RECENT ADVANCES IN HARVEST AND POST-HARVEST TECHNOLOGIES IN FISHERIES

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Training manual

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Seafood handling and curing techniques

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Introduction

The significance of fish as a powerful source of nutrients is well known. It's loaded with important components such as proteins, omega-3 fatty acids, vitamins and minerals which are incredibly important for the function 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 center, 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.

Food hygiene relates to "all 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 fishery 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 covering aspects related to the hygienic design of facilities on-board, during transportation, processing and distribution, to personnel hygiene, cleaning, sanitation and pest control.

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

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:

- Vessels must be designed and constructed so as to protect fish from contamination by bilge-water, sewage, smoke, fuel or other objectionable substances.
- 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.
- Fishing vessels should be designed and equipped with suitable holds, tanks or containers to preserve fresh fish and fishery products throughout the fishing period.
- Chilling devices must allow easy monitoring of temperatures.
- 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.
- All vessels must be kept free of pests using pest control devices.
- 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.
- 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 Domestic Market

Domestic markets play 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

Processing units aims towards value addition 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.

Proper Transportation

During transportation, measures should be taken to protect food from potential sources of contamination and damage likely to render the food unsuitable for consumption. Proper transportation maintaining low temperatures provide an environment which effectively controls the growth of pathogenic or spoilage microorganisms. Care during transportation includes:

- Construction of transportation vehicles and containers such that they can be easily cleaned and disinfected.
- All interior surfaces should be maintained clean, smooth and free of any objectionable odours.
- Vehicles and containers should be maintained at low temperature to ensure cold chain during transportation.

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

Curing techniques

Different processing and preservation methods: traditional and modern techniques including salting, drying, smoking, chilling, freezing, thermal processing, chemical treatments, as well as combination of two or more methods (referred to as hurdle technology) are used for fish preservation. Traditional methods of fish preservation include salting, drying, smoking, pickling, marination and fermentation, collectively known as Curing. Curing being the oldest and cheapest methods of fish preservation is still widely practiced in many parts of the World. These techniques are applied as single or in combination. In the current market situation both wet and dry cured fishery products have commercial importance. Advances have been made in this regard for process standardizations to meet the current demand of the market. Cured fish consumption is more practiced in areas where the availability of fresh fish is comparatively limited viz., interior markets as well as hilly areas. This method is also widely adopted in coastal areas when an excess catch is to be preserved for later utilization during the lean season or for marketing to other areas, thereby assuring its seasonal as well as regional availability.

Drying

The term 'drying' implies the removal of moisture by means of evaporation. Water being the essential component for all living organisms, its removal facilitates microbial retardation, arrest of autolytic activity as well as oxidative changes and hence can be used as a method of preservation. In any process of drying, the removal of water requires an input of thermal energy. The thermal energy required to drive off moisture can be obtained from a variety of sources, e.g., the sun or the controlled burning of oil, gas or wood, electrical heating etc. The thermal energy can also be supplied directly to the fish tissue by microwave electromagnetic radiation or ultrasonic heating. In fish, water constitutes about 70-80% and removal of this constituent to a level that arrests the unfavorable microbial and oxidative activities facilitates its effective preservation.

Drying phases

In foods, there exist three layers of water viz., an adsorption layer, a diffusion layer and a free layer. Water at the adsorption layer, also referred to as the bound water is tightly bound to the particle and hence does not take part in any chemical reactions. The second layer being the diffusion layer is less tightly bound and the third layer consists of free water which has all the properties of ordinary water. Free water involves in all chemical reactions and favors the growth of microorganisms and hence is important in the drying process. Water activity is the measure of the free water available and lowering of this water activity is essential for effective preservation.

During air drying, water is removed from the surface of the fish and water moves from the deeper layers to the surface. Drying takes place in two distinct phases. In the first phase, whilst the surface of the fish is wet, the rate of drying depends on the condition (velocity, relative humidity etc.) of the air around the fish. If the surrounding air conditions remain constant, the rate of drying will remain constant; this phase is called the 'constant rate period'. Once all the surface moisture has been carried away, the second phase of drying begins and this depends on the rate at which moisture can be brought to the surface of the fish. As the concentration of moisture in the fish falls, the rate of movement of moisture to the surface is reduced and the drying rate becomes slower; this phase is called the 'falling rate period'.

Constant rate drying phase

During this period the rate of drying is dependent on several factors:

Air temperature: At the beginning of drying, the heat energy required for evaporation is balanced by the heat supplied by the surrounding air. Warm air can provide more heat energy and, provided that the air speed and relative humidity will allow a high rate of water movement, the rate of drying will be increased.

Relative humidity of the air: The lower the relative humidity of air surrounding the drying area, the greater the ability to absorb water and the faster the rate of drying.

Air velocity*:* Air velocity has a positive relation with rate of drying. Better the speed of the air over the fish, the greater will be the drying rate. The air around fish consists of an immediate stationary layer above the fish, a slowly moving middle layer and an outer turbulent layer. On saturation of the immediate stationary air layer, the moisture passes into the slowly moving middle layer. The higher the air speed in the outer layer, the thinner the slow-moving layer, allowing more rapid movement of water away from the fish.

Surface area of the fish: the larger the surface area, the faster the rate of drying. By scoring and splitting the fish, the surface area increases relative to the weight/thickness resulting in the rate of drying to be faster.

Falling rate drying phase

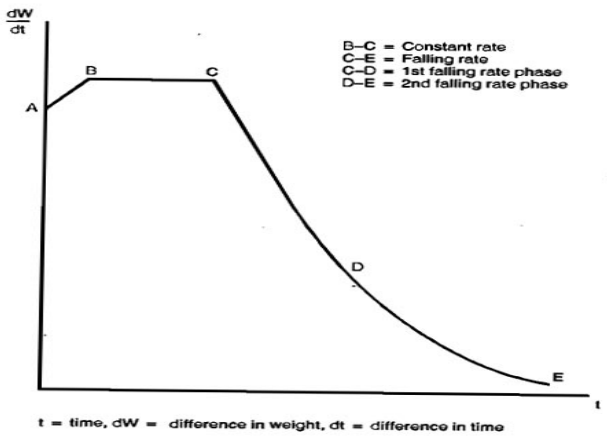
As drying progresses, the water evaporates from the fish surface and is replaced by the water from the interior of the muscles by diffusion. This process is comparatively slower which limits the drying rate and is referred to as the falling rate phase. Drier the product is, slower will be the diffusion of water to the surface. Several factors influence the rate of drying at this phase:

***Nature of the fish*:** a high fat content in the fish retards the rate of drying.

***Thickness of the fish*:** the thicker the fish, the further the water in the middle layers has to travel to reach the surface, slowing down the drying rate.

***Temperature of the fish*:** diffusion of water from the deeper layers to the surface is greater at higher temperatures.

***Water content*:** as the water content falls, the rate of movement to the surface layers is reduced.



Drying rate curve.

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

Methods of Drying

There are basically two methods of drying fish. The common and traditional method being sun drying which is done by utilizing the atmospheric conditions viz., temperature, humidity and airflow. 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. In sun drying, there is no control over the operational conditions and hence generally the losses viz., quantitative as well as qualitative ones, cannot be substantiated. Hence it is essential that the operations be controlled to get a product with superior quality as well as stability. Recently, the controlled artificial dehydration of fish has been developed so that fish drying can be carried out under controlled conditions.

Natural or sun drying:

In this type solar and wind energies are utilized as the source of energy.

- Drying on the ground
- Rack Drying
- Solar drying using Solar tent dryers, Solar cabinet dryers

Artificial / Mechanical Dryers

- *Hot air dryers*
  - Cabinet dryer
  - Tunnel dryer
  - Multi-deck tunnel
- *Contact Dryers*
  - Vacuum dryers
  - Rotary dryers
  - Drum dryers

Salting

Salting, one of the traditional methods of preservation is usually done alone or in combination with drying or as a pretreatment to smoking. The presence of sufficient quantities of common salt (sodium chloride) in fish can prevent or drastically reduce bacterial action. Salting amounts to a process of salt penetration into the fish flesh when fish is placed in a strong solution of salt (brine) which is stronger than the solution of salt in the fish tissue. Penetration ends when the salt concentration of the fish equals that of the surrounding medium. This phenomenon is known as osmosis. It is based on different factors like diffusion and biochemical changes in various constituents of the fish. This process facilitates preservation of fish by reducing the water activity. A concentration of between 6–10 % salt in the tissue together with the removal of some water from the tissue during the salting process will prevent the activity of most spoilage bacteria. If fish are salted before drying, less water needs to be removed to achieve preservation. A water content of 35–45 %, depending on the amount of salt present, will often prevent, or drastically reduce, the action of bacteria.

Salt

Source

Common salt, in its purest form consists of sodium chloride (NaCl). However almost all commercial salts contain varying levels of impurities depending on the source and method of production.

Based on the source as well as method of manufacture, common salt can be grouped as:

- ***Solar salt:*** prepared by the evaporation of sea or salt lake waters by the action of sun and wind.
- ***Brine evaporated salts:*** produced from underground salt deposits which are brought to the surface in solution form and is heat evaporated.
- ***Rock salt:*** obtained as natural deposits from interior rock mines which are ground to varying degrees of fineness without any purification.

Chemical composition

Commercial salts vary widely in their composition with best quality salt containing upto 99.9 % sodium chloride, whereas low quality salt may only contain 80 % sodium chloride. The main chemical impurities of commercial salts include calcium and magnesium chlorides and sulphates, sodium sulphate and carbonate, and traces of copper and iron. Apart from these, contaminants such as dust, sand and water may also be present in salt. Presence of calcium and magnesium chlorides even in small quantities tends to slow down the penetration of salt into

the flesh and hence their presence may lead to increase the rate of spoilage. Further magnesium chloride is hygroscopic and tends to absorb water, making the fish more difficult to dry and to keep dry. Calcium and magnesium salts give a whiter colour but tend to impart a bitter taste. Very often the consumer demands a whitish colour in salted fish products and small quantities of calcium and magnesium compounds in the salt are usually considered desirable. Excessive quantities, however lead to a bitter flavour and the dried product tends to be brittle which can cause problems during packaging and distribution. Trace quantities of copper in salt can cause the surface of salted fish to turn brown affecting the appeal of dried fish.

Microbiological purity

Many commercial salts, particularly solar salts, contain large numbers of salt tolerant bacteria (halophiles) and counts of up to 105/g have been recorded. A group of halophiles, also referred to as the red or pink bacteria, can be a problem in commercial fish curing operations as they cause a reddening of wet or partly dried salt fish. Halophilic moulds tend to grow on dried fish under favourable conditions causing the formation of dark patches called 'dun'. They tend to occur more frequently in rock salt.

Physical properties

Fine grain salt dissolves more rapidly in water and is preferred for making brines. However on direct application of fine grain salt on fish causes a rapid removal of water from the surface which becomes hard and prevents the penetration of salt to the inside of the fish, a condition referred to as 'salt burn'. Hence for dry salting, a mixture of large and small grain sizes of salt is recommended.

Types of Salting

- ***Dry salting:*** This is the most widely used method of fish curing. Dry salting is advisable for fishes of any size, except fatty fishes. The fish is gutted, beheaded or ventrally split open and the viscera removed followed by washing. Scoring is also practiced if the flesh portion is thick for facilitating better salt penetration. Salt is then applied in the ratio 1:3 to 1: 10 (salt to fish) depending upon the size of the fish. The fish is then stacked in clean cement tanks or other good containers layered with salt and weight is applied from top for better salt penetration. The fish is kept in this condition for 24-48 hours. After salting period, the fish is taken out, washed in brine to remove adhering salt and drained. It is then hygienically dried to a moisture content of about 25%. Yield of the product by this method is about 35-40% with a storage stability of upto three months under ambient conditions.
- ***Wet salting:*** The initial stages of processing and salting are the same as for dry curing. However, the fish kept in tank is allowed to remain in self brine till marketing without further drying. For marketing, as per the demand the wet salted fish is drained and packed in palmyrah leaf baskets or coconut leaf baskets. This method is particularly suitable for fatty fishes like oil sardine, mackerel etc. Wet salted fishes have short shelf stability with a moisture content of 50-55% and a salt content of around 25%.
- ***Pickle salting:*** Pickle curing is a type of wet salting where the fish is layered by granular salt 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.

- ***Kench salting:*** In this method, salt is rubbed on to the surface of the fish and stacked in layers of salt and fish. The self-brine formed is allowed to drain away. 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 flavours.
- ***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.
- **Quality issues in dried and salted fish**
- ***Pink/Red:*** Salt content prevents the growth of normal spoilage microflora in the fish but halophiles, which can survive at 12-15% of salt concentration, will survive. Halophilic bacteria are present in most of the commercial salt. A particular group of halophiles called Red / Pink cause reddening of wet or partially dried salted fish. These do not grow in brine or in fully dried fish. They are aerobic and proteolytic in nature, grows best at 36ºC by decomposing protein and giving out an ammoniacal odour. Spoilage appears on the surface as slimy pink patches. However these bacteria are not harmful in nature. Usage of good quality salt is recommended to avoid this condition. This spoilage is mostly found in heavily salted fish and absent in unsalted fish.
- ***Dun:*** In salted fish, brownish black or yellow brown spots are seen on the fleshy parts, referred to as “dun”. This is mainly caused by growth of halophilic mould called *Sporendonema epizoum*. This gives the fish a very bad appearance. Moulds usually grow at relative humidity above 75%. The optimum temperature for growth is 30-35 ºC. 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. Chemical method of prevention includes dipping the fish in a 5% solution of calcium propionate in saturated brine for 3-5 minutes depending upon the size of the fish.

- ***Salt burn***: A mixture of large and small grain sizes is recommended for dry salting of fish. If fine grain is used directly on the fish, salt burn may occur due to the rapid removal of water from the surface with no penetration of salt to the interior of the fish.
- ***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.
- ***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 hydroxy 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.
- ***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. Beetles attack when the moisture content is low and especially when the storage is for a long time. The commonly found beetles are *Dermestes ater, D frischii, D maculates, D carnivorous* and *Necrobia rufipes*. The larva does most of the damage by consuming dried flesh until the bones only remain. Mites are also an important pest, which are found infesting dried and smoked products. *Lardoglyphus konoi* is the commonly found mite in fish products. Infestation can be reduced by proper hygiene and sanitation, disposal of wastes and decaying matter, use of physical barriers like screens, covers for curing tanks etc, and use of heat to physically drive away the insects and kill them at 45 º C.
- ***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.

Conclusion

Drying and salting are age-old practices followed for seafood preservation on account of its simplicity and effectiveness. However, a major drawback with this traditional processing is the lack of standard operating procedures being followed which affects the quality of cured products. Moreover, there is a general a conception that drying/salting is a secondary method for preservation applicable for low value as well as inferior quality varieties. Efforts towards

effective and hygienic handling practices in the process chain, popularization of improved drying and packaging practices, and adequate extension services can facilitate better adoption of cured fishery products in the seafood sector. The recent market trends also indicate rejuvenation of this sector on account of the technical advancements being carried out in this area.

Suggested Readings

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Low temperature preservation of seafoods

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Introduction

Fish is regarded as an excellent source of nutrients with its richness in health beneficial omega-3 fatty acids, quality and easily digestible proteins with balanced amino acid profile, essential minerals and vitamins. These nutritional advantages offer considerable benefits to fish as a means to achieve nutritional as well as social security. Better awareness regarding this biomass as a potential source of nutrients has created increased interest in effective exploitation of these resources. However, their richness in nutrients as well as high moisture content increases its perishability, necessitating the processing and preservation of fish mandatory soon after harvesting. 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 ranges 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 k cal of heat and this will be sufficient to cool about 3 kg of fish from ambient temperature of 30^OC to 0^OC. Hence theoretically about 30% of ice is needed to bring down the temperature from ambient conditions to 0^oC. 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 its 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 center 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, white fleshed fishes, freshwater fishes, tropical fishes, flat fishes, thick skinned fishes have better storage stability than their counterparts viz., fatty fishes, dark fleshed fishes, marine fishes, temperate fishes, round fishes, thin skinned fishes, respectively.

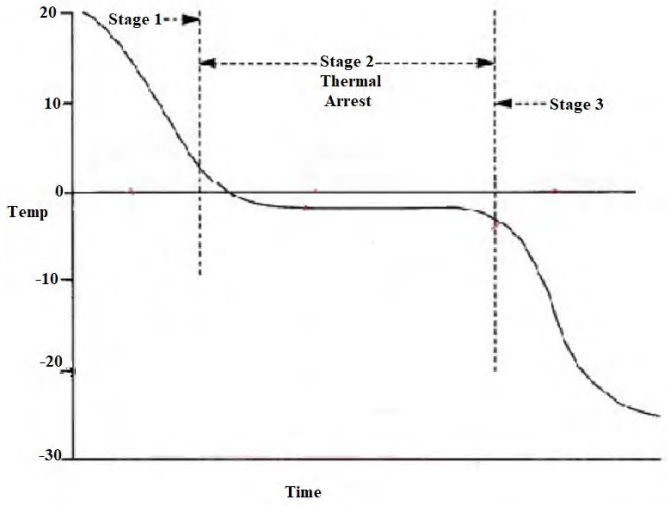
Quality Changes in fish during the chilling/icing

- *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 2ºC, 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 ssubcutaneous 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 ^{0}C but proceeds over a range of temperature. Thus, even at -30 ^{0}C, 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 occur 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 ^{0}C to -2 ^{0}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^{0}C to -5^{0}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 -30^{0}C such that the thermal centre of the fish attains -20^{0}C prior to its removal from the freezer. The time taken to lower the temperature of the thermal centre to -20^{0}C is termed as the freezing time. Based on this, most of the commercial freezers operate at temperatures of -35^{0}C to -40^{0}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 ttechniques have evolved with different modes of operation and the ffirst 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 ffreezing 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 –40 ºC. 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 easyy 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 clean-up 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 refrigerant, 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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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°C, and this thermal process lethality time is termed the F_0 value. Although there are other microorganisms, for example *Bacillus stearothermophilus*, *B. thermoacidurans*,and *C. thermosaccolyaticum*, which are *thermophilic* in nature (optimal growth temperature ~ 50–55°C) and are more heat resistant than *C. botulinum a* compromise on the practical impossibility of achieving full sterility in the contents of a hermetically sealed container during commercial heat processing, whereby the initial bacterial load is destroyed through sufficient decimal reductions to reduce the possibility of a single organism surviving to an acceptably low level. This level depends on the organism, usually *Clostridium botulinum*, which the process is designed to destroy. The time required to reduce the number of spores of this organism (or any other micro-organism) by a factor of 10 at a specific reference temperature (121.1°C) is the decimal reduction time, or *D* value, denoted D_0. The D_0 value for *Clostridium botulinum* spores can be taken as 0.25 minutes. To achieve a reduction by a factor of 10^{12}, regarded as an acceptably low level, requires 3 minutes at 121.1°C, and is known as the process value, or *F* value, designated F_0 so, in this case, F_0 = 3, which is known as a botulinum cook which is the basis of commercial sterility.

Thermal resistance of microorganisms

For establishing a safe thermal processing, knowledge on the target microorganism or enzyme, its thermal resistance, microbiological history of the product, composition of the product and storage conditions are essential. After identifying the target microorganism, thermal resistance of the microorganism must be determined under conditions similar to the container. Thermal destruction of microorganism generally 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.

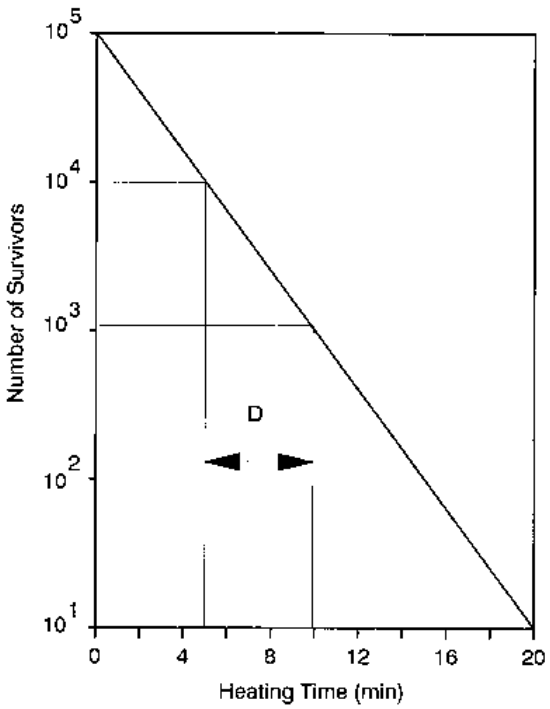

Fig 1. Survivor curve

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

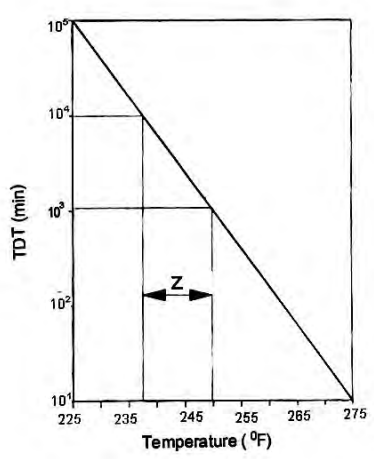

Fig. 2 TDT Curve

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

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

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

Thermal Process Severity or F_0 value

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

$t = D (\log No - \log N_t)$ 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 centre for food products heated by conduction method. Convection heat transfer involves the transfer of heat from one location to the other through the actual movement or flow of a fluid. The slowest heating point for convection heated products in cylindrical metal container is approximately $1/10^{th}$ up from the base of the container. Packaging material forms the most important component of thermal processed foods. It should be able to withstand the severe process conditions and should prevent recontamination of the product.

Containers for thermal processing

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

Glass containers

Glass is a natural solution of suitable silicates formed by heat and fusion followed by immediate cooling to prevent crystallization. It is an amorphous transparent or translucent super cooled liquid. Modern glass container is made of a mixture of oxides viz., silica (S_1O_2), lime (CaO), Soda (Na_2O), alumina (Al_2O3), magnesia (MgO) and potash in definite proportions. Colouring matter and strength improvers are added to this mixture and fused at 1350 – 1400 ºC 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, eg; beer can.

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

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

Aluminium cans

Pure aluminium of 99.5 to 99.7 % purity is alloyed with one or more elements like magnesium, manganese, zinc, copper etc. to obtain the desired composition. Aluminium alloyed with 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°C (sterilization), the thermal treatment has to be performed under pressure in pressure cookers, also called autoclaves or retorts. Simple autoclaves are generally vertical ones with the lid on top. Through the opened lid, the goods to be sterilized are loaded into the autoclave. The cans are normally placed in metal baskets. The autoclave and lid are designed to withstand higher pressures up to 5.0 bar. These types of autoclaves are best suited for smaller operations as they do not require complicated supply lines and should be available at affordable prices. Larger autoclaves are usually horizontal and loaded through a front lid. Horizontal autoclaves can be built as single or double vessel system. The double vessel systems have the advantage that the water is heated up in the upper vessel to the sterilization temperature and released into the lower (processing) vessel, when it is loaded and hermetically closed. Using the two–vessel system, the heat treatment can begin immediately without lengthy heating up of the processing vessel and the hot water can be recycled afterwards for immediate use in the following sterilization cycle. In rotary autoclaves, the basket containing the cans rotates during

sterilization which enhances the heat penetration resulting in reduced process time. This technique is useful for cans with liquid or semi-liquid content as it achieves a mixing effect of the liquid/semi-liquid goods. Water immersion retorts are also used in the industry for thermal processing which is advantageous over steam retorts due to its uniform temperature distribution as there is no possibility of forming air pockets in the retort which limits the heat transfer in steam retorts. At the final stage of the sterilization process the products must be cooled as quickly as possible by introducing cold water. The contact of cold water with steam causes the latter to condense with a rapid pressure drop in the retort. However, the overpressure built up during thermal treatment within the cans, jars or pouches remain for a certain period. During this phase, when the outside pressure is low but the pressure inside the containers is still high due to high temperatures there, the pressure difference may induce permanent deformation of the containers. Therefore, high pressure difference between the autoclave and the thermal pressure in the containers must be avoided. This is generally achieved by a blast of compressed air into the autoclave at the initial phase of the cooling. Sufficient hydrostatic pressure of the introduced cooling water can also build up counter pressure so that in specific cases, in particular where strong resistant metallic cans are used, the water pressure can be sufficient and compressed air may not be needed unlike in flexible retortable pouches. After thermal processing, the containers are washed with chlorinated potable water and stored for conditioning for 2 – 4 weeks. Conditioning helps in proper mixing of the ingredients with the fish products and helps in assessing the extent of thermal process severity. If the containers do not show any deformation, it indicates the effectiveness of the thermal processing.

The important steps in canning process are:

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

Encapsulation of bioactive ingredients: Application and challenges

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Introduction

Now-a-days the demand for healthy and nutritional food products is increasing worldwide. Today foods are intended not only to fulfil the hunger and to provide necessary nutrients for humans but also to prevent nutrition-related diseases and improve physical and mental health. In this regard, functional foods play an outstanding role. Functional foods are foods that enriched with functional ingredients to offer health benefits or to reduce the risk of chronic diseases beyond their basic nutritional functions. Bioactive in food are physiologically active components that provide health benefits beyond their nutritional role. Bioactive ingredients include proteins, vitamins, minerals, lipids, antioxidants, phytochemicals and probiotic bacteria. These bio actives are very sensitive and their application in food is a great challenge to the industry without affecting their properties. Encapsulation technology has proven to be an excellent method to protect the sensitive food ingredients and to develop the novel foods formulations with improved properties. Microencapsulation defined as a process of coating small particles of solids, liquids, or gaseous components, with protective coating material. Microcapsules or micron size ranged from 2-5000 μm. In the food industry, the microencapsulation process can be applied for a various purpose such as (i) to protect the core material from degradation and to reduce the evaporation rate of the core material to the surrounding environment; (ii) to modify the nature of the original material for easier handling; (iii) to release the core material slowly over time at the constant rate; iv) to prevent unwanted flavor or taste of the core material; v) to separate the components of the mixture that would react one another. Depends on the consumer needs, microencapsulation process has been improved constantly. As a result, it has become an example of a dynamic and technological intensive process method, characterized by a fast growth of patent in microencapsulation process and its applications, as well as by an increasing number of scientific research articles.

Overview of microencapsulation technologies

The material that is encapsulated is called as core material, the active agent, internal phase, or payload phase. The substance or material that is encapsulating the core is called as wall material, coating material, membrane, shell, carrier material, external phase or matrix. Two main types of encapsulates are reservoir type and matrix type. In reservoir type, the active agents form a core surrounded by an inert barrier. It is also called single-core or mono-core or core-shell type. In matrix type, the active agent is dispersed or dissolved in an inert polymer. Coated matrix type is a combination of first two (Fig.1).

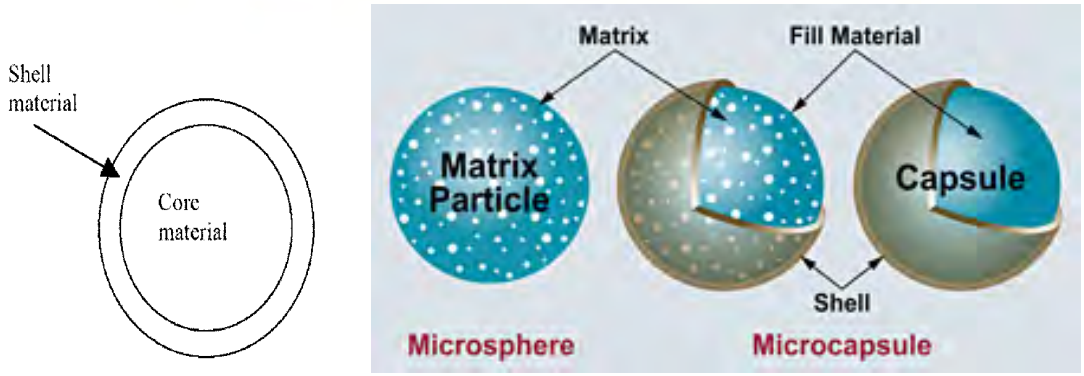

Fig. 1. Morphology of microcapsule

The microcapsules are prepared by a variety of methods. The microencapsulation process can be divided into physical and chemical process. Physical process includes spray drying, spray chilling, rotary disk atomization, fluid bed coating, coextrusion and pan coating. The chemical process includes simple and complex coacervation, interfacial polymerization and phase separation.

Spray drying: The general process of spray drying involves dispersion of a core material into a polymer solution, forming an emulsion or dispersion, pumping of the feed solution/emulsion, atomization of the mixture and dehydration of the atomized droplets to produce microcapsules. Depending on the feeding solution and operating conditions, the size of the microcapsules vary from 10–50 µm or large size particles of 2–3 mm with active load of 5–50 %.

Freeze drying: In this method, the emulsion is frozen at temperature between −90 °C and −40 °C and then dried by sublimation under low pressure. In general, less than 40 % of active load can be achieved by this method. Encapsulates made by freeze drying have particle size ranging from 1 to 100 µm. Advantages are Product with good resistance to oxidation Maintain the shape of microcapsule. Disadvantages are i) High energy use, the long processing time and the open porous structure obtained ii) Compared to spray-drying, freeze-drying is upto 30–50 times more expensive.

Coacervation: In simple coacervation, the oil component is usually dispersed in gelatin solution and then a pH adjustment causes the gelatin to coacervate and form a coating over oil droplets. The subsequent cooling step hardens the coating and encapsulates the oil. Complex coacervation uses two oppositely charged polymers and is one of the most promising technologies for stabilization of omega-3 oils by microencapsulation delivering highest pay load of 40–90%. In this method, the isolated coacervates might be dried by spray drying or fluid bed drying. Encapsulates made by coacervation have particle sizes ranging from 10 to 800 µm.

Fluid bed coating: In this method, process includes i) Preparation of coating solution, ii) Fluidization of core particles iii) Coating of core particle iv) Dehydrate or cool. Encapsulates made this method have particle size ranging from 5 - 5000 µm. Advantage of this method is uniform layer of shell material onto solid particles. Disadvantages are i) Control of air stream and air temperature is a critical factor ii) To achieve uniform coating droplets must be significantly smaller than core.

Extrusion: Process includes i) Preparation of molten coating solution Dispersion of core into molten polymer ii) Cooling or passing of core-coat mixture through dehydrating liquid. Particle size ranging from 200 - 5000 µm. Advantage is product shelf life is long (eg.5 years for extruded flavour oils). Disadvantages are i) Large particles formed by extrusion ii) Very limited range of shell material is available.

Liposome Entrapment: Major process involved are i) Micro fluidization ii) Ultrasonication iii) Reverse-phase evaporation. Encapsulates made this method have particle size ranging from 10 - 1000 µm. Advantages are Liposomes are mainly studied and used as advanced, pharmaceutical drug carriers and their use in foods. Disadvantages are i) Limited due to its chemical and physical instability ii) Low encapsulation yield

Microencapsulation of bio active ingredients

Encapsulation of omega-3 fatty acids

Omega-3 fatty acids belong to the family of polyunsaturated fatty acids that the body cannot synthesize, but are essential for multiple function in human health. Biochemically, omega-3 fatty acids which have their first double bond (unsaturated) in the third carbon from the methyl end. The most important omega-3 fatty acids are alpha linolenic acid (ALA, 18:3 n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaneoic acid (DHA, 22:6n-3). Due to its unsaturated nature, they are susceptible to oxidation and also produce hydroperoxides and off-flavours which are objectionable by consumers. To overcome the above-mentioned problems, the utilization of microencapsulation technique has been studied by various researchers.

Methods and wall material used for microencapsulation of omega-3 fatty acids

Methods: Spray drying, Freeze-drying, coacervation, Electro spraying, Spray granulation and fluid bed film coating etc.

Wall material: Gelatin, maltodextrin, casein, lactose, sodium caseinate, dextrose equivalence, highly branched cyclic dextrin, methylcellulose, hydroxypropyl methylcellulose, n-octenylsuccinate, derivatized starch/glucose syrup or trehalose, sugar beet pectin, gum arabic, corn syrup solids, egg white powder etc.

Encapsulation of polyphenols/flavors

Flavour plays an important role in food products which influences further consumption of foods and provide consumer satisfaction. Commercially available food flavors in liquid forms are difficult to handle or incorporate into food systems. However, many flavor constituents are very sensitive to oxygen, light, and heat. These problems can be solved by encapsulation. Several essential oils such as ginger, garlic, cinnamon, coriander, clove, peppermint, citrus peel, oregano, thyme, rosemary basil, eucalyptus and have been demonstrated various biological properties activities, including antioxidant, antimicrobial, antiviral and anti-inflammatory functions. Several researchers reported that plant polyphenols can slow the progression of cancers, diabetes, osteoporosis and reduce the risks of cardiovascular disease . Due their instability and unpleasant taste (astringency) which needs to be protected or masked before incorporation into food products.

Methods, wall material used for encapsulation of polyphenols

***Methods*:** Spray Drying, Coacervation, Co-crystallization, Freeze drying, Molecular encapsulation, Extrusion, Electrostatic extrusion

***Wall material*:** Maltodextrin, gum arabic, chitosan, citrus fruit fiber, colloidal silicon dioxide, maltodextrin and starch, sodium caseinate-soy lecithin, skimmed milk powder, whey protein concentrate, gelatin, Calcium alginate, chitosan, κ-carrageenan, etc.

Encapsulation of vitamins and minerals

Fat-soluble (e.g. A, D, E, K) and water-soluble (e.g. ascorbic acid) vitamins can be encapsulated by microencapsulation. Iron is one of the most important elements and plays a major role in human health and its inadequate consumption leads to iron deficiency. One of the ways to prevent this problem is fortification of food with iron. But, the bioavailability of iron is affected by interactions of iron with the food ingredients such as tannins, phytates and polyphenols. Microencapsulation can be used to prevent these reactions.

Methods and wall material used for microencapsulation of vitamins and minerals

***Methods*:** Spray drying, Spray cooling and spray chilling, Liposome entrapment, Extrusion, Fluidised bed coating, Coacervation, Molecular inclusion, Liposome entrapment

***Wall materials*:** Tripolyphosphate, cross-linked chitosan, starch, β-cyclodextrin, malto dextrin, gum arabic, Waxes, fatty acids, water-soluble polymers and water-insoluble monomers, soy lecithin, Maltodextrin (DE 7–10), lactose, fructo-oligosaccharide, Polymethacrylate, ethylcellulose, waxes, hydrogenated vegetable oil, stearin, fatty acids, emulsifiers, gums and maltodextrins etc.

Encapsulation of calcium

Soya milk contains much less calcium (12 mg/100 g) than cow's milk (120 mg/100 g), which is undesirable from a nutritional point of view. By encapsulating the Ca salt (calcium lactate) in a lecithin liposome, provides possible to fortify 100g soya milk with calcium up to 110 mg for obtaining calcium levels equivalent to those in normal cow's milk.

Encapsulation of enzymes

Enzymes are biomacromolecules or in other words complex protein molecules with specific catalytic functions and they regulate the chemical reactions needed for the human body. Because of their enormous catalytic power in aqueous solution at normal temperatures and pressures, enzymes are of great commercial and industrial importance. In the microencapsulation method, the enzyme is entrapped within a semipermeable membrane so that the activity of an enzyme is not affected (Table 5). But the movement of the substrate to the active site may be restricted by the diffusional limitations especially when large molecules like starch and proteins are used, which can have an adverse effect on the enzyme kinetics.

Methods and wall material used for microencapsulation of enzymes

***Methods*:** Liposome, Complex coacervation, Spray drying, Liposome entrapment

***Wall materials*:** Alginate, Chitosan/Cacl2 polyelectrolyte beads, Sodium alginate and starch, Chitosan, modified chitosan (water soluble), alginate, calcium alginate and arabic gum, α-amalase, Alginate, carrageenan etc.

Encapsulation of microorganisms

Probiotic bacteria are the live microorganisms that are confer a beneficial physiological effect on the host (humans or animals). These bioactive ingredients have been at the forefront of the development of functional foods, particularly in dairy products.

Wall materials used for microencapsulation of microorganisms

***Methods*:** spray-coating (fluid bed coating), spray-drying, extrusion, emulsion and gel particle technologies (which include spray-chilling).

***Wall materials*:** Alginate and its combinations, High-amylose corn starch, Carrageenan and its mixtures, Gelatin, Mixture of chitosan and hexamethylene di isocyanate etc.

Encapsulation of protein hydrolysate and peptide

Food protein hydrolysates and peptides are considered as a promising functional food ingredient. However, food application of protein hydrolysates and peptides can be inhibited by their bitter taste, hygroscopicity and interaction with the food matrix. These problems can be solved by encapsulation.

Methods and wall material used for microencapsulation of protein hydrolysate and peptide

***Methods*:** Spray drying, Coacervation, Liposome entrapment

***Wall materials*:** Soy protein isolate, gelatin, whey protein concentrate, alginate, maltodextrin, gum Arabic, carboxymethylated gum, pectin, Phosphatidyl choline, phosphatidyl glycine, lecithin, stearic acid and cupuacu butter

Application of microencapsulated bioactive ingredients in food and Pharmaceutical industry

Bioactive ingredients from aquatic secondary raw material has wide food and nutraceutical application. Details are given below.

Bioactive ingredients from aquatic secondary raw material: Fish protein hydrolysate, Fish protein Isolate and Fish protein concentrate, Fish gelatin, Enzymes, Fish collagen, Collagen peptide, Astaxanthin, Fish oil, Chitin, Chitosan and its derivatives

***Food Application*:** Functional ingredient in cereal products, simulated fish and meat products, beverages, soups, gravies, breads, cakes, Mayonnaise, stabilizer, thickener, or texturizer in foods, ingredient for the production of functional fishery products etc.

***Nutraceutical applications*:** Used as capsules, slow release matrices, sponges, scaffolds and "smart" hydrogels for treating obesity, cancer, blood glucose stabilization, weight management. and antihypertension etc.

Challenges and future prospects

Currently, the demand for nutraceutical product from marine source are increasing day by day. Apart from marine oil and protein, several bioactive ingredients from process discards have entered beverage market as functional and medicinal supplements. The successful seafood waste utilization and management is a great challenge for the seafood Industry and it requires appropriate eco-friendly reprocessing technologies that can convert all the valuable components present in the waste into valuable products. The major issues related to processing of secondary raw material is that lack of awareness in bioactive and nutraceutical ingredients from seafood waste, lack of cost-effective process to convert waste to value added products, finally inappropriate cold chain management from the source of generation to the point of conversion to valuable product. Hence, improved utilization of fish processing discards reduces bioactive ingredient loss and can help reduce the pressure on the environmental pollution.

Value Addition of Fishery Resources

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Introduction

The popularity of fish and fishery products in the domestic, as well as the international markets, is increasing day by day at a greater pace. Fish is considered as a superfood for humans because of its high-quality protein content, n-3 polyunsaturated fatty acids (PUFAs), minerals, vitamins, and other trace elements. **It is recommended to consume fish at least two times per week as part of a healthy diet. Apart from the nutritional and health benefits it offers, it plays an important role in building the economy as it is** one of the most traded commodities and a regular food item in the diet of a large population. To reach customers around the world, product diversification and adoption of international flavours preferably ethnic are of great importance. Better utilization of the available fishery resources especially underutilized catches can be attained by adding value which increases the utilization in an improved way by modifying the products in a convenient manner as demanded by the consumers.

Value addition is the enhancement added to a product before it is offered to the customers. It can be defined as "any additional activity that changes the nature of the product which leads to an increase in price at the time of sale". The category of value-added products includes 'Ready to Eat', 'Ready to Cook', 'Ready to Fry', 'Thaw and Eat', 'Heat and Serve', retail raw branded products, fishery pharmaceutical, and cosmetic products of high unit value in the export market. These products are gaining wide acceptance as modern customers prefer convenience products mostly ready-to-cook foods and of course due to the increasing trend of fast food gastronomy. Adding value to a product can be achieved through improving the existing market forms of the products, processing convenience food, and developing functional foods. Innovative products with multiple formats or shapes or dimensions, flavours, texture profiles, and new packages will attract customers without any difficulty. The process of value addition is of greater use because it increases the production and productivity, enhances the shelf life of the products, improves the safety of the food, reduces wastage and discards of the fishery resources, increases the utilization of all kinds of available fishery resources, satisfying the changing customer needs and demands, product and market diversification and better income through the sale of the products. Different categories of such value added products from fishery resources are discussed in this chapter.

Different product styles of fish

Raw and processed fish are marketed in different appearances, shapes, dimensions, and formats for attracting customers, convenience, and for the intended use. Chilled, frozen, and cooked fishes are available in a variety of such product styles in the modern market. A few examples of such product styles of finfishes are cleaned whole fish, drawn fish (only the entrails removed), dressed or pan-dressed fish (fins, head, and tail removed), steaks, fillets, sticks, butterfly style (dorso-ventral cut), chunks, cubes, etc. A few examples of the product styles of shellfishes are peeled and deveined shrimp, peeled, cooked and tail-on shrimp, headless

shrimp, shrimp head on (centre peeled), shrimp head on cooked (centre peeled), barbecue shrimp (beheaded, deveined, peeled with a bamboo stick pierced into the meat from head to tail portion), sushi (cooked butterfly shrimp), skewered shrimp (4-5 shrimps are arranged in a skewer in an inverted "U" shape), squid tubes, squid rings, live lobster, frozen lobster tails, whole lobster frozen or chilled, whole cooked and frozen lobster meat, whole or shucked molluscs, etc.

Chilled fish products

Chilled fish is an important value-added product that dominates the market in terms of revenue. Chilling is the most widespread and effective primary preservation method used for the short-term preservation of fish. The ice and fish are packed in alternative layers in the ratio of 1:1 (fish: ice, w/w) or the temperature is lowered by keeping the fish in slurry ice. The temperature is lowered near to 1 to 4^0 C which aids in preservation by arresting all enzymatic changes that take place inside the fish, killing mesophilic bacteria and inhibiting the growth of all spoilage bacteria. Chilling should be done quickly as possible after the harvest of the fish to get high-quality end products. The most common type of ice used is flake ice or crushed block ice. Prime quality chilled fishes usually fetch more price than frozen fish. The shelf life of the fish stored in a chilled condition depends on the shape, size, fat content, skin characteristics, etc. Round, small fatty fishes with thin skin will spoil faster compared to large, flat, lean fishes with thick skin. Generally, lean fishes will have a shelf life of 12-16 days, fatty fishes 5-8 days, prawns 8-10 days, and cephalopods 4-8 days. The application of modern packaging techniques such as vacuum packaging, modified atmospheric packaging, and active packaging considerably increases the shelf life of chilled fish products. Chilled sashimi grade tuna from bluefin, bigeye, and yellowfin tuna is a major delicacy in the international market. Other than raw fish, chilled processed fish products like smoked and marinated fishes are also available in the market.

Frozen fish products

Freezing is known as a modern method of preservation intended for the long-term preservation of fish at low temperatures. It is considered as a gentle method as the organoleptic qualities of the properly stored frozen fish are as good as fresh fish. During the process, the water in the fish is separated out from other food components and converted into ice. The water in fish flesh begins to freeze at about –1 ˚C. As the temperature drops below –1 ˚C, more water is frozen out and the concentration of salts in the remaining water rises so that its freezing point is lowered further. The ideal condition for fish to be frozen is –30 ˚C for 2 hours. Frozen fish fillets and steaks are popular in domestic markets whereas block frozen fish and individually quick frozen (IQF) fish play a major role in the international markets. In IQF technology, the fish is frozen individually in the highest quality possible. Moisture-proof thermoform moulded trays are best for such products to store at -30 °C. IQF Head on/Headless/Butterfly cooked/Blanched shrimp, IQF tray packed shrimp, IQF peeled Tail-on cooked shrimp, IQF marinated shrimp, Skinless and boneless fish fillets, IQF cooked/blanched squid/cuttlefish, Stuffed squid IQF tray packed, IQF tray packed lobster meat, whole cooked lobster, lobster tails, lobster meat, squid tubes, squid rings, fan tail, and round tail-on shrimp, stretched shrimp (Nobashi), skewered shrimp, boiled clam meat, etc. are few examples of frozen fish products popular in the market. The expected shelf life of frozen fish is 9 months to 2 years. Plate

freezing, air blast freezing, and cryogenic freezing are the other methods of freezing widely accepted by the industry.

