for non-food uses. Global fish consumption has increased from 9.0 kg percapita in 1961 to 20.5 kg in 2018, by about 1.5 percent every year. The fish consumption accounted for 17 percent of total animal protein, and 7 percent of all proteins, consumed globally (FAO 2020). Live, fresh or chilled fish are the most preferred items and utilized maximum (44 percent) for direct human consumption. The rest of production is processed, with 35% frozen, 11% in prepared and preserved forms, and 10% cured (FAO, 2020). Seafood is one of the most traded food commodities (USD 164 billion) in the world. Nearly, 75% of the seafood was imported by the developed countries in international trade and 50% was exported by developing nations.

Fish is considered as safe and healthy food for consumption. However, it is well known those microorganisms are present on fish surface, skin, gills, digestive tract and internal organs. Several outbreaks were reported in association with bacterial pathogens, biotoxins, histamine, viruses, and/or parasites by the consumption of raw or undercooked fish and fish products (Galaviz-Silva *et al.,* 2009). Both pathogenic and spoilage bacteria can be added to fish at any stage of transportation, handling, processing and storage. According to the U.S. Centers for Disease Control and Prevention (CDC), fish was considered as food category commonly implicated in food borne outbreaks involving single food categories (CDC, 2018). A total of 937 food borne outbreaks associated with fish were reported, resulting in 5,011 illnesses, 364 hospitalizations, and four deaths in past ten years in United States (CDC, 2018). The fish and fish products have been continuously implicated in food borne outbreaks, contributing 7% of total confirmed food borne-illness outbreaks over recent years (CDC, 2018). The significant increase in food borne outbreaks may be due to the rise of new nutritional trends which supports the consumption of raw or fresh foods. According to CDC 2014, there are 31 major pathogens are reported which can cause 32 diseases in human. The most common outbreaks associated

with consumption of fish is scombroid toxin or histamine, *Salmonella* spp. and *Clostridium botulinum, Clostridium perfringens.*

Bacterial Toxin

Food borne illness caused by the pathogenic bacteria is an important concern in seafood. The most common types of food borne illness in human are infection and intoxication. Food borne infections are caused by ingesting live pathogens that develop inside the body, generally in the intestine tract. Intoxication is a condition caused by swallowing preformed toxins i.e. toxins created by microorganisms in the food before it is consumed. Furthermore, a toxic-infection (also known as toxin-mediated infections), is caused by the ingestion of pathogens, which produce biologically active toxins in the small or large intestine. Both gram positive and gram negative bacteria can able to produce toxins. They can produce even single or multiple toxins. Toxin production as a result of (excessive) microbial proliferation can occur at any point in the food production chain. Even though the bacteria were killed during the food processing steps, the toxin remains resident and biologically active. The toxin production in food is influenced by extrinsic (e.g., temperature, humidity, atmosphere) and intrinsic (e.g., pH, aw, nutrients) properties , cell density, growth phase, cell stress, and injury .The ability of toxins production in humans to cause disease symptoms depends on several factors including strain pathogenicity, quality of toxin produced, physic-chemical characteristics of toxins, interactions with food components, metabolites produced by microorganisms, stability in food and in the human gastrointestinal tract, inherent (sub)clinical dose of toxins, mode of action, effect of acute and (sub)chronic exposure, and targets and receptors in the human body(Rajkovic *et al.*, 2020).

Types of Toxins

A bacterial toxin is a protein-based macromolecule that can cause toxic harm to a specific organ of the host (Iriarte *et al.*, 2001). Toxins can be divided into endotoxins and exotoxins:

Endotoxins: These are the components of Gram-negative bacteria's outer membrane; they are the most important antigen of the bacteria, and they are released into the medium during various processess such as lysis and cell division. This endotoxin can able to cause endotoxic shock and tissue damage.

Exotoxins: These are protein-derived macromolecules that the bacterium produces and then releases into the media. Depending on their mechanism of action, exotoxins are classified as follows:

Toxins Type I: These toxins alter the cells of the host's without internalizing in the cells;

for example, the superantigens produced by *Staphylococcus aureus.*

Toxins Type II: Within this group there are hemolysins and phospholipases; they cause pore formation and/or membrane destruction in the host cells. The pathogen can penetrate the host cell using this virulence factor. Eg: aerolysin and GCAT protein produced by *Aeromonas* spp.