Ethnic Fish Products

As fish is a highly perishable commodity preservation by various means is of utmost importance to extend its shelf life. A major portion of fish is consumed as fresh but still, a considerable portion is preserved. This will help to make the availability of fish in lean periods. Preservation helps to keep the fresh fish edible for a longer period of time. There are many age-old, region-specific practices to preserve the fish that include drying, salting, smoking, marination, and fermentation. All these traditional fish preservation techniques follow centuries-old indigenous knowledge of processing together known as curing methods. The customer demand for ethnic flavours and cuisines is ever increasing due to market expansion, globalization and hence they are upgraded as specialty food products.

Dry fish products

Drying is one of the widely used, oldest, and cheapest method of fish preservation in which the moisture content of fish is removed by evaporation to arrest microbial and enzymatic spoilage. Dried fishes with and without salt are popular in domestic markets as well as in overseas markets. In India, around 17-20 % of the total fish catch is converted to dried products. Fish drying can be done by natural and artificial means. Natural drying or sun drying is the process in which fishes are dried under sunlight. Here solar energy is used to evaporate the water in fish. Generally, fishes are suspended in bamboo poles or any other support or laid out flat on the open ground for getting dried. In artificial drying or dehydration, the fish is dried mechanically in an enclosed atmosphere under a controlled condition, unlike natural drying where we have no control over the environmental condition. At present, solar drying is of great demand, in which energy of the sun is collected and concentrated to produce optimum temperature for drying the fishes. The fish can be dried hygienically in solar driers without any energy cost even when the relative humidity is high. One simplest model is a solar tent drier. Solar driers with electrical backup, LPG backup, etc. are available in markets. The ideal temperature for fish drying is 44-55 °C. There is high demand for spiced and dried products, flavour incorporated products, coated and dried products in the modern market. Entrepreneurs are attracted to this business as it is highly profitable that requires less sophisticated machinery and storage facilities. The dried products can be stored in dry conditions at ambient temperature for a minimum of six months if properly dried and packed.

Salted fish products

Salting is a method in which common salt (sodium chloride) is used to preserve the fish. Salting is practiced as such or in combination with drying or smoking. The penetration of salt into the fish tissue removes the water inside, thus reducing the water activity which will help to inhibit spoilage by bacteria. Along with this, enzymes also get inactivated which further delays the spoilage. Generally, small-sized fishes are salted directly without removing head, fins, and entrails, unlike large and medium-sized fishes. For attaining proper salting and drying, the fish can be cut to butterfly-style, small pieces or scoring can be done to increase the surface area. Layer salting is preferred for medium and large-sized fishes whereas small-sized fishes can be salted by dip treatment for uniform penetration of salt through flesh. Fish to salt ratio for layer salting is in the range of 2:1 to 10:1 for big to small-sized fishes. Dip treatment can be done for 5-10 min in a 5 % brine solution.

Smoked fish products

Smoking is a very popular method of fish preservation, especially in the North-eastern states of India. Smoked fishes are known for their unique aroma, texture and its golden yellow colour imparted by wood smoke. The characteristic colour and flavour are imparted by the phenolic compounds present in the smoke. Heavily salted fishes were used to smoke for a longer period of time to get 'Hard cures'. This method combines salting, drying, and preservation by smoke components produced during the thermal breakdown of wood. Smoking of fish is usually done as an intermediate step in fish canning also. There are two categories of smoked products available, cold smoked and hot smoked products. Cold smoked products are usually made in traditional chimney kilns by smoking the fish for 36-72 hours at a temperature maximum of 40 °C. The fish is smoked and dried at 75 °C -80 ° C in case of hot smoking, unlike cold smoking this high temperature gives cooking partial sterilization effect on fish flesh. More conveniently, commercially available liquid smokes can be used to impart the aroma to fish products. Masmin of Lakshadweep is a very popular smoked fish product.

Marinated fish products

The value of fresh, frozen, salted, and dried fish can be increased by the process of marinating it with spices, sugar solutions, oil, plant extracts, acids like vinegar, fruit juice, and wine to enhance the flavour, tenderness, juiciness and also to extend the shelf life. These products are attracting customers because of their typical flavour and textural properties. Traditionally, acetic acid and salt were used for the marination process. Marinades are semi preserves, in which acetic acid inhibits microorganisms, giving characteristics succulence and tenderness. The addition of acid will favour the action of proteolytic enzymes and partial breakdown of protein into amino acids. The addition of salt aids in the extraction of salt out from the fish tissues and helps in coagulation of protein. The addition of plant extracts, spices, sauce, cream, oil, mayonnaise, etc. can increase the flavour and shelf life of marinades further. There are three types of marinades. Cold marinades or 'marinade proper', as the name indicates the process does not involve any heat treatment of fish or ingredients used. The entire processing and further storage take place at a temperature of 10-12°C. The product is having a shelf life of several months at chill storage. Cooked marinades or 'jellied products' are generally packed in a jelly. Here acid-salt treated fish is further heat treated for better preservation. Low pH is maintained to avoid harmful bacteria, especially *Clostridium botulinum*. The shelf life of such products is 6 months. In the case of fried marinades, the pre-treated fish with acid and salt is baked or broiled in oil with or without breading. Then this can be immersed in acetic acid or sauce. Higher temperature inhibits the growth of most bacteria. The shelf life can be up to one year if properly stored at 0-8 °C.

Fermented fish products

Fermented fish products are mainly popular in north-eastern states in India. They are upgraded as specialty fish products because of their unique aroma usually described as umami. Fermented products have a meaty flavour and they are rich in nutrients. The process of fermentation is an age-old practice of fish preservation in which complex protein molecules in the fish are broken down into simpler molecules by the action of organic catalysts, enzymes, or ferments which are stable at normal temperatures of storage. The method is suitable for both freshwater and marine fishes. Fermented products are of three distinct types, products in which

fish retains its original form e.g. cured fish, products in the form of paste, and products in the form of liquid that is fish sauce.

Fish pickle

Fish pickle is a widely accepted ethnic product commercially and a common product in households. Pickling is also a curing method in which edible products are preserved through anaerobic fermentation in brine or immersion in acid with spices. People relish this spicy adjunct with sour flavour as a food accompaniment to make the food palatable and appetizing. Vinegar is the preservative and flavouring agent used in fish pickles. Acetic acid aids in preservation by restricting the growth of spoiling microorganisms. Vinegar pickles are known as fresh pickles or quick pickles. The added salt in the pickle can actually add flavour to it, helps in extracting the excess water from fish, unlocking the flavourful juices, concentrating the juices, and ultimately gives a firm texture to the fish meat. The oil content in the pickle seals off the air from the pickle which helps to enhance the shelf life. The flavour can be improved by adding seasonings. The process of pickling enhances the shelf life to six months and more. Any fleshy fish can be used for preparing fish pickles like tuna and seer fish. It is important to maintain the pH of fish pickles below 4.5 to reduce microbial activity.

Mince based products

Mince is the edible fish meat that is separated from the inedible portions like the scale, skin, fins, and bones. It can be prepared by manual hand picking or by mechanical deboning technique. The fish mince serves as an intermediate stage for the preparation of a variety of value-added products. The fish mince devoid of inedible portions is consumer friendly in usage. Low value fishes with white meat are mainly preferred for the preparation to increase the utilization and demand of such resources by adding value to it. Fish mince-based products available in the market include fish sausage, fish sandwich spread, fish wafers/crackers, fish cookies, momos, papad, spring roll, samosa, fish flakes, fish spirals, etc.

Extruded products

The process of extrusion is one of the popular methods of processing wherein soft mixed ingredients are forced through a perforated die designed to produce products of the required shape, size, and texture. There is a greater demand for snacks and ready to prepare products in the market. In the process, small granular food or powdered particles are reinforced into large pieces. The process of Extrusion cooking or thermoplastic extrusion is considered as a High-Temperature, Short-Time (HTST) process, used mainly for developing cereal-based products rich in calories. The nutritional value of such products can be further increased by the addition of protein rich fishes. During the process material fed into the extruder gets compacted, softens, gelatinized, and/or melts to form a plasticized material. The combined effect of high temperature and mechanical shear causes gelatinization of starch and denaturation of protein. The technology is used to develop pasta, crackers, baby food, snack foods, dried soups, dry beverage mixes, etc. The utilization of low-value fishes can be enhanced through this technology to develop products stable at ambient temperature like fish kure.

Battered and Breaded Products

Battered and breaded products are convenient products of greater demand in which meat protein component is covered by a cereal-based coating. These products are also called as enrobed products or coated products as one food material is coated with another stuff. A coating is referred to as the batter and/or breading adhering to food after cooking. The external

coating forms a stable crispy layer retaining most of the sensory and nutritional quality of the fish product. Coating by battering and breading enhances the appearance, colour, flavour, texture, and nutritional value of the product. It also acts as a moisture barrier by minimizing moisture loss during frozen storage and microwave reheating. It seals the flavour in the product by acting as a sealant that prevents natural juices from flowing out. Wet coatings are referred to as batter. The batter is basically made from wheat flour or corn flour. Coating ingredients generally include polysaccharide, proteins, fats and hydrogenated oil, seasonings and water. A typical ratio of the batter mix to water is 1:2. There are three types of batter. Adhesion batters are mainly starch based that designed to adhere to the product whereas cohesion batters are mainly flour based which forms a shell around the product. Tempura batter is starch/flour based with a raising agent (sodium bicarbonate) for a puffy appearance, usually not followed by breading. Wide variety of bread crumbs are also available in the market like reclaimed and industrial bread crumbs. Deep fried coated products are ready to eat products, it can be par-fried/flash fried for storage (30 second at 190 °C) to cement the breading. The shelf life of stored products under frozen storage is 9-24 months. Fish finger, cutlets, balls, nuggets, coated shrimp, coated squid rings, coated bivalve products, coated fish fillets etc. are the most commonly available form of battered and breaded products. Coated Nobashi is a high value specialty product made from shrimp, literally means stretched shrimp. Nobashi is peeled, deveined tail on shrimp stretched by mechanical means. The length can be increased by about 1-2 cm depending on the size of the shrimp by making parallel cuttings at the bottom and applying pressure using simple mechanical devices. During the coating process, the product will have more pick up due to increased surface area and attract customers because of the aesthetic appearance.

Surimi based products

Surimi is a Japanese term for water washed fish mince. The fish mince devoid of any pigments or blood stains has excellent keeping quality with the added cryoprotectants. It is defined as mechanically deboned fish mince from white fleshed fish that has been washed, refined, and mixed with cryoprotectants for better frozen shelf life. The washed mince will be white in color and have a unique texture that often provides a viscoelastic nature to the end product. Surimi- based products form an important dish in Japanese cuisine. Due to its high gel strength, it is used as an intermediate product used for the preparation of a wide variety of value-added products. Most commonly white fleshed fish with very less fat content is chosen for the product preparation. Analogue products or “imitation products” or “fiberized products” and moulded products like fish ball form an important category under surimi-based products. These products are prepared to mimic the texture, flavor, and appearance of shrimp, crab or scallop even when they are prepared from the commonly available fish from the market. This involves the use of sophisticated technology for preparation and has not gained much popularity in the Indian market. Surimi-based products are popular in developed countries. Kamaboko is a traditional Japanese product prepared from surimi. It is a steamed cake made out of surimi. This product it is known by different names according to the regions of production, ingredients used, cooking method, and shape of the product. Chikuwa is broiled kamaboko in the shape of bamboo. Steamed Kamaboko is called Sumaki or Mushiita. Fried kamaboko is called Tenpura or Satsuma Age. Hampen is boiled kamaboko in a square shape.

Canned fish products

The growing popularity of safe packed seafood with enhanced shelf life has fueled the demand for canned fish globally. Canned fish products are ready to eat products. The process of canning or retorting is high temperature long term preservation method in which the food is preserved by the application of heat in a hermetically sealed container to obtain commercial sterility. The filling medium usually used in cans is oil or light brine. The double sealed robust cans maintain sterility throughout the storage period at ambient temperature. Canned tuna, herring, mackerel, and sardines are popular in the markets. Instead of metal cans, now canned products are more common in retort pouches. 3-ply laminated flexible pouches consist of polyester/aluminium/cast polypropylene is widely in use. Canned sardine in oil, tuna chunks in oil and brine, tuna flakes in oil, fish curry, etc. are a few examples of products available in the markets. The expected shelf life of canned fish is minimum one to two years.

Accelerated freeze dried products

Accelerated freeze drying is a novel technology of food preservation in which water from the frozen product is removed by the process of sublimation under vacuum. The method is expensive and finds easy acceptance in the case of high value food products. Properly processed freeze-dried products are comparable with the fresh material in case of flavour, colour, and nutritive value as there will not be product shrinkage, case hardening, thermal degradation of proteins, deteriorative changes in color or flavor and products will get rehydrated rapidly. Further, freeze dried products can be stored under ambient storage conditions without any additional cost for storage and it is convenient to use. The reported shelf life of freeze-dried products is more than two years. Instant fish soup mixes, prawn cakes, pre-cooked ready to serve salads are some products prepared using this technique having consumer acceptance. In India, freeze drying is employed for processing shrimp, squid rings, etc.

Seaweed incorporated products

India aspires to expand seaweed production to at least one million tonnes by 2050 considering the enormous potential of seaweed farming that can contribute to the blue revolution. Seaweeds getting more attention because of their nutrient reserves such as protein, essential amino acids, fibre, iodine, vitamin K and compounds having antioxidant and anti-inflammatory properties, such as polyphenols. Seaweeds are healthy, nutritious, and low-caloric food with low lipid content, rich in ω-3 and ω-6 polyunsaturated fatty acids making them an important food component in the diet at the present time. They are considered as an important part of food, animal feed, and fertilizers. Seaweed based snacks, cookies, biscuits, burgers, nutridrinks, fish soup enriched with seaweed bioactive compounds are novel products with high market potential. Sulphated polysaccharides with bioactive properties can also be extracted from seaweed.

Live Fish

There is a greater demand for live fish for food purposes and it usually fetches a high price as the freshness is ensured in the marketing. Consumers often demand smaller and medium-sized fishes in live form. Grouper, snapper, seabreams, seabass, red tilapia, reef fishes, air-breathing fishes, shrimps, lobster, crabs, clams, oysters, and mussels are examples of candidate fishes for live fish transport. Live fish trade of high-value fish is a lucrative business nowadays as it gives huge profit to the business. But the high rate of mortality of fish during

transport is a big challenge in the trade. The ways to improve the survivability of fish to be standardized for a continuous supply of fresh live fish to the consumers.

Speciality products from secondary raw materials

The term “fish wastes” in general indicates the non-edible portion of fish which includes the head, skin, bone, scale, visceral mass, and trimmings. Besides, fish species having mere or no market value, under-sized fishes as well as spoiled or physically damaged fishes will also be added to this category. By considering the potential for recycling, the term “fish wastes” has been replaced now as “rest raw material” and “secondary raw material”. These waste materials are having potential for recycling as they are good sources of high-quality protein, minerals, fat, etc. and thus they are important sources of different secondary products. The technology has a huge scope, as developed products can be used for human consumption, animal nutrition, and agricultural applications. Different secondary products such as fish meal, fish oil, squalene, collagen, gelatin, chitosan, hydroxyapatite, proteolytic enzymes, pigments, calcium, fish protein concentrate, etc. are of high value having wide acceptance market including the food industry.

Conclusion

Owing to the rich nutritional and health benefits, the demand for fishery products is on an increasing trend globally. The flow of new entrepreneurs with novel value-added seafood products all over the world makes the seafood processing and marketing sector more competitive every day. Value addition of the fishery resources is the pressing priority to utilize the available potential resources sustainably to increase profit without losing it. The modern market demands healthy, nutritious, and tasty convenient products. The value addition of fishery products has immense potential to uplift the livelihood of the stakeholders involved by expanding the array of products available in the markets.

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.

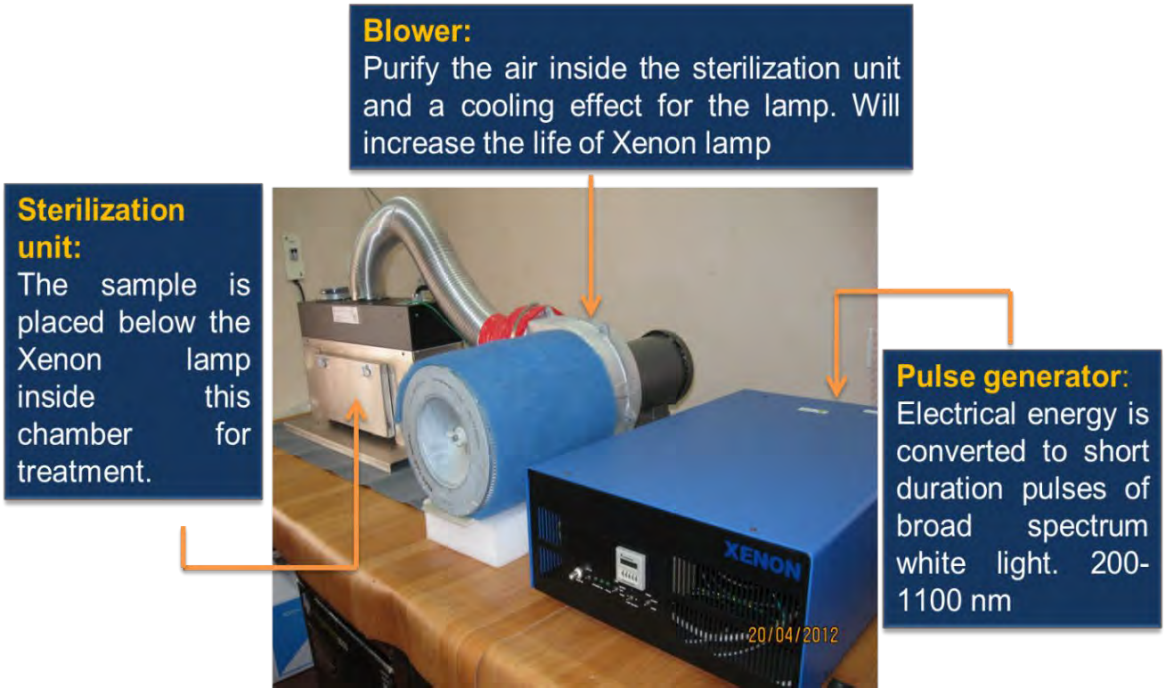

Figure 1: Pulsed Light Equipment of CIFT

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 25–65 ºC.
- 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.

Role of statistics in research

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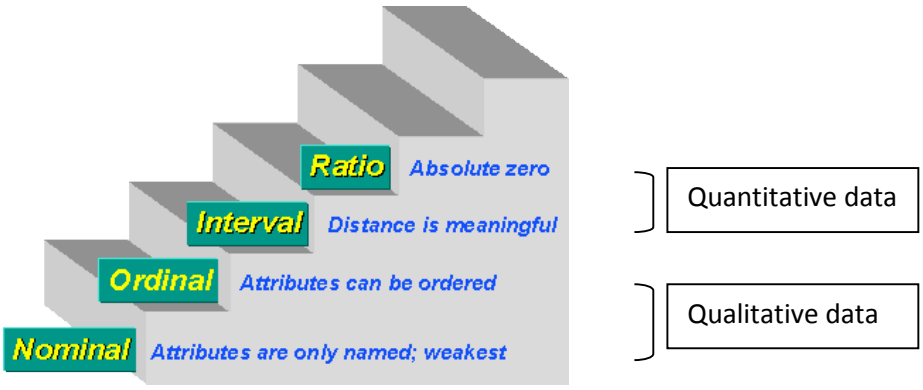
Descriptive Statistics

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, S.Ds 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

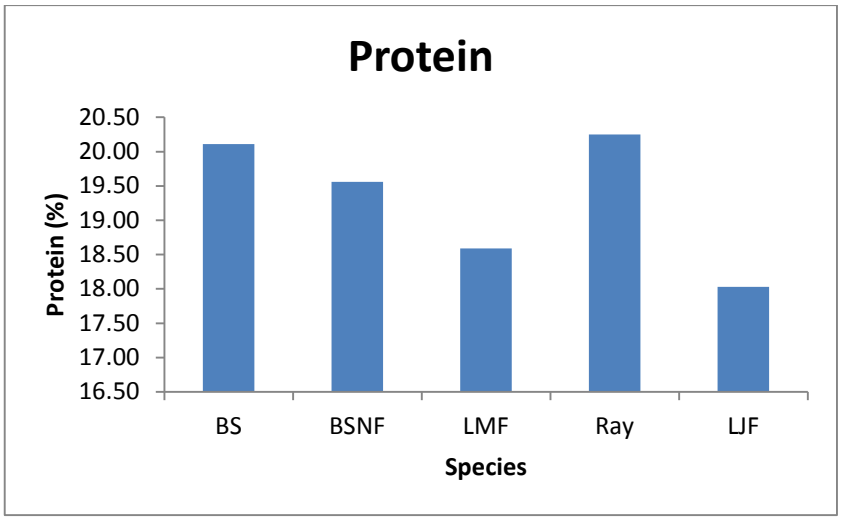
A frequency polygon represents the shape of the data. It can be conceptualized by connecting the midpoints of the classes at the height specified by the frequency.

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.

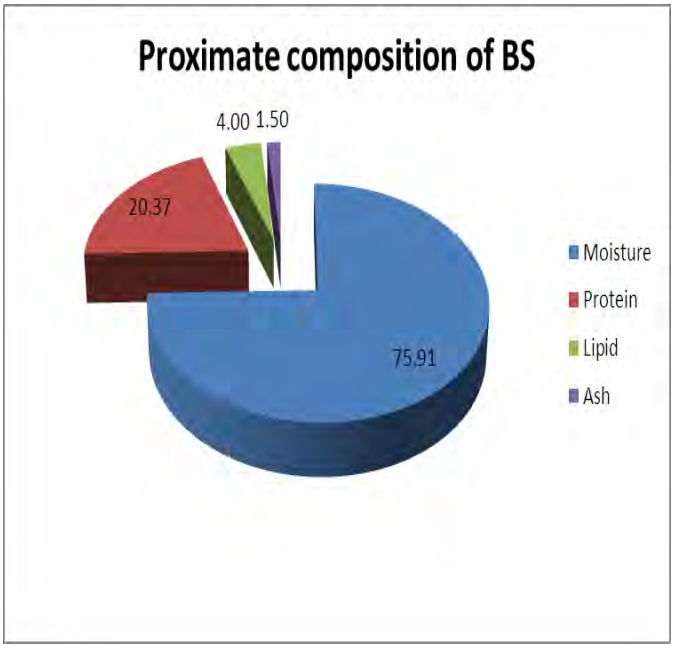
- The qualitative data is summarized in a frequency, relative frequency, or percent frequency distribution
- On the horizontal axis, the labels used for each of the classes are specified
- On the vertical axis, frequency is specified
- The bars are separated to show that each class is a separate category



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.

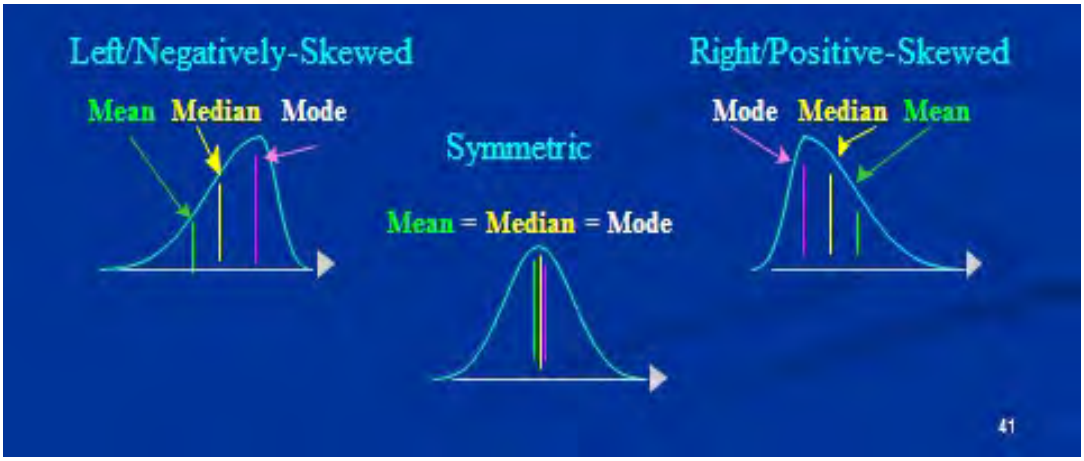
- Commonly used graphical device for presenting relative frequency distributions for qualitative data
- Use the relative frequencies to subdivide a circle (360^0) into sectors that correspond to the relative frequency for each class
- A class with a relative frequency of 0.25 would take $0.25(360) = 90^0$ of the circle

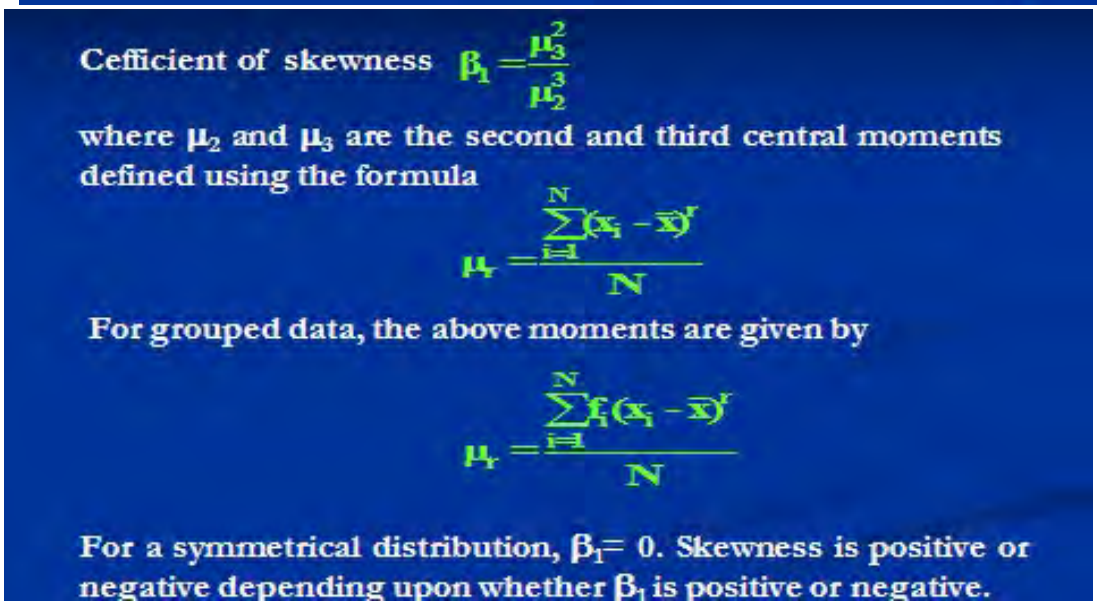


Distribution of a given data

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 $\boldsymbol{\beta_2} = \frac{\mu_4}{\mu_2^2}$)





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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Packaging of Fish and Fishery products

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1.0 Introduction

Packaging is crucial to our modern food distribution and marketing systems. Without protective packaging, food spoilage and wastage would increase tremendously. The advent of modern packaging technologies and new methods of packaging materials made possible the era of convenience products. In the past packaging emphasized the expectations of the producers and distributors but now it has shifted towards the consumer since they are becoming more demanding and aware of different choices to choose from. A food package usually provides a number of functions in addition to protection. Fish is one of the most perishable of all foods. The best package material cannot improve the quality of the contents and so the fish must be of high quality prior to processing and packaging. Different products have different packaging requirements and it is important to choose suitable packaging material accordingly. The intended storage conditions of the product, i.e., temperature, relative humidity and expected shelf life have to be known. Multilayered plastics are very popular since properties of different films can be effectively used to pack different products. The basic function of food packaging is to protect the product from physical damage and contaminants, to delay microbial spoilage, to allow greater handling and to improve presentation.

2.0. Types of Packaging Material

2.1. Glass

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

2.2. Cans

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

Polymer coated two-piece cans of 6 oz capacity (307 x 109) with a universal polymer coating can be widely used for a variety of products. The can is made of Electrochemically chromium coated steel (ECCS) plate with clear polyethylene terephtahalate (PET) coating on either side. The finished plate has a thickness of 0.19mm (0.15 mm of base steel + 20 µ PET

coating on either side). The cans are made out of the steel plate by draw and redraw (DRD) process. The chromium coating along with the PET coating provides the can with a smooth, greyish, glistening appearance in addition to act as a barrier between the product and the base steel. The bottom of the can is designed for better stackability so that it can be stacked vertically without risk of toppling on the shelf. This also helps to reduce the storage space requirement for the cans. These cans are found to be suitable for thermal processing of fish and fish products. These cans are having easy open ends. Metal cans are advantageous as packages because of superior strength, high speed manufacturing and easy filling and dosing. Disadvantages of metal cans are weight, difficulty in reclosing and disposal.

2.3. Paper

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

2.3.1. Paper board

Thicker paper is called as paper board. There is not a clear cut dividing line between the heaviest grade of paper and the lightest board. The lightest standard board is 0.19 mm thick and heavy papers are of 0.125 mm thickness. Paper boards are used for making corrugated fibre board cartons.

2.4. Polymer Packaging

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

2.4.1. Low Density Polyethylene (LDPE)

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

2.4.2. High Density Polyethylene (HDPE)

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

2.4.3. Linear Low-Density Polythene (LLDPE)

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

2.4.4. Polypropylene (PP)

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

2.4.4.1. Cast polypropylene (CPP)

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

2.4.4.2. Oriented, Heat set Polypropylene (OPP)

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

2.4.5. Polystyrene

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

2.4.6. Polyester

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

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

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

2.4.7. Polyamides (Nylon)

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

2.4.8. Polyvinyl Chloride (PVC)

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

2.4.9. Copolymers

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

2.4.10. Aluminium foil

Aluminum foil is defined as a solid sheet section rolled to a thickness less than 0.006 inches. Aluminum has excellent properties like thermal conductivity, light weight, corrosion resistance, grease and oil resistance, tastelessness, odourlessness, heat and flame resistance, opacity and non-toxicity. Aluminum foil free from defects is a perfect moisture and oxygen barrier. In all flexible packaging applications using aluminum foil where good moisture and oxygen barrier properties are important, the foil is almost always combined with heat sealing media such as polythene or polypropylene. It is the cheapest material to use for the properties obtained. Foils of thickness 8 to 40 microns are generally used in food packaging. Foil as such is soft and susceptible for creasing. Hence, foil is generally used as an inner layer.

3.0. Packaging of fresh fish

A suitable package for fresh fish should keep the fish moist and prevent dehydration, retard chemical and bacterial spoilage, provide a barrier against moisture and oxygen to reduce fat oxidation and prevent permeation of external odors. Generally baskets made of split bamboo, palmyrah leaf and similar plant materials were traditionally used for packing fresh iced fish. However, they do not possess adequate mechanical strength and get deformed under

stacking. The porous surface of these containers tends to absorb water and accumulate slime, creating an ideal breeding ground for spoilage bacteria, which can contaminate the fish. Even though washing cleans the contaminated surfaces of the container it has been shown to be ineffective in reducing the bacterial load significantly. Sharp edges of bamboo also cause bruises on the skin of fish. Used tea chests provided with 2.5 cm thick foamed polystyrene slabs inside have been found extremely beneficial for transport of fish over long distances up to 60 h duration.

Modern insulated containers are made of HDPE or polypropylene with polyurethane insulation sandwiched between the inner and outer walls of the double walled containers. They are durable and in normal use have a life span of over 5 years. Materials such as aluminium, steel and fibreglass are also used in the construction of insulated containers. Insulation properties of these containers depend on the integrity of the layer of insulation. Contamination of insulation layer with water drastically reduces insulation properties of the medium. An insulated corrugated polypropylene container which is the lightest of all packages is used for iced fish transport. It lasts for 5 trips and being of collapsible design and lightweight, return of empty container is very easy. The use of fibreboard containers for the transportation of iced fish and frozen fish showed that fish could be transported in good with effective insulation.

3.1. Packaging of frozen fish

World trade in frozen fishery products has been increasing every year. Fish being highly perishable transportation and storage of frozen fishery products requires a cold chain and these fishery products are to be stored at temperatures below –18° C. Fishery products are frozen at – 40° C. However cold storage temperature where they are subsequently stored varies from -30 to –18° C. The enzymatic activities bring about deteriorative changes like rancidity in frozen fish products. Exposure to low temperatures for a long time may result in freezer burns. Hence for extending shelf life and further storage, packaging is of absolute importance. To get a quality frozen product in perfect condition the package must provide protection against dehydration, oxidation, flavour and odour loss and physical changes. Evaporation of moisture from the surface of the fish may occur resulting in freezer burns. In order to overcome these problems suitable packaging is absolutely necessary. The advantages of packaging frozen fish are, prevention of dehydration, prevention of rancidity in fatty fishes, protection against contamination and physical damages, convenience of handling the product and using a portion of the product, retention of flavour and colour attractive appearance of the product and to allow pack for thawing without leaching.

3.1.1. Primary wrap for block frozen products

The material used as a primary wrap for contact with the food is mainly Low-density polythene (LDPE). This can be in the shape of a bag or a film. Usually 2 kg or 5 lbs fish is packed along with 10-20 % glaze. Glazing should be optimum at the recommended level, since this will add to cost and weight during packaging and transportation. Alternately, films of high molecular weight high-density polyethylene (HM-HDPE), which is not as transparent as LDPE film are also used being more cost effective. 100-gauge LDPE is used for wrap while 200 gauge is used for bag. The corresponding values for HDPE are 60 and 120 gauge. Polythene films should be of food grade conforming to IS: 9845 specifications.

3.1.2. Duplex carton/ Inner carton

There are four types of cartons used for packaging of seafood products, which are top opening, end opening, end loading and tray type. In top opening carton system filling is done from the top. This is mainly for filling larger pieces of fish and cephalopods. End opening type cartons are used when the product is smaller and free flowing, like packaging of fish curry or soup. Here the carton is coated with polyethylene on both the inside and outside. The end loading system feeds the product from one end into a horizontal glued carton. End flaps are heat sealed or closed by tucks in flap. End loading is suitable for products packed in aluminium /carton trays. Tray type cartons consist of cartons systems/ polypropylene trays, which are sealed with a lid and used for production of frozen pre-cooked food that will be heated and thawed in the package itself. To withstand heating, the board is coated with polypropylene.

The frozen blocks are wrapped in film and then packed in duplex cartons. A number of such blocks are packed in a master poly bag and then packed into master cartons. The carton should have details like net weight, type and size, name and address of the producer and the country of origin.

3.1.3. Master carton

In the case of frozen shrimps about 6 units of 2 kg each or 10 units of 2 kg each are packed into master cartons. Corrugated fiberboards are used for the packaging of frozen fish. They may be of virgin material and having three or five ply with liners. The cartons may be wax coated or supported with liner paper with higher wet strength to make it moisture resistant. The specifications for master carton vary depending upon the country or the type of pack.

3.1.4. Strapping and tying

Boxes are now mainly closed at the top and bottom by using cellophane tapes. They are also stapled or strapped by using polypropylene / high density/ rayon extruded straps. The straps are clipped or heat-sealed. The tensile strength must be great enough to withstand the load. For polypropylene the fluctuations in the tensile strength and elongation at break (%) at –20°C are comparatively less. Hence this material is most suitable when compared to HDPE where the tensile strength and elongation at break vary.

3.1.5. Packaging of Individually Quick Frozen (IQF) Products

Packaging requirements of IQF shrimps vary from those of block frozen. IQF shrimps are mainly packed for retail marketing in consumer packs ranging from 100g to 5 kg. An IQF pack has a single glaze on its surface and because of the larger surface area, they are vulnerable to several risk. Essential characteristics required for packaging materials of IQF shrimps are

- Low water vapour transmission rate to reduce the risk of dehydration
- Low gas/oxygen permeability, thereby reducing the risk of oxidation and changes in colour, flavour and odour
- Flexibility to fix the contours of the food
- Resistance to puncture, brittleness and deterioration at low temperatures.
- Ease of filling

IQF shrimps are filled in primary containers along with code slip and weighed. Bar coding is nowadays adopted which will depict various product and inventory details through a series of bars. Bar coding is compulsory for products imported to the EEC and US markets. The product is filled into primary pack which heat sealed and further it is packed in master

cartons for storage and transportation. The primary pack may be plastic film pouches (monofilm co-extruded film or laminated pouches). The unit pouches may be provided with unit/intermediate cartons or directly packed into master cartons. The unit/intermediate cartons are made of duplex or three ply corrugated fibreboard laminated with plastic film on the inside and outside to improve the functional properties as well as aesthetic value of the pack. The most functional cost-effective film has been identified as 10 μ biaxially oriented polypropylene (BOPP). Some duplex cartons are also wax-coated. One major requirement of the master carton is high compression strength to bear weight without damage to the product. Compression strength of 500 kg is the minimum recommended specification, which might give reasonable safety to the product. The cartons are made of 5 or 7 ply corrugated fibreboard.

3.2. Battered and Breaded fish products

This forms an important class of value-added products in convenience form. The battering and breading process increase the bulk of the product thus reducing the cost element. A number of value-added marine products both for export and internal markets can be prepared from shrimp, squids, cuttle fish, certain species of fish and minced meat from low priced fishes. The changes taking place during frozen storage of the value-added products are desiccation, discoloration, development of rancidity etc. Application of proper packaging prevents/retards these changes and enhances shelf life. Conventional packaging materials like flexible plastic films alone are not suitable for these products as they provide little mechanical protection to the products and as a result the products get damaged or broken during handling and transportation. Hence, thermoformed containers are commonly used for this purpose. The thermoformed trays produced from food grade materials are suitable for the packaging of value-added fishery products both for internal and export markets. Trays made of materials like PVC, HIP and HDPE are unaffected by low temperature of frozen storage and provide protection to the contents against desiccation, oxidation etc. during prolonged storage.

3.3. Dry fish

Traditionally, coconut leaf baskets, palmyrah leaf baskets, jute sacks and news paper baskets have been used for packing and transportation of dried fish. These containers only help in transportation of the fish. They do not protect or preserve the fish. The dry fish packed in such containers have a very short shelf life and is usually not of good quality. These fishes are often found to be rancid or have mould growth. Since the packaging is permeable, the product absorbs moisture and gets soggy. Hence these packaging materials afford least protection to the product. Plywood boxes and waxed corrugated cartons are also used for packing large quantities. High density polythene woven gusseted bags laminated with 100-gauge low density polythene are suitable for packaging dried fish. HDPE is impervious to microbial and insect attack. HDPE is a material which will not spoil even if it gets wet. It is hard and translucent and has high tensile strength.

Table.1. Bulk packaging materials and their properties

Type	Merits	Demerits
Waxed corrugated cartons	Handy, light, hygienic and presentable	Very delicate, Not foolproof against insects, rodents, moisture, breakage

Dealwood or Plywood boxes	Compact and strong, Larger quantities can be packed, handling, transportation and stacking are easy, Can be reused, Protection against damage	Comparatively heavy, Cost is high, Cheap wood not easily available
Bamboo baskets	Handy, light, Not costly	Very delicate, Not foolproof against insects, rodents, moisture, breakage
Gunny bag	Light, handy, cheap, proof against breakage	Not foolproof against insects, rodents, moisture, Not hygienic
Dried palmyrah and coconut palm leaves	Cheapest of all and readily available in the coastal regions of India	Not foolproof against insects, rodents, moisture, Not hygienic and does not give good appearance, Packing is laborious
Multiwall paper sack lined with 300-gauge LDPE	Hygienic, presentable and can be printed	Costly, polythene lining may break during handling and hence is not foolproof against insects, rodents, moisture
HDPE woven gusseted bags laminated with 100-gauge LDPE	Hygienic, presentable and can be printed, Stackable, can be packed uniformly	

In the consumer market the dried fish is packed in low-density polyethylene or polypropylene. Due to the high moisture content of about 35 % in certain salted fishes they are often attacked by microbes. Hence fish should be dried to a moisture level of 25 % or below. Packets of different sizes and weights ranging from 50g up to 2 kg and bulk packs are available. Nowadays monolayer and multilayer films, combination and co extruded films are used for bulk packing and consumer packaging of dry fish. Polyester polythene laminates and thermoform containers are used to pack dried prawns and value-added dried products.

Table 2: Consumer packaging of dry fish

Material Composition	**Merits**	**Demerits**
250 gauge low density polyethylene film	Cheap, readily available, good bursting and tearing strength and heat sealability	High water vapour and gas transmission rate, easy to puncture due to sharp spines, smell comes out. Shelf life limited.
250 gauge polypropylene film	Cheap, readily available, good bursting and tearing strength and heat sealability	High water vapour and gas transmission rate, easy to puncture due to sharp spines. Shelf life is limited.
300MXXT Cellophane/150 gauge LDPE	Very low water vapour and gas transmission rate, transparent,	Prone to easy attack by insects, costly.

	good bursting and tearing strength, heat sealability and long shelf life.	
12 micron plain polyester/150 g low density polyethylene	Very low water vapour and gas transmission rate, transparent, good bursting strength, puncture resistance & heat sealability. No insect penetration	Costlier
20micron Nylon laminated with 150 gauge polyethylene	Very low water vapour and gas transmission rate, transparent, good bursting strength, puncture resistance & heat sealability. No insect penetration	Costlier

In consumer packaging 100 to 700 gauge LDPE and PP were found suitable for storing dry fish. It also showed that dry fish when packed in films of higher gauge remained in good condition for a longer period. This is mainly due to the low water vapour transmission rate and oxygen transmission rate, which decrease with increase in thickness. In the case of overall quality 200, 300 and 400 gauge LDPE films also showed promising results. The advantages of low-density polythene are clarity, low water vapour transmission rate, good bursting and tearing strength and heat-sealing capacity. The main disadvantage is the high gas transmission rate which is undesirable in dried fish packaging because the smell dissipates to the surrounding atmosphere.

Dry shell on prawns are packed mostly in duplex cartons or polystyrene trays and then covered with a laminate film. This is mainly due to the fact the spines will puncture the packaging material. Polypropylene pouches of 300 gauge are recommended for salted fishery products with moisture content of 35% and above for obtaining a shelf life of 6 months. The advantages being good clarity, Low WVTR, good bursting strength and tearing strength. Currently laminate films of Polyester/polythene are mostly used for packaging of dried fish. Polyester films are capable of giving good mechanical strength and reverse colour printing can also be done. Polythene is heat sealable and has good food contact application. The keeping quality of dry fish can be enhanced in an air-conditioned room where the temperature and humidity is low.

Dry fish is irregular in shape and size leading to great difficulty in packing. The have spines and projections which may puncture the packaging materials. In the case of jute bags because of its permeable nature, salted fish may absorb moisture depending on the relative humidity of the environment. In the coastal place where RH is always above 80 % this invariably takes place making the fish wet. Thus, a suitable packaging material will ensure protection against migration of moisture and oxygen, and odour and insect attacks.

3.4. Accelerated freeze dried (AFD)

AFD products demand a very high price in the export trade. The final moisture content of AFD products generally is about 2 %. Low moisture content and large surface area make these foods extremely hygroscopic. Most dried products deteriorate when exposed to oxygen. Changes in colour may also take place as a result of bleaching. Light accelerates oxidative reactions and hence contact with light should be prevented. If proper packaging materials are not used there is every chance that the materials may undergo flavour changes due to the

oxidation of the product and also migration of flavour from the packaging material. Since, fish contains fat there may be also a chance of it taking up the taints from the packaging material. The particular structural properties of freeze-dried products lead to damage by mechanical means. The light porous nature causes them to be very fragile and easily prone to breakage during handling and transportation. Freeze dried products are also liable to damage caused by free movement within the package. Measures must be taken to fit the product compactly in the container, while leaving the minimum headspace for filling inert gas.

Rigid containers both glass and cans were used earlier for packaging of freeze dried products. However, now metallised polyester laminated with polythene or aluminum foil /paper/polythenes are used since they have low oxygen transmission rate and water vapour transmission rate. Most of the packages are filled with an inert gas. The product can also be packed under vacuum to give better protection against damage.

3.5. Packaging of thermal process fish products

Retort pouches consist of three or four layers consisting of an outer polyester layer, a middle aluminum layer and an inner cast polypropylene layer. Aluminium foil is the barrier layer which gives the product a longer shelf life. Polypropylene has a high melting point of about 138°C and is used as the inner layer to provide critical seal integrity, flexibility, strength, taste and odour compatibility with a wide range of products. The different layers are held together with adhesives which are usually modified polyolefins such as ethylene vinyl acetate (EVA). Some pouches contain polyvinylidiene chloride, ethylene vinyl alcohol or nylon instead of the aluminium layer to permit viewing of the product. These are foil free laminated materials. These plastics are good barriers to oxygen molecules but are not complete barriers and therefore the shelf life is reduced. There are mainly two types of retort pouches viz, preformed and pouches which are made from laminates on the process line. Preformed retort pouches are more commonly used and they are filled manually or by using automatic filling machines. Sauces and curry products are packed instantaneously in pouches that are produced from laminated rolls which are simultaneously formed, filled and sealed. In case of products with solid contents, either pouch is filled with solids together with some liquid and sealed using a vacuum sealing machine. Once the product is filled and sealed it is then subjected to temperatures of 121.1°C with counter pressure so that the cold point or slowest heating point within the food reaches the predetermined time temperature integral.

3.6. Fish pickles

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

3.7. Fish soup powder

Fish soup powder is a speciality product containing partially hydrolysed fish, protein, carbohydrates, fat and several other seasonings including salt. The product is hygroscopic and hence the selection of the package assumes great significance. Appropriate package developed

for such products are 12-micron plain polyester laminated with LDPE-HDPE co-extruded film or 90-100 micron LD/BA/Nylon/BA/Primacore multilayer films which ensure a safe storage of the product up to six months.

3.8. Extruded products

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

3.9. Surimi and surimi-based products

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

3.10. Fish Sausage

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

3.11. Glucosamine hydrochloride

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

3.12. Chitin and Chitosan

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

3.13. Fish Hydrolysate

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

3.14. Fish Meal

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

3.15. Fish oils

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

Fish oil is also marketed for regular oral dosage in the form softgel capsules. The shell is made of gelatin, water, glycerol or sorbitol. The process of encapsulation is by using the rotary die encapsulation process. The encapsulation process is a FFS operation. Two flat gelatin ribbons manufactured on the machine are brought together on a twin set of rotating dies that contain recesses in the desired size and shape, these cuts out the ribbon into a two-dimensional shape, and form a seal around the outside. At the same time a pump delivers a precise dose of oil through a nozzle incorporated into a filling wedge whose tip sits between the two ribbons in between two die pockets at the point of cut out. The wedge is heated to facilitate the sealing

process. The wedge injection causes the two flat ribbons to expand into the die pockets, giving rise to the three-dimensional finished product. After encapsulation, the soft gels are further dried depending on the product. They are then further packed in glass or plastic bottles. The soft gels are also packed as blister packs.

3.16. Fish silage

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

3.17. Shark fin rays

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

Suggested Reading

1. Gordon L. Robertson (2006). Food Packaging -Principles and Practice, CRC Press, Taylor and Francis, Boca Raton, FL.
2. Kerry. P.J (2013). Advances in meat, poultry and seafood packaging. Woodhead Publishing Ltd. UK.

Fish preservation by smoking

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Introduction

Smoking is an ancient method of food preservation, which is also known as smoke curing, produces products with very high salt content (>10%) and low water activity (~0.85). Smoking is a process of treating fish by exposing it to smoke from smouldering wood or plant materials to introduce flavour, taste, and preservative ingredients into the fish. This process is usually characterised by an integrated combination of salting, drying, heating and smoking steps in a smoking chamber. The drying effects during smoking, together with the antioxidant and bacteriostatic effects of the smoke, allow smoked products to have extended shelf-life. Smoked seafood includes different varieties like, smoked finfish and smoked bivalves. Many of the smoked products are in the form of ready-to-eat.

Developments of modern food preservation technology, such as pasteurization, cooling/refrigeration, deep-freezing, and vacuum packaging, have eclipsed the preserving functions of many traditional methods including smoking. Nowadays, the main purpose of smoking has been shifted for sensory quality rather than for its preservative effect.

Depending upon how the smoke is delivered into the food and smoking temperature, four basic types of smoking can be defined: hot smoking, cold smoking, liquid smoking, and electrostatic smoking. Hot smoking is the traditional smoking method using both heat and smoke, which usually occurs at temperatures above 70 °C. For smoked fish and fisheries products, a minimum thermal process of 30 min at or above 145 °F (62.8 °C) is required by FDA (2001). Therefore, after hot smoking, products are fully cooked and ready for consumption.

Hot smoking

Torry smoking kiln was introduced in the early 1960s by United Kingdom's Torry Research Station. The Torry smoking kiln is considered as a model for the modern smokers/smokehouses by enabling the precise controls of the heating temperature, air ventilation, and smoke density. Some recently designed smokehouse may also be equipped with more precise time and temperature controls, humidity control, and product internal temperature monitor probes. Thus, the products produced by the modern smokehouses are much more uniform than those produced with traditional smokers. Hot smoking is typically not a single process. Several other steps such as brining, drying and smoking are also involved to produce a product of good quality.

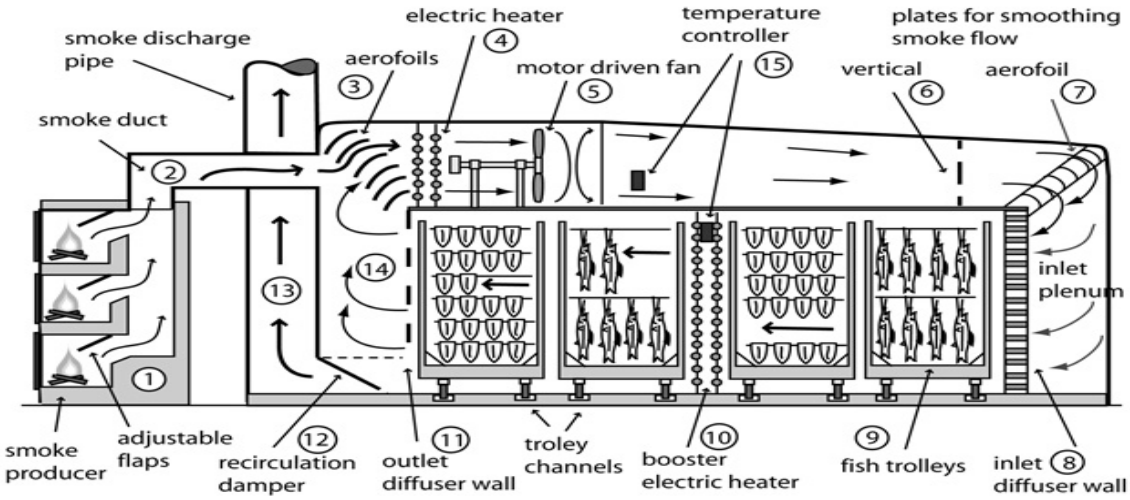

Fig. Illustration of the hot smoke airflow in the Torry smoking kiln

Cold smoking

Fish can also be subjected to cold smoking. Temperatures of cold smoking typically do not exceed 30 °C. Thus, cold smoked products are not cooked and typically heavily salted. Compared to the traditional hot smoking, cold smoking runs longer, has a higher yield and retains the original textural properties much better than the hot-smoked ones. Cold smoking of varied fish species has been reported, including rainbow trout.

Liquid smoking

Liquid smoke is smoke condensate that is dissolved in a solvent, such as water or oil (Maga, 1988). Liquid smoke can be used directly on products by dipping or spraying. It is rapid and much easier to achieve a uniform smoke flavour than traditional cold and hot smoking processes, although the flavour and colour from the traditional smoking cannot be exactly duplicated (Varlet et al., 2007). Some potential harmful ingredients (e.g. polycyclic aromatic hydrocarbons, PAHs) in the nature smoke can be separated out and excluded from the liquid smoke (Chen & Lin, 1997). Other advantages of liquid smoke include easy modification, application to food items that traditionally are not smoked, lower operation cost, and less environmental pollution (Abu-Ali & Barringer, 2007). However, the application of liquid smoking may be expensive compared to other methods. Liquid smoking of fish species had been reported on swordfish, salmon and rainbow trout.

Electrostatic smoking

Electrostatic smoking is another rapid way to smoke. In the electrostatic smoking, fish are sent into a tunnel where an electrostatic field is created. Smoke particles are given a positive charge and deposit onto the surface of the fish which are negative charged. Although this procedure will change the composition of the smoke, the efficiency of smoking is still higher than that of the traditional smoking. It can also be operated continuously. The smoke compound ratio in the vapour phase may be modified by the electrostatic field, which results in increased level of carbonyl compounds (Ruiter, 1979). Factors that may influence the electrostatic smoking operation include the skin thickness, presence of scales, and subcutaneous fat amount (Maga, 1988). This operation may present safety problems to employees. Applications of electrostatic smoking have been reported mainly in salmon and herring.

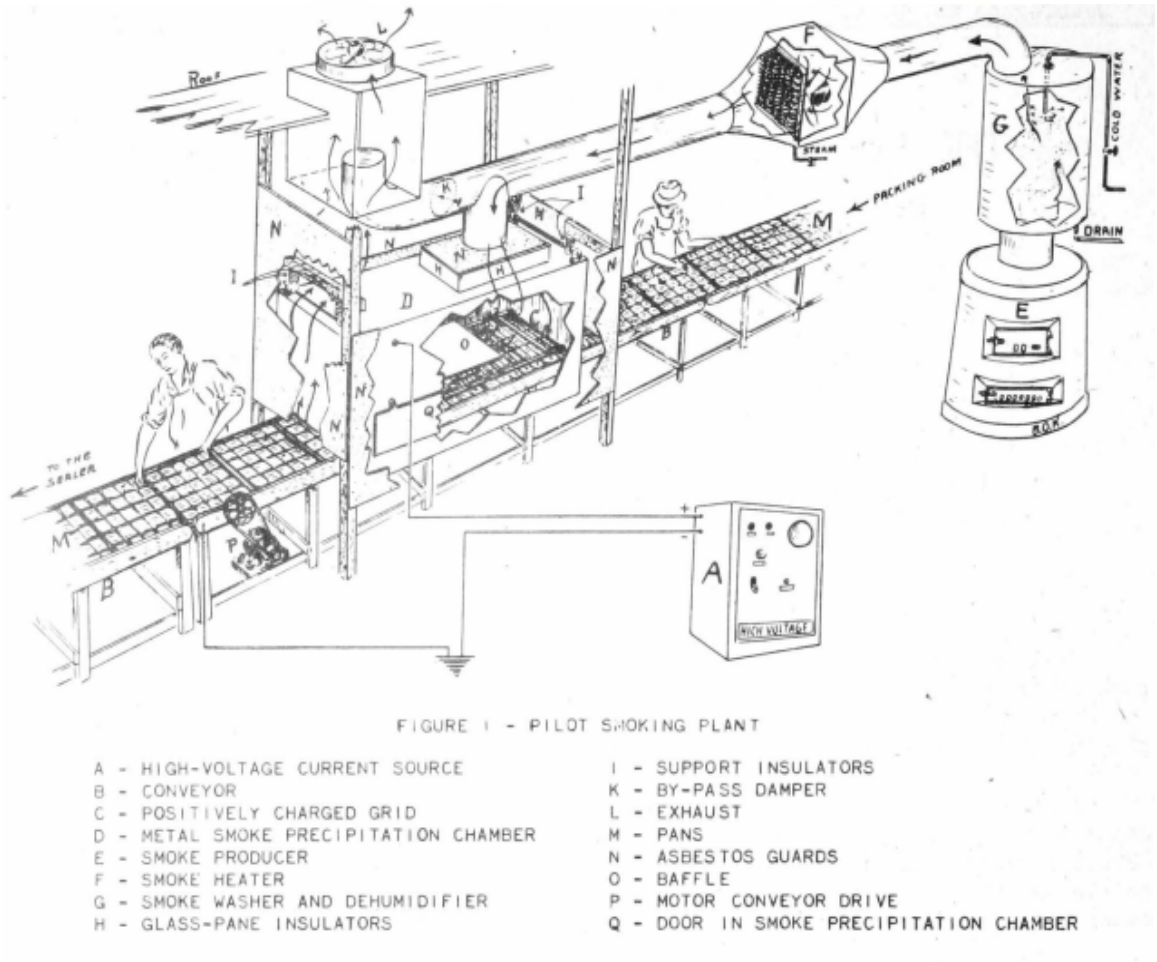

FIGURE 1 - PILOT SMOKING PLANT

A - HIGH-VOLTAGE CURRENT SOURCE
B - CONVEYOR
C - POSITIVELY CHARGED GRID
D - METAL SMOKE PRECIPITATION CHAMBER
E - SMOKE PRODUCER
F - SMOKE HEATER
G - SMOKE WASHER AND DEHUMIDIFIER
H - GLASS-PANE INSULATORS
I - SUPPORT INSULATORS
K - BY-PASS DAMPER
L - EXHAUST
M - PANS
N - ASBESTOS GUARDS
O - BAFFLE
P - MOTOR CONVEYOR DRIVE
Q - DOOR IN SMOKE PRECIPITATION CHAMBER

Fig. Schematic diagram of Electrostatic smoking with basic components.

Hot smoking of fish

Good smoked products can only be obtained from good raw material (Dore, 1993). In addition, control of the smoking procedures plays an equal importance in the production of good products. From raw material preparation to final product storage, smoking includes several operations, such as brining, drying, smoking, packaging and storage.

Brining

This is the stage when the flavours and spices are introduced into the fish. Cleaned fish are submerged under a prepared brine solution for a certain amount of time. A brine time less than 12 hours at 3.3 °C (38 °F) is recommended to minimize the possible spoilage in the fish (Lee, 1977). Salt is an important ingredient to be delivered into the fish tissue at this stage as well as a key hazard analysis and critical control point (HACCP) preventive measure for smoked fish. Not only does it bring the taste but also reduces the water activity (a_w) in the product, so that bacterial growth can be inhibited in the smoked fish.

Of all the bacteria that can exist in fish products, *Clostridium botulinum* is a major concern for vacuum or reduced packaged fish products. *C. botulinum* is a strictly anaerobic, gram positive bacillus bacterium. The vegetative cells and their neurotoxins can be easily destroyed by heat (less than five minutes) at 85 °C. However, their spores are very resistant to heat and can survive for up to 2 hours at 100 °C (Caya, 2001). Thus, prevention of botulism from hot smoked fish products depends on the destruction of all *C. botulinum* spores or inhibition germination of the spores that may be present in the products.

Water phase salt (WPS) is used to measure the amount of salt in the fish products. The WPS is calculated as (FDA, 2001):

$$WPS = \frac{\%Salt}{\%Salt + \%Moisture} \times 100$$

The higher the WPS value, the less the availability of the water. When sodium chloride is the only major humectant in the cured food, the relationship between the aw and WPS can be express as (Ross & Dalgaard, 2004):

$$a_w = 1 - 0.0052471 \cdot WPS\% - 0.00012206 \cdot (WPS\%)^2$$

or

$$WPS\% = 8 - 140.07 \cdot (a_w - 0.95) - 405.12 \cdot (a_w - 0.95)^2$$

Current regulations require at least 3.5% WPS in the loin muscle of the vacuum packaged smoke products; at least 3.0% WPS if at least an additional 100 ppm nitrite exists in the vacuum packaged product; air packaged smoked fish products must contain at least 2.5% WPS (FDA, 2001).

Several salting methods are available to deliver the salt into the fish. The most common techniques used by the industry are dry and brine salting. Dry salting is widely used in low fat fish. Basically, fish are put into layers with dry salt separating each layer. Water removed by salt is allowed to drain away. Periodical reshuffling of the layers may be necessary to make sure all the fish get uniform salting and pressure. Muscle fiber shrinks more during dry salting than brine salting (Sigurgisladottir et al., 2000b). Thus, dry salting of fish typically results in over-dried fish and low yield. A better quality and higher yield is usually obtained from brine salting.

Fish are brine salted by completely being covered in a prepared brine solution for a certain time period. The brine solution can have a salt concentration from relatively low to saturated levels. Brine salting is also used widely for most fatty fish since oxygen cannot oxidize the fish fat easily. Some modern processors inject the brine to speed up the process, therefore lowering the cost and minimizing the chance of fish deterioration. Salt is distributed evenly in the fish when injection brine is used. A higher brine yield can be obtained through injection brine as compared to brine or dry salting. Flavour ingredients can also be incorporated into the injection solution. However, the injecting brine operation has to be carefully controlled to avoid contamination delivered by the needles into the previously sterile flesh. Brine salting is still one of the most widely used salting methods for smoked fish. Efficiency of salt penetration into the fish tissue is affected by several factors, such as species, physiological state of fish (rigor), fish quality (fresh/frozen) fish dimension (thickness), brine concentration, brine time, brine to fish ratio, brine temperature, fat content, texture, etc.

After brining, fish have to be rinsed with clean water to remove the brine solution on its surface because a harsh, salty flavour can develop due to residues of brine solution.

Drying

It is widely known that reducing the water activity (a_w) will result in a reduction of microbial activity. The a_w is defined as:

$$a_w = p / p_0$$

where p is the vapour pressure of the product, and p_0 is the vapour pressure of pure water at the same temperature (Olley, Doe, & Heruwati, 1989).

For ideal solutions (real solutions at low concentrations), water activity can be calculated from the formula:

$$a_w = n_1 / (n_1+n_2)$$

where n_1 is the number of moles of solvent, and n_2 is the number of moles of the solute.

This relationship may become complex due to the interactions between moisture and the fish tissue and also the relatively high solute concentration involved in cured fish. Drying of the fish can still be simulated with the formula in a way that drying the fish will cause a decrease in n_1 and an increase in n_2, which finally decreases the a_w.

A certain amount of moisture has to be lost from fish after brining; so that water activity (a_w) can be decreased and a good texture can be obtained at the end of the smoking process. Drying of fish occurs at the early stage of smoking process. An air flow is applied on the fish; so that moisture in the fish tissue can migrate to the surface and leave the fish by evaporation. The temperature, relative humidity and velocity of the air flow are keys to the rate of drying. Drying with a low relative humidity air at high velocity may not drive the moisture out of the fish fast. If the temperature is too high fish surface may be hardened at the beginning of drying resulting in a blocking layer to the inside moisture migration. The hardened surface may also prevent smoke penetrating into the tissue, which decreases the preservative effects of the smoke. Tissues under the hardened surface will tend to spoil from inside.

Drying at temperatures below 70 to 80 °C was recommended to minimize the damage to protein quality in fish (Opstvedt, 1989). Drying also influences the quality of finished smoked fish product.

Smoking

Smoke is generated from the incomplete combustion of wood at certain temperatures followed by thermal disintegration or pyrolysis of high molecular organic compounds into volatile lower molecular mass (Eyo, 2001). Smoke is composed of two phases: a particulate or dispersed phase and a gaseous or dispersing phase. The major parts of dispersed phase are particles in the droplet form having an average diameter of 0.196 to 0.346 µm (Maga, 1988; Wheaton & Lawson, 1985). These particles are mainly tars, wood resins, and compounds with high or low boiling points. The dispersed phase is the visible part of the smoke. The dispersing phase is responsible for flavouring, colouring, antioxidative, and bacteriostatic roles of the smoke (Hall, 1997). The composition of the dispersing smoke phase is complicated, many of which have yet been identified. More than 200 components have been identified. The most abundant chemicals found in smoke are carbonyls, organic acids, phenols, alcohols, and hydrocarbons.

Quality and composition of the smoke are affected by several factors, such as combustion temperature, wood type, moisture content of wood, air ventilation rate, and wood size.

Cellulose, hemicellulose and lignin are three main components in wood and their contents and compositions vary in different types of wood. Cellulose levels are fairly consistent among different species. Softwoods have higher lignin content than hardwoods. Hardwoods typically contain more hemicellulose than softwoods. Decomposition of hemicellulose happens at the early stage of smoking and produces furan and its derivatives as well as aliphatic carboxylic acids, which drops the pH in the smoked product. Softwoods also contain more resin acids than hardwoods, which typically introduces unpleasant flavor to the fish. Hardwoods, such as hickory, oak, cherry, apple and beech, are preferred in most situations over the softwoods for smoke generation. This is because hardwoods tend to produce more phenols and organic acids which contribute to the flavor and preservation effect of smoking (Hall, 1997).

The amount of air present during the production of smoke also influences the results of wood pyrolysis. Lower temperature and less air produce a smoke with more flavoring and preserving substances. While a higher temperature and more air burn the woods into carbon dioxide and water. Smoke production can be influenced by the size of wood. Wood can be used as chunks, chips or sawdust forms. However, their combustion rates will vary if same ventilation rate is used. Sawdust produces more smoke than chunks or chips due to its self-smoldering effect, which blocks the access of oxygen. Fish is also more likely to be charred with less smoke when chunks or chips are used. Most modern smokers use continuously fed sawdust to maintain a consistent production of smoke.

Although people like the flavour and taste of the smoked product, there are concerns about the negative side of smoked products, which are mainly focused on the carcinogenic substances found in the smoke: the polynuclear aromatic hydrocarbons (PAHs). PAHs are composed of multiple fuzed benzene rings. It can be thermally produced by either high temperature pyrolysis or from the incomplete combustion of materials containing carbon and hydrogen. Up to 100 PAHs compounds have been either identified or detected (Maga, 1988). The level of PAHs can be reduced by decreasing the combustion temperature since the PAHs content was found to change linearly from 5 to 20 µg/100g in temperature range 400 to 1000 °C (Eyo, 2001). Indirect smoking like liquid and electrostatic smoking also significantly reduces the PAHs amount.

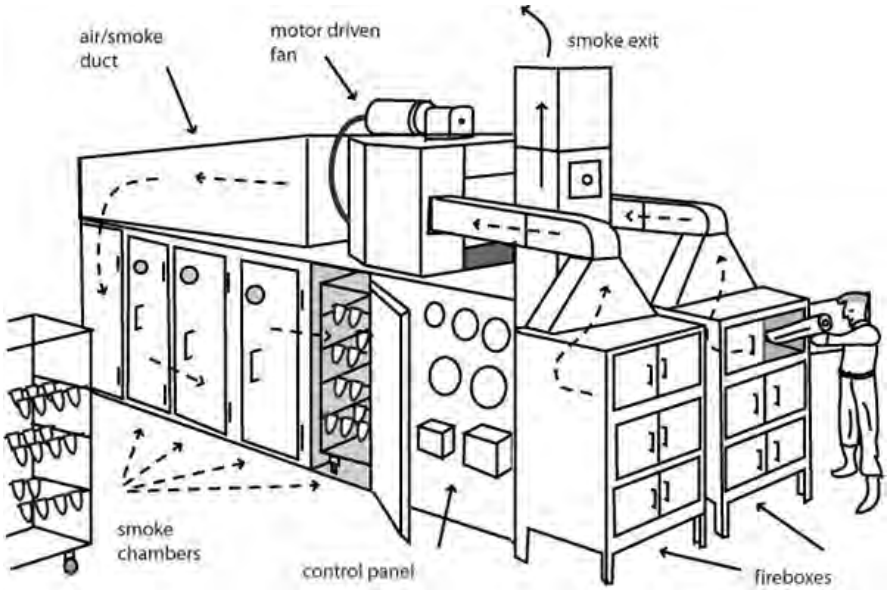

Fig. Smoking kiln

Potential hazards associated with smoking of fish

1. **Biological hazards**

Generally, Cold smoking will typically reduce the level of microorganism by 90 to 99 %. But after the cold smoking there is no such steps to eliminate or reduce the level of microorganisms. Typical temperature used for cold smoking is 22-28º C. However, this temperature is not sufficient to eliminate the risk from *Listeria monocytogens*, a gram positive, facultative anaerobic, psychrotropic bacteria causing deadly septicaemia, meningitis, spontaneous abortion, and foetal death in adult human beings. Specific high-risk categories like persons with altered immune system, pregnant ladies, old aged persons etc. will be more susceptible to listeriosis followed by accidental inclusion. Comparatively high temperature used in hot-smoking process and long-time of exposure to that temperature (60-70˚C for 2-3 h) can inactivate the *L. monocytogens* effectively, provided the raw material is not extra-ordinarily contaminated with the bacteria prior to processing. At the same time listericidal process should be validated to ensure that the treatments are effective and can be applied continuously. But the hot smoked products are susceptible to post-process contaminations from many of the micro-organisms due to improper handling and storage of the products. Sufficient heat treatment, proper hygienic handling and cold chain maintenance during distribution can reduce the risk of biological hazards in smoked fish and fishery products.

Another important biological hazard associated with storage of smoked fish is *Clostridium botulinum.* The toxin produced by *C. botulinum* can lead to botulism, serious illness and death to the consumer. Even a few micrograms of intoxication can lead to ill-health with symptoms like weakness, vertigo, double vision, difficulty in speaking, swallowing and breathing, abdominal swelling, constipation, paralysis and death. The symptoms will start within 18-36 h after consumption of the infected product. By achieving proper salt concentration in processed fish, proper refrigeration during storage and reduced oxygen packaging like Modified Atmosphere Packaging (MAP) and vacuum packaging of the products can prevent the occurrence of *C. botulinum* in smoked fish and fishery products, especially type E and non-proteolytic types B and F. Salt along with smoke effectively prevents the toxin formation from type E, B and F.

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

2. **Chemical hazards**

1. Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are large class of organic compounds containing two or more fused aromatic rings made up of carbon and hydrogen atoms. Incomplete combustion (pyrolysis), during smoking can lead to formation and release of PAHs into the smoked product. Some of them are carcinogenic and mutagenic substances causing serious health issues to the consumers.

Processing procedures such as smoking, drying, roasting, baking, frying and barbecuing/grilling can lead to formation of PAHs in food items. Many reports indicate that individual PAHs in smoked fish can go up to a level of 200µg/Kg. Among the 33 PAHs evaluated by the scientific committee on Food (SCF, 2002) of EU, 15 were found to be having mutagenicity/Geno toxicity in somatic cells of experimental animal in-vivo. They are benzo[a]anthracene, benzo[b]-, benzo[j]- and benzo[k]fluoranthene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, cyclopenta[cd]pyrene, dibenz[a,h]anthracene, dibenzo[a,e]-, dibenzo[a,h]-, dibenzo[a,i]-, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene. The carcinogenic and genotoxic potentials of PAH are largest among the high molecular weight PAH, i.e. compounds with 4 rings or more. Among that benzo[a]pyrene regarded as potentially genotoxic and carcinogenic to humans. They can cause long-term adverse health effects following dietary intake of PAH.

The PAH contamination in smoked products can be significantly reduced by using indirect smoking process instead of direct smoking of the fish. In indirect smoking, the smoke generated in an external smoking kiln, under controlled conditions, is used for smoking process. The smoke produced can be even, washed before coming into contact with the food material processed. In addition to that, use of lean fish for smoking, and cooking at lower temperature for longer time can also reduce the PAH contamination significantly. If the smoke condensate is used for smoking, usage of smoke condensate from reputed reliable resources approved by competent authority can effectively reduce the occurrence of PAH contamination in the final product. The formation of PAH in smoked fish can be minimised by following Code of Practice for the Reduction of Contamination of Food with Polycyclic Hydrocarbons (PAH) from Smoking and Direct Drying Processes (CAC/RCP 68-2009) given by Codex Alimentarius Commission. EU No.835/2011 specifies that maximum level of benzopyrene, and PAH4 (benzo[a]pyrene + chrysene+ benz[a]anthracene+benzo[b]fluoranthene) should be 2µg/Kg wet weight and 12µg/Kg in meat of smoked fish and fishery products, 5µg/Kg and 30µg/Kg in smoked sprats and 6µg/Kg and 35µg/Kg in smoked bivalve mollusc respectively.

2. Histamine:

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

3. Biotoxins:

Biotoxins causing a number of food borne diseases. The poisoning due to biotoxins are caused by consuming finfish/shell fish containing poisonous tissues with accumulated toxins from plankton they consumed. Paralytic shellfish poisoning (PSP), diarrheic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), and neurotoxic shellfish poisoning (NSP) are mostly associated with shellfish species such as oysters, clam and mussels. The control of

biotoxin is very difficult. They cannot be destroyed by any of the processing methods like cooking, smoking, drying or salting. Environmental monitoring of plankton and proper depuration process of the bivalves only can reduce the occurrence significantly.

3. Physical Hazards

Presence of parasites like nematodes, cestodes, trematodes and any other extraneous matter can be considered as physical hazards. Particular attention needs to be paid to cold smoked or smoke-flavoured products, which should be frozen before or after smoking if a parasite hazard is present.

4. Other potential hazards associated with smoking of fish

If wood or plant material is using for smoking of fish, there is a chance of presence of natural toxins, chemicals, paint, or impregnating material in plant or wood used which may result in imparting undesirable odour in processed products. This can be prevented by using sufficiently dried wood or plant material for smoke generation, judicious selection of the species of wood or plant and not using woods having mould or fungus growth for smoking process. Moreover, the material for smoking should be kept in a clean dry place during storage to prevent any kind of contamination, till the usage.

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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.

Chitin and its derivatives

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Introduction

Chitin is a natural polymer and secures the second position in terms of its abundance only next to cellulose. Cellulose accounts to 35-45% of biomass composition available on the earth. Chitin, a Greek word for 'envelop', was discovered in 1811 as a substance occurring in mushrooms. Chitin are synthesized by crustaceans, molluscs, insects, and fungi to the extent of about 100 billion tonnes every year. In spite of its greater abundance, among the natural polymer, chitin is the most underexploited one.

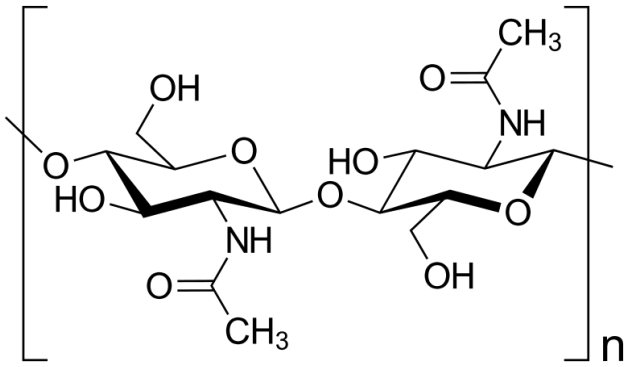


Fig.1 Chemical structure of chitin

Biosynthesis pathway of chitin

The pathway of chitin synthesis has been confirmed in insects and fungi. It is a complex process with a number of sequential bioprocess which varies with the organisms. Chitin synthesis follows hexosamine pathway (HP). The steps involved along with the initial compounds, metabolic enzymes involved and final products are presented in Table 1 and the schematic pathway is presented in Figure 2 as presented by Hou et al. (2021). The chitin polymeric chains synthesized are extruded through the cell membrane into the extra-cellular space wherein the polymer chains assemble to form chitin nanofibrils.

Table 1. Sequence of reaction in hexosamine pathway involved in chitin synthesis

Reaction steps	Initial compound (s)	Enzyme involved	Final product
1	Sugars such as glucose, glycogen or trehalose	Phosphorylase kinase	Glucose-1-phosphate
2	Glucose-1-phosphate	Hexokinase	Glucose-6-phosphate
3	Glucose-6-phosphate	glucose-6-phosphate isomerase	fructose-6-phosphate
4	Fructose-6-phosphate	Glutamine fructose-6-phosphate amino transferase	Glucosamine 6-phosphate
5	Glucosamine 6-phosphate	Glucosamine-6-phosphate N-acetyl transferase	N-acetylglucosamine-6-phosphate.
6	N-acetyl- glucosamine-6-phosphate	Phosphoacetylglucosamine mutase	N-acetyl-glucosamine-1-phosphate.
7	N-acetylglucosamine-1-phosphate +UTP	UDP-N-acetylglucosamine pyrophosphorylase	UDP-N-acetylglucosamine
8	UDP-N-acetylglucosamine	Chitin synthase	Poly- N-acetylglucosamine

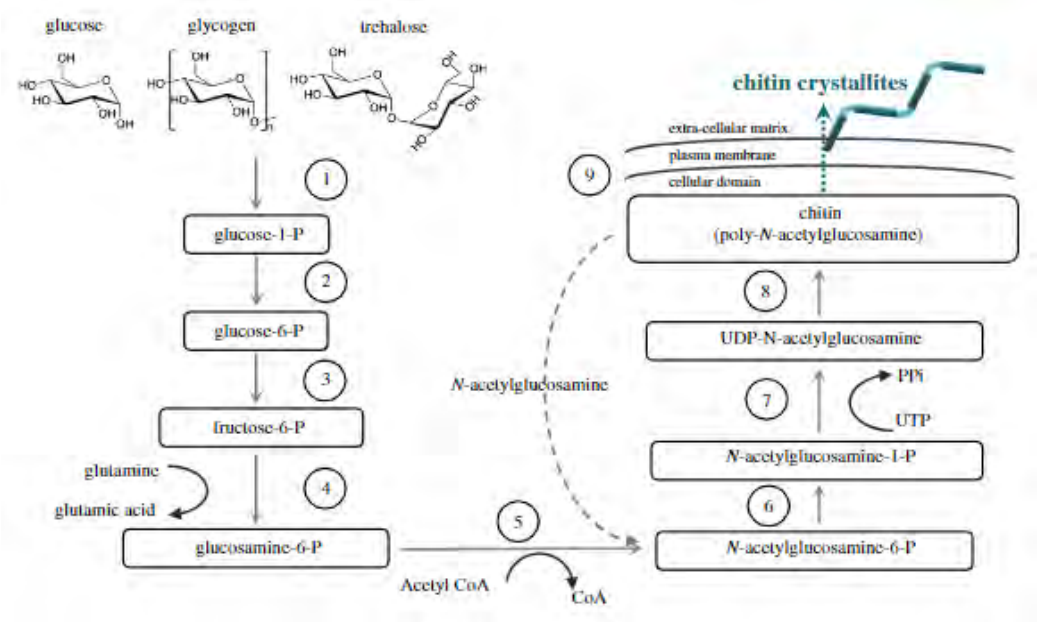


Figure 2. Steps involved in biosynthesis path way of chitin in fungi and insects (Ref: Hou J, Aydemir BE, Dumanli AG. 2021)

Structure of chitin

Chitin: Chemically it is a linear aminopolysaccharide linked by glycosidic bond (β ,1-4 linkage). Chitin has got structural similarity with cellulose. The hydroxy group at position C2 of cellulose is replaced by an acetamido group. Based on the fibre/chain orientation, chitin is found in three different polymorphic forms namely α-chitin, β-chitin and γ-chitin.

- α-chitin (most common form) – Chains are parallel and adjacent polymer chains are always in the opposite direction. A strong network dominated by intrachain hydrogen

bonds between the groups of C═O···NH and C═O···OH within a distance of 0.47 nm. Additional inter-chain hydrogen bonds bind the hydroxymethyl groups.

- β-chitin - all chains are parallel and in the same direction. The network is strong and dominated by intrachain hydrogen bonds. No additional inter-chain hydrogen bonds found in this conformation.
- γ-chitin – Two adjacent chains are parallel, and unidirectional while third one is in opposite direction.

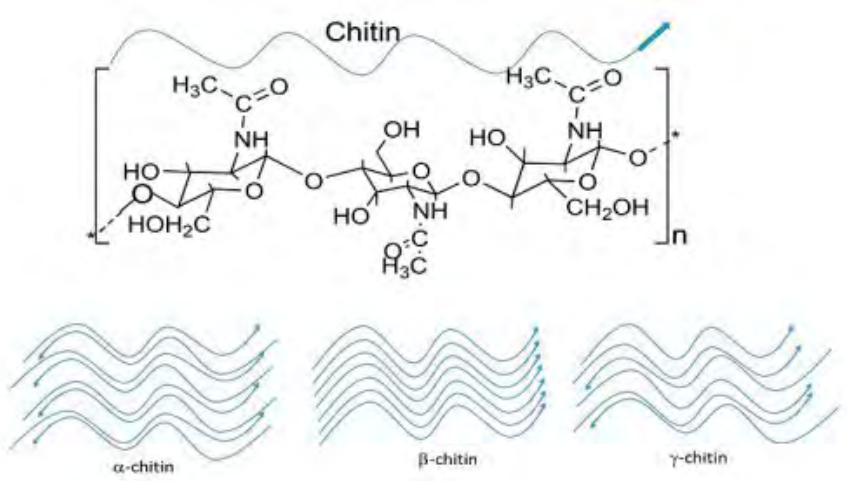


Figure 3. Polymorphic forms of Chitin (Roy et al., 2017)

Major sources of chitin of aquatic origin

- Shrimp shell waste
- Crab shell waste
- Lobster shell waste
- Acetus
- Squid pen
- Cuttle bone

Shrimp processing shell waste serve as a major source of industrial chitin production in countries like India where the shrimp is major processed seafood for export market.

Composition of shell waste

The shell waste contains water, protein, minerals, chitin, lipids and other minor compounds including pigments. In terms of quantity, aforementioned components tend to vary due to various intrinsic and extrinsic factors. To generalize the composition, the following table can be considered.

Table. 2 Proximate composition of shell waste

Constituents	Quantity
Water	65-70%
Protein	9-12%
Fat	0.5-3%
Minerals (Ash)	8-13%
Chitin	3-5%

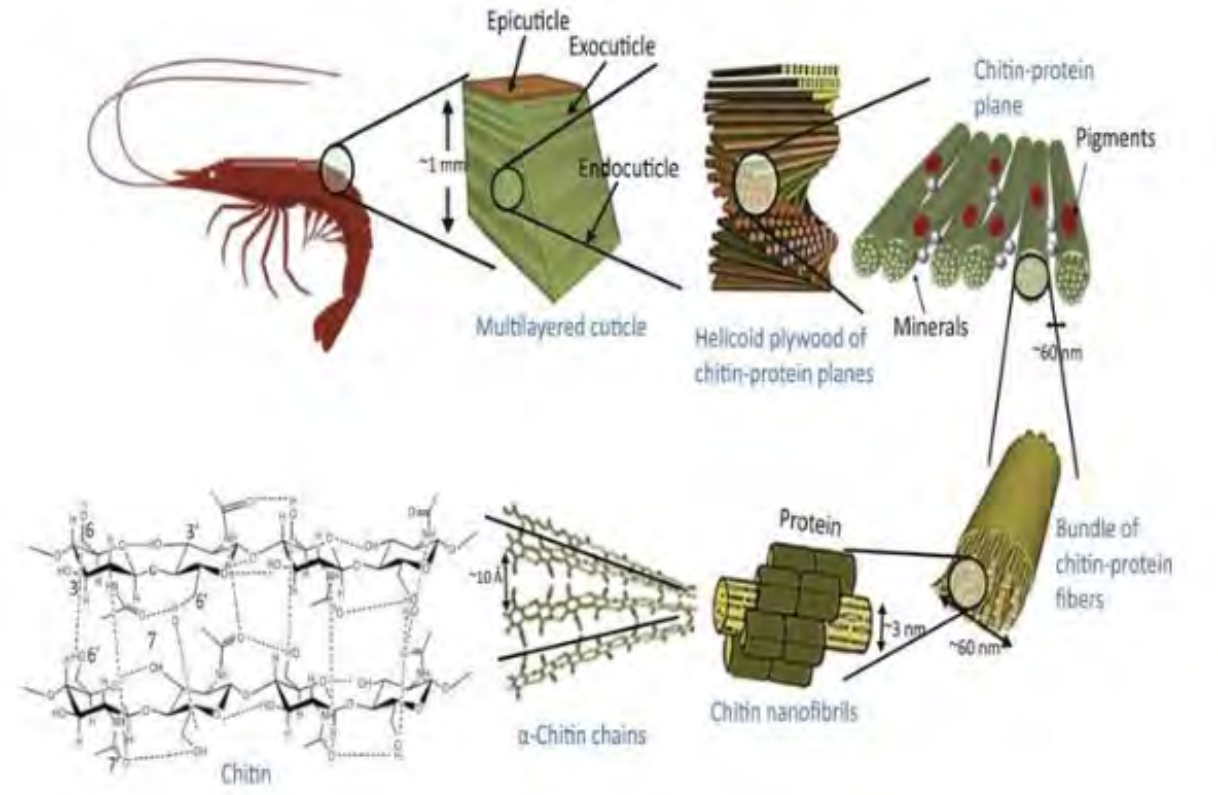
Shell structure – Inter linkage of the components

Shrimp shell is a multi-layered natural compositee containing epicuticle, endocuticle and exocuticle. In between these three layers, there is a membraneous layer. Crustacean shell established to have twisted plywood or Bouligand pattern. Chitin is found mainly in the inner layer of the skeleton, surrounded by a layer of protein. The middle layer consists of chitin and minerals, while the upper layer consists of proteins and minerals.

Epicuticle:

- Outermost layer which is thin and waxy
- Consists of long chain hydrocarbons, esters of fatty acids, and alcohols
- Exo and endocuticle:
- Multi-layered composite tissue
- Consisting mainly of chitin with various proteins
- Chitin and protein polymers are linked through covalent bond.
- Chitin-protein fibrils are biomineralized with calcium carbonate
- Spacing between the fibres is filled up with proteins and biominerals

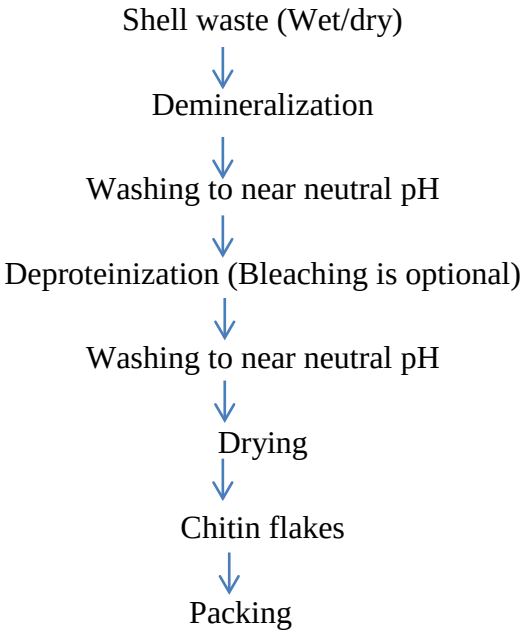
Long-chain chitin molecules are bound into fibrils (3 nm in diameter and 300 nm in length) at the molecular level, which are always embedded in a matrix of proteins by covalent bonds, hydrogen bonds, and molecular interactions, to form fibers (about 60 nm in diameter) that further assemble in parallel into bundles to form horizontal planes. [22] These planes are stacked in a helicoid fashion to construct different layers.



Chitin process

The process for chitin production basically aims to eliminate other chemical constituents like proteins and minerals. For removing these constituents, conventionally

chemical process is employed using diluted acid and alkali for demineralization and deproteinization, respectively. The general process flow is presented in the flow diagram.



Major unit operations in the chitin process

1. **Raw materials**

The quality chitin is influenced by the type of raw material, size and part of the raw material, delay in processing or state of raw material, size of the particle (if it is ground) and any other pretreatment given to the raw material in order to preserve when it is abundant or under certain circumference for example drying the shrimp shell waste and later using it for chitin production.