Toxins Type III: These toxins are known as A/B due to their binary structure. Fraction B binds to the receptor of the cell and fraction A has enzymatic activity, which, depending on the toxin and its mechanism of action, will cause cell damage ; for example, the Shiga toxin produced by *Escherichia coli* O157:H7, the Cholera toxin (Ctx) produced by *Vibrio cholerae*, and the Anthrax toxin produced by *Bacillus anthracis*

The exotoxins produced by bacteria play an important role in the pathogenesis of diarrheal illness, inducing excessive liquid secretion without the destruction and death of intestinal mucosal cells. These toxins are generically referred to as enterotoxins (Hernández-Cortez *et al.,* 2017)

Toxins produced by pathogens involved in foodborne diseases are as follows:

- *Bacillus cereus,*
- *Clostridium botulinum,*
- *Clostridium perfringens and*
- *Staphylococcus aureus.*
- *Pathogenic Escherichia coli*
- *Vibrio cholera*
- *Shigella* spp.
- *Yersinia enterocolitica*

Bacillus cereus

Bacillus cereus is one among the *Bacillus* spp. that has been identified as the most frequent cause of foodborne illness. *B. cereus* is commonly found in many raw and unprocessed foods and the presence of low numbers of *B. cereus* in raw foods is regarded normal, while the numbers more than 5 log CFU/g (or per mL) are considered as a hazard to food safety (Sanchez-Chica, *et al.*, 2020). *B. cereus* usually found in rice, pasta, dairy, meat and seafoods. Food poisoning due to this organism may occur when foods are prepared and held without adequate refrigeration for several hours before serving. The *B. cereus* spores can withstand heat processes, and germinated vegetative cells can multiply and produce toxins under ideal conditions. Therefore, in order to inactivate *B. cereus,* suitable time/temperature profile must be developed, which will be often specific for specific foods as well as maintain cold chain due to psychotropic character of some strains of *B. cereus* (Webb *et al.*, 2019).

B. cereus toxins cause two distinctly different forms of food poisoning—the emetic or vomiting type and the diarrheal type. The emetic type is an intoxication caused by the presence of emetic toxin, cereulide, in food. Cereulide intoxication is characterized by the quick onset of symptoms (0.5 to 6 hours), which include nausea, vomiting, and occasionally abdominal cramps and/or diarrhoea, which normally resolve within 24 hours. The Intoxication/infection dose is ca. 10 μg/kg−1 bw, 0.01μg/g[1] of food (produced by *B. cereus* of more than 10^5 CFU/g food, depending on the strain, food and condition. The diarrheal type is produced by the synthesis and release of protein enterotoxins in the small intestine after consumption of viable *B. cereus* vegetative cells and/or spores. Hemolysin BL (Hbl), nonhemolytic enterotoxin (Nhe), and cytotoxin K are known to be implicated in this syndrome. They are all heat labile, pH sensitive, and proteases sensitive proteins, which is why preformed toxins in food typically do not result in foodborne intoxication (Rajkovic *et al.*, 2020). The symptoms of diarrheal type are characterized by the onset of watery diarrhea, abdominal cramps, and pain occurs 6-15 hours after consumption of contaminated food. Nausea may accompany diarrhea, but vomiting rarely occurs. The heat toxin stability of diarrheal type is 5 min. at 56 ºC whereas emetic type(cereulide): 90 min at 121 ◦C.

Control measures: Proper hygiene and appropriate temperature control should be maintained throughout the production and storage. Optimization of heat process and temperature control

to prevent spore germination and multiplication of vegetative cells of *B. cereus*, quick chilling methods to cool foods below 7.2° C within 4hrs of preparation should be followed.

Clostridium botulinum

Clostridium botulinum is a dangerous food poisoning organism and it produce a very deadly, exotoxin (neurotoxin) when grows in food. The food poisoning caused by this organism is known as 'botulism'. *C. botulinum* is an anaerobic, gram-positive, spore-forming rod shaped bacteria. The spores of *C. botulinum* are highly heat resistant. Seven different toxins i.e. A to G are known to exist. Nausea, vomiting, fatigue, headache, paralysis, difficulty to talk, double vision and sound in the ear are the usual symptoms. Symptoms develop within 18-36 h of consuming infected food. Death occurs due to respiratory failure. Mortality rate is very high (10 – 50%). This organism is found throughout the environment and found in the intestinal tract of fish, gills and viscera of crabs and shell fish. It can survive in normal cooking temperature and grows in vaccum packed and MAP. Botulism is the problem in home canned foods or canned foods that are improperly sterilized. Botulism is also reported from smoked, salted and fermented fish.