2. **Demineralization**

Shell waste contains huge amount of minerals particularly as calcium carbonate. During demineralization carbon-di oxide is liberated. In the commercial production of chitin, demineralization is practiced as first step as it softens the material and makes further operations like handling easier. The extent of demineralization is affected by type of acid, strength of acid, raw material to solvent ratio and duration of demineralization. Generally, hydrochloric acid is used in industries as it is relatively cheaper than other acids. However, depends on the properties and applications aimed, the aforementioned process variables can be modified. Normally this unit operation is performed without any heat processing at room temperature. The completion of demineralization is ensured by testing the formation of effervescence from few pieces of shells in diluted acid solution (HCl).

3. **Deproteinization**

Deproteinization from demineralized shells is carried out using diluted alkali. As mentioned in the demineralization, the strength of alkali, type of alkali, alkali to raw material ratio, duration of deproteinization influence the extent of deproteinization. Generally, sodium hydroxide is the most preferred and cost effective in deproteinization. Both thermal and room temperature process can be employed. Heat assisted process is shorter than the cold process. However, the polymer quality is relatively better in room temperature process.

4. Washing

Use of water in chitin and chitosan production is enormous. In between the demineralization and deproteinization process many number of washing cycle is required to achieve the near neutral pH. The requirement of water depends on the initial quality of water indirectly the source of water. More alkaline water like bore-well is required in high quantity. Seawater also can be used for washing the demineralized and deproteinized shell. Washing can also be performed after neutralization. However, one should take care to remove the salt formed by giving wash using fresh water to keep the residual mineral in the chitin and chitosan low

5. Drying of chitin

Wet chitin i.e. demineralized and deproteinized shell is subjected to drying under sun in open concrete drying yards or poly-house tent dryer. In 5-6 h the drying is completed. However, other drying methods can be employed. Compare to all the drying methods, open sun drying improves the color of chitin because photo degradation of pigments present in the wet chitin. Drying under sun required large area of land. Hence, alternative effective drying technologies with lesser space occupation need to be developed.

Chitin derivatives

Chitin is an intermediate product used for producing many derivatives having wide applications. Among them, glucosamine hydrochloride is the most demanded one because of its use in arthritic supplement formulations. Second most important derivative which has received greater attention is chitosan. Similarly, there are salt derivatives like chitosan sulphate, chitosan lactate, chitosan-HCl, Chitosan acetate etc. Water soluble chitosan is another important derivative has received attention. As there are functional group in the structure of chitin like hydroxyl, amino acetyl as well as free amino group in chitosan, many numbers of derivatives can be manufactured through various chemical reactions.

Other products like chitosan sponges, chitosan hydrogel, electro spun nanofibers are all receiving interest for their medical uses. Recently chitooligosaccharides is another derivative of chitosan produced through chemical hydrolysis or using specific as well as non-specific enzymes has found uses as nutraceutical as well as for agricultural applications like immunostimulants

For Further readings

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Trawls and trawling

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Introduction

Bottom or demersal trawling continues to be one of the most important fishing methods of the world. In India, more than 35,230 trawlers of various sizes ranging from 9 to 24 m LOA with engine power ranging from 45 to 450 hp @ 2000 rpm are in operation. Trawl is a bag net towed through water to filter out fishes, the mouth of which is kept open horizontally by means of a beam or otter boards and vertically by means of floats, kite and sinkers. Horizontal mouth opening is also affected by dragging the net from two boats known as bull trawling or pair trawling. The main principle of trawling is the movement of the net underwater filtering the water through the mesh in the netting, without either permitting the fish to escape or gilling them. Trawl net is fabricated using polyethylene netting after cutting and shaping the panels as per the design.

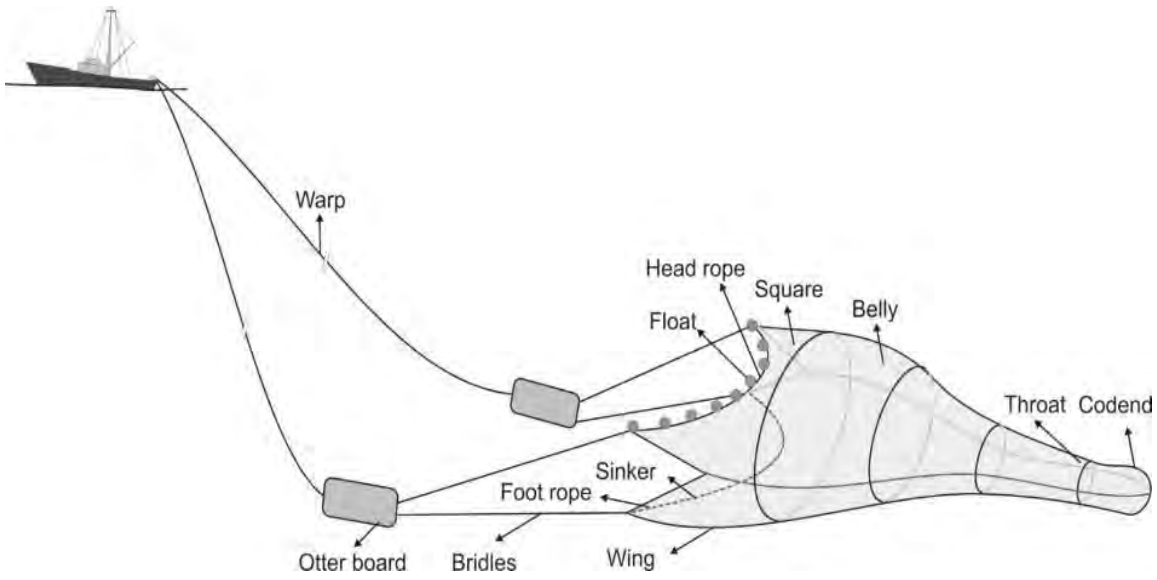


Fig.1. A demersal trawl in operation

In India, trawling was first attempted during exploratory surveys conducted from S.T. Premier off Bombay coast in 1902. Several designs of demersal trawls have been introduced in Indian fisheries in subsequent years.

The most important issue in this sector is the excess capacity in terms of number of trawlers. The size of the trawlers has also increased over the years. Since the introduction of Chinese engines in the Indian waters, the horsepower of the vessel has also increased tremendously and as a result of these changes, there is tough competition out at sea within and between the sectors, which is leading to overexploitation of the resources.

Classification of trawls

Trawls are classified based on the device used for mouth opening, number of panels used for fabrication, depth of operation and based on target species.

1. **Beam trawl**

Beam trawl was the forerunner of all trawl gears. In beam trawls, mouth of the net is kept open using a rigid and curved metal frame with a shoe at the bottom known as beam. This is the simplest method of bottom trawling practiced mainly in the North Sea for flatfish and shrimps. Since the shoe penetrates the seabed and the marks remain for a long period, beam trawling adversely affects the bottom ecosystem. Due to the plowing effect, resistance is high resulting in more fuel consumption than otter trawling. Moreover, a large net requires a large beam which is very difficult to safely handle onboard a fishing boat.

2. **Otter trawl**

In otter trawls, the most popular method of trawling, the mouth opening of the net is achieved by the attachment of two otter boards, through bridles, on each side of the net. The towing warps are attached to these boards at an angle so that while towing the water forces acting on them tends to diverge them resulting in the opening of the net mouth.

3. **Pair trawls**

In pair trawling or bull trawling the net is towed by two boats cruising on a pre-arranged parallel course and speed. The distance between the two boats is also maintained constant, so that the diverging warps keep the mouth of the net open. The main advantage of this method is that a much larger net can be used, as two boats are engaged. As the vessels are operated from a distance from each other scaring effect due to vessel noise is also minimal. Pair trawling is banned in many countries as it generates huge quantity of bycatch.

Trawl types based on number of panels:

Two-seam nets have only two major parts i.e., upper and lower panels and these two are seamed together laterally to form the two seams. The upper part invariably includes the overhang or square. The Cross-section of the net is elliptical in shape and since the vertical opening is comparatively less, these nets were mainly operated for shrimps. Presently all the trawls are two seem.

Four-seam nets are having upper, lower and two side panels with or without overhang. The Cross section of the net is rectangular in shape and hence the vertical opening of the trawl may be influenced by the width of the side panels.

Six seam nets have six panels and cross-section of the net generally acquires oval shape. The six and eight-seam nets are designed to have more vertical openings and hence suitable for catching fishes.

Design of trawls

The efficiency of a trawl mainly depends on the symmetry of the construction of the body and mouth configuration. A trawl is designed in such a way that (i) it offers minimum resistance during tow (ii) total drag matches the available towing force of the trawler, (iii) it achieves maximum mouth opening, and (iv) offers the least hindrance to the movement of fish within the net towards the codend. While designing new gear, different factors have to be taken into consideration such as strength and elasticity of webbing, resistance to the water flow, weight and bulk, speed of operation, cost of materials and conditions of fishing ground. A selective, environment-friendly and energy-efficient trawl system is generally the aim of the design process. Important design considerations in the design of trawl gear involve biological and behavioural characteristics of the target species; fishing conditions in the trawling ground where the system is to be used; and characteristics of the fishing vessel from which the gear is

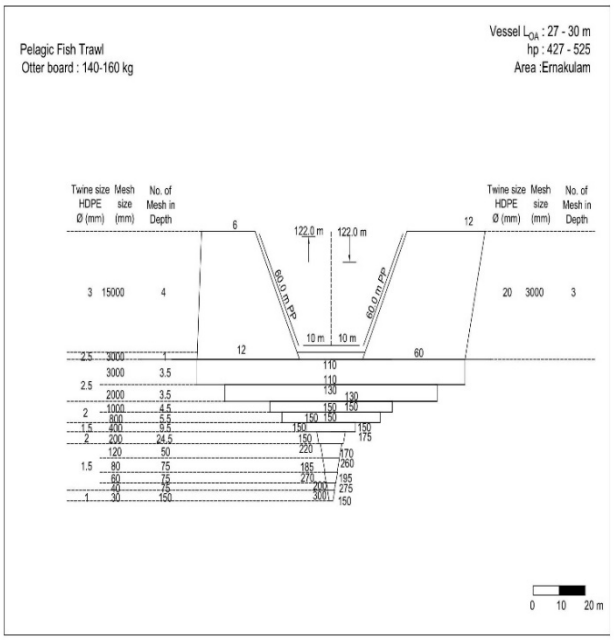
to be operated. The length of the trawl is measured along the last ridges (side lines) from wing (jib) end to tip of codend and it varies from 1.1 to 1.5 times the head rope length. The right size of a trawl for a particular vessel can be selected according to the total twine surface area or by comparison with a trawl of the same type used by a vessel in the same horsepower. Design drawing of the trawl net is prepared to provide all information relating to the size, shape, material and construction using recognized nomenclature and symbols, prior to the fabrication of the net.

Shrimp trawls

Shrimp trawls in the Cochin coast are smaller than fish and cephalopod trawls, with head rope lengths ranging from 50.0 to 53.50 meters and polypropylene is used as a rope material. A diameter of 14 mm for the head and foot ropes were used on the Cochin coast, HDPE twines with a diameter of 0.5 to 2.5 m are used for the fabrication of the net. The shrimp trawl mesh size ranged from 1000-1500 mm at the wing end to 18-25 mm at the codend (Table 1). The most common shrimp trawls cochin coast is poovalan vala, and karikkadi vala (Fig.12, Fig.13)

Cephalopod trawls

Cephalopod trawls have a head rope length of 106.0–114.0 m and are made of HDPE webbing with a diameter of 1.0–3.0 mm. The wing region has a mesh size of 4000-15000 mm, whereas the codend has a mesh size of 20-40 mm. The head rope and foot rope were made of Poly Propylene rope with a diameter of 14 to 16 mm. (Table 1). Trawl net designs used most commonly along the coast of Cochin coast is Kanava vala, a cephalopod trawl that targets cuttlefish is the most prevalent cephalopod trawl on the Cochin coast. (Fig.14, Fig.15, Fig.16)



A large mesh fish trawl design from Kochi

Trawling operation

Demersal trawls can be operated from a few meters to more than around 1000 meters in the sea. The demersal trawl is designed and rigged to have bottom contact during fishing and is, depending on the bottom substrate equipped with different kinds of ground rope rigging. This is for the purpose of shielding the lower leading margin of the trawl from ground damage

whilst maintaining ground contact and easy move on the bottom. The trawlers must have sufficient towing force for towing the gear and require a winch or mechanical hauling system. However, in some small-scale operations hauling is done manually. The methods adopted for demersal trawling are beam trawling, side trawling, stern trawling, double rig trawling, bull trawling and multi-rig trawling. Stern otter trawling is the most popular method in India.

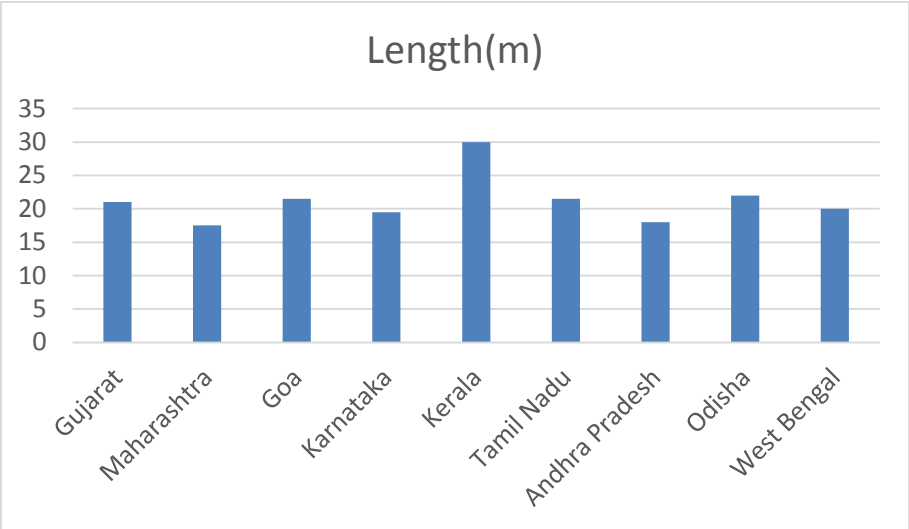
1. Beam trawling:

On arrival at the fishing ground, the beam trawls are hoisted on the booms which are then swung out. The operation is undertaken while the fishing vessel sails on a straight course. When hauling, the net is heaved in until it is at the boom tips. The cod end is taken by the line attached to the cod end strap and the catch is emptied out directly.

2. Otter trawling:

The Vigneron-Dahl system was introduced during 1920s where the otter boards were attached to the wings by means of sweep lines and bridles. This helped in increasing the effective swept area and thus increased the catch due to the herding effect of sweep lines and otter boards. In larger trawls, in addition to the weight on the foot rope, iron bobbins or rubber discs are attached depending upon the nature of the fishing ground. The towing warps are provided with markers at distinct intervals for facilitating the release warp, in small-scale operations. In large scale operations it is hydraulically or electrically controlled with metering arrangements. The length of warp released in bottom trawling depends on the depth of the fishing ground and nature of sea bottom. The ratio of depth of fishing ground and the warp released is known as scope ratio or in other words, it is the warp-length ratio. The length of warp to be released is generally (i) 5-6 times the depth in shallow waters below 50 m, (ii) 4-5 times the depth in off shore waters of 50-100 m, (iii) 3- 4 times the depth in deep waters of 100-200 m and (v) 2-3 times the depth in the deep sea of 200 m and more. The speed at which the trawl is towed over the bottom range from about 2 to 2.5 knots for slow swimming species to 3- 4½ knots for fast swimming fish. Towing a particular trawl too slowly may cause the otter boards to close together, providing insufficient spreading power to the net which tends to sag onto the bottom. Towing too fast may result in the net lifting off the bottom and floating which may lead to fouling of gear. Winches are used to pay out and haul the warps. The winches have two drums, one for each of the two warps; an additional drum is provided for the operation of the try net in shrimp trawling. In larger trawlers, single drum split winches are used for each of the warps. Hauling speeds could vary from 30 to 60 m.min-1. Stern ramps are provided in larger stern trawlers, which facilitate the shooting and hauling up of the large trawl gear with less manpower. In large trawlers net drums are used to haul up, pay out and store the sweeps, bridles and net with its rigging. The factors such as (i) availability of fish (by using echo-sounders, fishery charts and fishery forecasts), (ii) depth and nature of sea bottom of the fishing ground, (iii) current and wind speeds are to be taken into consideration before the commencement of fishing operation. On reaching the ground, the warps are attached to the net and the codend is closed properly. The codend is the first part to be released, followed by the main body of the net. The vessel steams forward slowly releasing the net and the otter boards. The winch is stopped after releasing few meters of the warp to ensure the proper spreading of the bridles and otter boards. The gear is then lowered to the desired fishing depth by releasing sufficient length of warp. The net is dragged for a duration of about 1 to 4 hours, depending on the concentration of catch. The net is hauled by heaving in the trawl warps evenly on to the

winch drums, until the otter boards reach the gallows. Sweeps and bridles are then hauled up followed by the main body of the net and finally the codend. In small trawlers, the sweeps and the net are shot and hauled in manually and sweeps may remain connected to the otter boards. In large trawlers, a Kelley‘s eye, independent wire and back-strop is used for facilitating the hauling of the sweep lines and net on to the net drum after the otter boards have reached the gallows.



Length of trawlers in different states of India

Conservation Strategies

Large mesh trawls: In these trawls, the front trawl sections are made using large mesh panels which results in a reduction of trawl resistance. The reduced drag permits greater trawling speed and operation of a large trawl with the available installed engine power. Trawls with a mesh size up to 5 m is now under operation in Kerala. Such a trawl uses only 3 large floats for lifting the headline.

Rope trawl: In rope trawl, the front trawl sections are replaced by ropes, which as in the case of large mesh demersal trawl, results in a reduction of trawl resistance with the same advantages as in large mesh trawls. Finfishes are retained due to the herding effect of the ropes.

High opening trawls: High opening demersal trawls are designed to harvest off-bottom fishes, which are beyond the reach of conventional demersal trawls, along with bottom resources. It has been reported that the large mesh high opening trawls offer 18% lesser resistance compared to conventional bottom trawls which in turn results in utilization of lesser horsepower.

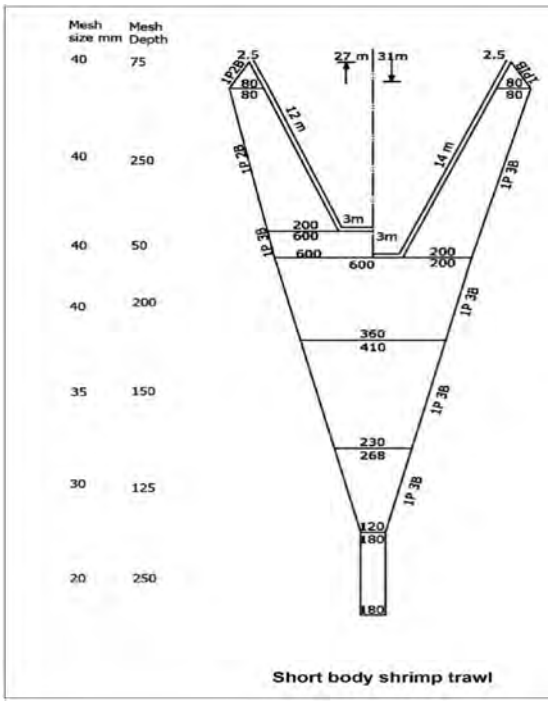
High-Speed Demersal Trawls: Commercial exploitation of active fishes with low population density fishery resources requires high-speed trawling. High-speed demersal trawls (HSDTs) have been developed with light material, large meshes, smooth tapering along the belly facilitating even distribution of stress along the framing and strengthening ropes facilitating smooth filtration and herding.

Bulged belly trawl: In the bulged belly design, wide side panels are provided to increase the vertical opening, and at the same time tapering of the belly is streamlined so as to improve herding and filtration efficiency. The improved bulged belly trawl fitted with tapering jibs consistently landed better shrimp catches. Technological Strategies Increasing awareness on responsible fishing methods has resulted in studies to improve the selectivity of the trawls. Size

selectivity in bottom trawls can be achieved by controlling the mesh size and shape. Species selectivity can be achieved using separator panels and grids by making use of the behavioural differences in species in the fishing area.

Separator trawls: It is designed to separate shrimp from fishes based on the difference in their swimming behaviour. Insertion of a horizontal panel in the separator trawl, separates the fish and shrimp catch, leading them to separate codends. The selection process of this device is based on the fact that shrimps which are usually distributed close to the bottom move to the lower codend while the high swimming species usually end up in the upper codend. Separator trawls reduce the sorting time, as the catch is landed in a pre-sorted condition.

Short body shrimp trawl: CIFT has developed and successfully field tested a 27 m shrimp trawl with a relatively short body and large horizontal spread suitable for selective retention of shrimp. The width and length of the trawl funnel have been reduced by increasing the tapering ratio and the vertical opening of the mouth has been reduced to eliminate bycatch. Because of the larger horizontal spread of the mouth, the effective sweep area is more, which is the most vital requirement for a shrimp trawl. Trials carried out along the coastal waters off Cochin with a prototype of short body shrimp trawl reveal considerable reduction in the catch fish due to the behavioral difference of the targeted species.



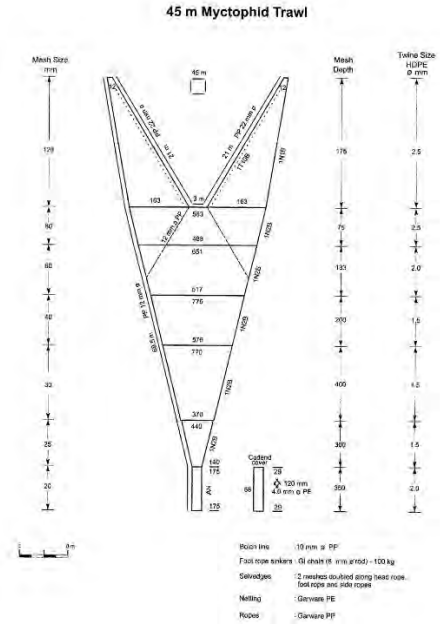
Short body shrimp trawl

Cut-away top belly shrimp trawl: A shrimp trawl without a top belly has been developed and field tested at CIFT. Results reveal that considerable reduction in the quantity of bycatch landed. The net was able to cover more area within the stipulated speed and time due to reduced drag.

Semi-pelagic trawls: 27m four panel CIFT-SPTS in combination with high aspect ratio Suberkrub otter boards weighing 85kg each with front weights are designed to catch fishes, which are up to 4 m above the ground, with minimum impact to the sea bottom.

Krill trawl: Krill (*Euphausia superba*) is a small crustacean found in the Antarctic waters of the Southern Ocean. Large trawls with small mesh inner lining is operated in Antarctic waters for krill fishing.

Mesopelagic trawls: Mesopelagics are small fishes in the size range of 3 to 30 cm inhabiting the disphotic oxygen minimum zone in world oceans in the depth range of 200 to 1000 m. Large trawls are used in Oman and South Africa for commercially harvesting mesopelagic mainly for making fishmeal and fish oil.



Mesopelagic trawl

Environmental impact of bottom trawling

Bottom otter trawls interact physically with the bottom sediment, which might result in the removal or damage of sedentary living organisms (including seaweed or coral) and in the case of uneven bottom surface displacement of stones or other larger objects. On flat sandy/muddy bottom, the sediments might be whirled up into the water masses and suspended. The short and long-term impact on the bottom environment is still poorly documented. The major negative impact of bottom trawling is the capture and discarding of huge quantity of juveniles of fishes and other aquatic organisms.

Conclusion

Trawls are non-selective fishing gears creating ploughing effect on the sea bottom leading to the destruction of the benthic ecosystem. In trawl design and improvement, the aim should be to produce a trawl system which can selectively and efficiently catch the target fish, eliminating juveniles and other aquatic organisms with minimum environmental impacts. Since trawling is an energy-intensive fishing method, development of low drag trawl systems to save energy and cost of operation is imperative. Resource-specific trawls like semi-pelagic trawls should be popularized to minimize the impact on ecosystem. Excess capacity in terms of a number of trawlers, size, engine power and trawl efficiency are major issues that need to be addressed to make trawling economical and sustainable.

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Bycatch issues in the inland capture fisheries of India

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Introduction

Total world inland capture fish production was 11.63mmt in 2016 and China was the leading nation followed by India with 1.46 mmt. Inland waters include freshwater and brackish water bodies in the form of rivers, reservoirs, lakes, backwaters, mangroves, estuaries, tanks, ponds, paddy fields, wetlands, etc. India has vast inland resources in the form of rivers and canals, 1,97,024 km; reservoirs,3.15 million ha; ponds and tanks, 2.35 million ha; oxbow lakes and derelict waters, 1.3 million ha; brackish water, 1.24 million ha and estuaries, 0.29 million ha. Inland water bodies include fresh water and brackish water areas. The river systems of the country are classified into five groups namely Ganga, Brahmaputra, Indus, Peninsular east coast river systems and west coast river systems. It comprises of 14 major rivers, 44 medium rivers and several small rivers and streams.

Fishery resources include 2546 species so far listed 73 (3.32%) belong to the cold freshwater, 544 (24.73%) to the warm fresh waters, 143 (6.50%) to the brackish waters and 1440 (65.45%) to the marine ecosystem. Lakhs of people are engaged fishing and allied activities and earn their livelihood from the inland waters in our country. Currently, these water bodies are under stress due to dam construction, siltation, pollution, land reclamation, water abstraction, etc., which adversely affected fish production and fishery collapsed in several water bodies. Ganga action plan launched in 1986 with the main objective of pollution abatement, to improve the water quality by treatment of domestic sewage and industrial chemical wastes is a glaring example. Excess capacity and destructive fishing practices are other major reasons for declining fishery resources in inland waters.

Chilika Lake

Chilika lake

Since the capture fish production from the marine waters are declining inland sector is in the focus. Further aquaculture activities, especially shrimp and carp farming are taken up in a big to meet the increasing demand for fish.

Pulicat Lake

Pulicat lake

Among the native fauna most of the fishes are permanent dwellers and others are migrant species coming from the marine or fresh water bodies. Most of the fishes are native species and others are exotic which are accidentally or otherwise introduced into the system. Exotic species are harmful to the native fauna. Occurrence of African catfish in the inland water bodies is a good example. Immediately after the flood in Kerala fishermen had a good catch of several exotic fishes like paccu, gourami and arapaima.

Fishing craft

Variety of fishing craft are in operation in the inland waters, which include a piece of log or an inflated rubber tube to motorized FRP boats, depending on the type of fishing and nature of water body. In reservoirs bamboo raft, coracles and inflated tubes are common. In larger water bodies like Pulicat , catamarams are used for cast netting and motorized FRP canoes are used for seine netting. In Chilika lake, sail is used for wind-assisted navigation in wooden canoes.

Catamaram in Pulicat lake

Raft

Bamboo poles are tied together with help of rope keeping all the lower end of the trunk towards the stern side. These rafts are about 6-10 m in length and 1.5 to 5.0 m wide. It is

operated with the help of bamboo poles or oars in the sluggish rivers, floodplain lakes and in some reservoirs. The life span of this raft is about 1 to 2 years. Wooden raft and banana rafts are also made in some areas.

Fig.3. Bamboo raft

Coracles

Coracles (Fig. 5 and 6) are primitive, light, bowl-shaped boats with a frame of woven grasses, reeds, bamboo or saplings covered with sheets. Coracles are mainly used in reservoirs and backwaters in the southern regions of the country. Coracles are about 2-2.5 m in diameter with the greatest diameter across the centre. The bottoms of the boats are covered with few layers of plastic gunny bags or with plastic sheets and are tarred to make it waterproof. Coracles are steered and propelled using a single paddle.

Fig.4. Coracle of a migrant fishermen family from Karnataka

Canoes

Dugout canoes are mainly made from a single large log by scooping out the wood with the help of a small hand spade. The length of this boat ranges from 4 to 8 m. In shallow water bodies, it is operated either by a bamboo pole or by an oar by 2 to 3 persons. Fishing gears like traps, gill nets and hook and lines are operated from this canoe. Plank-built canoes are predominantly used in rivers and reservoirs. They are of different types and vary widely in size and shape depending on where they are used and the type of fishing to be carried out. These types of canoe are operated by oar and in the case of shallow water bamboo poles are also used. Sometimes canoes are provided with arch-shaped roofing made of bamboo mat or polythene sheet which provide shelter to the fishermen. Coat tar, indigenous preservatives and FRP sheathing is used in canoes to extend the life. FRP canoes are also used for fishing in the inland waters. Its smooth finish and light weight enables the fishermen to manoeuvre easily in the river.

Fishing craft in Ganges (Credit: H. KOLDEWEY)

Fishing gears

Diversity of fishing gears are more in inland waters than in the sea. Hook and line, cast net, traps, drag nets, gill nets and seine nets are the most popular gears. Hand picking and other primitive tools like spears and arrows are still in use in some pockets. Nylon monofilaments gillnets are the most predominant fishing gear across the sector. Fish traps are usually made of natural biodegradable materials, whereas all kinds of nets are made synthetic materials. Proliferation non-selective fishing gears like small mesh gillnets, seines and stationary bag nets is a major concern in most of water bodies.

Seine nets are roughly rectangular in shape without a distinct bag and are set vertically in water; to surround the school of fish generally pelagic. Shore seine is a large net operated near the bank of a river, reservoirs or beels. The net usually has two wings and a middle landing part. The net is payed in the form of an arc from the shore using a boat and a number of fishermen pulls the net from the shore. The foot rope of the net always touches the bottom and the net is pulled towards the shore and the fishes are collected from shore. Do-Dandi of Ganga river, Bori of Gujarat and Gorubale of Karnataka and Pattuvala or Chavittu vala of Kerala. Tana jaal, Ghayala jaal, Raja-rani jaal,Gheesa jaal, Ber jaal, Chati jaal, Ghon jaal, Moshori jaal, Fesi jaal, and Pet-kasi jaal operated in the north eastern regions are some shore seine nets of the country.

Large seine net in Pulicat lake

Boat seines are also operated in inland water bodies. Its construction is similar to the bag net and is operated from boats. The net is released from one or two boats to form an arc. After

encircling the fish, the net is hauled from the boat. Buro jaal and Koni jaal are single boat seines operated in backwaters of West Bengal. Pesi jaal is another small boat seine. operated in Assam. Patua-jaal is a boat seine operated in Chilika lake for small clupeids and beloniforms.

Stow net is a bag net conical in shape similar to a trawl net. It is known by different names in different regions. The mouth of the net is fastened to the opposite river banks against the current using ropes or wire ropes. The upper edge of the net mouth is kept open with the help of bamboo poles fixed at both ends of the wing and near the mouth region of the net. The fishes are collected in the cod end as the current of water takes the fish inside the net. These nets are used only when there is sufficient flow of water. Baghjaal and Bion jaal of Assam are examples of stow nets.

Push nets are operated in shallow water bodies. It has a 'V' shaped bamboo frame to which the webbing is attached. The net is pushed through water by man wading and during operation it scraps the bottom. It is hauled at frequent intervals. Some scoop nets have a cod end to facilitate collection of catch. The net is also operated from boats. Pelni of Narmada, kamjaal and kursung jaal of Assam, Schiki of Hoogly and Kuppu valai of Tamil Nadu are some examples.

Stick held drag net is operated in Orissa, Madhya Pradesh, Andhra Pradesh and Kerala. Mesh size of the gear ranges from 10-15mm. Webbing is fixed to bamboo stick of 70cm to 90cm length at regular intervals to form a pouch. The net is dragged by two persons in shallow areas which are devoid of bottom obstruction. While hauling the net fishes are driven into the net from both sides by splashing water with one hand. A drag net thandevala with two poles on either side of the rectangular mouth are operated in backwaters of Kerala.

Scoop net or small bag nets with rectangular mouth or circular mouth with frame used to scoop fish out of water. Net is operated in beels, backwaters and other inland water bodies. Vadivala and koruvala of Kerala Bachra jaal and hatjaal of Assam are some examples Trawl fishing has been carried out on experimental basis in reservoirs and rivers. Otter trawling has been tried in Hoogly estuary, Hirakud reservoir, and in Gandhisagar reservoir. Operations of mini trawl in Kerala has been recommended as an active fishing method in reservoir for the control / capture / elimination of cat fishes, uneconomical fishes and trash fishes. It is not recommended in rivers.

Hand operated dredges (kuthi vaaral) are used in backwaters in Kerala to harvest clams. The dredge is made of slightly inwardly curved horizontal plate of about 50 cm length having about 40 spikes pointing downward at the lower edge of the plate. To this curved plate an arch shaped bamboo frame of about 30 cm height at the center is attached. A small bag net of about 50 cm length is attached to this frame. The net and the dredge are attached to a wooden pole of approximately 10 m length. The dredges are operated by two or more fishermen using two canoes.

Lift net is a sheet of net, usually square, but may sometimes be conical, is stretched either by everal rods, ropes, or a frame. The fishing principle is to keep the net submerged for an interval of time and then pull it rapidly out of water so as to catch any fish, which happen to be over it. A variety of nets, employing the above principle of fishing, are operated in inland water bodies. A lure and lift net techniques is practiced in Tamil Nadu.

Hand lift net operated along the shore in shallow waters. Four corners of the net are attached to poles tied at the center and is operated by dipping and quickly lifting the net out of water. Panjaal of Assam khora jai, kabjai and pah jaal are lift nets operated from boat or flat forms

built in shallow waters of Brahmaputra. Kacha of Tamil Nadu, kurli of Punjab, Arippuvala and hoop nets of Kerala, Maharashtra and Tamil Nadu and Jamdajaal of Gujarat are examples.

Falling gear is usually a cone-shaped net or other devices, which is dropped to cover aquatic animals and enclose them. Generally, they are hand-operated in shallow waters, but some are operated from a boat. The stick-held cast net is an example. The principle is to catch the fish by covering from above. The gear is cast over the area where the fish is available and the trapped fish are caught by hand. Cover pots, lantern net and plunge baskets are examples

Cast net is found throughout India. Cast nets are conical bag-shaped net. It is the most widely used gear in the inland sector by a single fisherman. Three types of cast nets are operated in inland waters viz. with closing strings, with peripheral pockets and without strings, pockets and hauling rope. Iron sinkers are fixed in the lower periphery of the net. The net is thrown in a circular fashion over the water and due to the presence of sinkers the net sinks to the bottom. It is then hauled up with the help of the hauling rope tied to the apex of the net. Fishes that come within the area covered by the gears enter the pockets while hauling. The cast nets vary in their sizes. Based on the size and different mesh size, the nets are named differently. The cast nets are mostly made of PA multifilament. Khewali jaal of Assam, chakar jaal of Gujarat and veesuvala of Kerala are some examples of cast nets.

Gill nets are long walls of webbing hung vertically in water that are either set in one spot or allowed to drift with the current (Fig. 27). Gill nets are used in rivers, reservoirs, beels and other inland water bodies. Gill nets can be operated in the bottom, midwater or surface targeting desired fish. These nets are also used as encircling gear. It is highly selective and can be used judiciously by using the optimum mesh size to capture the right size of the fish. Gill nets are also named by the target fish they capture. Gochail jaal of Allahabad, thangadi of Hoshangabad, kuto jaal of Hoogly, current jaal, langi jaal and phansi jaal of Assam and ozhuku vala of Kerala are examples. The rampant use of very thin polyamide monofilament materials, discarded and lost nets in the inland water bodies could lead to ghost fishing and can also cause environmental and ecological problems. Proper selection of mesh sizes, hanging ratio, and mode and time of operation can make gill net an eco-friendly, low energy and sustainable fishing method.

Traps are passive fishing gears into which the fish can enter voluntarily in such a manner that the entrance then becomes a non-return passage of the device. Trap fishing is highly fuel-efficient both in terms of returns and biomass per unit of fuel consumed. Traps can fish continuously during day and night with periodical checking and the organisms can be retrieved alive without any damage. Traps are mostly made of bamboo, Palmyra fibres, coconut tree, coconut leaves etc. Kankada khadia and Khonda screen traps in Chilka lake, Orissa Chempally koode of Kerala, Kumini of Madhya Pradesh, Sepa and Dingora of Assam are some examples of fishing traps.

Fish barriers are long leaders of converging screens erected in shallow waters to lead the fishes into the chambers fixed in the end. Net barriers are slowly replacing the bamboo barriers as these are cost affective and saves labour and lasts longer than the bamboo screens. The gear consists of leaders, gathering ground, channels and filter platforms. The leaders guide the fish into the trap. The length varies from 10 to 50 m depending on the width of the river stream or canal. Water seep through the platform, leaving the fish. These gears are very effective in capturing nearly all fish moving downstream. The fish reaching inside the barriers are captured by using lift nets. Roak used in river Yamuna in Agra during summer to catch major carps,

jano khonda or disco net of Chilka lake, banamara and betamara of northeastern states are some examples.

***Hook and line fishing*:** Different lines such as hand line, pole and line, set line lone, drift line, long line, drop line, multiple baited lines, etc are also operated in inland waters. Some lines are operated without bait.

***Purse net*:** It is a semi-circular purse net extensively used in catching Hilsa (Fig. 17). The net consists of an elliptical frame by tying two-split bamboo on either side or a bag shaped net attached to it. The net with its mouth opened vertically is towed along the river bottom by 1 to 2 fishers while being steered by 2 more. The frame of the net consists of two long slender arched bamboo strips about 6 to 7 m long tied together at both the ends in the form of hinges. To this frame is attached a rounded bag shaped net having a mesh of 22 to 70 mm made of PA about 3 to 3.5 m deep. The mouth is kept open by a brick, iron ball or a stone weight of 1.5 to 4.0 kg tied to the center of the lower lip. There is a feeler cord fixed to the upper portion of the net to transmit the disturbance caused by the entrance of fish. The stout haul rope is paid out to the desired depth. This haul rope passes through a ring or Y-shaped piece of wood in the upper lip and attached to the middle of the lower lip immediately above the weight. Net is operated from a boat moving with the current. When any fish enters the net it causes certain jerk which is felt by the fisherman holding the rope, which immediately close the net by pulling the rope and haul the net. Illishashangala jaal' and karal shangala jaal are very popular purse nets in the lower Brahmaputra, the former for hilsa and the latter for migratory carps. This net is also seen in West Bengal.

Brush parks are the most common fishing method employed in the beel (Fig.18). These parks mainly act as shelter areas. Two different types of brush parks locally known as katal / jeng and pit / chek, are erected in the beels of Assam. Katal fishing or katalmara is a method, which is extensively used in the beel fisheries of Assam. Katals are prepared by erecting tree branches in the bottom with a collection of water hyacinth, in the form of a circle. Pit / chek is a very large brush park (0.5 to 2.0 ha) erected in beels heavily infested with floating water hyacinth. Similar type of bush parks known as Phooms are seen in Loktak lake, Manipur. Fishes take shelter in this. During winter when the water level goes down, katal is surrounded using screen or net. Fishes are collected after removing the weeds

In the case of ***drive-in-nets***, the technique of this fishing method is to drive the fishes into fixed fishing gear from a distance. Sometimes gill nets are used for this purpose. The operation is done in the shallow areas. Scare lines can be made by inserting tender coconut leaves into the twists of a long coir rope or with broken pieces of bricks and thin strips of turtle shell similar to a stick held seine net. The net is fixed in the form of "U" and the fishes are driven into the net using the scare lines. In the final stage of operation of the net two ends are brought together and the confined fishes are captured. Beppevala in rivers of Kerala, gopal jaal in Allahabad, sone jaal and tik tiki khedani of Assam are examples.

Above described are major fishing gears and methods of inland waters in India and there may be some other indigenous fishing methods in certain pockets, which is likely to be insignificant in terms of catch or employment. Major issues in the sector are given below.

- Habitat degradation due various anthropogenic activities
- Siltation

- Land reclamation
- Profuse weed infestation
- Aquatic pollution
- Construction of check dams/barricades
- Destruction of mangrove forest
- Sand mining
- Water abstraction in smaller water bodies
- Invasive predators/exotic species
- Large scale prawn seed collection from natural water bodies for farming
- Destructive fishing methods
- Bycatch/discards
- Climate change

Towards Sustainable Fishery

Excess capacity and over exploitation are major problems. Licensing of fishing craft and gear is required with periodic checking to control destructive fishing practices. Small meshed gears and use of mosquito net for fishing gear making should be banned. Gillnet with less than 90mm mesh size should not be used for hilsa fishing. Huge quantities of juveniles and post larvae are being landed in the stationary bag nets including juveniles of priced fishes like hilsa and pomfret. Such gears should be phased out or replaced with more selective gears. A buyback scheme can be introduced to purchase the licence of destructive gears. Completely ban the destructive fishing technique like blast fishing, electrical fishing and fishing using poison and chemicals. Trading of juvenile fishes need to be discouraged. Almost all gillnets are presently made of very thin nylon monofilament. Within 1-3 moths time the net get damaged and it is discarded as the fishermen usually does not mend the monofilament nets. The discarded non-biodegradable nets in the water bodies leads to ghost fishing.

CIFT has optimised mesh sizes for different gears based on the extensive field trials conducted in different water bodies and the recommendations have been communicated to the respective States for enacting. As the fisheries resources in open water bodies are common wealth, people utilising the same have the responsibly to conserve the same to prevent Tragedy of the commons proposed by British economist William Forster Lloyd. Responsible fishing practices using optimised fishing gears developed by CIFT should be adopted. It is believed that self-regulation by the fishermen and community managing the resources is better than master and slave approach for sustainable fishery.

Fisheries management measures for sustainable fishery in inland waters

1. Fishing capacity regulation/ license for craft and gear
2. Prevention of destructive fishing gears and practices
3. Mesh size regulation
4. MLS for inland fishes
5. Observing closed season and closed areas
6. Discouraging use of mosquito nets/ destructive fishing gears
7. Community pond/cages for fattening live juveniles of fishes landed in fishing gear
8. Species enhancement in selected water bodies
9. Prevent habitat degradation process

10. Banning of fish seed collection from natural waters
11. Stocking and ranching
12. Restoring connection between isolated ponds and open water bodies for facilitating breeding migration
13. License for all aquaculture units to control the introduction of exotic predatory fish

By-catch reduction devices for trawls

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Introduction

Global fish production reached an all-time high in 2016, estimated at 171 million tonnes, with the capture fishery contributing 90.9 million tonnes and the rest from aquaculture. With this high recorded production, the world fish supply reached a record high of 20.3 kg per capita in 2016. The record growth has been due to the increase in aquaculture production, whereas the global marine fisheries production has reached a plateau during the last decade and is now hovering around 80 million tonnes. It is estimated that about 33.1% of assessed fish stocks are overfished and the stocks which were fished at biologically sustainable levels decreased from 90 percent in 1974 to 66.9 percent in 2015 (SOFIA, 2018), and the percentage of assessed stocks that are underfished is estimated now as only 7%. The trends are really ominous and unless measures to ensure sustainability are not considered, there is no further potential for an increase in marine capture.

Though there are different dimensions to the problem of marine capture, growth overfishing and recruitment overfishing, due to illegal fishing using illegal methods and gears is often a big issue. The problem of using non-legal gears often with smaller mesh sizes and designs that are regionally not appropriate is rampant in many parts of the world. However, trawling has been implicated the most due to the generation of bycatch and damage to the ecosystem structure and function, due to its non-selective nature and destruction to the bottom fauna and flora. Adding to the complexity is the exponential increase in the number of trawlers in the tropics over the years.

The importance of reducing bycatch and minimizing the ecological impacts of fishing operations has been emphasized by scientists and fishery managers and recognized by fishermen. Trawl fisheries in different parts of the world are now required to use bycatch reduction devices as a result of legal regimes introduced by the governments. The Code of Conduct for Responsible Fisheries (CCRF) (FAO, 1995), which gives guidelines for sustainable development of fisheries, stresses the need for developing selective fishing gears in order to conserve resources, and protect non-targeted resources and endangered species.

Bycatch from harvesting systems

The term bycatch refers to the non-targeted species retained, sold or discarded for any reason (Alverson et al., 1994). Target catch is the species that is primarily sought after in the fishery and incidental catches is the retained catch of non-targeted species and discarded catch is that portion of the catch that is returned to the sea due to economic, legal or personal considerations. Global bycatch by the world's marine fishing fleets was estimated at 28.7 million t in 1994, of which 27.0 million t (range: 17.9-39.5 million t) were discarded annually and shrimp trawling alone accounted for 9.5 million t (35%) of discards annually (Alverson et al., 1994). In 1998, FAO estimated a global discard level of 20 million t (FAO, 1999a). Average annual global discards, has been re-estimated to be 7.3 million t, based on a weighted discard

rate of 8%, during 1992-2001 period (Kelleher, 2004). Davies et al. (2009) redefined bycatch as the catch that is either unused or unmanaged and re-estimated it at 38.5 million tonnes, forming 40.4% of global marine catches. The recent global estimates of bycatch are 9.1 million tonnes, with highest contribution from bottom trawls of about 4.2 million tonnes, with tropical shrimp trawl fisheries contributing the most.

The reduction in bycatch discards globally, in recent years could be attributed to (i) increased use of bycatch reduction technologies, (ii) anti-discard regulations and improved enforcement of regulatory measures, and (iii) increased bycatch utilization for human consumption or as animal feed, due to improved processing technologies and expanding market opportunities. Also, equally important as the issue of bycatch is the un-quantified impacts of different fishing systems on the ecosystem, with active fishing gears like trawls causing the most damage. FAO has brought out international guidelines on bycatch management and reduction of discards, in view of its importance in responsible fisheries (FAO, 2011). Life underwater (14th Goal) among the Sustainable Development Goal (SDG) has different targets for sustainable use of fisheries resources.

Trawl bycatch, in the tropics is constituted by a high proportion of juveniles and sub-adults, particularly of commercially important fishes, which needs serious attention in the development, optimization and adoption of Bycatch Reduction Technologies (BRD).

Bycatch reduction devices

Devices developed to reduce the capture of non-targeted species during trawling are collectively known as Bycatch Reduction Devices (BRDs). These devices have been developed taking into consideration variations in the size, and differential behavior pattern of shrimp and other animals inside the net. Different types of bycatch reduction technologies have been developed in the fishing industry around the world (Prado, 1993; Brewer et al., 1998; 2006; Eayrs et al., 1997; Broadhurst, 2000; CIFT, 2007; Eayrs, 2007; Boopendranath, 2007; 2009; 2012; Boopendranath et al., 2008; 2010a; 2010b; Kennelly, 2007; Broeg, 2008; Boopendranath & Pravin, 2009; Pravin et al., 2011; Suuronen et al., 2012).

BRDs can be broadly classified into three categories based on the type of materials used for their construction, *viz*., Soft BRDs, Hard BRDs, and Combination BRDs. Soft BRDs make use of soft materials like netting and rope frames for separating and excluding bycatch. Hard BRDs are those, which use hard or semi-flexible grids and structures for separating and excluding bycatch. Combination BRDs use more than one BRD, usually hard BRD in combination with soft BRD, integrated into a single system. Designs that reduce the non-targeted catch either by taking into account the behavioural difference of the species or by excluding the catch entered also can be considered as BRDs, though the term is commonly used for devices that are attached to trawls to reduce non-targeted catch.

Use of BRDs is one of the widely used approaches to reduce bycatch in shrimp trawls. Some of the advantages in reducing the amount of unwanted bycatch caught in shrimp trawls by using BRDs are (i) Reduction in impact of trawling on non-targeted marine resources, (ii) Reduction in damage to shrimps due to absence of large animals in codend, (iii) Shorter sorting times, (iv) Longer tow times, and (v) Lower fuel costs due to reduced net drag (Boopendranath et al., 2008; Boopendranath & Pravin, 2009). The effects of BRD installation on total drag of the trawl system and hence on fuel consumption has been reported to be negligible (Boopendranath et al., 2008).

Soft Bycatch Reduction Devices

The soft Bycatch Reduction Devices use soft structures made of netting and rope frames instead of rigid grids, prevalent in hard BRDs, for separating and excluding bycatch. Based on the structure and principles of operation they are classified into five categories viz., (i) Escape windows, (ii) Radial Escapement Section without Funnel, (iii) Radial Escapement Section with Funnel, (iv) BRDs with differently shaped slits and (v) BRDs with guiding/separator panel. Soft BRDs have advantages such as ease of handling, low weight, simplicity in construction and low cost, compared to hard BRDs.

Hard Bycatch Reduction Devices TED

Various designs of hard BRDs are in operation around the world which includes (i) Oval grids, oval-shaped metallic grids with exit openings like Georgia-Jumper, Saunders grid, Thai Turtle Free Device (TTFD), Oregon grate, CIFT-TED, Seal Excluder Device and Halibut Excluder Grate; (ii) Slotted grid BRDs which provide slots for the passage of non-targeted organisms such as Hinged grid and Anthony Weedless; (iii) Bent grids in which grid bars and grid frame are bent at one end near the opening such as Juvenile and Trash Excluder Device (JTED), NAFTED; (iv) Flat grid BRDs such as Nordmore grid, Wicks TED, Kelly-Girourard grid, and EX-it grid.

Fisheye BRD is considered an important hard BRD around the world. There are several design variations of fisheye BRD such as Florida Fish Eye (FFE) used in the Southeast US Atlantic and in the Gulf of Mexico. Other designs in this category are Snake-eye BRD used in North Carolina Bay, Fish slot, Sea eagle BRD and Popeye Fish excluder or Fishbox BRD.

Hard BRDs also include TEDs like NMFS hooped TED, Fixed angle TED and Cameron TED (Oravetz and Grant, 1986; Prado, 1993; Mitchell *et al*., 1995; Talavera, 1997, Rogers *et al*., 1997), Matagorda TED, Georgia-Jumper, Super Shooter, Anthony Weedless, Jones TED and Flounder TED (Talavera, 1997; Mitchell *et al*., 1995; Dawson, 2000; Belcher *et al*., 2001; CIFT, 2003) that are devices used for the conservation of Sea turtles.

Semi-flexible BRDs

Semi-flexible BRDs made of semi-flexible or flexible materials such as polyethylene, polyamide and FRP are used in the North Sea brown shrimp fishery, Polyamide grid devices provided with hinges to facilitates operation from net drums have been used in the Danish experiments in the North Sea shrimp fishery and Polyamide-rubber grid design are used in Denmark.

BRDs with guiding or separator panel

Guiding or separator panels are used to achieve separation of the bycatch by using differences in their behaviour or size. BRDs with guiding panels lead the fishes to escape openings, making use of the herding effect of the netting panels on finfishes. The shrimps are not subjected to herding effect and hence pass through the meshes towards the codend. BRDs with separator panels physically separate the catch according to the size, with the use of appropriate mesh size. Shrimps pass through the panels to the codend while bycatch such as fishes and sea turtles are directed towards the exit opening Fig: (1).

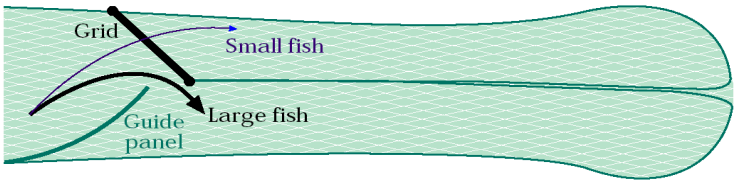

Fig. 1 Separator panel BRDs

BRDs in India

A number of BRDs have been developed and field tested in India. The BRDs evaluated include hard BRDs *viz*., Rectangular Grid BRD, Oval Grid BRD, Fisheye BRD and Juvenile Bycatch Excluder cum Shrimp Sorting Device (JFE-SSD) and soft BRDs *viz*., Radial Escapement Device (RED), Sieve net BRD, Separator Panel BRD and Bigeye BRD (Boopendranath et al., 2008). The efficacy of square mesh codends for selective fishing is widely reported and the selection parameters for a large number of fishes have been derived. The conceptual simplicity and the ease of installation of square mesh codends make its adoption much easier in the small-scale fisheries. The mesh lumen (opening) of the diamond meshes tend to close during fishing due to various forces acting on the net, whereas the square meshes remain open and retain their shape, thus allowing non-targeted catch like small fish and juveniles to escape through the mesh openings. Studies using square mesh codends in India, have demonstrated improvements in the selection properties. (Kunjipalu,1994; Boopendranath and Pravin, 2005; Madhu et al., 2016).

Improved trawl designs like the CIFT-Off Bottom Trawls System CIFT-OBTS, short body shrimp trawl, Cut-away Trawl belly and separator trawls also are found to significantly reduce non-targeted catches due to its design features.

Conclusion

Studies using bycatch reduction devices have shown to reduce the incidence of bycatch in trawling considerably. Different BRD designs have been tried and the efficacy of a particular design depends on the composition of bycatch in the area. Experimental trials for optimization are needed before the designs are released for field trials among the fishers for adoption. A small loss in revenue, as a result of reduced bycatch, is often negated when the overall future gain is considered in the fishery as a result of increase in the yield per recruit from the stock. Benefits like subsidies in the fishery can also be linked with the adoption of good practices in the trawl fishery.

Use of BRDs for resource conservation is one of the many strategies for sustainable harvest of the fishery resources. Adherence to the norms in the marine fisheries regulations acts (MFRA), reduction of fishing effort (in terms of capacity and size of the vessels and gear), spatial and temporal fishing area restrictions and strict monitoring, control and surveillance are required for the gear based technical measures like BRDs to be effective.

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Indian Deepsea Fishing: Status and Challenges

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Introduction

Deepsea fishing takes place at great depths namely Exclusive Economic Zone and high seas. Ever since, the expansion of the fisheries sector following industrialization in capacity attained a multi-fold increase, and venturing into new areas for fishing has also become a focal point. This leads to conflict in the area of fishing as the sea adjoining the land is commanded by the land itself. Hence, the jurisdiction has always been a hurdle for fishery managers and the concept of EEZ, territorial waters and contiguous zone were introduced globally. These regulations are also applied to the conservation of resources. Recent development in the fisheries sector required expansion and the deep-sea resources were targeted as a new area of development. Technological advancements have prepared the sector to face much higher challenges. The onset of FAO International Guidelines for the Management of Deep-sea Fisheries and High Seas led to the adoption of specific recommendations to follow in the scenario.

The Indian economy has been supported by the fisheries sector since industrialization and the sector contributes significantly to the export market. Also, the nutritional security and employment generation of the country depends on it greatly. Even though, the country is unable to reach the annual per capita fish consumption of 11 kg /year, the present per capita consumption is around 9 kg, which shows the need for an immediate additional nutritional requirement for the country. Blessed with a huge coastline of 8129 km, India holds the right to explore the Indian Ocean up to 200 nautical miles from the coastline and our production has reached 3.05 million tonnes in 2021against the projected potential of 4.41 million tonnes.

Studies concluded that the fishery resources being harvested are mostly from the coastal waters and more than 90% of the catch is obtained from within 50-meter depth. It leads to increased fishing pressure in the nearshore waters. As there are plenty of uncharted areas of high potential, exploitation at deeper waters with increased capacity is recommended by many fishery managers. Though keeping high expectations in the exploitation of resources, management strategies are crucial in ensuring sustainable fishing practices.

Indian deepsea fishing is ongoing since the introduction of the industrial strategy called the First Five Year Plan (1951-56) where chartering ventures were invited from foreign countries. The government also encouraged the mechanization of indigenous fishing vessels with motor power. One of the outcomes of this mechanization programme was the design popularly known as Pablo boat. Twelve standard designs of wooden fishing boats in the size range of 7.67 to 15.24 m were developed and introduced by ICAR-CIFT, Cochin which gave a major boost to the mechanization program of Indian fisheries. By the end of 60’s, about 3000 indigenous boats were mechanized with the ability to venture deep into the sea. Maritime Zones of India Act,1981 enforced the first regulation of fishing by foreign vessels in Indian waters and paved the way for the deep-sea fishing policy in 1991. Though it was practised for a considerable long time till 1997 and additional licenses were not given due to protests from

local fishermen. From 200-2001, the EXIM policy by the Ministry of Commerce and Industry again introduced a Special License Scheme to invite foreign vessels into the Indian EEZ followed by the first set of regulations issued by the GOI that allowed specific fishing practices in the deep sea such as long lining and purse seining for tuna, squid jigging and hand lining, mid-water pelagic trawling and trap fishing. The Guidelines also defined deep sea fishing (fishing activities beyond 12 nautical miles from the shore line i.e. the Territorial Waters) and deep sea fishing vessels (fishing vessels of 20 meter overall length and above). In 2004 hook and line fishing and pole and line fishing were also incorporated under the resource-specific fishing methods. It is reported that up to 200 vessels were exploiting offshore tuna resources, and deep-water species such as shrimp and lobster as per the charter/joint venture system which was existed at the beginning of 1990s. Following the 1996 abolition of the charter/joint venture system, numbers of industrial scale vessels operating in the EEZ came down to below 60, but have subsequently picked up again under the guidelines on deep-sea fishing, promulgated by Government in 2002.

Major Deep Sea resources

Based on the report of 2010, Revalidation Committee, the total potential of oceanic waters is estimated at 216 500 tonnes, including Yellowfin tuna (37%) and Skipjack tuna (46%). Other major species include Bigeye tuna, Billfishes, Sharks, Barracuda, Dolphin fish, Wahoo, etc., and comprise about 17 per cent of the total. From the species composition, it is clear that the primary objective of exploring oceanic fishery should be to exploit quality Yellowfin tuna resources and complement this with skipjack tuna and other resources such as Bigeye tuna and Billfishes. India is still a small player in global tuna fisheries. Except the Lakshadweep group of Islands, there is hardly any organized tuna fishery in India. Synonymous with tuna fishing, the Lakshadweep Islands abound in skipjack followed by yellow fin. Fish aggregating devices such as ‘payao’ were introduced in Lakshadweep for increasing tuna catch and have performed well. Similarly, the Lakshadweep Administration is introducing larger fishing vessels (12- and 17-meters overall length) for increasing tuna catches from its waters. Baitfish fishing also forms an important component of the pole and line tuna fishing of Lakshadweep and could become a constraint in future if not managed sustainably.

In the Bay of Bengal, the Andaman and Nicobar Islands offer some of the best tuna fishing grounds in the Indian EEZ. However, due to lack of capacity and weak forward and backward linkages prevailing in the Islands, tuna resources from the Andaman and Nicobar waters have largely remained unexploited. Since the oceanic tunas are migratory in nature, the tunas that could have been caught by the Indian fleet in the Andaman and Nicobar waters mostly get harvested in the EEZs of the neighbouring countries or in the high seas by the fleet of the distant water fishing nations. Simultaneously, the small-scale fishing sector, especially off the coast of Visakhapatnam and in some coastal districts of southern Tamil Nadu has also ventured into tuna fishing. These initiatives include the targeting of Skipjack and Yellow fin tunas (particularly in Vishakapatnam) using troll line, hand line, gill nets and hook and line. In southern Tamil Nadu (Nagapattinam area), large floating devices are being developed to aggregate tuna and tuna-like species. Tuna fishing on the east coast is seasonal and takes place for about 7-8 months (August -March). Further, in Nagapattinam and other fishing centres located on Palk Bay, fishers are also seriously considering converting their trawlers into long liners and moving offshore for fishing tuna and tuna-like species.

In the indigenous expertise on offshore fishing for tuna and tuna-like species, the Toothoor-based (in Kanyakumari district of Tamil Nadu) artisanal fishermen deserve particular mention. The Toothoor deep sea fishermen are not only fishing in different areas of the Indian EEZ (mostly in the Arabian sea), but also in Areas Beyond National Jurisdiction (ABNJ). Since 2006 onwards, MPEDA has also initiated conversion of trawlers into tuna long liners and most of such conversions have taken place in southern districts of Tamil Nadu.

Categories of deep-sea fishing fleet of India

The deep-sea fishing fleet in India can be broadly categorized under four heads. The first comprises fishing trawlers converted to tuna long liners under a scheme implemented by the MPEDA. The second category includes the vessels of 20-meter OAL and above brought through the Letter of Permission (LOPs) issued by the Department of Animal Husbandry, Dairying and Fisheries (DAHD&F), Ministry of Agriculture. The deep sea going fishing vessels of Thoothoor in Kanyakumari district form the third category. These vessels also have a collective called the Association of Deep Sea Going Artisanal Fishermen (ADSGAF). The fourth category of vessels is from Visakhapatnam and they also fish in the deeper waters off the coast of Andhra Pradesh. These vessels apart from deeper waters of our EEZ also carry out fishing in the area beyond our national jurisdiction i.e. international waters.

Issues in deep sea fishing industry

1. **Policy limitations**

Introduction of deep-sea fishing vessels under charter policy was targeting the export market alone, as opined by the local fishermen. Without proper monitoring, many vessels have approached nearshore waters and resulting in conflict between the artisanal sector and the mechanized sector weakening the financial stability of the domestic market. Recommendation of buffer zone, opening off shore completely for joint venture and foreign vessels until domestic fishermen attain capacity, uniform ban on monsoon fishery have made the imbalance in the resource exploitation as they have created agitation among fishermen.

2. **Marketing hurdles**

Indian fish marketing is still facing problems due to the weak linkage between the consumer and the producer. Middlemen interventions are still playing at large by controlling price spread has been demanding government interventions. Also, not all deep-sea resources are marketed due to differential demand as tuna, shrimp, sharks are having better acceptance. The price disparity between primary auction and retail price has been varying highly in case of high demanded species also.

3. **Capital investment and recurring cost**

Deep sea fishing is an expensive venture compared to coastal fishing due to the increased scouting and market unpredictability. Limited schemes from the government are not found to be reaching many fishermen due to the lack of financial capacity by the fishermen.

4. **Demanding skilled fishermen**

In India, when the deep-sea fishing sector is not organized this is well known that there will be no or adequate manpower with technical competency. Almost all the deep-sea boat owners surveyed felt that the longer duration of fishing in this sector is a major limiting factor for the non-availability of Skilled manpower.

Recommendations

1) Training to improve the skills of deep-sea fishermen to achieve a better income

2) Financial assistance along with the current plan of conversion of trawlers to deep sea longliner cum gillnetter.
3) Policy interventions to improve the fishing scenario and to attract more fishermen to venture into deep sea fishing.
4) Direct market support to ensure demand for the deep-sea commodity throughout the season.
5) Direct marketing of the deep-sea commodity by regulating the middleman intervention and also constituting fisheries societies to ensure minimum market price for the commodities.

Conclusion

Fishing rights and the responsibilities it entails in the deep-sea sector have been a vexing issue since the early 80's due to sectoral conflicts. While there is enormous potential for the exploitation of oceanic larger pelagic from the pelagic region of deeper wares and non-conventional resources from the mesopelagic realms of deeper waters, it is essential to develop value-added products for domestic and export markets. It is also essential to create awareness of the edible qualities and the nutrient values of the non-conventional resources among the public through various print and electronic media so as to generate a free market for many such deep-sea resources. Research and development programmes should be strengthened through projects on exploratory deep-sea surveys for pelagic, mesopelagic and bathypelagic resources and their tropic and population dynamics. Many targeted deep-sea 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 the deep-sea 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.

Trap fishing in India

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Introduction

Nature and mode of operation of fishing gears have a significant role in making fishing responsible and sustainable. Fishing in a responsible and sustainable way is one of the important challenges faced by the fishing industry. Fishing is an energy-intensive process that results in higher operation expenses especially in capture fisheries. Under Section 8.6 under Article 8: of Code of Conduct for Responsible Fisheries envisages the importance of optimum use of energy for responsible and sustainable fisheries. Among passive fishing methods, Trap fishing is one of the energy-efficient age-old fishing methods and it has been widely practiced throughout the world in both tropical and temperate seas (Hawkins, et al., 2007). Pots and traps are gears which make the entry of the aquatic species easy and make the escapement difficult due to special designs. The parts of traps which prevent the escapement may be chambers, flaps, narrow paths, funnels etc. Enormous designs of pots and traps exist throughout the world. Based on the abiotic and biotic factors, pots and traps differ regionally in size, design, operation etc.

Pots and traps

According to FAO, traps are large structures fixed to the shore. Pots are smaller, movable traps, enclosed baskets or boxes which are deployed from any craft. In India, the usage "Pot" is not much common and the fish trapping devices are generally termed as "Traps". Traps are generally operated in the area where other types of fishing gears cannot be operated due to uneven bottom or submerged obstacles. The advantages of trap fishing are

- Trap fishing is economic and low energy is required when compared to the active fishing method. They are highly fuel efficient both in terms of f returns and biomass per unit of fuel consumed (Wilimovsky and Alverson, 1971, Mohan Rajan, 1993).
- Organisms caught in the trap can be retrieved alive in an undamaged condition
- Traps can fish continuously day and night and require only periodical tending (Pravin et al., 2011)
- They can be left in the sea during unfavorable weather conditions and can be collected when favorable conditions set-in.
- Capital investment is relatively low and many traps show a high degree of selectivity.

Mechanism & Type of fish trapping

In India, based on the area of operation, pots and traps are classified mainly into pots and traps of marine and inland sector. The inland traps and pots are very common and popular throughout the country. Even though various marine fish traps are operated for livelihood subsistence, organized marine trap fishing exists only in the Southern coast of the country, especially in Tamil Nadu. Rectangular-shaped trap for fin fishes and semi cylindrical-shaped

design for lobsters are the most widely used trap designs in India. Depending on the level of modernization, traps are also classified into traditional traps and modern traps. Plunge baskets, box traps, filter traps, aproned filter traps screen barrier, bamboo screen barrier, net barrier, *Chemballi koodu*, *chevu*, Kalava traps, lobster traps, crab traps etc are some of the examples for the traditional trapping systems (Remesan, 2006, Remesan and Ramachandran, 2008). Details of some of the important traditional traps (Marine sector) are described below.

Marine Fish traps

The traditional fish traps operated along Gulf of Mannar, Palk Bay and south coast are known as *koodu*. These traps are mainly used for catching perches and perch like fishes. Fishers from Rameswaram evolved extremely elaborate stellate form of this traps with a roomy side chamber in each of the arm and even with 5 entrances of the interior. These traps are made of splinters of babul tree or with thin bamboo reepers or palmyrah leaf stalk fibers (Meenakumari, 2009). The meshes are hexagonal in shape with each side of the mesh having a length of 3-4cm. The length of the trap varies from 60-150cm, breadth from 60 to 120cm and height from 15 to 45 cm.

Kalava traps

Kalava traps are operated for kalava and perches. They are used in rocky sea bottom and submerged reefs in depth ranging 60-150m along the west and east coast of India. Traditional Kalava traps are known as Rameswaram type traps. Modified modern kalava traps are also operational in various part of the country. These rectangular traps made of 10mm dia MS rods with strengthening ribs. These rods are joined together with coil hinges so as to facilitate the collapse of the trap when not in use.

Lobster traps

Spiny lobsters are traditionally caught from the south coast of India with traditional lobster traps. These traditional traps are called as Colachal traps. They are heart-shaped/arrow-headed trap locally fabricated with biodegradable materials. By understanding the shortcomings and operational difficulties of the traditional traps, ICAR-CIFT has developed and popularized modern lobster trap for this region (Meenakumari et al., 2009). These traps were accepted by fishermen (Fig.1) due to their special design and durability.

Gargoor fish traps, Caribbean traps (arrowhead, "Z", "S", etc.); round traps, rectangular traps;"D"-shaped traps, collapsible traps, pelagic fish traps, North Atlantic cod pots, plastic multipurpose traps are some of the common designs used throughout the world. In trap fishing, fishes are caught by attracting (using bait or any other attractant) or forcefully directing to specially designed traps or trapping area by utilising the behaviour of the targeted species. The diversity of fish traps designs ranges from natural structures like rocks and corals to specially designed species-specific traps (Slack-Smit, 2001). Based on the nature of catching mechanism, tarps are classified in to various categories viz. Barrier type, Habitat traps, Tubular traps, Mechanically operated traps, Basket traps , Large open traps, Aerial traps etc.

Targeted species

Most of the fishes, crustaceans and cephalopods can be caught with traps and pots. The catch rate of the trap fishing depends on the distribution and assemblage of the targeted species in the fishing ground also the behaviour of the fishes. In India, shallow-water reef and estuarine fish and shellfish are commonly caught with traps and pots, Most pots and traps used in the tropics have been designed for fishing in reefs, rocky areas and on the rough bottom. The fish,

cephalopods and crustaceans taken include snappers, emperors, groupers, parrot fish, surgeon fish, squirrelfish, angelfish, tropical rock lobsters and others. Pot fishery is widespread in mangrove creeks and estuarine areas for various crabs (mud crabs, swimmer crabs, spanner crabs, etc.), adult prawns (giant freshwater prawn) and a number of offshore shrimps. Various types of squid and octopus are also trapped in most tropical waters

Factors considered during the fabrication of fish traps.

The cost for material and the charge for fabrication of fishing traps should be made minimal, by using locally and easily available materials. The material used for the construction should be durable and should be able to withstand the physical stress of the fishing environment. If the traps are for marine use, the material used should be sturdy in sea water or it should be coated or treated with suitable anti corrosion agent. By using biodegradable materials, ghost fishing can be prevented in the event of losing the trap during operation. The design should be simple and easy to set and haul. The gear should be easy to carry in the vessel and should not have any complex structures, projections or attachments. The catch quantity can be improved by using a greater number of traps. For this stackability of the gear plays an important role. If the traps are of light weight and collapsible, a greater number of gears can be accommodated in boat or vessel. The design should be selected based on the biological characteristics of the targeted species like morphology, feeding and swimming behavior, niche etc.

Parts of a typical fishing trap

A typical fish trap consists of the following parts.

Main frame skeleton (rib): frames are the main skeleton or ribs of trap. Usually, strong materials prevent the traps and pots from losing their shape during fishing. Wood, bamboo or metal are the commonly used materials for the fabrication of main ribs,

The outer covering: This part may be with bamboo slits; synthetic meshes or metallic webbings. In traditional pots, coconut or palms leaves are used. The selection of material is mainly based on the traditional usage, cost and availability.

Funnel (entrance): funnel or entrance is the major part of a trap. These are the entrance to the trap. The number of funnels varies depending on the design of the trap. The entrance may be single or multiple. Studies show that a greater number of funnels increases the catching efficacy of the gear.

Door: Doors are referred to the catch collecting area**.** Some designs may be provided with, an area where the meshes can be opened and closed for collecting the catch

Escape gaps: An Escape vent ensures responsible fishing. These are the gates for the escapement of juveniles entering inside the gear (Fig 1). Escape gaps are common in lobster traps in many parts of the world, but not in India.

Bait area: normally bait will be provided in the trap to attract the fishes. Bait will be fixed in the main chamber of the trap with suitable bait bags or chambers. Small pelagic fishes, slaughter house waste and small animals are commonly used as bait for attracting the fishes. Even artificially formulated bait can be used in traps.

Ballast: In the area with higher tidal flow or current, suitable weights need to be provided in the traps to prevent losing of traps. Ballast is normally used in the traps constructed with light weight material. Ballast also helps to maintain the original posture of the traps during operation.

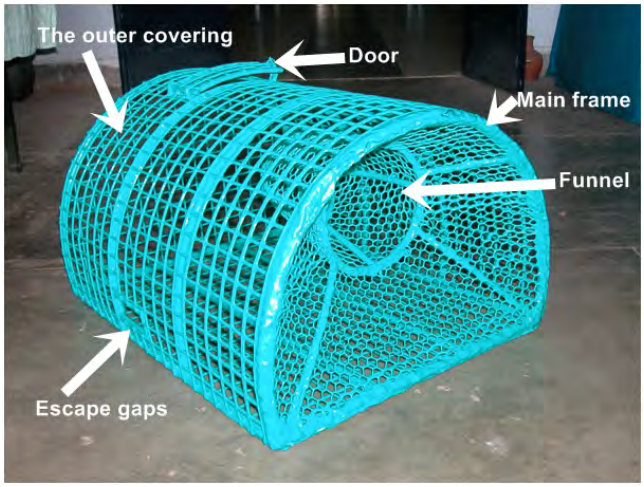

Fig 1. Modern lobster trap (ICAR-CIFT Design)

Operation of traps

Before operating traps, we should have some basic idea on the type of targeted fish, bait, post-harvest handling, storage of catch and market for the harvest. (Slack-Smit, 2001). Simple trapping and potting can be carried out from small boats or canoes or from large vessels. The efficiency of fishing with pots or traps can be improved by the use of equipment like power winches, haulers etc. Once the fishing grounds are fixed, traps can be setup at any time of a day.

Buoys or floats are normally attached to mark the location of the traps. There will be a buoy line attached to the traps/pots for the operation. Proper rigging is essential for the successful operation of the gear. The type and size of the buoy and the length of the buoy line varies based on the area of operation. Normally the length of float line is kept as one and half to twice the water depth of the fishing ground. The length of the line can be increased if the water current is higher at the fishing site. Bright coloured flags, radar, reflectors and even radio beacons are used in advanced trap designs for easy identification. Traps can be operated as single or in series (Slack-Smit, 2001).

Traps and pots can be operated with or without bait. In the case of habitat traps, there will not be provision for the bait attaching area. Funnel shape and positioning of the bait play important role in catch rate. Normally, centre of the traps is the ideal location for attaching the bait. The position of the bait can be optimised by fishers by continuous trial and error method. Depending upon the targeted species, waste from poultry slaughter house, fish and shrimp waste, molluscan meat, wheat flour mix etc can be used as bait. Quality of a good bait include effectiveness to attract targeted species, easy to attach in the gear, long lasting, local availability, low cost etc.

Soaking time also depends on the targeted species and its behaviour. It also depends on the species abundance at the fishing ground. Soaking time varies from few minutes to two to three days while 12- 24 hours is ideal soaking time. After suitable soaking time, traps can be hauled onboard. This can be done either by hand or by mechanical hauler. After collecting the catch, re-baiting can be done and traps can be deployed again in the same or different location.

Ghost fishing in trap sector

Due to bad weather condition, gear conflicts, physical condition of the fishing ground, entangling of large marine animals etc. there will be a chance to get lost or abandon the fishing

gear during operation. These lost or discarded fishing gear which are no longer under a fisherman's control known as derelict fishing gear (DFG), can continue to trap and kill fish, crustaceans, marine mammals, sea turtles, and seabirds. The most common types of DFG to ghost fish are gillnets and pots/traps. Ghost fishing can impose a variety of harmful impacts, including: the ability to kill target and non-target organisms, including endangered and protected species; causing damage to underwater habitats such as coral reefs and benthic fauna; and contributing to marine pollution (NOAA, 2015). To prevent the ghost fishing in traps fisheries, the following steps can be adopted.

- Using proper ballast and anchoring mechanism
- Always operate traps in good weather condition
- During unfavourable conditions, remove traps from fishing ground
- Select suitable site for the installation of traps
- Always provide escape vent or escaping mechanism in the design.
- Use of biodegradable meshes in specific locations

Conclusion

In India, marine trap fishing is an age-old artisanal fishing technique that is confined to the southern states, especially in Tamil Nadu. Traps are highly energy efficient low-cost fishing gears with high size selectivity. Trapping allows some control over the species and sizes of the catch. The trap entrance, or funnel, can be regulated to control the size of fish that enter. Fresh and live catch ensure premium price to the fishers. Once the traps are set, the fishers can operate other gear or engage in other works to increase their income. There is enormous scope for modernizing the traditional fish traps with the most efficient designs and durable gear materials. In the context of energy conservation and responsible fishing techniques, trap fishing in the artisanal sector needs to be promoted.

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Seine fishing in India

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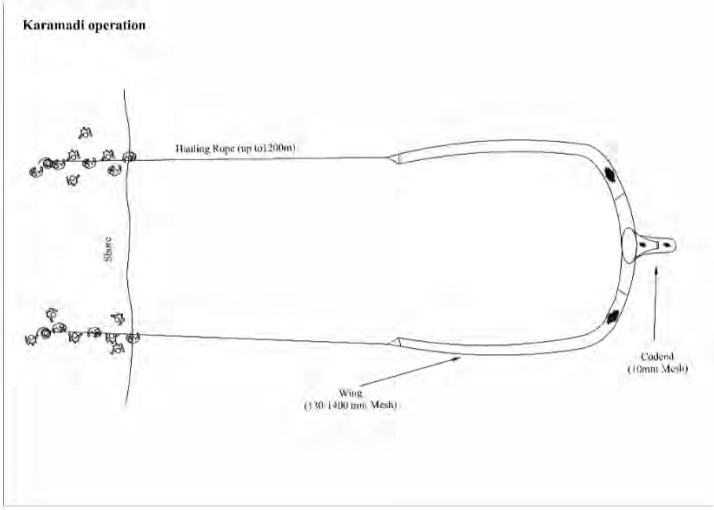
Introduction

Seine nets were used by Greeks in 3rd millennium BC and later Romans employed a large gear referred to as "sagena", which was later introduced to several other countries (Brandt, 2005). It is a rectangular shaped long net to encircle a fish school in shallow coastal waters around a certain area. According to FAO (2001) seine net is a very long net with or without a bag in the centre, which is set either from the shore or from a boat for surrounding a certain area to operate with two long ropes fixed to its ends for hauling and herding the fish. The International Standard Statistical Classification of Fishing Gear (ISSCFG) classifies seines into two major categories such as beach seines and boat seines where boat seines are again classified into Danish seines, Scottish seines and Pair seines. From the age-old days, seines like *ayilakollivala, arakollivala, choodavala, discovala, deppavala, ringvala, kudukkuvala, thanguvala, kollivala, koruvala, mathikollivala, paithuvala* were operational in the south coast of India (Pillai et al., 2000; Edwin et al., 2015).

Beachseine

Beach seine is a seine net operated from the shore composed of a bunt (bag or loose netting), two long wings extended with ropes for towing the gear to the beach and the head rope with floats remains on the surface while the footrope remains attached to the bottom, forming a barrier which prevents the fish in shallow coastal waters from escaping once enclosed (FAO, 2001). Beachseines of India are generally classified into two (a) with codend and (b) without codend. In Kerala beachseines are locally known as *kambavala/karamadi* which have a separate bag like codend whereas in Maharashtra, Goa and Karnataka, *rampani/rampon*the traditional beachseine is without a specific codend (loosely hung meshes only).

A typical beach seine has two long ropes (hauling rope) on either end of the net. One group of fishers remain onshore holding one end of the hauling warp and the second group carries the gear on a vessel along with the other end of the hauling rope. When the fish shoal is sighted the second group encircles the shoal at a distance away from the starting point and returns back to the shore. The hauling ropes are then hauled simultaneously from the shore by two groups of fishers by keeping a distance between them. When the hauling starts, the two groups of fishers come closer as the codend almost reaches the shore. The depth of operation varies from 2-18 m.

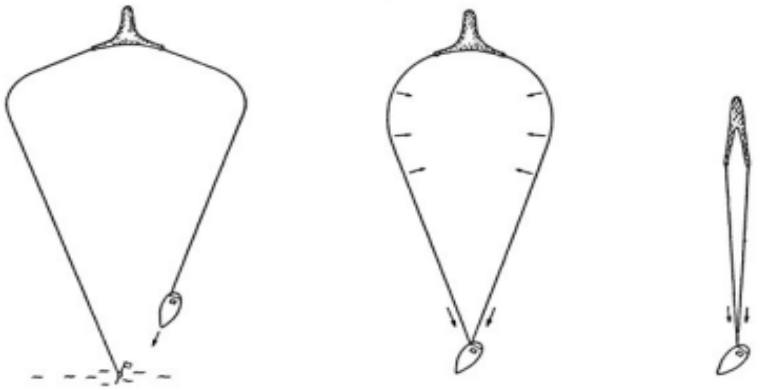


Design of a typical beach seine from Kerala

Boat seine

The boat seines consist of a conical netting body, two relatively long wings and a bag. An important component for the capture efficiency of boat seines is the long ropes extending from the wings, which are used to encircle a large area. Many seine nets are very similar in design to trawl nets. Frequently, however, the wings are longer than on trawls. The foot rope is usually a fairly heavy rope weighted with lead rings or hanging lead ropes. The seine ropes are made from synthetic fibre ropes with a lead core or from a combination of ropes.

The whole gear is encircling a large area in more or less a triangular pattern. The net is hauled back by the anchored boat, which is done by hauling the two drag lines simultaneously with the help of the winches, first relatively slowly and increasing to a larger hauling speed when the net is nearly closed. The use of an anchor is often referred to as Danish seining. Fish inside the ropes are frightened into the forward-moving path of the seine net where they are subsequently overtaken by the net and captured. Another boat seine technique is similar but is not using an anchor. Instead, the boat is kept stationary during haul back with the propeller. This technique is often referred to as Scottish seining or Fly dragging. Mainly demersal and pelagic species are targeted by boat seine. Seine nets are operated both inland and in marine waters. The catching area depends on the length of the ropes; catching depth is shallower than 50 m in lakes and down till 500 m in marine waters. The technique is most efficient on the flat and smooth bottom when long ropes (2 500 m) can be used. Boat seines are also used in rougher grounds, but then with shorter ropes. In some areas boat seines are used to catch schooling fish off the bottom. The impact on living resources is similar to that for trawls as small meshes in the codend may result in capture of undersized fish and sometimes non-target species.



Operation of Boat seine

Seines are normally operating throughout the year while the peak season is during the monsoon & post-monsoon seasons. Operational time varies from 1-5 h. Target species for seine fishing are coastal pelagics and the major catch includes mackerel, sardines, lesser sardines, anchovies, silverbellies, halfbeaks, full beaks, trevallies, herrings, pomfrets, silver whitings, lizard fish, ribbon fishes, squid, shrimp etc. Motorised and non-motorised wooden/ fibre reinforced plastic sheathed plywood boats are used for the operation of seines.

Key issues in seine fishing

The use of a small codend mesh size in the range of 6-10 mm results in high juvenile incidence in the catch. Juvenile incidences of about 50-80% were reported from different states of India. Diamond mesh in codend which on hauling tends to close preventing the juvenile

escapement from the gear. Currently, no regulations are in place for traditional seine fishing. Use of this non-selective gear in ecologically sensitive areas like coral, seagrass, seaweed ecosystems, etc. reported a large occurrence of bycatch including sponges, starfishes, sea cucumbers, sea horses, coral rubbles, seagrasses, mollusc, ascidians, sea anemones, etc. Lack of regulations and poor post-harvest fish handling practices are the other issues in seine fishing in India.

Recommendations

- To reduce the incidence of juveniles, the existing codend mesh size of 6-10 mm has to be increased to 22 mm to help the escape of juvenile fish from the gear.
- During the hauling as the diamond mesh tends to close up preventing the escapement of juvenile fish, the use of square mesh at the codend region is recommended.
- Beach seining has to be avoided and regulated in ecologically sensitive areas (coral reef, seagrass, seaweed, mangrove, etc).
- Handling practices have to be improved with respect to unloading the catch on a clean surface for ensuring hygiene/quality.
- Creating awareness among fishers on the issues and their solutions.
- The Department of Fisheries may develop a reliable database on beach seining as it forms an important secondary livelihood for fishers.

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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) motorised vessels, (iv) mechanised 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 dug out canoes, coracles and catamaran are artisanal crafts.

Fig.1 Artisanal coracle-reservoir/ river fishing

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

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 wheelhouse and accommodation are provided in these vessels. In general, simple traditional fishing is carried out from these vessels.

Fig.4 Traditional fishing boat - Andamans

Motorised vessels: Motor is used for the propulsion of these vessels. Fig.5 shows a 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.

Mechanised 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, life-saving, 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**

Source: 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

Multi-purpose fishing vessels

Trawler

Uses trawl gear for catching fish from the sea. This vessel has a main engine fitted with a reversible reduction gearbox for propulsion. The trawl winch powered by the main engine handles the trawl gear. The gallows fitted in aft are 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

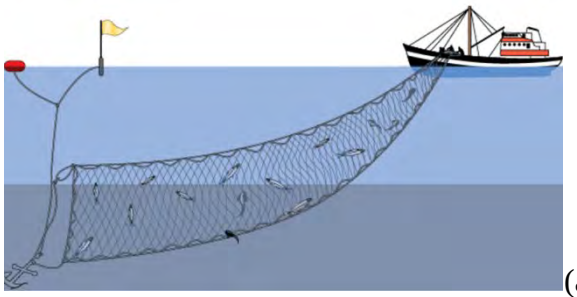
Seiner

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 the operation of the surrounding and seine nets. To assist in fish school spotting observation crows nests are fitted 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 fish collected in the purse, a brailer is provided. OBM and IBM type Ring Seiners have 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 the main deck. A 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 Pole and line vessel in Lakshadweep

Dol netter

Dol nets are fixed bag nets that 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. The below figure shows a Dolnetter.

Fig.11 Dol netters

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.

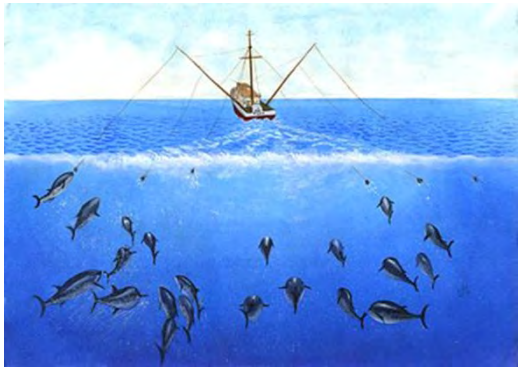


Fig.12 Troller

Multi-purpose vessels

These are vessels that 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 operates 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 F.V.Sagar Harita vessel of CIFT

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 most typical fishing vessels with additional accommodation for trainees. Fig. 14 M.V.Prashikshini training vessel of CIFNET.

Fig 14 M.V.Prashikshini training vessel of CIFNET

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

Energy use optimization and innovations in fishing

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Introduction

In India fisheries sector is promises 14 million employment and income generation. Fishing has been an ancient occupation. It directly contributes approximately 10% of the total animal protein intake by humans. As far as per capita consumption is concerned, global fish consumption is growing at an average rate of about 1.5 percent per year. It was 9.0 kg in 1961 which touched 20.5 kg in 2018 (FAO, 2020). India is one of major fish producing countries in the world. It has an Exclusive Economic Zone (EEZ) of 2.02 million sq.km, a long coastline of 8,118 km and two major groups of Islands with rich and diverse marine living resources. Fishing operation is an extremely energy-intensive process which depends on fossil fuels which are non-renewable and releases high amount of carbon dioxide, contributing to greenhouse gases (GHGs). 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. 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). Global greenhouse gas emissions would be significantly higher if inland fisheries had to be replaced with other forms of animal protein production. Fossil fuel is a prime factor in operational expenses, which can be reduced in order to increase economic feasibility and make fishing operation sustainable. Gradual increment in fuel price is a matter of concern as value wise fuel cost accounted for 60-70% of the total operational expanse. 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 (Parker et al., 2018).

During the last decade, the price of fuel and other energy sources was on a rising trend. In 2001, fuel was estimated to account for 21% of the value of total landed catch, whereas in 2008 this increased to about 50%. Profitability and livelihoods are potentially highly sensitive to energy costs (FAO, 2015). In Indian marine fisheries, the boosted fishing effort and efficiency in the last five decades has led to considerable increase in fuel consumption, which is equivalent to CO2 emission of 0.30 million tons (mt) in the year 1961 to 3.60 MMT in 2010. The CO2 emission has increased from 0.50 to 1.02 t for every t of fish caught during the period. There are 1,99,141 fishing vessels operating in marine fisheries sector of India out of which mechanised, motorised and artisanal vessels contributes about 36.5%, 36.9% and 26.6% respectively, which accounts for about 75%, 23% and 2% of the total catch landed. (CMFRI, 2018). 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 from fisheries were not given importance as compared to other

sectors for emission in India, however, the contribution of fisheries sector is negligible which roughly may be <1% to global GHG emission (Tyedmers, 2004).