C. botulinum has four groups, as well as seven antigenic variations of botulinum neurotoxins (A–G). Botulinum toxin type A, a neurotoxin with a high fatality, is about 1,000 times more toxic than tetanus toxin. Types A, B, E, and F are mainly involved in botulism in humans, while types C and D are mainly involved in animals. *C. botulinum* type E is most common in seafoods and considered as a major concern because it can grow at very low temperatures 3.3°C and produces little noticeable evidence of spoilage. *C. botulinum*-proteolytic (mesophilic bacteria) belongs to group I, while *C. botulinum*-non-proteolytic belongs to group II (psychrophilic microorganisms). Group I produces heat-resistant spores, which are inactivated by the "Botulinum cook" (121°C/3 min) applied to canned goods with low acid content; neurotoxins generated in this group include A, B, F, and H. Group II produces spores that are moderately heat resistant, and the neurotoxins produced are B, E, and F. Group II can able to grow and produce neurotoxin at refrigeration temperatures, as low as 3.0 ◦C, and is a concern in minimally processed refrigerated foods. Foods involved in botulism are fruits and vegetables, meats, fish, and miscellaneous combined foods (Peck, 2005). Intoxication/Infection dose is 1 µg/kg b.w. orally, for 70 kg man 0.09 to 0.15 µg intravenously or intramuscularly, 0.70 to 0.90 µg

inhalationally. The toxin stability is 80ºC for 10 min (function of pH and other factors); exact values are also toxin dependent. Substances in food such as divalent cations and organic acid anions protect the toxin from heat.

Clostridium perfringens

Clostridium perfringens is an anaerobic pathogen which can able to produce several toxins and cause enterotoxic diseases in humans and animals. Food poisoning caused by *C. perfringens* may occur when foods such as meat or poultry are cooked and held without maintaining adequate heat or refrigeration before serving. The illness is a self-limiting gastroenteritis with an incubation period of 8-15 hours and duration of 12-24 hours. The symptoms, which include intense abdominal cramps, gas, and diarrhea, have been attributed to a protein enterotoxin produced during sporulation of the organism in the intestine. (Toxico-infection)

C. perfringens are estimated to be the second most common bacterial causes of foodborne illness in the US, causing one million illnesses each year. *C.perfringens* strains are classified into seven groups A, B, C, D, E, F and G based on the different toxins it produces (alpha, beta, epsilon, and iota). The alpha, beta, epsilon, and iota, are responsible for the tissue lesions and the host's death and are considered to be major toxins. Alpha toxin: The alpha toxin, found in type A strains of *C. perfringens* causes gas gangrene and also hemolysis in infected species. Beta toxin: This lethal toxin is found in *C. perfringens* type B and type C strains. This toxin also results in necrosis by way of increased blood pressure, which is brought on by the presence of catecholiamine. Epsilon toxin: This toxin is produced by type B and type D strains of *C. perfringens*. It is isolated from animals, particularly sheep, goats, and cattle, but rarely from humans. Similar to the other toxins, epsilon toxin creates pores in tissues, which can result in leaked potassium ions and fluid leakage. Iota toxin: The iota toxin is produced solely by type E strain of *C. perfringens* and is known as an AB toxin. The iota toxin can cause tissue death in infected individuals. Among the seven groups, *C. perfringens* type F is commonly involved in foodborne toxico-infections. *C. perfringens* type F carries the a-toxin gene and the cpe gene and produce CPE (*C. perfringens* Enterotoxin) single polypeptide of approximately 35 kDa upon sporulation, but do not carry the structural genes for /]-toxin, .:-toxin, or t-toxin (Mi, Li and McClane, 2018; Rood *et al.,* 2018). The Infection / Intoxication dose is 10^6 to 10^7 CFU/g of food (ingested vegetative cells produce CPE during intestinal sporulation). The toxins produced

usually in the small intestine of the host. The heat stability of toxin is at 60 ◦C for 5 min and pH 5 to 10.

Control measures: Prevention from cross-contamination of cooked foods. Cleaning and sanitizing food contact surfaces after being used for raw products is an effective way to control.

Staphylococcus aureus

Staphylococcus aureus is Gram positive, non-motile, facultative anaerobic, spherical non-sporing cocci, arranged in grape-like clusters. The primary habitat of *Staphylococcus aureus* is man**.** This organism is found in sweat, eargum, tears, throat, ulcers, boils and nasal cavities**.** Fish caught from the open sea doesn't contain *Staphylococcus aureus* when the material is taken onboard and handled by workers, contamination takes place**.** So its presence in seafood / food indicates lapse in maintaining personal hygiene