In most fisheries energy inputs are required to propel fishing vessels and deploy fishing gears. 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 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. *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. Fuel use varies usually with type of fishing and level of effort, which is one of the key cost components. 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 Thomson (1988) and Allsopp (1989) 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 (Endal, 1980; Nomura, 1980; Aegisson & Endal, 1993). 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 (Gulbrandson, 1986). 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 (Aegisson & Endal, 1993). For large trawlers, 90% fuel consumption accounts during active trawling operation (Anon, 1984a). Percentage of fuel cost in the operational expenditure of trawlers may vary between 45% and 75%, depending on engine power and duration of voyage (Iyer et.al. 1985; Verghese, 1994; Shibu, 1999). Boopendranath (2000) studied 674 trawlers belong to the length class of 13.1-14.0 m LOA and were installed with 99-106 hp engine with about 20% of the vessel had steel hull and remaining wooden hull. Author reported gross energy requirement ranged from 31.40 GJ/t fish for wooden trawlers to 36.97 GJ/t fish for steel trawlers.

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. According to a study based on 210 wooden gillnetter-cum-liners operating from Cochin harbour of length class of 9.1-11. 0 m LOA and majority were installed with 89-99 hp inboard engines the average fuel consumption was 0.36 kg fuel/kg of fish. Among the operational inputs, fuel contributed 95% of the gross energy requirement (Boopendranath, 2000). A study conducted by Vivekanandan et al. (2013) suggested that the larger mechanized boats emitted 1.18 t CO_2/t of fish caught, and the smaller motorized boats (with outboard motor) 0.59 t CO_2/t of fish caught. Among the mechanized craft, the trawlers emitted more CO2 (1.43 t CO_2/t of fish) than the gillnetters, bagnetters, seiners, liners and dolnetters (0.56–1.07 t CO_2/t of fish).

Purse seining: Purse seining is one of the most aggressive and efficient commercial fishing methods for capture of shoaling pelagic species (Sainsbury, 1996; Ben-Yami, 1994a and 1994b). 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 mechanised purse seine operations is low compared to trawling, gillnetting and lining operations (Boopendranath and Hameed, 2013).The gross energy requirement/t of fish were 5.54, 5.93 and 6.4, for 156 hp wooden seiner, 156 hp steel seiner and 235 hp wooden seiner, respectively (Boopendranath, 2000). 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. Tyedmers (2001) reviewed over an approximately 20-year period (early 1980s to late 1990s) and found about 330 L of fuel used to catch per t of catch in a crab trap.

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-year-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 (FAO, 2014). Small scale fisheries require less capital investment and energy for operation (Ben-Yami and Anderson, 1985). Boopendranath (2000) reported number of OBMs vessel in operation was estimated to 16466 nos. and combined power of about 252590 hp and average power of 15.4 hp per vessel of operation period 5 hours per day for 200 days in a year, total fuel consumption by traditional motorised sector in Kerala was estimated was 139x106 L/year, and specific fuel consumption of 0.55 L hp/L. In the context of Indian scenario assuming an average of 15.3 hp per OBM and

one OBM per craft, the total fuel consumption by the motorised fishing fleet of India, would be approximately $269x10^6$ L/year. Among all fish harvesting systems, mechanised trawling is the most energy intensive operation and traditional non-motorised gillnetting is the most energy efficient having the lowest gross energy requirement. Out of non-motorised systems, stake nets have comparatively high energy intensive. Among motorised operations, ring seines have a lower gross energy requirement per ton of fish landed. Gross energy requirement of mechanised seine was found more compared to mechanised seine, this may be due to lower fuel efficiency of outboard motors compared to inboard diesel engines. 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.

Energy and its input linkage

The primary energy elements are fuel for propulsion, and for larger vessels, power supply for a range of ancillaries. The relationship between fishing effort, fishing methods, distance to fishing grounds, vessel speed and fuel efficiency of hulls, engines and propulsion systems are all key factors. Linking with stock conditions and market values, these are all reflected in operating costs, the profitability of fishing and the level and choice of activity. In most forms of fishing activity, fuel costs have direct implications for viability. Types of fishery, conditions of fishing and market prices will all determine the impact of fuel prices, as will specific conditions of the fishing enterprise. Impacts of rising fuel prices and reduced profitability, including the value of capital assets used in the sector, can extend widely. Shorter-term changes can be accommodated by scrapping older or more inefficient vessels, selling and writing down capital values (and hence financing and depreciation costs). Poor profitability will inhibit the building of new fishing vessels, and decrease fleet size even at global level. In the absence of external actions, such as fuel subsidies or market interventions, rising fuel prices will drive out unprofitable fishing businesses, and will tend to reduce fleet size. Depending on the nature of the fishery, this may reduce output, or improve vessel yields and overall economic and fuel-use performance. However, various forms of inertia – time lags in market responses and shorter-term support actions – might delay these changes.

Indian context

In Indian context, several constrain which are acts as a hurdle in fuel use reduction and optimisation. To address this issue ICAR-CIFT has taken many initiatives in the field of energy use reduction. The ICAR-Central Institute of Fisheries Technology (ICAR-CIFT) set up in 1957 is the national institute in the country where research related to fishing and fish processing is undertaken. The institute started functioning at Cochin in 1957. As a contribution to the nation's fishing sector, ICAR-CIFT focuses on basic, strategic and applied research in developing fuel efficient fishing vessels, responsible fishing gears, designing innovative implements & machinery for fishing, Eco-friendly technologies for responsible fishing and low-energy fishing technologies for the traditional sector.

Contributions of ICAR-CIFT in green harvesting

ICAR-CIFT has developed and disseminated standard designs of fishing vessels in the size range 7.67-15.24 m, suitable for various types of fishing under the Indian conditions and appropriate gear systems for trawling, seining, gillnetting, lining and trapping. Modernisation of fishing vessels and development of fuel-efficient designs have been undertaken and newer craft materials have been introduced to reduce the cost of operation and increase the income of

fishermen. Significant contributions of the Institute in recent years in harvest sector include the following:

1) Fuel efficient fishing vessels

19.75 m fuel efficient multipurpose fishing vessel; Sagar Harita: The fishing vessel, Sagar Harita, a 19.75 m long fuel-efficient multipurpose fishing vessel designed by Fishing Technology Division of ICAR-CIFT. The vessel has met all the requirements of the Indian register of shipping (IRS) and ICAR-CIFT. This new generation energy efficient green fishing vessel is equipped with the latest technology viz. solar panels, aiming to promote green energy and reduce the carbon foot prints. The solar panels fitted on the vessel cater the energy requirement for navigational lights, cabin lights etc. The vessel also incorporates an optimized hull design with a bulbous bow, fuel efficient propeller design and improved sea keeping characteristics. Modern tools and techniques including software simulation and model testing have been used for the refinement of the design.

15.5 m deep sea fishing vessel; Sagar Kripa: ICAR-CIFT has taken initiative to develop fuel efficient fishing vessels in view of high expenditure incurred in mechanised fishing operations. A 15.5 m multi-purpose deep sea fishing vessel Sagar Kripa with steel hull was designed and developed with energy saving features. These include optimized hull design, optimized installed engine power, fuel efficient propeller and propeller nozzle. The commercial trials by the fishing boat operators have saved about 17% of the fuel cost.

ICAR-CIFT Sun Boat (Solar boat): ICAR-CIFT developed Sun Boat is another mile stone in this area, as it does not require fuel for propulsion with zero emission for fishing and recreational purposes. The sun boat is capable of running for 2.5 to 3.0 hours after complete charging and attains a speed of nearly 4.0 knots in calm water. Considering the 240 days of fishing in a year the fuel saved compared to an equivalent diesel-powered boat is Rs. 48,000. This boat runs without any fuel cost and pollution. It has clean FRP surface with wider space and low rolling during fishing and a canopy for protection from rain and sun. The boat has navigational lights which facilitate fishing in early morning and also in the night. The traditional small fishing boats are not fitted with navigational lights.

FRP pedal boat for fishing in reservoirs and rivers: Majority of the inland fishing vessels are using fossil fuel for propulsion. Petrol and kerosene are widely used for this purpose. The engine fitted on these vessels pollutes the water body through the exhaust. In order to solve this issue as well as to reduce the operational expenses of the inland fishers a 3 m, FRP pedal boat design was developed and constructed. This boat requires no fuel and zero carbon emission. A light weight type foldable canopy is fixed on top for protection from rain/sun.

2) Energy saving trawling technologies

Trawling is an active fishing method in which a bag shaped fishing gear is towed from mechanized fishing vessel. It is known to be one of the most energy intensive fishing methods.

Low drag trawls: In excess of 60% of the total resistance in the trawl system is known to be contributed by netting alone. Fuel consumption during trawling is directly related to the drag of the gear system. Substitution of large meshes in the front trawl sections has been reported to reduce the drag of the trawl system by about 7% and hence reduces fuel consumption in trawling. The reduced drag permits greater trawling speed and/or operation of larger trawl with the available installed engine power. Adoption of optimised towing speed, thinner twines and large mesh to reduce twine surface area are found to bring down the drag and hence the fuel

consumption. Conventional trawls made of HDPE are with more drag due more twine surface area and weight of webbing. Ultra-High Molecular Weight Polyethylene is a stronger material compared to HDPE, which permit to use thinner twine for trawl fabrication. UHMWPE low drag trawl developed by ICAR-CIFT revealed that average reduction of drag was 15% with 13% average reduction in fuel consumption and average 7.5% reduction in operational expenditure compared to HDPE trawls.

Square mesh codend: Use of small diamond mesh codends is one of the reasons for high drag resistance produced while dragging of trawl net. The excess drag produced by fishing net leads to more fuel consumption. Diamond shape mesh tends to decrease size of mesh lumen while dragging the net, this is due to hindrance in water filtration. Square mesh retains its shape while dragging and allow normal water filtration which consumes less fuel due to less drag. In addition, it is one of the measures to reduce bycatch in the trawl net. Based on the studies carried out by ICAR-CIFT, Gujarat had adopted square mesh codends in trawlnets operated along its coast. Similarly, based on the studies conducted by ICAR-CIFT along Sindhudurg coast, Maharashtra state has implemented the mandatory use of square mesh codends in trawls since 2018. The recommendations of ICAR-CIFT have been incorporated in the amendment of the Marine Fisheries Regulation Act (MFRA) of the Kerala state and use of 35 mm square mesh codend (for fish trawl) or 25 mm square mesh codend (for shrimp) is made mandatory.

Large Mesh Purse Seine and power block for purse seine operations: Purse seining is one of the most efficient and advanced commercial fishing methods. It is aimed mainly at catching dense, mobile school of pelagic fish and includes all elements of searching, hunting and capture. Introduction of large mesh purse seines facilitated by ICAR-CIFT has led to the revival of small mechanized purse seine fishery. The purse seiners were also targeting the same resource in the coastal waters. With the introduction of large mesh purse seine, the fishermen could go to deeper and farther waters targeting large pelagic like tunas, seer fish, pomfrets and large mackerels thus reducing the competition and fishing pressure in the coastal waters. The large mesh helps in minimising fuel consumption.

Cambered otter boards: Otter boards are known to contribute 20-25 % of the total drag of the trawl system. Introducing camber in otter board design is known to reduce resistance of the boards considerably, by increasing the hydrodynamic efficiency of the boards. ICAR-CIFT has introduced high aspect ratio, cambered otter boards for semi-pelagic trawling. Introduction of camber in otter boards reduces the drag of the trawl system by 4% with accompanying savings in fuel.

V-form otter boards: The V-form otter boards are hydrodynamically efficient and have very inherent stability. It is made of steel and do not utilize wood in their constructions. These boards do not plough or dig into the bottom and will tide over smaller bottom obstacles, thus becoming suitable for trawling in uneven and rocky grounds. V-form boards are cheaper and safe in shooting and hauling if properly rigged with a longer service life of 5-6 years. V-form type otter boards have become popular among trawler fishermen of southern India and Gujarat, since its introduction.

V-form double slotted otter board for energy conservation in trawl systems: V-form double slotted otter board is another milestone in the purpose of fuel use reduction. Comparative trials with the conventional V-form otter boards (without slots) showed that with less engine rpm

trawling could be carried out and fuel consumption reduced by approximately 2-3 liters/h of dragging compared conventional V-form otter boards of same size and weight

Eco friendly/energy efficient trawls for off bottom resources: Resource specific trawls for off bottom resources have comparatively low impact on the benthic biota. ICAR-CIFT off bottom Trawl System (ICAR-CIFT OBTS) has been developed as an alternative to shrimp trawling in the small-scale mechanized trawler sector, after extensive field-testing for resource and energy conservation.

Low energy and eco-friendly harvest technologies for the inland fisheries and traditional marine sector: Appropriate craft designs and improved gear designs such as optimised gill nets, lines and traps have been developed and introduced for the inland fisheries. Improved and durable lobster traps with escape window for juveniles have been developed as substitute for traditional traps of short life span and low efficiency, for harvesting of spiny lobster. The rich tuna resources of the Lakshadweep waters are under-exploited as the fishing operations are still limited to traditional pole and line method. ICAR-CIFT has introduced large mesh gill nets and monoline (monofilament long lines) in Lakshadweep waters, for targeted fishing of Tunas, Billfishes, Seer fishes, Carangids and Perches, in an effort to diversify fishing methods and improve catching efficiency.

Use of non-conventional fuel viz. LNG/CNG/Solar energy: The ICAR-Central Institute of Fisheries Technology, Kochi, in collaboration with Petronet LNG Limited, Kochi Terminal, and Kerala State Development & Innovation Strategic Council (KDISC) is going to launch LNG Driven fishing boat first time ever in the country as a pilot project. The experimental fishing vessel of ICAR-CIFT, MV Matsyakumari has been selected as a pilot vessel for the purpose and necessary equipment has been installed in the vessel. Since the use of LNG in fishing vessel is first of its kind in the country, it will be a boon to the fishing industry and oceans as far as green environment is concerned.

Current estimates of fuel use and cost: Tyedmers et al., (2005) proposed annual fuel use of about 50 million m^3, 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 (CO_2) into the atmosphere at an average of 1.7 kg of CO_2 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 CO_2 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 CO_2 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: 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. 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. Gulbrandson (1986) reported that 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 by Wileman (1984), 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. (Wileman, 1984); The Oil fish project (1981-84), Nordforsk. 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 (Gulbrandson, 1986; Aegisson & Endal, 1993). 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 (Boopendranath, 2009).

Conclusions

Global fishing practice is highly varied. Harvest is a process in which capture of aquatic animals takes place using a variety craft and gear, mostly vessel-based fishing gears. The major forms and quantities of energy inputs used in fishing operation also vary extensive. Most of the fisheries, particularly large-scale fisheries, over the past half-century, input of energy/fossil fuel dominate energy profile. Cost and use of fuel is a significant issue in the capture fishery sector. It represents significant input cost in most of the fishing operations, except for non-motorized sector. Impact of fuel price is verifying with economic conditions and location, generally developing countries, distant-water fishing and poorer market conditions will have and greater impacts. The data on fuel consumption can be extrapolated to get the idea about fleet, levels of fuel used, its cost and also help in better management of fishing fleets along with resource and efficiency considerations. The long-term solutions for the problem of fuel conservation in fishing is, restoration and enhancement of coastal fishery resources by promotion of responsible fishing practices, removal of excess fishing fleet (capacity) and resource conservation monitoring and implementation of related rules along with other enhancement strategies.

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Nano technology and its applications in fisheries

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Summary

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 synthesized 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.

Introduction

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 football 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.

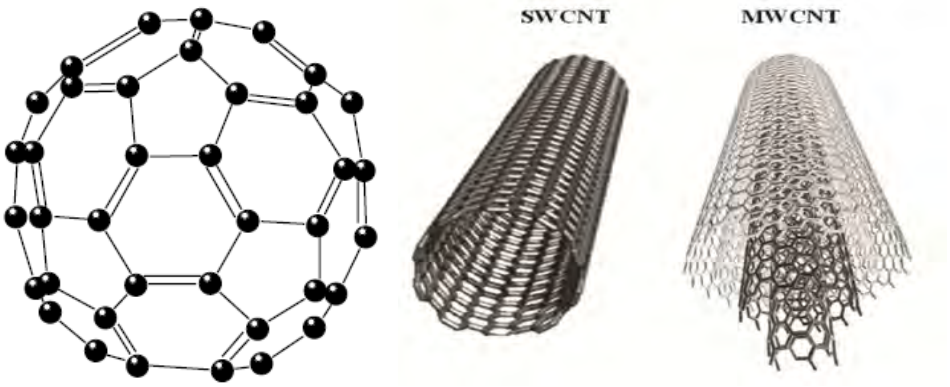

Fig 1. A) Fullerene C60 molecule B) SWCNT and C) MWCNT.

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 analyser with zeta potential etc.

Characterisation 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.

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 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 thermochromic sensors, colourimetric sensors and electrochemical sensors for detection of contaminent 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 synthesised 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 analysing 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 1. 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 articifical 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 was 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 are considered first settled microbes over marine exposed surfaces. The measurement was 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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Recent Developments in Gillnet Fishing

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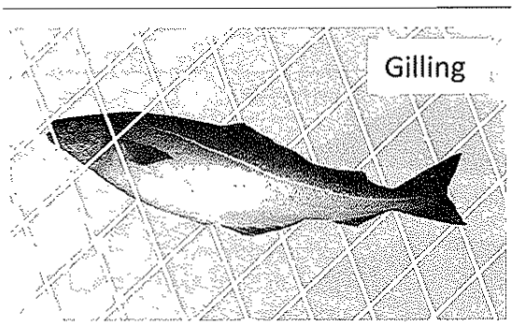
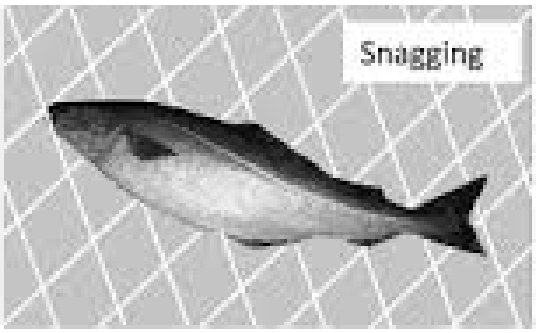
Introduction

Gillnets, an efficient, selective type of passive fishing gear operated world wide both in inland and coastal waterbodies. Simplicity in design, construction, operation and low operating costs, the ability to operate from different vessel types make it a favourite choice among artisanal fishers . The gear is a vertical wall of netting, which is kept erect in water by means of floats and sinkers. Among the various fishing methods, it consumes between 0.15 and 0.25 kg fuels compared to trawl (0.8kg fuel/kg) to catch 1 kg of fish (Brandt, 1984, Gulbrandson, 1986). Gillnet is a highly selective gear compared to other gears. It can be operated in the surface, column or bottom layers of the water column in inland, coastal and deep seas. Gillnets of varying mesh sizes, target a variety of fishes such as sardine, mackerel, anchovies, tuna, shark, seer fish, prawns, lobsters etc. Gillnet operations contribute to more than 15% of total landings of India (Sathianandan, 2013). Currently in India, gillnets provide livelihoods for an estimated 0.86 million people in fisheries, and contribute significantly to fish catches, income and food security, as well as the local and national economy (Thomas *et al*., 2020)

Mechanism of capture in gillnets

Gilling is the basic mechanism of fish capture in gillnet where in the mesh size is selected in such a way that the fish can only partly penetrate the mesh and on sensing the obstruction it tries to pull back (Pinngue He, 2010). In its struggle to free itself the twine slips back over the gill cover and prevents the fish from escaping. Thus, the fish is gilled and hence called ‘gillnet’. Fishes are also caught in gillnets by (1) snagging, when the fish is held tight by the twine of the mesh around its head, (2) wedging, when the fish is held tight around its body, and (3) entangling when the fish is held in the net by the teeth, opercular spines or other protruding appendages of the body without actually entering the mesh (Fig 1.).





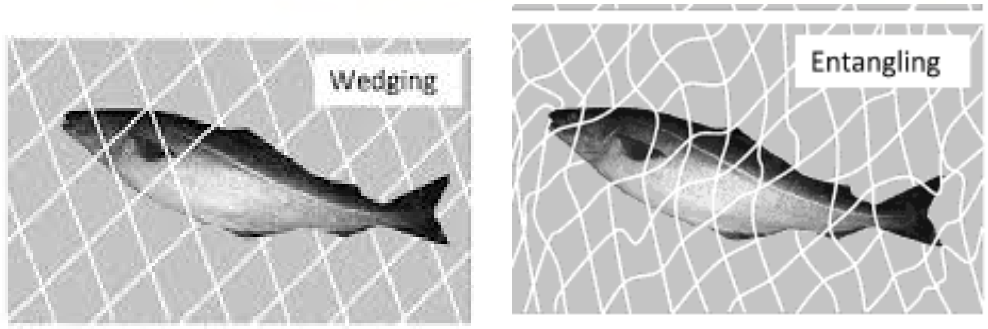


Fig 1: Mechanism of capture in gillnets (Source**:** Pingguo He. 2010.)

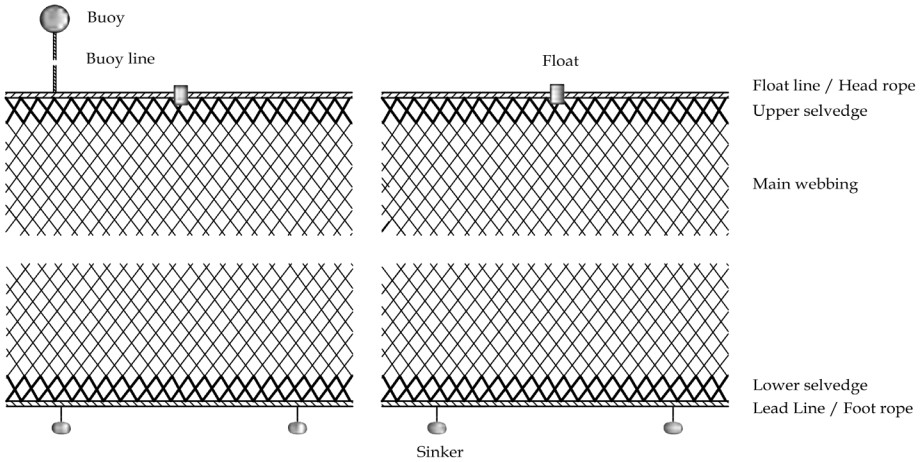
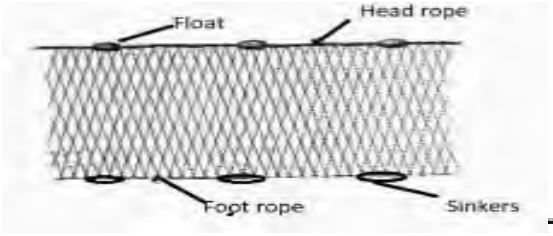


Fig 2: Typical design of gillnet (Source: Thomas., 2010)

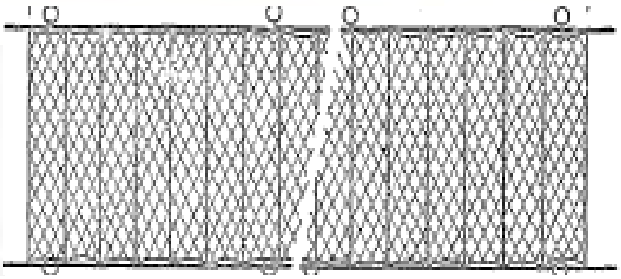
A typical simple gillnet consists of a main netting panel of specific dimensions, twine size and mesh size, selvedge (top and bottom), float line, lead line, gavel line/ side ropes, floats, sinkers, buoys and buoy lines depending on the target fishery (Fig.2). Selvedge, generally of thicker material than the main netting is provided along the edges to give protection to the main webbing during handling and operation. Floats are attached either directly to the head rope or to a separate float line, which runs along with the head rope. Sinkers are also attached likewise, either to the footrope or to a separate sinker line. Buoys attached through buoy lines to the head rope are for adjusting the floatation of the mounted net. The required numbers of units are tied end to end depending on the size of the target species and area of operation.

Types of gillnets

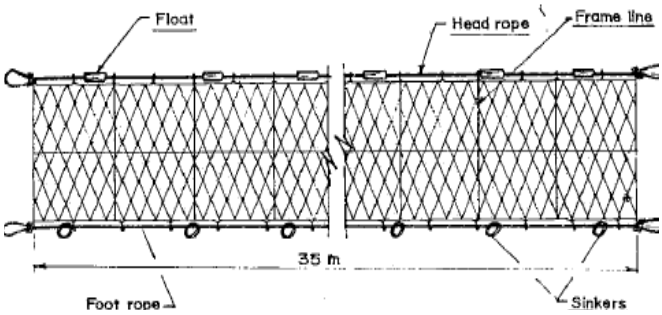
Gill nets can be classified into different groups depending upon the type of construction, area of operation, fish targeted and method of operation. Based on construction there are single walled and multiple walled gillnets. Simple gill nets , vertical line gill nets and frame nets are single walled nets (Fig 3) while trammel nets (double or triple walled) come under the multi walled nets. The vertical line nets are simple gill nets, which are divided into different sections by passing vertical lines from the head rope to the foot rope through the meshes of the webbing. Frame nets are single walled nets whose slackness is increased by attaching vertical and horizontal lines between the main lines dividing the main webbing to compartments of 1 to 1.5 sq.m.



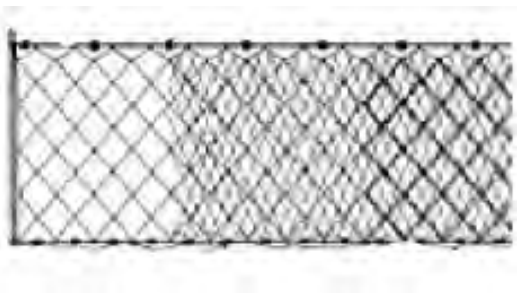
Simple gillnets



Vertical line gill nets



Framed gillnets



Trammel net

Figure 3: Types of gillnets based on construction (Source: Kuriyan. 1971**)**

Trammel nets are triple walled nets having a loosely hung center wall of small mesh netting which is bordered on each side by tightly hung walls of large open meshes. Fish swimming through the outer meshes encounter the center netting and push their way through the opposite outer meshes. Fish become trapped in the resulting pockets that are formed (Fig 4). The outer meshes on one side of the net must be a mirror image of the outer meshes on the opposite side. Semi trammel nets are same as that of trammel nets except that only one layer of outer webbing is present instead of two.

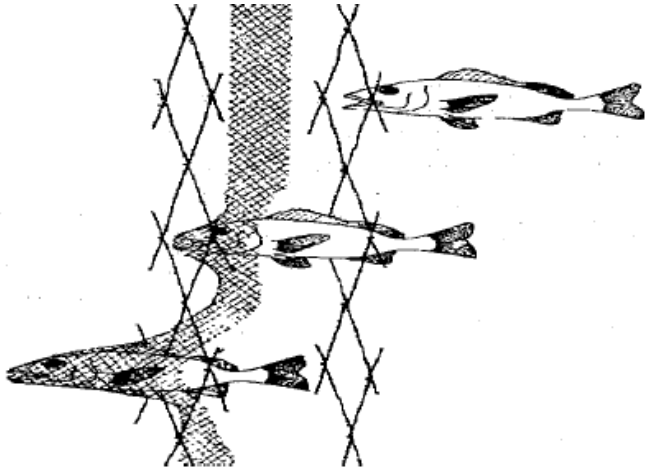
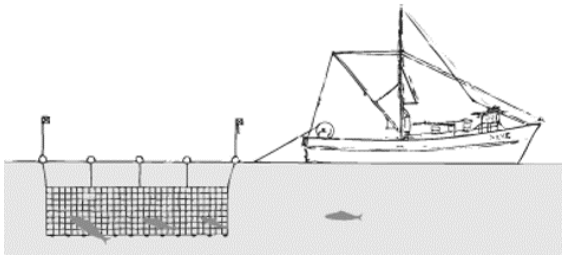


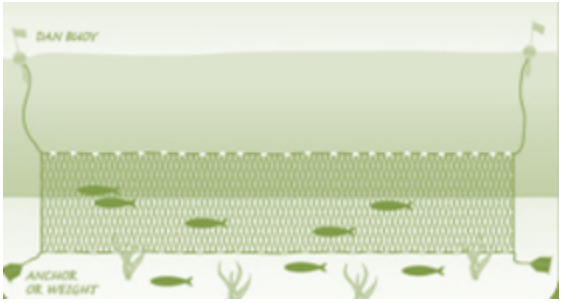
Figure 4 : Method of capture in trammel net (Source: Thomas., 2010)

Depending on the method of operation there are 1) drift gill nets Drifting freely according to wind force or current and kept more or less vertically by floats on the headrope and sinkers on the foot rope. They may be attached at one end to the boat which is fishing them, or they may be left to drift free of the boat 2) Set gill nets which are set in water either surface subsurface or bottom and anchored or stalked to sea bed by means of anchors or stakes

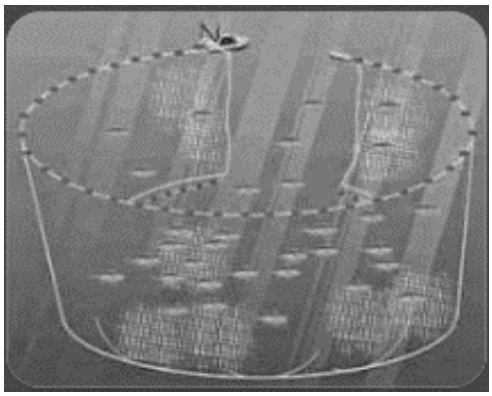
to prevent them from moving with water 3) Encircling gillnets where the fishes are surrounded and driven from the centre by noise and other means (Fig 5).



Drift gillnet



Set gillnet



Encircling gillnets

Figure 5: Types of gillnets based on operation

Based on area of operation, which is dependent on the depth of water column at which they are operated gillnets are classified as surface, column and bottom gillnets. Based on target species nets are also classified viz; nets for anchovy, lesser sardine, sardine, mackerel, prawn, mullet, crab, lobster, pomfret, hilsa, ghol, seer, tuna, shark, catfish, perch, snapper, rock cod etc.

Design aspects

The design of a gillnet depends on target species, its characteristic body shape, behaviour and swimming layer. The main parameters to be considered while designing a gillnet are: (i) size of mesh in relation to the size of the targeted fish, (ii) diameter of the twine in relation to mesh size, (iii) hanging coefficient (looseness of the net, (iv) visibility of the net, (v) softness of the material and the (vi) buoyancy and ballast given. The mesh size is the most critical factor as it selects the fish by body size or shape. Gillnet is the only gear in which the mesh itself serves the dual function of catching fish and selecting the fish to be caught. The mesh size, the material the net is made of, its thickness and colour and the hanging ratio of the nets perform these two functions. Any fish which is too small for the mesh size will be able to slip through the net and escape, while any fish that is too large on the other hand will not pass through and be able to escape the way it came (Thomas, 2010).

Recent Developments in gillnet fishing

Introduction of synthetic fibres

Introduction of man-made synthetic fibres in the late 1950s replaced the natural fibres used for the fishing gears mainly due to their highly positive properties such as highly non-biodegradable nature, high breaking strength, better uniformity in characteristics, high abrasion

resistance, low maintenance cost and long service life. Earlier, nettings used to be fabricated manually, which is laborious and time consuming while the introduction of synthetic fibres paved way for machine made nettings which revolutionized the fishing industry. Introduction of nylon monofilament material in early 1990s was a remarkable technological intervention adopted instantly by fishers. By late 1990s it became very popular and by early 2000 it almost replaced all gillnet types except large mesh nets targeting large pelagics. Monofilament nets last hardly for a season (2 – 6 months) and unless properly discarded, these nets will end up in ocean adding to plastic pollution, ALDFG and ghost fishing (Thomas, 2019).

Motorization and Mechanization

Introduction of out-board motors (OBMs) and mechanisation of propulsion in fishing vessels revolutionized the Indian gillnet fishing industry. The increase in vessel size, engine power, volume of net deployed per operation, fishing time and soaking time all of which collectively increased the total fishing effort. Over the past 6 to 7 decades, there has been a substantial increase in the fishing effort by all the three gillnet categories viz., non-motorized, motorized and mechanized sub-sectors. In India, the length and depth of gill net increased from 150x3 m in 1950s to 18000x20 m at present. Currently, the mechanized gillnetters categorized as small (<12.0 m L OA) medium (12.1-16 m L OA) and large (16.1 -24.6 m L OA) with 60, 120 and 193 hp engines respectively, are deploying large net fleets of 5 to 16 km long and 8 - 20 m deep (weighing upto 3 tonnes). In the non-motorized sector; and motorized sub- sectors also, there was corresponding increase in net volume (Thomas, 2019). However, intensification of fishing capacity through use of very large volume of nets extending to 100s of kilometres gave more chances of non-target organisms including cetaceans and turtles getting entangled in the nets during fishing as well as through ghost fishing by lost nets.

Optimum mesh size

Gillnets were considered as resource specific, eco-friendly having very low environmental impacts as the sea bed interaction is bare minimum in most circumstances. Besides, being a highly selective gear catching a narrow size range of fishes, it was considered as a very responsible fishing gear till two to three decades before. However, these attributes given to gillnets started losing by early 1990s due various issues associated with gillnets such as incidental catch of juveniles and non-target species due to loosely hung drift gillnets and use of multi-mesh and non-optimum mesh sizes. Many coastal states of India have come out with minimum mesh size regulation for gillnet fishery under the Marine Fishing Regulation Acts while Kerala has enacted it for seven gillnet types.

Maximum allowable dimensions of gillnet

The deployment of long nets and extensive use of monofilament gillnets by Indian fishers, pause high risks of gear loss and consequent ghost fishing in Indian waters. Monofilament nets last hardly for a season (2 – 6 months) and unlike nylon multifilament, it is difficult to mend monofilament netting. Once these nets or their parts are either abandoned, lost, or otherwise discarded (ALDFG) into the marine environment causes considerable threat to marine species and also add to marine plastic pollution. The uncontrolled increase in volume of gillnet, demands restriction as it may give more chances of entanglement and ghost fishing also. Though mesh size regulation is enacted by many maritime states, maximum allowable dimension (length and hung depth) of gillnets is not specified by any of the states. Kerala, for

the first time in the country, amended the KMFRA Act and Rules in 2018, and brought out regulations on the dimensions of the gear for gillnets targeted for seven important commercial fishes. The maximum dimensions prescribed for small mesh gillnets are 2000 m length x 10 m hung depth and for large mesh gillnets are 5000 m length x 18 m hung depth (Thomas, 2019).

Minimum legal sizes of fishes

For the first time in the country, Kerala state has prescribed minimum legal size for 58 species of fish and shellfish to be landed. By following optimum mesh size, minimum size of fish to be landed by gillnets can be decided. Gillnets being highly size selective, strict adherence to optimum mesh size for specific fishery would help in reducing juvenile by catch.

Biodegradable netting

As synthetic fibres are non- biodegradable, the environmental threats it causes due to ghost fishing is an important problem. Entanglement and subsequent mortality of non-target and endangered species due to derelict and lost gillnets can be lessened using easily degrading materials (e.g., thinner net twine diameter and weaker material) which reduces the floatation capacity of lost gillnet, which in turn decreases the vertical profile of nets and allow larger organisms to break free of the gear and escape (Gilman *et al*., 2010). Carr *et al*. (1992) tested degradable plastic plates for attaching floats to the headrope of gillnet. Biodegradable gillnets made of polybutylene succinate (PBS) resin blended with polybutylene adipate-co-tere-phthalate (PBAT) resin have been widely studied (Bae *et al*., 2012).

Measures to reduce interactions/entanglement of marine mammals and turtles in gillnets

Marine mammal/turtle entanglements in gillnets are a widely reported problem worldwide. Chances of entanglement are more in surface drift nets. Acoustic pingers and alarms are used to reduce marine mammal bycatch in gillnets and other fishing gears (Koschinski *et al*., 2006). Technical modifications in gillnets such as acoustically reflective nets or incorporating reflective components such as barium sulphate or metal compounds into the nets can help cetaceans to detect gillnet and avoid becoming entangled (Larsen *et al*., 2007). Attachment of visual mitigation measures like shark shaped silhouettes and light sticks and light emitting diode lamps in gillnets have shown reduction in number of turtles caught (Wang *et al*., 2013). Making the nets more visible especially the upper portion by using thicker twine, attaching corks, colouring the net etc. will help to reduce turtle interactions. Other technical measures include increasing net hanging ratio, using buoyless floatlines and/or reducing the number of floats (Gilman, 2010).

Conclusion

Gillnets have great scope in sustainable harvesting of resources, being a highly size selective gear. Enforcement of regulations by proper monitoring and surveillance is necessary for the continued harvesting of resources in a sustainable way. If proper care is taken to responsibly design and operate, gillnetting can continue to be a very sustainable fishing method.

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Identification of fishing gear materials

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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.

Polyolefines: 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 terepthalic 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

The 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 pelagics in Maharashtra region of India.

Ultra high 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 ultra-high 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 fibre 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 upto 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.

Identification of synthetic fibres

Identification of synthetic fibres by appearance alone is not easy and correct. 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.

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.

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 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	+	o	o	o
Sulphuric acid/H_2SO_4 (97-98%) 30 minutes at room temperature	+	+	o	o
(1) Dimethylformamide/HCON $(CH_3)_2$ 5 minutes boiling	+	+	o (2)	o (2)
Formic acid/HCOOH (96-100%) 30 minutes at room temperature	+	o	o	o
Glacial acetic acid/CH_3-COOH 5 minutes boiling	+	o	o	o
Xylene/C_6H_4 $(CH_3)_2$ 5 minutes boiling (inflammable)	o	o	+	+
Pyridine 30 minutes at room temperature	o	o	o	o

Source: Klust, 1982.

+ = soluble, o = 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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Fish for health and prosperity

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As far as India is concerned, the successful outcome of green revolution has answered the challenges of food security due to rapid growth in population. But considering the fact that 35% of Indian population falls still below the poverty line emphasizes the need to recognize fisheries as an important sector of the National economy for meeting the food and nutritional security. In the days ahead, "blue revolution" will be the buzzword to meet the challenges of food and nutritional security.

Fish and fishery products form an important food component for a large section of world population. They represent 15.6 % of animal protein supply and 5.6 % of total protein supply on a worldwide basis. Fish is the primary source of animal protein for over one billion people of developing countries. It is estimated that 60% of people in developing countries obtain 40-100 % of the animal protein in their diets from fish [Lowe *et al*., 1998). Protein.lipids and bioactive compounds from seafood's have unique features that differ from those of land animals. The uniqueness of fish protein is due to its excellent nutritive value, high digestibility and presence of all essential amino acids. In general, fish flesh contains 60-84% water, 15-24% protein, 0.1-22 % fat and 1-2% minerals. Seafood serves as a rich source of polyunsaturated fatty acids [PUFAs], especially omega-3 PUFAs, minerals and vitamins [Fierens and Corthout *et al*., 2007].

Fish is a health food, with relatively lesser taboos connected to it, unlike meat. World over fish is considered as a delicious item and in nutritional point of view, it is the balanced diet one can easily think of, when consumed along with cereals. A health food should contain all the principal constituents like carbohydrates, proteins, lipids, minerals, vitamins etc. in the right proportion. Detailed biochemical composition of all important Indian food fishes (including proximate composition, fatty acid composition of body and liver oils, content of important minerals, amino acid composition of muscle proteins etc from fresh water, brackish water and marine and deep sea waters have been compiled and reported by the Central Institute of Fisheries Technology (Gopakumar *et al* ., 1997). People are now more health conscious. Diets low in fat and cholesterol with high vitamin and mineral contents are often preferred, especially in the affluent west. For a healthy lifestyle, fish is a good starting point. Importance of fish as a source of high quality, balanced and easily digestible protein is now well understood. For the affluent it is the best health food with curative properties whereas for the less privileged section in developing nations it is the only source of high-quality protein available at affordable cost and in sufficient quantity.

Fish plays a major role in human nutrition. Fish and shellfish form an important part of the human diet, both of the poor and of the wealthy. Good quality fish is an extremely safe food. Meat products are viewed as unsafe after the incidences of diseases like mad cow disease. Fish is a versatile, tasty and easy to prepare food. Consumers are increasingly demanding for

natural food stuffs, which contain no chemical residues and are not genetically manipulated. Fish is organic and is considered as wild, and for the same reason safer, though of late farmed fish has posed minor problems of harmful residues.

For thousands of years, fish has been an important part of the human diet. The ancient Assyrians, Romans and Chinese were famous for their fish farming. During the past decades per capita consumption of fish has gone up globally. Fish is the diet of the poor fishermen, which meets most of their nutritional requirements

Researchers all over the world have repeatedly emphasized the beneficial effect of eating fish, after conducting systematic research for many years. In recent years, the link between fish oil and heart disease has been the subject of thousands of scientific papers. The whole story began following the discovery that coronary heart disease, while being one of the biggest killers in the world, is practically unknown among the Eskimos. The investigations found that their diet is mostly fish based and is rich in long chain n –3 poly unsaturated fatty acids (Lee and Lip 2003; Von Sehachy and Dyerberg,2001). Eskimos also have a reduced tendency to blood clotting and longer bleeding times compared to other people (Krishnan, *et al.* 2001). Medical researchers carried out detailed investigations and showed that men who ate fish once or twice per week were protected against coronary heart disease (Ite *et al.* 2004: Eokkila *et al.* 2004). An increase in fish oils in the diet results in a marked reduction in blood cholesterol and triglyceride levels and also thrombosis problem (Bjerregaard *et al.*, 2004).

Lipid content in fish varies between species as also within the species depending on many factors. Fish with fat content as low as 0.5% and as high as 18-20% are common. Squalene and wax esters are other components found in unusually high concentrations in certain fish. The fatty acid composition of marine lipids is much more complex than others. Lipids of fish and other aquatic animals contain high proportion of highly unsaturated long chain fatty acids. Fatty acids with carbon chain varying from 10 to 22 and unsaturation varying from 0–6 double bonds are common. Among the saturated acids palmitic and stearic acids are the important ones and in the monounsaturated group, palmitoleic and oleic acids are the major constituents. Among the polyunsaturated acids arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the major components. In Central Institute of Fisheries Technology, marine, fresh water and brackish water fishes were screened for evaluating their fatty acid composition and in the flesh. Fish and shellfish from tropical waters were analysed for their cholesterol content, showing higher levels in shellfish compared to fish (Mathew *et al.*, 1998).

Fish oils have no effect on the levels of low-density lipoprotein cholesterol (LDL); but they do raise high-density lipoprotein (HDL) by about 10%. HDL is a protective type of lipoprotein since it takes excess cholesterol away from the tissue and returns it to the liver. Diseased heart muscle is susceptible to bouts of irregular electrical activity (arrhythmias), which are potentially lethal and often cause sudden cardiac arrests. There is evidence from animal studies that increasing fish oil in the diet helps to reduce cardiac arrhythmias (Sellmayer *et al.* 2004; Covington 2004). Fish oils improve the functionality of cell membranes, which helps in proper signal transmission. Fish oil inhibits platelet aggregation, which also reduces the risk of heart disease (Vanschoonbeek *et al.* 2004). Raised blood pressure is known to be a

major risk factor in coronary heart disease. Most studies on the effects of fish oil given as dietary supplements have shown modest reductions in blood pressure, especially in hypertensed people (Aguilera *et al.* 2004; Wilbuurn *et al.* 2004; Maano *et al.* 1995).