Staphylococcus aureus is considered as one of the major food borne pathogen responsible for food poisoning outbreaks worldwide. They are enterotoxin producing pathogenic bacterium and occurring as commensal flora of humans (Alves *et al.,* 2014). They have a great significance in food industry due to the ability of certain strains to produce heat stable enterotoxin and other virulence factors which are responsible for staphylococcal food poisoning (SFP). (Argudin *et al.,* 2012; Tango *et al.,* 2015). Symptoms of SFP include nausea, violent vomiting, and abdominal cramping, with or without diarrhea within 2-4hr of consumption (Chen *et al.,* 2018). The minimum amount of toxins required to have symptoms is about 1ng/g of food. SFP is widely reported on protein rich foods such as meat, dairy and fish products which have extensive manual handling, inadequate heating and inappropriate storage (Adam and Moss 2007). The bacteria can be killed by heat treatment, but toxin produced is very heat resistant and remain in food even after cooking, which can cause food poisoning

SEs (Staphylococcus enterotoxins) belongs to a great family of staphylococcal and streptococcal pyrogenic exotoxins, characterized by common phylogenetic relationships, structure, function, and sequence homology. SEs function not only as potent gastrointestinal toxins causing emesis but also as superantigens that stimulate nonspecific T-cell proliferation. (Rajkovic *et al.,* 2020). To date, 26 SEs and enterotoxin-like types have been described: enterotoxins A (SEA), B (SEB), C1 (SEC1), C2 (SEC2), C3 (SEC3), D (SED), E (SEE), G (SEG), H (SEH), I (SEI), J (SElJ), K (SElK), L (SElL), M (SElM), N (SElN), O (SElO), P (SElP), Q (SElQ), R (SER), S (SES), T

(SET), U (SElU), W(SElW)), V (SElV), X (SElX), and Y (SElY). Enterotoxins are encoded in prophages, plasmids, or chromosomal pathogenicity islands.

The location of the SE genes on mobile genetic elements presents an additional risk factor in *S. aureus* food intoxication, due to possible horizontal gene transfer (Cafini *et al*., 2017; Lindsay, 2014). The transfer of genetic elements in *S. aureus* has contributed to strain variability and enhanced virulence. It is well known that *S. aureus* strains usually carry more than one SE encoding gene. The stability of toxin is SEA: 3 min at 80 ◦C, 1 min at 100 ◦C; SEB 87 min at 99 ◦C. Stable at wide range of pH and resistant to gastric pH.

Control measures: Adequate control over the health and hygiene of fish handlers. The fish has to maintain at low temperature (below 5°C) during handling and processing. Minimize time/temperature abuse of seafood, especially after cooking

Pathogenic Escherichia coli

E. coli is Gram-negative, rod-shaped, non-spore forming facultative anaerobic bacteria. It is commonly found in the gut of humans and warm-blooded animals. Pathogenic strains of *E. coli* are transferred to seafood through sewage pollution of the coastal environment or by contamination after harvest. Similar concerns occur if contaminated ice used for preservation or the utensils contaminated with *E. coli.* Improperly cleaned boat deck, and containers used in onboard trawlers can also act be source of contamination. There are six categories of pathogenic *E. coli* which include Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E.coli* (EPEC), Enteroinvasive *E.coli* (EIEC), Enterohemorrhagic E. coli (EHEC, Shiga toxin-producing *E. coli* or STEC), Enteroaggregative *E. coli* (EAEC or EAggEc) and Diffusely adherent *E. coli* (DAEC). Among these Shiga toxin-producing *E. coli* (STEC) has been associated with severe foodborne outbreaks of major public health importance in the last years. STEC produces toxins, known as Shiga-toxins because of their similarity to the toxins produced by *Shigella dysenteriae*. Shiga toxins (Stx) can be divided into two categories: Stx1, which is identical to the toxins produced by *Shigella dysenteriae* 1, and Stx2, which is around 60% similar to Stx1. Production of one or more Shiga toxins is essential to cause disease, but the production of Stx2 is more closely linked to the severity of the disease such as hemolytic uremic syndrome (HUS) and HC (Farrokh, *et al.*, 2013). STEC strains can be classified as O157 and non-O157. Serotype O157:H7 is the most common serotype involved in severe infections resulting to HUS and HC,

and it has been linked to the majority of large-scale outbreaks of STEC infections. Symptoms of STEC are severe diarrhea, stomach cramps, and vomiting. Diarrhea is often bloody without fever. Symptoms typically appear 3-4 days after eating contaminated product, but can range from 1-10 days. STEC can grow in temperatures ranging from 7℃ to 50°C. A recent study found that *E. coli* O157 strains possess inherent genetic mechanisms which enable growth at low temperatures (< 15 ℃), compared to non-pathogenic *E. coli* (Vidovic *et al.*, 2011). Some STEC can grow in acidic foods, down to a pH of 4.4, and in foods with a minimum water activity (a_W) of 0.95.