As stated earlier, fish oils are rich sources of the essential fatty acids eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3). Both EPA and DHA fall into a larger category of polyunsaturated fatty acids (PUFAs). Approximately 50% of the fatty acids in lean fish and 25% in fattier fish are polyunsaturated fatty acids. In contrast, the polyunsaturated and saturated fatty acids in beef are 4 – 10 % and 40 – 45 % respectively. EPA and DHA reduce vasoconstriction by competing with arachidonic acid for the enzyme cyclo-oxygenase (Sametz *et al.* 2000). EPA, the main n-3 acid is converted by platelet cyclo-oxygenase to thromboxane A3, which is only a very weak vasoconstrictor, unlike thromboxane A2, which is formed by the action of cyclooxygenase on arachidonic acid, the n-6 acid and is a strong vasoconstrictor (Tapiero *et al.* 2002; Akiba *et al.* 2000). The American Heart Association recommends including fatty fish at least twice a week in the diet (Kris-Etherton *et al.* 2002; Krauss *et al.* 2000). Institute of Human Nutrition in New York also recommends eating plenty of fish. Italian study involving 985 people who survived heart attacks, also proved the beneficial effect of fish oil (Tavani *et al.* 2001). The new slogan in the west is that a tuna sandwiches a day keeps heart problems at bay (Mozaffarian *et al.* 2004; O'Neill 2002). It is also stated that if a person wants to reduce the risk of heart attack by more than 20 %, he has to eat a tuna sandwich just once a month. No wonder they say, "Seafood is heartfood".

Recently the inhibitory role of n-3 PUFAs in the development and progression of a range of human cancers have been established by researchers, world over. Studies have found that the anti-tumor effect of EPA is mainly related to its suppression of cell proliferation (Pham and Ziboh 2002; Yuri *et al.* 2003). The effect of DHA appears to be related to its ability to induce apoptosis or cell death (Baumgartner *et al.* 2004; Chiu *et al.* 2004). The dietary n-3/n-6 fatty acid ratio, rather than the quantity administered, appears to be the principal factor in the anti-tumor effect of n-3 PUFAs.

Apart from heart disease and cancer, fish oil is proved to be effective for preventing wide variety of diseases. In several observational studies, low concentrations of n-3 PUFAs were predictive of impulsive behaviours and severe mental depression (Ruxton 2004; Freeman *et al.* 2004). The importance of PUFAs in the maintenance of insulin in the blood has also been proved in experiments (Holness *et al.* 2004). Clinical and biochemical studies have shown that fish oil, and to a lesser extent fish can be used as a source of n-3 fattyacids in the treatment of rheumatoid arthritis (Ruxton 2004; Remans *et al.* 2004). Supplementations with fish oils can markedly reduce inter leukin – 1 beta production and results in a significant reduction in morning stiffness and the number of painful joints in arthritis patients. Studies have shown fish oil to be effective in the treatment of acute respiratory distress syndrome (Pacht *et al.* 2003), psoriasis (Mayser *et al.* 2002), and multiple sclerosis (Nordvik *et al.* 200) also. Older people who eat fish at least once a week may reduce their risk of Alzheimer's disease by more than half (Yazawa 2004; Morris *et al.* 2002). Other diseases which are reduced due to the consumption of PUFAs include primary Raynaud's disease (DiGiacomo 1989; Swanson 1986),

gastric ulcer (Olafsson *et al.* 2000; Manjari and Das 2000) and Crohn's disease (Geerling *et al.* 2000).

Along with fish oils, proteins in fish are also having positive role in reducing blood cholesterol (Ait Yahia *et al.*, 2004). Recent studies have shown that fish proteins have a clear protective effect in diabetic renal diseases (Mollsten *et al.*, 2001). Fish proteins are having high biological value, as they contain all essential amino acids in the right proportion. Plant proteins, although rich in certain essential amino acids do not always offer all essential amino acids in a single given food. Legumes lack methionine, while grains lack lysine. Fish protein is also an excellent source of lysine as well as the sulphur-containing amino acids, methionine and cysteine. Amino acid scores of fish protein compares well with the FAO reference pattern. In the studies conducted in the Central Institute of Fisheries Technology, Kochi, it was seen that the amino acid composition of the protein is crucial in determining its hypocholesterolemic properties. The alanine/proline ratio in a protein was found to be the significant factor determining its hypocholesterolemic properties (Ammu,K., *et al*.,1994).

Protein content of fish muscle ranges between 16 and 20% depending on the species of the animal, the nutritional condition, and the type of muscle. The crude protein calculated on the basis of the total nitrogen content represents proteins and other nitrogenous compounds, such as nucleic acids, nucleotides, trimethylamine (TMA) and trimethylamine oxide (TMAO), free amino acids, urea, etc**.** Protein from fish is easily digested, with most species showing a protein digestibility greater than 90%. The chemical score or amino acid score compares a food's amino acid pattern to that of whole egg protein. The chemical score of finfish is 70, an indication of its high quality, beef is 69 and cow's milk is 60. The protein efficiency ratio (PER) another measure of protein quality of fish is around 3.5, which is much higher than beef (2.30) and milk proteins (2.5) and close to that of egg (3.92). Fish is a good dietary source of taurine, a non-protein amino acid with multiple functions like neurotransmission in the brain, stabilization of cell membranes and in the transport of ions such as sodium, potassium, calcium and magnesium (Franconi *et al.*, 2004; Birdsall, 1998; Del Olmo *et al.*, 2000). Nutritional quality of protein is generally determined by factors like essential amino acid composition, digestibility and biological value. Fish protein is rated high in all the above qualities and is considered as a good dietary protein in all respects.

In general, both water soluble and fat-soluble vitamins are present in fish. Fat soluble vitamins A, D, K and E are present in fish in varying amounts-often in higher concentrations than in land animals. The number of vitamins and minerals is species-specific and can vary with season. The flesh of lean white fish, such as cod, haddock, and pollock, contains from 25 to 50 IU of vitamin A per 100 g, while in the fatty species such as herring, there is from 100 to about 4500 IU of this vitamin in 100 g of meat. The content of vitamin D in sardines and pilchards and in tuna is in the range of 530 to 5400 and 700 to 2000 IU per 100 g, respectively. The contents of vitamin E in the edible parts of fish and marine invertebrates range from about 0.2 to 270 mg/100 g. Fish is a good source of B vitamins. The red meat has higher content of vitamin B than white meat. Fish liver, eggs, milt and skin are good sources of Thiamine (B_1), riboflavin (B_2), pyridoxine (B_6), folic acid, biotin, and Cyanocobalamine (B_{12}).

Fish also contributes appreciable amounts of dietary calcium, iron and zinc. Fish contains copper and those who relish fish bones get a fair share of calcium and phosphorous. Salt-water fish are rich in iodine. The iodine in marine fish ranges from 300-3000 µg/kg. Fish is a good source of almost all the minerals present in seawater (Nair and Mathew, 2000). The total content of minerals in the raw flesh of fish and aquatic invertebrates is in the range of 0.6 to 1.5 % of wet weight. Certain seafoods such as snails and tuna are good source of the macro mineral magnesium. Seafood, especially tuna, is an important source of the essential antioxidant trace element selenium, which provides protection against heavy metal poisonings and a variety of carcinogens. Functioning cooperatively with vitamin E, selenium is also a vital factor in protection of lipids from oxidation as part of the enzyme glutathione peroxidase, which detoxifies products of rancid fat. The carbohydrate content of finfish is insignificant, but certain shellfish store some of their energy reserves as glycogen, which contributes to the characteristic sweet taste of these products.

When we consider the beneficial effects of dietary fish, vegetarianism in dietary habits does not seem to be wise. When one decides to become an obligate vegetarian and cuts out meat/dairy/fish out of diet, he decides to cut out some of the major nutrient's body needs on a daily basis for effective functioning. The argument that fishes lives in unhygienic habitat and polluted waters is also not valid as pollution is a universal phenomenon, affecting air, land and water Fish is the heart food which gives you both satisfaction and health and it is the word for nutritional security.

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Seaweeds nutrition facts and health benefits

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Abstract

Seaweed, a salubrious natural resource is being increasingly explored for culinary aspects. Seaweeds encompass a good source of antioxidants, dietary fibres, essential amino acids, vitamins, PUFA, minerals and phytochemicals. The presence of structurally diverse bioactive compounds with valuable nutraceutical properties makes seaweed a vital ingredient for functional food development. The incorporation of seaweed bio-actives in high demanded fortified food would provide an opportune approach. Basic nutritional components/ ingredients present in seaweed can be extracted and utilized for supplementation purpose. The review briefly describes the nutritional and functional potential of the seaweed.

Keywords: Seaweed, Salubrious, Nutraceutical, Food Development

Introduction

The consumption of marine foods is increasingly gaining attention due to awareness among people in regard to diet and health (Granato et al., 2020). Currently, several marine based foodstuffs have been developed and marketed, offering enhanced health benefits (Annunziata & Vecchio, 2011). Seaweeds are relatively unexplored and promising sources of novel molecules like peptides and carbohydrates possesing nutraceutical properties (Lafarga et al, 2020). Further seaweeds are a potent source for the phycocolloid industry (agar, carrageenan, fucoidan and algin). The fame of seaweed in the international trade is peculiarly for phycocolloids and products of laminarin and fucoidans.

Seaweeds are primitive plants, macrophytic algae which lacks true roots, stems and leaves belonging to genera Chlorophyta, Phaeophyta and Rhodophyta (Fouda et al., 2019) representing a diverse group of approximately 10,000 species (Makkar et al, 2016). They are cultured in abundance in the coastal areas of Tamil Nadu, Gujarat, Lakshadweep, Andaman and Nicobar Islands. Only few of these species are utilized for food applications, mainly as food additives or flavouring materials, particularly in Asian countries and is served in meals of Japanese approximately to 21% (Yoshinaga et al., 2001). Seaweeds are apt for human as well as animal feeds and are taken in various forms like raw salad, soups, meals and condiments in China, Japan, USA, France and Chile, etc (McHugh, 2003). Edible seaweeds are considered to be a good source of antioxidants, dietary fibers, essential amino acids, vitamins, phytochemicals, PUFAs, and minerals. The marine macro- and micro- algae are considered one of the excellent natural antioxidants and antimicrobial (Cox et al., 2010; Chen et al., 2009), vitamins (A, B1, B2, B3, B5, B7, B9, B12, C, D and E) and minerals (Ca, P, Na, K and I) sources along with polysaccharides holding dietary fibres with prebiotics and biological activities of potential medicinal value (Dhargalkar and Pereira, 2005; Smit, 2004).

It is believed that seaweed bioactive components significantly increase the health status if they are consumed throughout life (Biesalski et al., 2009). Apart from therapeutic properties, seaweeds are gaining importance because of their ability to act as texture modifier i.e. stabilizer, texture enhancer, viscosity modulator, gelling agents, etc. in various food products.. Due to consumer demand towards natural and safe substrate, emphasis on several plant based products is on the eye for "green" additives. The polyphenolic compounds from seaweed have well documented antioxidative and antimicrobial properties which helps in prevention of spoilage due to oxidation and food borne pathogens (Gupta & Abu-Ghannam, 2011).

The incorporation of seaweed bioactives in fortified food provides an opportune approach (Kadam and Prabhasankar, 2010). Seaweed based functional food products like alginate and carrageenan powder, fucoidan fortified phyto-complexes, alignin, minerals, vitamins, ß-carotene along with seaweed protein powder, etc. covers a confined niche in the market. Seafood constituents, seaweeds i.e. macro and microalgae can be added in various food stuffs prepared from meat, dairy, fish, vegetables, fruits, etc. to make them more functional, thus improving their health promoting characteristics. (Jimenez-Colmenero, 2007; Mikami & Hosokawa, 2013). For instance, bioactive peptides from seaweeds like algal fucans, galactans and alginates exhibits anticoagulant, anticancer, and hypercholesterolemic activities (Lordan, Ross, & Stanton, 2011). The beneficial properties of seaweeds are ascribed to the complex phytochemicals compounds comprising phenolic compounds, sulphated polysaccharides and organic acids which exhibits antioxidant, antimicrobial, anticancer and antiviral activity (Apostolidis et al., 2008; Liu, 2003). Antioxidants prevent oxidation by transforming free or peroxy radicals into non-radicals by donating electrons and hydrogen, chelating transition metals and dissolving thereby generating peroxidation compounds (Enrique & Lester, 2002). The use of antioxidants is an effective way to minimize or prevent lipid oxidation in foods, retarding the formation of toxic oxidation products, maintaining nutritional quality with prolonged shelf life. Synthetic antioxidants (BHA, BHT, THBQ) and antimicrobials (sodium benzoate, sodium nitrite and sorbic acid) are used extensively for safety and quality control in food industry. However, the toxicity regarding use of synthetic antioxidants and antimicrobials is well documented which force the food processing sector to switch towards use of natural preservatives (Andarwulan et al., 2010; Ayaz et al., 2008).

Biochemical composition of seaweed

The detailed chemical composition of seaweeds is not well known like terrestrial plants (Kadam and Prabhasankar, 2010), but these are considered one of the excellent source of natural antioxidants and antimicrobials (Cox et al., 2010; Chen et al., 2009), vitamins (A, B_1, B_2, B_3, B_5, B_7, B_9, B_{12}, C, D and E) and minerals (Ca, P, Na, K and I) along with polysaccharides holding dietary fibres with prebiotics and bioactives of potential medicinal value (Dhargalkar and Pereira, 2005; Smit, 2004).

Seaweeds are rich source of protein with all essential amino acids. The proteins content varies from 10-40% (w/w) dry weight based on the season and species (Murata and Nakazoe, 2001) with the highest in red seaweed. Certain green seaweed species like Ulva, Caulerpa contains high level of arginine and glycine (Fouda et al., 2019). The free amino acid section of seaweed is predominated by taurine, alanine, amino butyric acid, ornithine, citrulline, and hydroxyproline (Holdt and Kraan, 2011). Among all the groups of marine algae, highest concentration of taurine is reported in red algae. Among the proteins present in seaweeds,

lectins, a hemagglutinin protein binds with carbohydrates and actively takes part in host-pathogen and cell to cell interactions, identifying and binding carbohydrates to exert functional effects (Mori et al., 2005). Seaweed peptides (inactive in the amino-acid sequence of the parental protein) obtained through the enzymatic digestion process have displayed biological and mineral binding activities (Smit, 2004).

Studies conducted reveals that seaweeds have slightly elevated levels of total fibre (not readily digested in gut) compared to terrestrial foodstuffs comprising mainly alginates, carrageenan and agar (Brownlee et al., 2005) depending on the type of seaweed. Serving of 8 g seaweed provides around 12.5% of daily fibre needs i.e. 24 g/day. Marine algae have 10-100 times higher mineral content than vegetables (Nisizawa 2002) and can be labelled as supreme natural source of minerals (chiefly due to their habitat and the diverse minerals absorption from marine environment), but their large amount consumption can be detrimental. Accumulation of calcium is higher, moreover, sodium and potassium (Na:K ratio is below 1:5), iron, copper levels are also relatively higher than terrestrial foodstuffs like meats, spinach, etc. and can be considered as good minerals supplement. Ash content in seaweeds is reported upto 55% on dry weight basis (Rupérez 2002). The main source of lipids in seaweeds are glycolipids followed by phospholipids (Bhaskar et al. 2004, Khotimchenko, 2005) and content is relatively lower as compared to marine organism, constituting 4.5% on a dry weight basis which varies with the season and environmental factors. They are rich in fatty acids with essential fatty acids as well as omega fatty acids, moreover, among all the seaweeds red algae and green algae contains approximately 33 and 29 fatty acids respectively (Fouda et al., 2019). With the decrease in environmental temperature accumulation of polyunsaturated fatty acids (PUFAs) is common and the cold waters aquatic species generally contain larger quantities of PUFAs (Narayan et al. 2006). For instance, the number of phospholipids in various red seaweed species varies from 10% to 21% of the total lipid (0.5–2.6 mg/g on dry weight basis). Besides, the phenolic content varies from <1% to 14% (dry seaweed biomass) with the highest content in *Ascophyllum* and *Fucus* i.e., 14 and 12% respectively (Mabeau and Fleurence 1993).

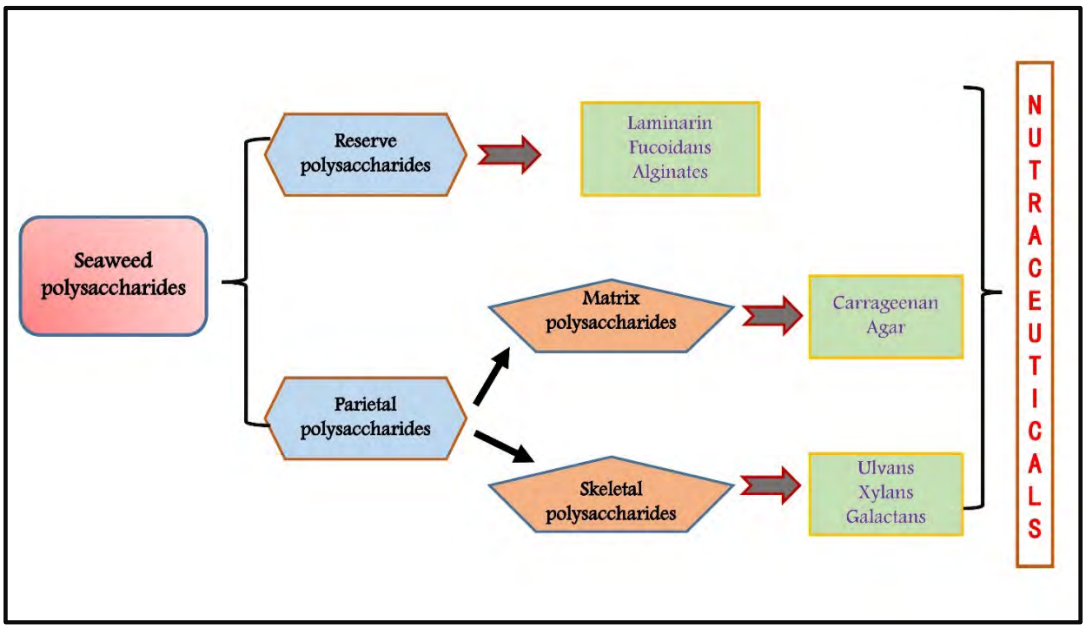

Fig 1. Seaweed derived nutraceuticals

Table 1. Bioactive compounds and Polysaccharides from various seaweed species

S.No	Bioactive compounds	Seaweed species	Reference
1	Total polysaccharides	*Saccharina latissima, Sargassum pallidum*	Rengasamy et al. 2014b, Murata and Nakazoe, 2001; Ye et al. 2008; Athukorala et al. 2007, Kumar et al. 2008, Holdt et al. 2011
2	Carrageenan	*Chondrus crispus, Eucheuma cottonii*	Fernandez et al. 1989; FAO, 2008; Hayashi et al. 2008; Carlucci et al. 1997; Caceres et al. 2000, Rodrigueza and Montaño, 2007
3	Agar	*Gracilaria cornea, Gracilaria domingensis*	FAO, 2008; Fernandez et al. 1989
4	Algins/alginic acid	*Laminaria digitata, Laminaria hyperborea*	Jensen and Haug, 1954; Morrissey et al. 2001; Kim and Lee, 2008; MacArtain et al. 2007; Jensen and Haug, 1956
5	Mannitol	*Saccharina latissima, Laminaria hyperborean, Sargassum mangarevense, Ascophyllum nodosum:*	Zubia et al. 2008, MacArtain et al. 2007
6	Phycarine	*Laminaria digitata*	Mayer et al. 2007
7	Porphyran	*Porphyra umbilicalis*	MacArtain et al. 2007, Plaza et al. 2008, Noda, 1993
8	Fucoidan	*Fucus vesiculosus, Ascophyllum nodosum*	Berteau and Mulloy 2003; Li et al. 2008a; Zhao et al. 2008
9	Laminarin	*Fucus vesiculosus, Laminaria hyperborea*	Deville et al. 2004; Shanmugam and Mody 2000; Miao et al. 1999
10	Ulvan	*Ulva lactuca, Ulva rigida*	Lahaye 1998; Angell et al. 2014, Mata et al. 2016, Onda et al. 2017
11	Floridoside	*Porphyra umbilicalis, Palmaria palmata*	MacArtain et al. 2007
12	Xylans	*Caulerpa lentillifera, C. racemosa, Bryopsis maxima, C. anceps, Halimeda cuneata*	Lahaye et al. 2003, Kiyohara et al. 2006
13	Total protein	*Undaria spp., Sargassum spp.*	Bird et al. 1993
14	Lectins	*Ulva sp., Eucheuma amakusaensis*	Kawakubo et al. 1997, 1999, Sugahara et al. 2001; Liao et al. 2003, Mori et al. 2005; Matsubara et al. 1996;

15	Phycobilliproteins	*Palmaria palmata, Gracilaria tikvahiae*	Chronakis et al. 2000; Wang et al. 2002
16	Phlorotannins	*Cystoseira trinodis, Fucus serratus, Ascophyllum nodosum, Halidrys siliquosa*	Sathya et al. 2017; Gager et al. 2020.
17	Carotenoids	*Palmaria, Sargassum horneri, Cystoseira hakodatensis,* and *Undaria pinnatifida*	Yuan 2008, Terasaki et al. 2012
18	Halogenated compounds	*Laurencia spp., Plocamium and Chondrococcus spps., Asparagopsis* and *Bonnemaisonia*	Dembitsky and Srebnik 2002, Blunt et al. 2003, Knott et al. 2005
19	Sterols	*C. crispus, Laminaria, Undaria, Palmaria* and *Porphyra*	Sanchez-Machado et al. 2004, Whittaker et al. 2000
20	Phenolic Acids	*Dasycladus vermicularis, Ascophyllum nodosum, Bifurcaria bifurcata,* and *Fucus vesiculosus*	Agregán et al. 2017, Farvin et al. 2013
21	Bromophenols	*Rhodomela confervoides, Polysiphonia morrowii* and *Ulva lactuca*	Fan et al. 2003, Ko et al. 2019, Flodin et al. 1999, Shi et al. 2010
22	Flavonoids	*Porphyra yezoensis, Padina arborescens and Acetabularia ryukyuensis*	Yoshie et al. 2000, Ismail et al. 2016
23	Mycosporine-Like Aminoacids (MAA)	*Rhodophyta spp., Rhodomonas baltica, Porphyra and Rhodomonas marina*	Llewellyn et al. 2010, Stengel et al. 2011

Antioxidant potential of seaweed

Seaweed are considered to be a rich source of antioxidants. Over last few years, the natural sources of antioxidant extracts from seaweeds have been well-developed in various countries.

For instance, carnosine and glutathione are reported in certain seaweed species though these antioxidant peptides are usually present in animal muscle (Harnedy and FitzGerald, 2011). Lipid oxidation, free radical chain mechanism is the main reason for the development of ROS and off-flavors. The chain mechanism involves three steps (initiation, propagation and termination) initiation leads to the formation of free radicals i.e. peroxyl radicals, propagation wherein radicals react with MUFA/PUFA to form lipid hydro peroxides and finally termination where the two peroxyl radical react to produce a non-radical species. Antioxidants inhibits the initiation or propagation reactions by deactivating or scavenging free radicals, thereby detaining the lipid oxidation. The potential antioxidants reported in seaweeds are fucoxanthin, astaxanthin, phlorotannins (polyphenols), phospholipids, flavonoids, bromophenols, polysaccharides, etc. The phenol rings in polyphenolic compounds act as electron traps and accounts towards multifunctional properties like hydroxyl radicals, peroxy radicals or superoxides scavenger, powerful metal chelator (Santoso et al., 2004). Phlorotannins present in seaweeds are reported to be more potent than plant polyphenols (Wang et al., 2009). Several studies have reported a high correlation between total phenolic content and antioxidant activity (Chew et al., 2008; Wang et al., 2009).

Chew et al., 2008 reported the antioxidant activities of three different seaweed varieties (*Padina antillarum*, *Caulerpa racemosa* and *Kappaphycus alvarezzi*) in-vitro by various assays i.e. DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing antioxidant power), FIC (ferrous ion chelating) and BCB (β-carotene bleaching) and found high antioxidant activity with BCB assay for all the three seaweed varieties and recommended to use them as preservatives efficiently and a number of seaweed species have the potential to be used in pharmaceutical industries (O'Sullivan et al., 2011). Souza et al. (2012) conducted a study to isolate polysaccharides from *Gracilaria birdiae* with aqueous extraction at 90 °C and antioxidant activity evaluation through DPPH assay. It was found that the extract possess remarkable antioxidant activity and this is attributed due to the presence of sulphate groups (Qi et al., 2005; Wang et al., 2009). Kuda et al., 2005 documented strong antioxidant activity in linoleic acid peroxidation assay (22 mg catechin equivalents/g dry sample) using water extract of *Scytosiphon lomentaria*.

Incorporation as antioxidant in food

Seaweeds and their extracts are nowadays being added to foods to take advantage of their health beneficial properties. Presence of high bioactive peptides assisting high antioxidant activity in seaweeds is reported frequently now adays (Admassu et al., 2018, Wada et al., 2015). Addition of seaweeds in various meat and meat based products is well documented. Cofrades et al. (2008) and López et al. (2009) reported the use of *Enteromorpha*, *Himanthalia elongata*, *U. pinnatifida* and *Porphyra umbilicalis* in meat products as well as in cereal based products. Application of fucoxanthin, from *U. pinnatifida* on ground chicken breast resulted in decreased TBARS value (Sasaki et al., 2008). The effect of carotenoid pigment, fucoxanthin on lipid peroxidation and meat colour in ground chicken breast using *U. pinnatifida* was studied and resulted decrease in the TBARS value along with decreased L* value and increased a* and b* values thereby presenting its potential to be used as an ingredient for the improvement of the appearance and shelf life of chicken meat and its products (Sasaki et al., 2008).

A study was conducted by Athukorala et al. (2005) to assess the antioxidant activities of *G. filicina* extract using linoleic acid and fish oil as substrates at 65°C for 168 hrs which resulted in delayed onset of oxidation. In addition, Siriwardhana et al. (2004) reported that the antioxidative effect of *H. fusiformis* methanolic extract on fish oil and linoleic acid was superior to BHT and BHA. In addition, study by Devi et al., 2011 to assess the reducing power, antioxidant activity and total phenolic content of crude methanol as well as diethyl ether extracts of Indian seaweeds i.e. *Halimeda tuna*, *Turbinaria conoides* and *Gracilaria foliifera* was done and reported highest total phenolic content and total antioxidant activity of 1.231 mg GAE/g and 1.675 mg GAE/g respectively in *T. conoides* extract. Thus, it can be concluded that seaweeds are promising alternatives to replace synthetic antioxidants by providing an environmentally safe and nontoxic source to use in functional foods and pharmaceutical industries. Andrade et al. (2013) examined the bioactive compounds from 18 macro-algae in ethanol extracts using gas chromatography-mass spectrometry (GC-MS) off the Portuguese coast. Indian brown seaweed, *Sargassum marginatum* incorporated pasta was developed by Prabhasankar et al. (2009) and reported that with the increase in concentration of seaweed the reducing power of pasta improved and DPPH radical scavenging activity of seaweed-incorporated pasta was higher in cooked form as compared to raw one. Pindi et al. 2017 studied the effect of incorporation of *Kappaphychus alvarezii* in chicken sausages as an antioxidant

source containing 2%, 4% and 6% *Kappaphychus alvarezii* during chilled storage and found that the presence of seaweed powder reduced L* i.e. lightness and increased a* i.e. redness with better physicochemical properties compared to the control. The effect of laminarin and fucoidan fortification at different concentration (0.01%, 0.1%, and 0.5%) on the quality characteristics of fresh and cooked pork patties was studied by Moroney et al., 2013 and reported that the lipid oxidation was reduced in fucoidan compared to laminarin fortified pork patties. This can be attributed due to higher free radical scavenging activity (i.e. presence of anionic sulphate group) of fucoidan.

Moreover, utilizing the synthetic antioxidants is correlated with probable toxicity as well as side-effects, like carcinogenesis (Dellarosa et al. 2015), besides, due to consumer awareness and demand towards selection of food fortified with natural and organic source the use of artificial or synthetic antioxidants has declined. Still, only a few natural food antioxidants are commercially available on the market. Hence, seaweeds and their extract can be used in convenience food, functional foods, value added foods, imitated foods in order to increase their stability and antioxidant potential.

Various forms of seaweed utilization:

Seaweeds can be utilized in various form, right from consuming in raw form to formulation of meat products along with dairy, bakery and confectionary products. Apart from food industry it has wide acceptance in bioengineering (biofuel, bio-plastics, etc.), pharmaceuticals, cosmetics and chemical industry. A pictorial representation is provided below to understand the wide array of seaweed utilization.

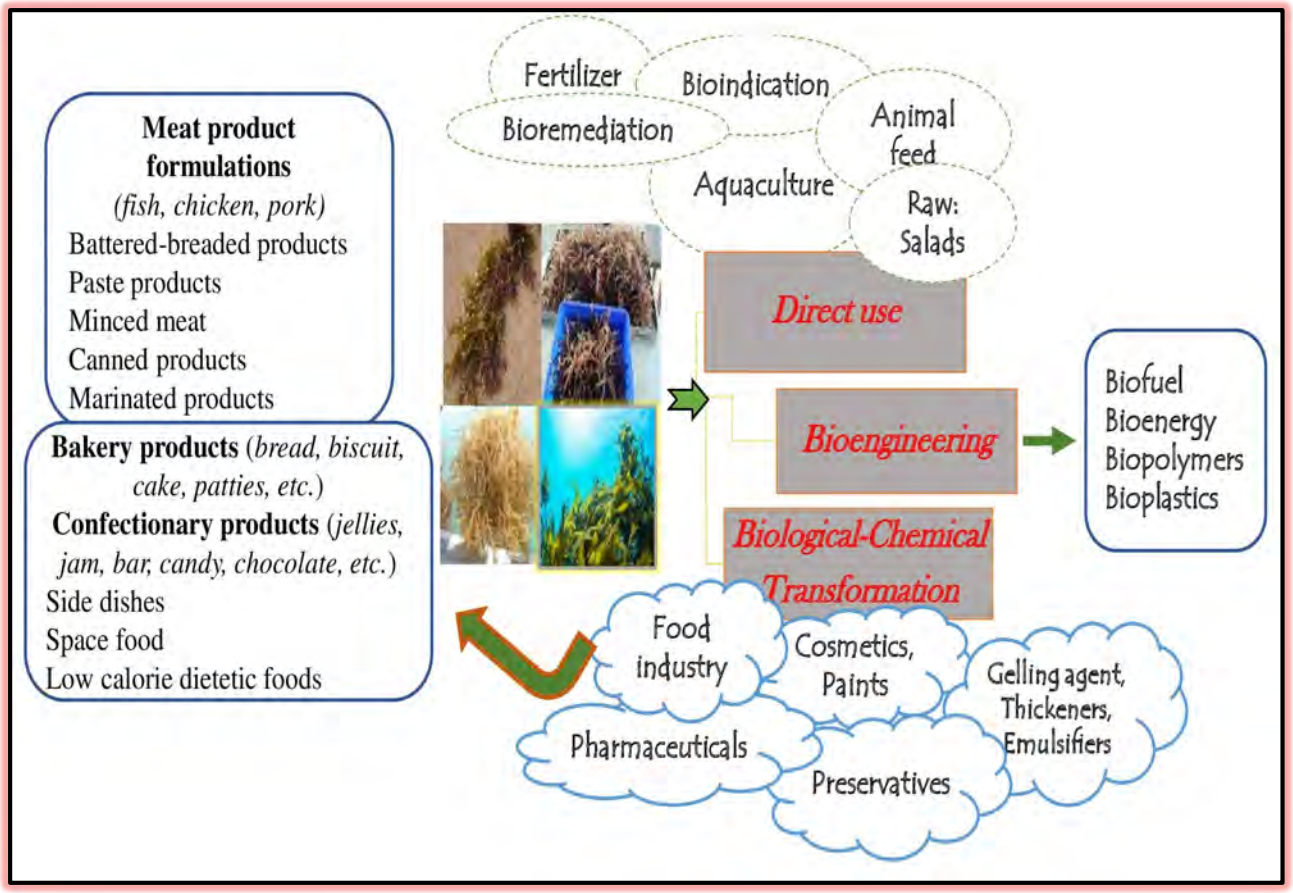

Fig 2. Utilization of Seaweed resources

Antimicrobial action of seaweeds extracts and their addition to food

Seaweed extracts are often regarded as interesting natural sources with potent antimicrobial properties (Pinteus et al., 2015). Expression of antimicrobial activity possessed by either natural extract or synthetic compound is usually clear, but the mechanism for this action is only moderately understood. The phenolic compounds attack the organism's cell wall and membrane thereby results in the release of intracellular constituents and possess numerous invasive targets which could lead to the inhibition of bacteria. In addition, interfere with the membrane functions such as electron transport disruption, uptake of nutrient and protein, synthesis of nucleic acid along with enzyme activity (Bajpai et al., 2007; Schulz et al., 1992). A continuous focus is developed towards seaweed and their extracts in the direction of their use as a source of natural products with antimicrobial activities for the treatment of many infectious diseases and is well deliberated (Abu-Ghannam et al., 2013, Safhi, 2014). The expansion of drug resistant pathogens is a momentous threat towards successful treatment of microbial diseases, thus, exploring new molecules and extracts with potential antimicrobial activities is pursued (Himejima & Kubo, 1991).

Phloro-tannin extracts are potent pharmacological substitute for treating wide array of microbial infections (Lopes et al., 2013). Phlorotannins interaction with bacterial proteins possibly plays an important role in the bactericidal action of phlorotannins (Nagayama et al., 2002) and the mechanism appears similar to terrestrial tannins comprising inhibition of extracellular bacterial enzymes, deprivation of growth substrate or direct inhibition of oxidative phosphorylation (Wang et al., 2009). The antimicrobial action of seaweed based bioactive compounds like terpenes, phenols against various gram positive and negative bacteria is very well documented (Kim et al., 2008, Gupta et al., 2010). Ultrasound-assisted laminarin showed marked inhibition of bacterial growth against *S. aureus*, *L. monocytogenes*, E. *coli*, and S. typhimurium (Kadam et al., 2015).

1, 8-dihydroxy- anthraquinone isolated from the red algae *Porphyra haitanensis* showed strong inhibition of the cell growth in the logarithmic phase towards S. *aureus* (Wei et al., 2015). It pointed for further investigation of 1, 8- dihydroxy-anthraquinone as a natural seaweed product in food safety control and drugs. Kolsi et al., 2015 found strong antimicrobial activities of organic extracts (hexane, ethyl acetate, methanol) obtained from various species pheophytea, chlorophytea and magnoliophytea of investigated the antibacterial and fungicidal activities of marine macroalgae and magnoliophytea from the Tunisia coast against human pathogenic bacteria, yeast and fungi (*Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, *Agrobacterium tumefaciens*, *Pseudomonas aeruginosa*, *S. aureus*, *Micrococcus luteus*, *Saccharomyces cerevisiae* and *Aspergillus niger*). Three extracts from five pheophytea, five cholorophytea, and three magnoliophytea species were prepared using hexane, ethyl acetate and methanol and the results revealed strong antimicrobial activities in all the extracts with maximum in brown algal extract and this can be used to treat certain human diseases. In addition, the ethanolic extract of *Gracilaria fisheri* has shown immuno-stimulant and anti-microbial activity against V. harveyi in *Peneaus monodon* (Kumaran et al., 2010).

Laminarin at 0.1% inhibited 70-90% adhesion of L. monocytogenes, S. typhimurium and V. parahaemolyticus to human enterocyte like HT-29-Luc cells (Kuda et al 2015). Nagayama et al 2002 reported an MBC (Minimum bactericidal concentration) of 0.79 µmol/ mL with E. kurome against Campylobacter jejuni. Furthermore, antibacterial activity of E. cava

against *Staphylococcus aureus* and *Salmonella* strains at MIC (Minimum inhibitory concentration) values of 125–250 µg/mL were reported by Choi et al. 2010. In addition, eckol and ampicillin in combination showed synergistic and additive effect (Choi et al. 2010). Kim et al. (2010) tested the toxicity of fucoidan in Sprague-Dawley rats (1350 mg/kg, bw/day) for 4 weeks and observed no significant differences in groups matched by gender in relation to body weight, urinalysis, ophthalmoscopy, hematology as well as histopathology. Additionally, the toxicity studies carried out by Vidal et al. (1984) on two metabolites of *Caulerpa* spp. i.e. caulerpin and caulerpicin and found that the 2 metabolites are non-toxic. Terpenoids like mertensene, violacene (Argandona et al., 2000), aplysiaterpenoid A, elatol, etc. (Bianco et al., 2013) have not been verified with toxicological studies till yet hence their safety profile in vitro and on animal species can be studied well. Markable inhibitory actions on antiinfluenza A virus (IAV) both in vivo and in vitro by carrageenan and its sulphated derivatives was reported by Wang et al (2012). Kappa/iota carrageenan possessed strong potential anticoagulant activity in low concentration and this is mainly due to the monosaccharide composition along with the position, number and distribution of sulphate groups along galactan chain (Yermak et al. 2012).

Seaweed as Polysaccharide or dietary fibre source

Marine seaweeds are ample source of numerous bioactive compounds including polysaccharides representing diversity in cell wall and structural polysaccharides in monosaccharide composition, configuration (absolute and anomeric), linkages i.e. glycosidic linkages, molecular mass and the availability of functional groups. Cell wall of seaweed is mainly comprised of polysaccharides accounting approximately 50% of dry weight basis (Stiger-Pouvreau et al. 2016) varying in the biochemical composition with species and environmental factors too (Rioux and Turgeon, 2015). Fucales and Laminariales family of brown seaweed contains Laminarin, a storage polysaccharide moreover, sulphate group containing polysaccharides namely sulphated polysaccharides like ulvan, fucoidan, carrageenans are present in green, brown and red seaweeds respectively (Costa et al., 2010). They are extracted from raw material and later purified either by preparative chromatographic techniques or chemical treatments. Algal/seaweed matrix cell wall polysaccharides can be labelled as phycocolloids or phycohydrocolloids due to good water solubility which creating a colloid system in aqueous media forming gels and films with suitable conditions (i.e. negatively charged groups, intramolecular and intermolecular junctions/linkages and their interactions), mostly the films are formed by solution casting and solvent evaporation (Lahaye, 2001). These have a wide range of application in biological as well as biomedical field due to their bio availability and bio compatibility (Venkatesan et al, 2015 b).

High fibres and mineral content in seaweeds promotes their use as a means to enhance the properties of various foods especially meat and meat products because they are deficient in fibre and contain surplus sodium which may be detrimental for the human health. The total dietary fibre in most of seaweeds is slightly higher than the terrestrial plants, it varies between 33-62% (dry weight basis) and are rich in soluble fractions (Dawczynski et al., 2007; Lahaye, 1991). Addition of seaweed as a functional ingredient helps to overcome certain scientific/technological glitches associated with low-salt meat products along with imparting the nutraceutical advantages and thereby popularizing seaweeds in diet among non-seaweed consuming population. Moreover, polysaccharides in seaweed possess various biological activities along with providing the textural properties (Balboa et al., 2013). In addition, they

provide plentiful hydrocolloids located in the cell wall and their content is influenced by several factors like harvest period, species, extraction method, etc. having significant impact on the functional properties of polysaccharides (Rioux & Turgeon, 2015) and are usually employed for stabilizing emulsions, viscous behavior, gelation, suspensions and foams and controlling crystal growth (Chapman, 2012). The key hydrocolloids are agar, alginates, and carrageenan. Agar has been used since the 17th century in Japan (Armisen, 1995) and is chiefly extracted from Gelidium and Gracilaria (McHugh, 2003). The cell wall of Gelidium and Gracilaria hold around 20-30% and 15-20% of agar, respectively (Freile-Pelegrín et al., 1995). It is principally used for its thickening and gelling properties. Carrageenans, commonly identified as carbohydrate antigens, has the potency to promote the growth of connective tissues. *Chondrus crispus* and *Gelidium cartilagineum* yields agar and carrageenan in higher concentrations. The most extensively produced algal polysaccharides are alginates, also known as alginic acid or algin. They are extracted from the cell walls of brown seaweeds (Kohajdová & Karovičová, 2009) and are mainly composed of 1,4-β-D-mannuronic acid and α-L-guluronic acid. This composition may differ from species to species. Unlike agar, alginates do not melt at high temperatures, and form cross linked gels002E

Conclusion

Seaweed stands as relative unexplored novel molecules for their utilization as functional foods and nutraceuticals. However, narrow market niche coverage can be seen for seaweed-based functional food, still their success is awaited, hence, fortifying the foods of high consumer acceptance with seaweed bioactive compounds can offer an opportunity to disseminate health benefits of seaweeds. Although immense literature is available on the bioactive properties of seaweeds, only few studies were conducted on their candid use in food products. Therefore, this area needs to be well researched to assess the full nutritive potential of seaweeds.

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Bioactive compounds from Marine Sources

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Introduction

Bioactive compounds are phytochemicals, which can modulate metabolic processes, promoting improved health to humans. Bioactive compounds have multiple biological effects including antioxidant, antimutagenic, anticarcinogenic, antiallergenic, anti-inflammatory and antimicrobial activities. Marine sources serve as a rich source of functional materials such as collagen, gelatin, polyunsaturated fatty acids, polysaccharides, pigments, enzymes, vitamins and minerals. Bioactive compounds from marine sources have been a major effect on many research groups in the world. However, marine sources are still considered as a relatively unexploited source of functional materials. Microalgae are one of the most promising sources for developing eco-sustainable production of natural bioactive metabolites.

Since oceans occupy more than 70 % of the earth surface, their high level of biodiversity makes them a logical target for looking for natural products. Marine bioactive constituents can be obtained from various marine organism includes animals, sponges, ascidians, mollusks, sea anemones, and seaweeds. Among the marine sources, seafood wastes and seaweed were considered an important source for the extraction of bioactive compounds at industrial production. Currently, seafood wastes were utilized to produce fish oil, fish meal, fertilizer, pet food and fish silage in India. However, recent research works were mainly focused for bioactive compounds such as bioactive peptides, collagen, omega 3 fatty acids oligosaccharides, enzymes for biotechnological and pharmaceutical applications. The majority of bioactive compounds from marine sources were made up of protein, lipid and polysaccharide. The procedure for isolation of bioactive compounds and their sources is mentioned in Figure 1 and Table 1

Protein based bioactive compounds

Proteins are complex polymers made up of a combination of 20 different amino acids coded by the genetic (DNA) code and several other amino acids.

Marine Proteins

Fish protein is the second major constituent after water in fish and present in the range of 16-18 %. The quality and the wholesomeness of the fish is determined by fish protein. The fish proteins are classified as myofibrillar, sarcoplasmic and stroma protein. Sarcoplasmic proteins are water soluble proteins include enzymes, pigments, heme proteins, myoglobin, hemocyanins and antifreeze proteins. Stroma proteins are structural proteins or connective tissue proteins insoluble in salt solutions. The myofibrillar protein is responsible for the structural organization of the muscle and account for about 65 – 75% of total fish muscle proteins. They are soluble in high ionic strength salt solutions.

Fish muscle proteins and microalgae contains all the essential amino acids in close to the right proportions for humans. Spirulina, for example, has high protein content (60 % to 70 %), with great balance of the essential amino acids and bioavailability. Spirulina appears to be

one of the most important microalgae used by humans. A daily supplement of Spirulina is believed to reduce allergy symptoms in human being.

Collagen

Collagen molecules, composed of three a-chains intertwined in the so-called collagen triple-helix, adopt a 3D structure that provides an ideal geometry for inter-chain hydrogen bonding. The triple-helix of collagen is approximately 300 nm in length, and the chain has a molecular weight of approximately 10^5 kDa. The triple helices are stabilized by the aforementioned inter-chain hydrogen bonds. Fish have lower concentrations of imino acids (proline and hydroxyproline) compared to mammalian collagen. Total or partial separation of the chains due to destruction of the hydrogen bonds, causing loss of the triple-helix conformation, and following denaturation, the polymers exist in a coiled form.

Fish collagen have numerous applications such as, pharmaceutical/biomedical applications (as anchor in glass, beads for cell culture, biomaterial for vascular prosthesis, microparticles for subcutaneous injection, scaffold in tissue regeneration, as feed/food (gelatin,glue), cosmetics, and to produce collagen hydrolysates (used in oral administration).