Control measures: The only effective method of eliminating STEC from foods is to introduce a bactericidal treatment, such as heating (for example, cooking or pasteurization) or irradiation. Basic good food hygiene practices have to be followed during handling and processing of foods.

Vibrio cholerae

V. cholerae are Gram-negative, coma shaped, aerobic, motile rods, non-spore forming bacteria. *V. cholerae* can be divided into two major groups: the cholera-causing strains of serogroups O1 and O139, and non-O1/non-O139 *V. cholerae*. The non-O1 strains do not cause diarrhoea as severe as cholera but they frequently cause extraintestinal infections. The main virulence factor of *V. cholerae* O1 (Ogawa, Inaba, and Hikojima serotypes, Classical and El Tor biotypes) and O139 is CTX toxin (Cholera toxin). It is a potent enterotoxin and causes toxico-infections in humans. It activates the adenylyl cyclase; increases the levels of intracellular cAMP promoting fluid and electrolytes secretion in the intestinal epithelium, causing diarrhea. This toxin can be identified by the presence of the ctxAB gene. Symptoms includes profuse diarrhea, after an incubation period from 2 h to 5 days; stools have the appearance of rice water, there is dehydration and electrolyte imbalance, which can lead to death. The pathogen is shed in their feces for 7–14 days, which is a very serious source of contamination since it is possible to infect others. The disease is occasionally spread through eating raw or undercooked shellfish that are naturally contaminated.

Control measures: Proper disinfection of contact surfaces. Avoid cross contamination of cooked products and strictly maintain the personal hygiene of seafood/food handlers

***Shigella* spp.**

Shigella belongs to the family Enterobacteriaceae. They are gram-negative, non-motile, and facultative anaerobic bacteria and classified in four serogroups, A (*Shigella dysenteriae*), B (*Shigella flexneri*), C (*Shigella boydii*) and D (*Shigella sonnei*). The disease caused by *shigella* is known as 'shigellosis', and S. *dysenteriae* is responsible for the more severe forms of shigellosis. *Shigella* can be transmitted through direct contact (person-to-person) or indirectly through contaminated food and water, ice, contact surface, files or food handlers who are carriers of this organism. Shigella is naturally found in the intestinal tract of humans. The virulence factor found in Shigella spp., is shiga toxin (Stx), which is commonly found in *S. dysenteriae* serotype 1 and closely resembles Stx in Shiga toxin-producing *Escherichia coli* (STEC). It is a heat labile exotoxin. It acts by inhibition of protein synthesis causing the death of susceptable cells.

Control measures: *Shigella* contamination can be controlled by strictly maintaining the personal hygiene of workers. Good sanitary and handling practice has to follow during food processing or storage. Avoid time/temperature abuse and cold chain should be maintained. Identify and avoid carriers from food operation and monitor for exclusion of pest.

Yersinia enterocolitica

Yersinia enterocolitica is naturally found in a wide range of foods, water, animals, and soil. They are a biochemically diverse group capable of surviving and developing in refrigerated temperatures. In terms of food safety, the ability to multiply at refrigeration temperatures is quite important. It is a gastrointestinal pathogen and cause illness in humans particularly in in young children, are fever, abdominal pain, and diarrhea, which is often bloody. In adults, in addition to symptoms resembling appendicitis, severe parenteral forms may appear, such as erythema nodosum, or micro abscesses in internal organs. It is transmitted via the feco-oral route by the consumption of contaminated food or water. *Y. enterocolitica* can able to produce heat-stable enterotoxins and play a key role in the pathogenesis of yersiniosis (Samoraj, 2022). The invitro conditions required to produce enterotoxin in *Y. enterocolitica* strains are 26 ºC and 37 ºC, pH7-7.5. *Y. enterocolitica* produce enterotoxins after reaching the final part of the small intestine. The Yersinia stable toxins (enterotoxins) produced by *Y. enterocolitica* are biologically and

antigenically similar to STX1 (Shiga Toxin I) enterotoxins produced by *E. coli*. Enterotoxins provoke diarrhea, which is the main cause of mortality in yersiniosis

Detection Methods

The toxins produced by the bacteria are the most important virulence factor of foodborne pathogens and a major contributor of foodborne related diseases. They are proteins or peptides that vary from one another in terms of their size, structure, toxicity, toxicological end points, solubility, and stability, primarily in relation to the types of food matrix. These differences influence the characteristics of required detection methods. The commonly used methods used for detection and quantification methods for toxins in foods are bioassay method (whole animal assay and cell culture assay), immunological method (Enzyme-linked immunosorbent assays and reversed passive latex agglutination assay), mass spectrometry, and molecular assays.

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