Gelatin

Gelatin is the denatured form of biopolymer derived by thermal hydrolysis of fibrous protein collagen. It is the principal constituent of animal skin, bone, and connective tissue. Gelatin is produced via the partial hydrolysis of native collagen. Gelatin is slightly differed from collagen in its chemical composition. The triple helical structure of collagen made up of three α-chains, whereas gelatin comprises three different chains viz., α-chain, β-chain and γ-chain. α-chain (one polymer chain), β-chain (two α -chains covalently crosslinked), and γ-chain (three covalently crosslinked α-chains). Gelatin is mainly composed of three amino acid repeat motif, Glycine-Proline-Hydroxyproline. The functional properties of gelatin and stability of triple helix are mainly governed by the proline and hydroxyproline content.

Gelatin is used in several applications as an emulsifier, stabilizer, wetting agent, fining agent, biodegradable packaging films, microencapsulating agent due to their functional properties such as viscosity, gel strength, gelling and melting points. Apart from food industries, it also used in photographic, pharmaceutical and cosmetic field. Nowadays, aquatic animal sources are gained interest for gelatin production because of several hindrances such as religious constraint, disease and vector transmitting medium of terrestrial animal source.

Marine Peptides

Peptides are important bioactive natural products which are present in many marine species. These marine peptides have high potential nutraceutical and medicinal values because of their broad spectra of bioactivities. The beneficial effects of marine bioactive peptides include scavenging reactive oxygen species (ROS) and preventing lipid peroxidation. In the last few years, different studies have isolated, characterized and purified bioactive peptides from different marine sources with anti-oxidant potential. Peptides present in enzymatically digested protein hydrolysates exhibited different physiochemical properties and biological activities.

Bioactive peptides generally include 3 -20 amino acid residues, and their biological activities are based on their molecular weights and amino acid sequences. Antimicrobial peptides usually have less than 50 amino acids, of which about 50% are hydrophobic and have a molecular weight of below 10kDa. The antioxidant activity of peptides influenced by the

hydrophobicity/hydrophilicity, amino acid sequences, degree of hydrolysis, and molecular weight of peptides.

Bioactive peptides or protein hydrolysates can be extracted and isolated from the protein of the marine species by various methods in industrial-scale production. Organic solvent extraction method was used traditionally, but it is a time-consuming, expensive and environmental unfriendly technique. Nowadays, better extraction techniques like supercritical fluid extraction, pressurized solvent extraction, microwave-assisted extraction, ultrasound-assisted extraction, pulsed electric field-assisted extraction and enzyme-assisted extraction are preferred. After the extraction procedure, the proteins are subjected to hydrolysis by which the proteins are hydrolyzed into bioactive peptides. Enzymatic hydrolysis is preferred in the nutraceutical and pharmaceutical industries in order to avoid harsh chemical and physical treatment and preserve the functionality and nutritive values.

Amino Acids

Seafood muscles are abundant in taurine, glutamic acid, glycine, proline, alanine and arginine. Fish is a good source of taurine a conditionally essential amino acid that has been shown to be involved in certain aspects of human development. It is assumed that consuming muscle proteins from fish are high in certain amino acids may improve human nutrition by boosting the nutritional value of foods.

Free amino acids usually interact with free radicals but the most efficient are the onces that can easily give away hydrogen atoms which include the amino acids having nucleophilic sulfur-containing side chains - cysteine and methionine or aromatic side chains (Tryptophan, Tyrosine, and Phenylalanine). This implies the specific compounds responsible for bioactivity of fish amino acids are Cysteine, Methionine, Lysine, Taurine, Tryptophan, Tyrosine, and Phenylalanine. Also, Glutamic acid, Proline, Glycine, Alanine and Arginine.

Minerals

Fish frames and bones would be a great potential source of high quantity minerals. In the total mass of fish bone nearly 60-70% is made up of minerals such as calcium, phosphorous and hydroxyapatite. Consumption of small fish along with bones in the regular diet will prevent the calcium deficiency. Since fish bones are the good source of hydroxyapatite, it can be extracted from fish processing waste. It is mainly used in medical and dental field as a bone graft material for produce artificial bone.

Enzymes

Presence of several proteases in fish viscera make it as a good source for the digestive enzyme viz., pepsin, trypsin, chymotrypsin and collagenase. Most of these enzymes were exhibit high catalytic activities even at low concentration. The internal organs would be used for extracting the enzymes in large scale.

Natural Pigments

The photosynthetic pigments are bioactive compounds that are able to capture solar energy. They are used by autotrophs for photosynthesis. For macroalgae, the major pigments are carotenoids and chlorophylls. These pigments are formed by algae, plants, fungi, and other microorganisms; however, humans and animals require ingesting them in their diets. Dietary carotenoids have nutritional and therapeutic importance since they act as provitamin A, which is converted into vitamin A. Carotenoids are known to be active agents for the protection against cancer, Cardio vascular disease, and macular degeneration. Microalgal formation of

carotenoids, including β-carotene and astaxanthin, is an active area of research as they can be present at relatively high concentrations. β-Carotene is one of the major natural colorants and it has been employed to a vast spectrum of food and drinks in order to enhance their aspect. Moreover, β-carotene with intense antioxidant properties helps to reduce the harmful effects of free radicals, which have been related to various life-threatening conditions, such as different kinds of cancer, CHD, premature aging, and arthritis.

Lipids and fatty acids

The long-chain omega-3 fatty acids such as eicosapentaenoic acid (EPA, C20:5) or docosahexaenoic acid (DHA, C22:6) are the most common omega-3 fatty acids generated from marine sources which have been well documented as essential for human health. Humans are incapable of synthesizing PUFAs with more than 18 carbons thus, they should get them from food. Seafood are the major sources of long-chain PUFAs, although the synthesis actually occurs in the algae eaten by the fish. The amount and composition of these oils depend on the species, season and location of catching sites. The long chain fatty acids help to regulate the blood clotting and blood pressure, and develop function of the brain and nervous systems. They also decrease the risk of many chronic diseases such as arthritis, diabetes and obesity. Moreover, PUFAs regulate inflammatory responses by producing inflammation mediators called eicosanoids (Lordan and others 2011). The rate of omega-3 to omega-6 of macroalgae is close to ideal, therefore they are used as dietary complement as part of a balanced diet.

Sterols

Another class of lipids from marine sources is the sterol compounds. ergosterol, clionasterol, fucosterol and cholesterol are some of the sterols present in the seafoods. Cholesterol is the major sterol in fish, shrimp and lobsters. Fucosterol, chondrillasterol, and sargasterol are found in brown algae and cholesterol has been found in red algae.

Marine polysaccharides

Marine polysaccharides including alginate, porphyran, fucoidan, chitin, and chitin derivatives, are used as down regulators of allergic responses. Polysaccharides isolated from algae that are mostly sulfated exhibit anti-infammatory activity in vitro and in vivo, which attributes to their structure and physicochemical characteristics.

Chitin

Chitin is the second most important natural polymer in the world. The main sources exploited are two marine crustaceans, shrimp and crabs. Chitin and its derivatives is the major by product from crustacean processing. Chitin or poly (β-(1→4)-*N*-acetyl-D-glucosamine) is a natural polysaccharide. This biopolymer is synthesized by enormous number of living organisms and it belongs to the most abundant natural polymers, after cellulose. In the native state, chitin occurs as ordered crystalline microfibrils which form structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast.

Chitosan

Chitosan is the most important derivative of chitin. The term chitosan usually refers to a family of polymers obtained after chitin deacetylation to varying degrees. In fact, the acetylation degree, which reflects the balance between the N-acetyl glucosamine and D-glucosamine residues, differentiates chitin from chitosan. When the Degree of acetylation is lower than 50%, the product is named chitosan and becomes soluble in acidic aqueous solutions. Chitin can be converted to chitosan by enzymatic preparations or chemical process.

Chemical methods are used extensively for commercial purpose of chitosan preparation because of their low cost and suitability to mass production.

Chitin and chitosan offer a wide range of application from the agriculture to pharmacy industry due to its specific properties like bioactivity, biodegradability, chelation ability, absorption capacity and film forming ability. Although the chitin and chitosan are known to have very interesting physicochemical, functional and biological properties in many areas, their molecular weight and their solubility property restrict their usage. Chitosan, which is soluble in acidic aqueous media, is used in many applications (food, cosmetics, biomedical and pharmaceutical applications). Unfortunately, all chitin and chitosan are not applicable in all sectors owing to its high molecular mass, high viscosity and, thus, low absorption for in vivo applications. The effectiveness of chitosan in various applications appears to be dependent on the degrees of acetylation. Recent studies on chitosan derivatives like Water soluble chitosan, chitooligosaccharides have drawn considerable attention, since the products obtained have been easily water soluble and also possess versatile.

Chitooligosaccharides

The depolymerised form of chitosans is called as chitosan oligomers or chitooligomers, or chitooligosaccharide (COS). COS has been paid great interest in pharmaceutical and medicinal applications due to their high solubility and non-toxicity.

Carboxy methyl Chitosan

Carboxy methyl chitosan (CM-chitosan) is the most fully explored derivative of chitosan. This derivative is water soluble in a wide range of pH, only if prepared from a fully acetylated chitin.

Hydroxy propyl Chitosan

Hydroxypropyl chitosan (HPCS), a kind of water-soluble functional derivative of chitosan, is obtained by means of etherification through propylene oxide at the C6/C3 position under alkali conditions. Application of HPCS includes drug delivery, tissue engineering and wound healing.

Phosphorylated Chitosan

Through phosphorylation chitosan is converted to the form of Phosphorylated Chitosan. This derivative is important due to its interesting biological and chemical properties and it also exhibits bactericidal and osteoinductive properties.

Glucosamine hydrochloride

Glucosamine in the form of glucosamine sulphate, glucosamine hydrochloride, or N-acetyl-glucosamine is extensively used as a dietary supplement in the treatment for osteoarthritis, knee pain, and back pain, and a critical evaluation indicated that glucosamine is safe and does not affect glucose metabolism.

Glucosaminoglycans

Glycosaminoglycans (GAGs) are heteropolysaccharides consist of a repeating disaccharide unit without branched chains in which one of the two monosaccharides is always an amino sugar (N-acetylgalactosamine or N-acetylglucosamine) and the other one is a uronic acid. It possesses significant antioxidant and antihypertensive properties and could be utilized as natural preservative ingredient in functional foods and in pharmaceutical industry.

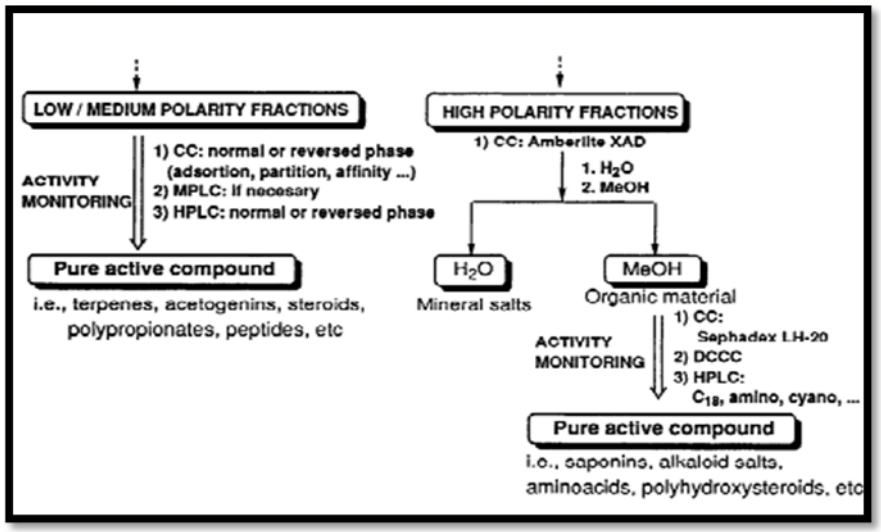

Figure 1: Procedure for isolating bioactive compounds from low/medium and high polarity fractions (adopted from Ricardo Riguera, 1997).

Table 1: Potential bioactive compounds obtained from different marine sources

S.No	Marine sources	Bioactive compounds
1	Sponges	Peptides
2	Marine microorganism	Protein, Vitamin B, Vitamin E and natural pigment
3	Seaweed	Peptides, Amino acids, Sterols, polysaccharide, vitamins, minerals
4	Cnidarians	Phenolic compounds
5	Bryozoans	Alkaloids
6	Molluscs	Proteins, Polypropionates
7	Tunicates	Peptides, Alkaloids
8	Echinoderms	Sterols, Alkaloids, natural pigments
9	Marine fishes and marine mammals	Fish oil, PUFA,Vitamins, Minerals
10	Crustaceans	Chitin, chitosan and its derivaties, pigments, minerals

Conclusion

Marine resources offer important bioactive molecules that have advantages on the human body. They can be applied in many fields such as the drug, cosmetic, and food industries. Functional foods can easily be developed from marine products since they are widely available and they have the ability to prevent certain diseases and cure some illnesses. Various kinds of seafood are consumed as nutritionally beneficial food. The sea offers an enormous resource for finding novel compounds, and it is considered as the largest remaining reservoir of natural molecules that may be used as functional ingredients in the food industry. Consequently, efforts should be made to develop marine functional foods responsibly, since their consumption could result in a decrease of the occurrence and gravity of chronic diseases.

Bioactive based feed formulation for aquaculture applications

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Introduction

Worldwide the demand for the fish and fishery product is continuously increasing due to many health beneficial aspects (Delgado et al. 2003, FAO 2010). Concerns about food security are of significant importance to emerging nations like India, where a substantial percentage of its population lives in poverty and where a large portion of total family expenditure is spent on food (Pradeepkiran, 2019). Fish and fishery product are known for the protein sources with all the essential micronutrients. To meet the present and future demand the culture sector has adopted innovative intensive and super intensive farming techniques. However, intensified farming has improved the production and productivity of fish, on the other side, risk of disease outbreaks in the culture system is also higher and leads to production and economic losses. As the sector has adopted intensification, requirement of quality feed ingredient for feed formulation has increased. As per the recent survey, the aquaculture sector in India is facing serious crisis with abrupt increase in cost of shrimp and fish feed due to unexpected increase in the prices of the key feed ingredients such fishmeal, soyameal, fish oil etc. The prime ingredient in the aqua feed sector is fishmeal, the cost has gone up due to non-availability of fish preparation of fishmeal. Fishmeal is being considered as one the gold standard ingredient due to the presence of quality protein with all essential micronutrients and also known to have unknown growth promoters which helps fish and shellfish growth. As the cost of all the essential feed ingredients are going up and anticipating the adverse effect of intensification in culture system, bioactive compound isolated from marine and agri based source having bio function properties like antioxidant, antimicrobial, growth promoters and immune system modulators have been used in the aquaculture system.

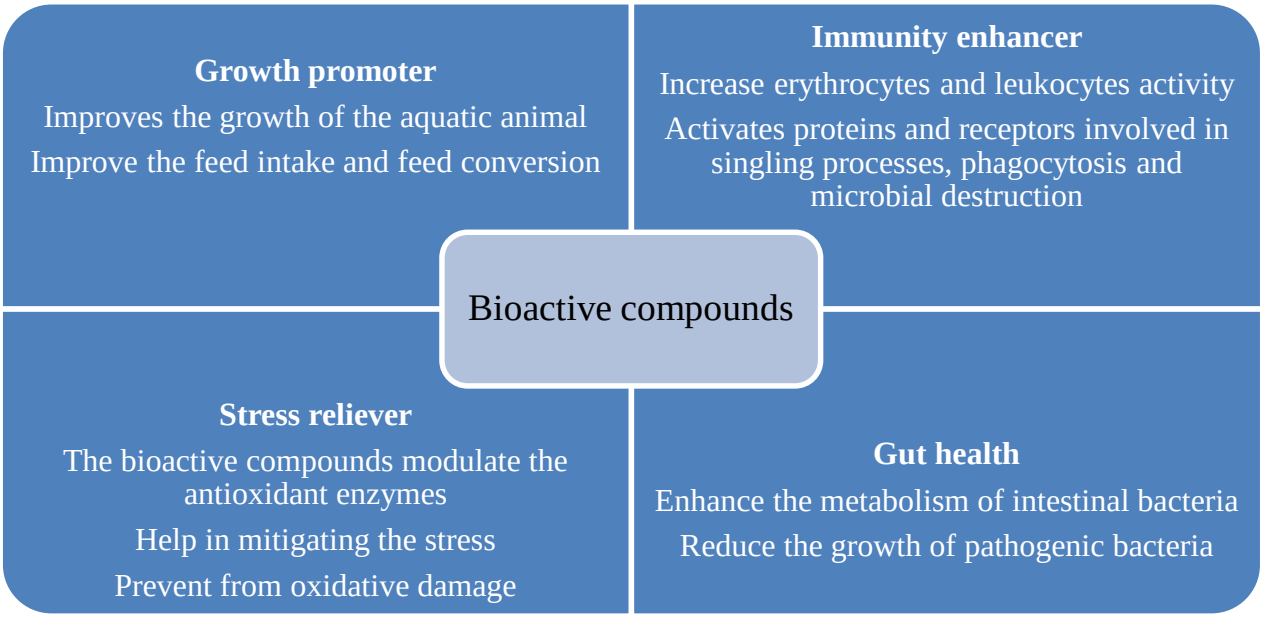
Bioactive ingredients obtained from agri source

Huge amount of waste is being generated from the cultivation and processing of agricultural products. Waste generated from the source includes husk, seeds, leaves, roots and stems, these can be considered as source of bioactive compounds (Veneziani et al., 2017). Bioactive compounds such as phenolic complexes (flavonoids, coumarins, xanthones, chalcones, stilbenes, lignins), terpenes (essential oils and carotenoids), dietary fiber (β-glucans, fructooligosaccharides, galactooligosaccharides), glucosinolates, etc (Vermerris and Nicholson, 2008, Ferreira et al., 2017, Leyva-López et al., 2020). These bioactive compounds are proven to have several biological properties, such as antioxidants, immunostimulants, and microbiota modulators. In addition, they also have properties like antibacterial, antiparasitic, antiviral, anti-inflammatory, anticancer, and antihypertensive effects (Leyva-López et al., 2020).

Due to rapid growth and huge demand for the fish and fishery product, the farming communities across the globe have adopted intensification in the culture system. Additionally,

other farming factors, including poor diet, poor water quality, changes in temperature and pH, may leads to stress resulted in reduced immunity in the aquatic animals, further may lead to rapid spread of infectious diseases. In the recent past, the aquaculture sector started using the bioactive compounds from medicinal plants as feed additives to enhance the immune response in the fish and shellfish. Most consumed cereals across the globe are corn, rice, wheat, and sorghum. As the waste generated from the cereal processing sector is huge and contain valuable bioactive compounds. Lot of researchers are working on converting bio-waste into functional feed ingredients. Corn and sorghum residues have been used a dietary supplement to boost the antioxidant efficiency in fish. Corn silk extract is good source of flavonoids such as luteolin, formononetin, maizine, and apigenin, incorporation of corn silk extract as feed additive as proven to lower the lipid peroxidation in the liver of Nile tilapia. In general, these polyphenolic compounds have ability to neutralizing reactive oxygen species and modulates antioxidant enzyme activities in the living system.

Role of Bioactive compounds in aqua feed



Bioactive compounds from marine source

Fish, shellfish, macroalgae, microalgae, and other varied organisms make up marine ecosystems (Thorpe et al., 2000). Marine habitats are frequently referred to as one of the richest treasure houses of biomolecules and these biomolecules from marine habitats may possess bioactive properties like antioxidant, antimicrobial, antiviral, antiparasitic, anti-inflammatory, antifibrotic, and anticancer activity. Due to their bio-functional properties, these bioactive compounds find its applications in field of pharmaceutical, nutraceutical, biomedical, and cosmetic industries (Barrow and Shahidi, 2007). As the demand for the fish is increasing constantly and culture sector are adopting new methods of farming to meet the present and future requirements. Meanwhile, fish processing sector tends to generate huge quantity of waste which includes head, skin, trimmings, fins, frames and viscera, and these generated biomasses can be utilized for the extraction of bioactive compounds (Dekkers et al., 2011).

Fish protein hydrolysate: Fish protein hydrolysates find application as fish feed ingredient or as a supplement to replace fishmeal. Fish protein hydrolysate are found to have desirable bio-functional properties like antihypertensive, antithrombotic, immune modulatory and

antioxidative properties. Various studies have shown that dietary supplementation of fish protein hydrolysate had positive influence on growth performance and immunity parameter in fish and shellfish (Quinto et al., 2018, Tejpal et al., 2021).

Chitin and chitosan

Chitin is natural biopolymer and it is a cationic amino polysaccharide composed of *N*-acetyl-d-glucosamine with β (1 → 4) glycosidic bonds between each monomer. Similar to chitin, chitosan is also a biopolymer and it consists of d-glucosamine units obtained during the deacetylation of chitin by adopting hot alkali treatment (Beaney et al., 2005, Se-Kwon, 2010). Chitin and chitosan are mainly present in many aquatic, terrestrial organisms and also found in some of the microorganisms. Bio-waste generated from aquatic and terrestrial source can be used as raw material for production of chitin and chitosan (Tokura and Tamura, 2007 and Se-Kwon, 2010). These biomaterials are reported to have wide range of applications. The chitin was found to improve the growth performance, feed conversion and modulates the immunity of the aquatic animals. However, inclusion of chitin at excess in feed had adverse influence in the aquatic animals. Whereas chitosan, an interesting bioactive polymer, found to have application in aqua feed sector as biomaterial for encapsulation of bioactive compounds. In addition, chitosan supplementation in the feed improves growth performance in aquatic animals.

Pigments

Pigments such as astaxanthin, fucaxanthin, melanin etc. are available in the marine source and found to have bio-functional properties. Generally, waste generated from the shellfish processing waste contain good amount of carotenoid pigments such as astaxanthin and canthaxanthin. The supplementation of these pigments will increase the palatability of the feed and improve the immunity of aquatic animals. Carotenoids, caramel, curcumin, and spirulina are feed pigments that are majorly available in the market.

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Recent advances in designing delivery systems for marine bioactive compounds

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Introduction

The concept of delivery systems is gaining significant attention in various fields such as pharmaceuticals, nutraceuticals, cosmetic industry etc. Delivery systems are mainly being used to encapsulate, protect or to impart controlled/sustained release of the active ingredients. Nutrient based delivery systems is an emerging research sector globally owing to the increased consumer demands for tailor made functional foods. The development of functional foods often involves the process of identifying, isolating, purifying, characterizing biomolecules and their subsequent incorporation in food systems. However, the development of functional foods is not an easier task; it involves several challenges such as loss of physico-chemical stability, solubility, bioavailability, melting point etc. Realising the major scientific and technological challenges associated with the formulation of functional foods, various efforts have been taken by researchers and nutritionists to battle the issue. Development of suitable delivery systems can be a promising solution for designing nutraceuticals by playing a major role in the encapsulation, protection and sustained release of biomolecules (Lekshmi et al., 2018).

Designing delivery systems for bioactive nutrients is an arduous task as several factors need to be taken into consideration. Some of the aspects that need to be considered are given below:

1.Delivery system intended for food application should be fabricated only from biomaterials which are of food-grade quality, safe and biodegradable in nature. It should also have Generally Recognized As Safe (GRAS) status (Augustin and Hemar 2009).

2. The material used for the development of delivery systems should be easy available, economically feasible and the benefits gained out of the encapsulation should outweigh the additional cost incurred in the process (McClements et al. 2007; Gutiérrez 2018).

3. The incorporation of the delivery systems should not adversely affect the physical, chemical, textural and sensory quality of the final product. That is, it should be compatible with other ingredients of the food matrix (Joye et al. 2014).

4. The delivery system developed should be robust. It should be physically and chemically stable and can offer the biomolecule considerable protection from any sort of degradation processes.

5. Ideally, the delivery system should have high loading capacity and retention of the biomolecule. Loading capacity refers to the amount of bioactive substance present per unit mass of the encapsulation material (McClements 2015).

6. Delivery system developed should ensure the sustained and targeted release of the biomolecule in response to a specific environmental stimulus such as pH, enzymatic action, temperature or ionic strength (Shegokar and Müller 2010).

Types of Delivery Systems

Delivery systems for functional food formulation can be developed from a variety of food-grade biopolymers such as proteins, lipids or carbohydrates etc. The biopolymers will be

used either singly or in combination to increase the functionality. The properties of biomolecule to be encapsulated and the nature of the surrounding food matrix has to be taken into account while selecting biopolymers. Adequate knowledge about the molecular structure of the biopolymer is also essential as it in turn determines the functionality of food systems. The different types of delivery systems used in food industry are discussed below:

Protein based delivery systems

Proteins are generally preferred for the development of delivery systems owing to their high nutritional value, biodegradability, economical and GRAS nature. They are also reported to have excellent functional properties including emulsification, gelation, water binding capacity, foaming etc. (Elzoghby et al. 2012). Its structural diversity attributed due to the multiple functional groups present in the primary sequence of polypeptides makes it an excellent candidate for the delivery of bioactives over a wide range of platforms such as hydrogels, micro and nano particles, molecular coacervates, emulsion droplet stabilization, films etc. (Chen et al. 2006). Protein based delivery systems are relatively simple to prepare, economical and deliver both hydrophobic and hydrophilic bioactives. Proteins can be obtained from various sources such as bacterial, fungal, plant and animals and among this, the latter two are commonly employed for food applications (Elzoghby et al. 2012). Gelatin, collagen, elastin casein, albumin and whey proteins are some of the commonly used proteins of animal origin for functional food applications.

Carbohydrate Based Delivery Systems

Carbohydrates which account for calorific value, sensory and textural properties form a significant component of many food systems. It is considered as suitable carrier for many nutraceuticals owing to its biocompatibility, biodegradability, structural versatility, site digestion properties. The presence of functional groups makes it an excellent candidate for the development of delivery systems as it can interact with a wide range of bioactive compounds of both hydrophobic and hydrophilic nature. Carbohydrate based delivery systems can be categorized into four main groups based on their source such as plant origin (e.g., starch, gum Arabic, guar gum, pectin), animal origin (chitin, chitosan), algal (agar, carrageenan, alginate) and microbial origin (xanthan gum, dextran, cyclodextrin etc.) (Kosaraju 2005). Polysaccharides can be further categorized based on their charges such as neutral, anionic and cationic.

Lipid Based Delivery Systems

Lipid based delivery systems are reported to have better encapsulation efficiency and low toxicity than other delivery systems. In general, four major lipid-based delivery systems are being used, namely, nano emulsions, nanoliposomes, solid lipid nanoparticles (SLN) and nanostructure lipid carriers (NLC). Among the various types of lipid-based delivery system, liposomes are widely being used in the food research industry. They are reported to possess wide range of benefits such as (1) can be produced from materials of natural origin (2) used for production, entrapment, release of compounds having wide range of solubility such as water-soluble, lipid-soluble, and amphiphilic materials (3) deliver and release their load in the target site inside and outside the body. They are widely used in food industry for making formulations of antimicrobials, enzymes, lipophilic vitamins, oils and minerals.

Mixed Delivery Systems

Of late, there is an increasing trend in application of mixtures of polymers instead of individual ones with a view to improve and broaden the application range and functionality. Among the various biopolymer mixture studied so far, protein–polysaccharide complex is considered more advantageous, because of their higher chemical and colloidal protection. The formation and stability of protein–polysaccharide complex is found to be affected by a number of factors such as pH, ionic strength, and biopolymer concentration, charge distribution, molecular weight, temperature, pressure, etc. In such cases, the sequence of biopolymers adsorption onto the interface determines the structure and stabilizing properties of the mixed protein- polysaccharide complex. Two phenomena can occur during the mixing of proteins and polysaccharides in a liquid medium depending on the pH and isoelectric point (1) the attractive interactions can lead to the formation of soluble and insoluble complexes and (2) the repulsive interactions can separation of the two biopolymers from each other (Weinbreck et al. 2004).

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Application of dryers in the fishery sector

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Introduction

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 overcatch, 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

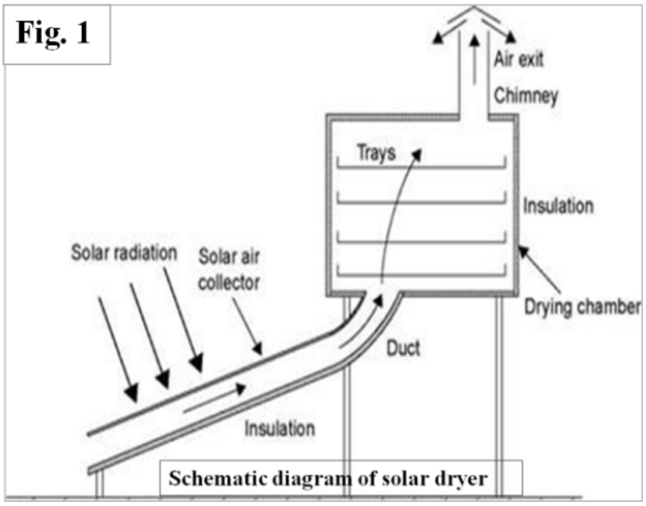

Fig 1. Schematic diagram of basic solar dryer

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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Fundamentals of Heat and Mass Transfer and Design and Development of a General-Purpose Dryer

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Introduction

Heat and mass transfer is a significant and consolidated branch of physics and engineering. Heat and mass transfer studies aims to understand the specific transport phenomena occurring at the fluid boundaries during different unit operations such as cooling, heating, boiling, condensation, evaporation, distillation, drying, frying, etc. Many of these thermal processes are aided by simultaneous heat and mass transfer. It is necessary to understand about heat and mass transfer, to design and develop the processing equipments, to bring out desired changes in the product, and to improve on the equipment's thermal efficiency.

Heat transfer

Heat transfer may be defined as flow of energy from one body to another body by virtue of temperature difference between them. The net flow of energy always occurs from high temperature body towards low temperature body and this flow of heat stops the moment temperature of both the bodies are equal.

Modes of heat transfer

Heat transfer is the study of transmission of thermal energy from a high temperature region to a low temperature region on account of temperature difference. The rate of heat transfer is directly proportional to the temperature difference between the heat exchanging regions/bodies. Three different modes of heat transfer are conduction, convection and radiation. In the conduction and convection modes, heat flows from high temperature to low temperature region/body, whereas, in radiation mode, transfer of heat takes place from both the bodies towards each other. However, net transfer of heat is always from high temperature body to low temperature body.

Conduction

Conduction is a process of heat transfer from a high temperature region to a low temperature region with in a body or between different bodies which are in direct physical contact. In heat conduction, energy is transferred due to exchange of molecular kinetic energy. As the temperature in one region of a body increases, kinetic energy of molecules in that region also increases as compared to that of the molecules of adjacent low temperature region. High energy molecules transfer a part of their energy by impact in case of fluids or by diffusion in case of metals to low energy molecules, thereby resulting in increase in their energy levels, hence temperature.

The basic law of heat transfer by conduction was proposed by the French Scientist J. B. J. Fourier in 1822. One dimensional heat conduction rate equation described by the Fourier Law is written as:

$$q_x = -kA\,\frac{dT}{dx} \quad \text{or} \quad q''_x = -k\frac{dT}{dx}$$

where,

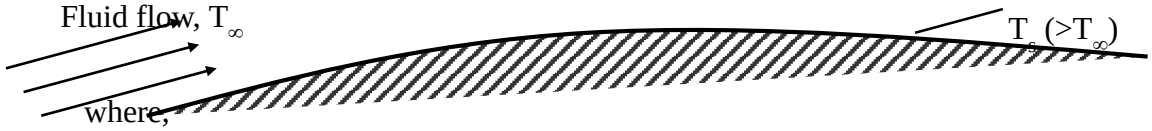
q_x = heat rate in x-direction (W)
$q"_x$ = heat flux in x-direction (W/m^2)
T = temperature (°C or K)
A = area normal to heat flow (m^2)
k = thermal conductivity of material (W/m-K)

Convection

Heat transfer by convection occurs when a fluid (liquid and gas) comes in contact with a solid through direct contact and a temperature difference exists between them. Heat transfer by convection occurs under the combined action of heat conduction and mixing motion. When a fluid comes in contact with a hot surface, energy in form of heat flows by conduction from hot surface to the adjacent stagnant layer of fluid particles, thereby increasing their temperature and internal energy. Due to increase in temperature, density of the fluid particles decreases and they become lighter as compared to the surrounding fluid particles. The lighter fluid particles move up to a region of lower temperature with in the fluid where they mix and exchange a part of their energy with colder fluid particles. Simultaneously, the cold fluid particles move downwards to occupy the space vacated by hot fluid particles. This upward and downward movement of hot and cold fluid particles continues till temperature of the fluid and the surface becomes equal. The upwards movement of hot fluid particles and downward movement of cold fluid particles is called convectional currents. If the convectional currents are set up only due to density differences, then the heat transfer process is termed as natural or free convection. If the convectional currents are caused by some external means such as blower, fan, pump etc. then heat transfer process is called forced convection. It is virtually impossible to observe pure heat conduction in a fluid because as soon as a temperature difference is imposed in a fluid, natural convection currents will occur due to resulting density differences.

Convective heat transfer rate is governed by Newton's law of cooling and is expressed as:

$$q'' = h(T_s - T_\infty) \quad \text{or} \quad q = hA_s(T_s - T_\infty)$$



where,

$q"$ = heat flux normal to surface
q = heat rate from or to surface A_s
T_s = surface temperature
T_∞ = freestream fluid temperature
A_s = surface area exposed to fluid
h = convection heat transfer coefficient (W/m^2-K)

Radiation

Heat exchanged between two bodies or mediums, which are separated and are not in contact with each other is called radiation heat transfer. Radiation heat transfer does not require presence of an intervening medium between the two bodies as in case of conduction and convection and it takes place most effectively in a vacuum.

Thermal radiation is the energy emitted by a body in the form of electromagnetic waves due to changes in the electronic configuration of the constituent atoms or molecules of the

body. When electromagnetic waves come in contact with a body, energy is transferred to the body as thermal energy which is partly absorbed, reflected and transmitted. Energy emitted per unit area as thermal radiation is called emissive power of a body and the maximum energy emitted as radiation by a body at a particular temperature is governed by Stefan-Boltzmann law which is expressed as:

$$E_b = \sigma A T_s^4$$

At a given temperature, maximum radiations are emitted by an ideal emitter called black body. The energy emitted by non-black bodies are less as compared to that of the ideal body when both the bodies are maintained at same temperature. Energy emitted by a non-black body maintained at temperature 'T' is given as:

$$E = \varepsilon \sigma A T_s^4$$

where,

E = Energy emitted per unit time (W)

A = Surface area ($m^{2)}$

σ = Stefan-Boltzmann constant = 5.67×10^{-8} W/m^2K^4

ε = emissivity ($0 < \varepsilon < 1$) of surface

T_s = surface temperature in absolute units (K)

Heat exchangers

A heat exchanger is a device used for efficient transfer of heat between a hot and cold fluid when it is required to heat up a cold fluid or cool down a hot fluid. Heat exchangers are used in wide range of applications such as:

- Heating and air conditioning systems
- Automobile radiators
- Cooling of internal combustion engines by a coolant
- Boilers and condensers of a power plant

Heat exchangers are generally classified on the basis of following parameters.

- Nature of Heat Exchange Process
  - Direct contact
  - Regenerative
- Relative Direction of Flow of Fluids
  - Parallel flow
  - Counter flow
  - Cross flow
- Mechanical Design of Heat Exchanging Surface
  - Concentric tubes
  - Shell and tubes
  - Multi-shell and tube passes
- Physical State of Heat Exchanging Fluids
  - Condenser
  - Evaporator

Logarithmic mean temperature difference (LMTD)

In a heat exchanger, thermal potential, responsible for heat exchange between the hot and cold fluids changes throughout the process. The LMTD is the maximum mean temperature

difference that can be achieved in geometry of heat exchanger for any given set of inlet and outlet temperatures.

$$\text{LMTD} = \frac{(T_{hi} - T_{ci}) - (T_{ho} - T_{co})}{ln\frac{(T_{hi} - T_{ci})}{(T_{ho} - T_{co})}}$$

where,

T_{hi} = Temperature of hot fluid at inlet

T_{ci} = Temperature of cold fluid at inlet

T_{ho} = Temperature of hot fluid at outlet

T_{co} = Temperature of hot fluid at outlet

The heat transfer rate in a heat exchanger at particular length is calculated by considering the thermal potential at that length which is expressed as:

$$Q = UA\ (T_h - T_c)$$

The overall heat transfer in a heat exchanger is calculated by using average thermal potential and is represented by the term LMTD. Therefore, the overall heat transfer I heat exchanger can be defined as:

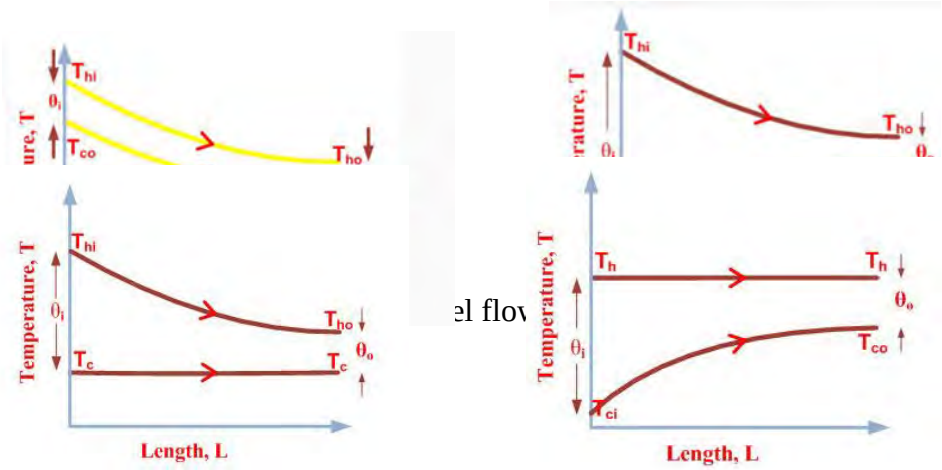
$$Q = UA\ (\Delta T_m)$$

where,

U = overall heat transfer coefficient (W/m^2-K)

A = heat transfer area, m^2

ΔT_m = LMTD



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Mass transfer

Mass transfer is defined as the transfer of material from one homogeneous phase to another with or without phase change. It is a complex phenomenon occurs in almost all unit operations. For example, transfer of solute in extraction process, transfer of water molecule in humidification. Drying is another complex process which involves simultaneous heat and mass transfer. Mass transfer is caused by differences in concentration of the substances between two regions. The mass transfer will continue till the concentration differences between two regions exist and will stop when equilibrium is obtained.

Mass transfer basically deals with transport of species:

- within a medium, for example sugar dissolves in a cup of tea to sweeten the entire cup of tea
- across an interface, for example from one medium to another i.e. spreading of food odour in the entire house

There are different types of mass transfer happens during a process. They are diffusion mass transfer, convective mass transfer and phase change mass transfer. Diffusion mass transfer is again classified into molecular diffusion and eddy diffusion. Eddy diffusion happens when one of the fluids in mass transfer is in turbulent condition. Molecular diffusion again classified as ordinary diffusion, in which the diffusion happens due purely due to concentration gradient. Thermal diffusion is due to temperature gradient, pressure diffusion is due to pressure gradient, and forced diffusion is due to the application of mechanical forces.

a) Molecular diffusion in gases

Two gases A and B, separated by a semipermeable membrane, Gas A moves towards chamber B and gas B moves towards chamber A. Concentration of A with distance towards chamber B and B towards A is termed as C_A and C_B. Variation in concentration of component with distance in the system called concentration gradient. Movement of molecule A or B occurs due to concentration gradient known as molecular diffusion.

Molecular diffusion is explained by Fick's law is expressed as:

$$N_A \propto -\frac{dC_A}{dx}$$

Negative sign indicates that concentration decreases with distance.

$$N_A = -D_{AB}\frac{dC_A}{dx}, \text{ for molecule A}$$

$$N_B = -D_{BA}\frac{dC_B}{dx}, \text{ for molecule B}$$

where,

D_{AB}, D_{BA} = Diffusivity of A in B & diffusivity of B in A, respectively (cm^2/sec)

N_A & N_B = Rate of diffusion (g moles/cm^2/sec)

b) Diffusion through stationary, non- diffusing gas

Movement of molecules from liquid or film on drying solids, occurs to a non-diffusing gas. Molecule A is moving from the surface to atmosphere due to concentration gradient in partial pressure, but B is not moving towards the surface. Therefore, rate of mass transfer of A takes place by molecular diffusion and bulk flow.

c) Molecular diffusion in liquids

According to Fick's law, for diffusion in liquid is expressed as:

$$N_A = -D\frac{dC_A}{dx}$$

For equimolar counter diffusion,

$$N_A = -D\frac{C_{A2} - C_{A1}}{x_2 - x_1}$$

where,

C_{A1} and C_{A2} = concentration of A at point x_1 and x_2

Convective mass transfer

The major driving potential for mass transfer process is concentration gradient. But in practical situations, the convective mass transfer in fluids cannot be neglected. The governing equation for convective mass transfer is similar to convective heat transfer equation and is expressed as:

$$m_b = h_{mc}C_{b1} - C_{b2}$$

where,

m_b is the diffused mass component of 'b'
h_{mc} is mass transfer coefficient of component 'b'
C_{b1} and C_{b2} are mass concentrations of component 'b'

Drying

Removal of water by evaporation is known as drying through the application of heat. It can also occur under natural atmospheric conditions. If the material is exposed to air at given temperature and humidity, the material will either lose or gain water until an equilibrium condition is established. Drying results in weight reduction, occupy less space, reduced shipping or transportation or storage costs, convenience in handling, provide definite properties, prevents microbial growth, arrest enzymatic reactions, stops other chemical reactions and helps to improve shelf life.

Drying is a simultaneous heat and mass transfer process occurs due to water vapor pressure gradient. Drying happens in three stages. Initially the moisture from the surface will have enough water to get evaporated, and this will continue till the rate of evaporation from the product surface will be equal to rate of moisture migration to the surface. This is called constant rate drying period. Constant drying rate period lasts till critical moisture content is reached. Then the drying rate decreases as the fraction of wet surface area decreases, is called first falling rate period. At the end of the first falling rate period the fraction of wet surface area decreases to zero. Finally, the subsurface evaporation occurs until the equilibrium moisture content is reached. This stage is known as second falling rate period. During drying, the moisture transfer occurs due to capillary movement, molecular diffusion, thermal diffusion, pressure diffusion and hydrodynamic flow.

Dryers

There are different methods of drying practiced for agricultural commodities. Open air sun drying is traditional method. Many of the developing countries still practice open sun drying method to dry fish and fishery products. It is one of the cheapest methods of drying, in which heat energy is freely available, renewable and abundant. Sun drying involves direct exposure of a commodity to solar radiation and the uses the convective power of the natural wind for drying products. Since the drying conditions are uncontrollable and depends upon weather conditions, it may take longer time for drying and yield inferior quality products. The open conditions may cause contamination with dust, insects, pests and microorganisms.

In order to overcome the drawbacks of open sun drying methods, mechanical dryers with electric heating systems are developed. But this involve running costs due to high electricity consumption and are not recommended due to exploitation of non-renewable sources of energy. Hence, the recent efforts are made to improve the open sun drying and has been led to adoption of solar drying method which is one of the best solutions to the overcome the drawbacks of open sun drying. Solar drying involves a design to capture and magnify the heat from the sun, as well as to help protect the material from infestation of dusts, insects, pests and other foreign bodies. Shorter drying times reduce the risks of spoilage or microbial growth. Even for many centuries farmers were using open-sun drying, solar drying has been taken over open sun drying, as it is more effective and energy efficient. Solar dryers use solar energy which is a renewable energy and freely available. It is clean and green energy and therefore the effective utilisation of solar energy in drying process makes the dryer operated at low cost with

maximum energy efficiency. Solar dryers are classified as direct dryers, indirect dryers, greenhouse solar dryers, hybrid solar dryers, solar dryers with energy storage systems etc.

Solar dryer can perform drying only during sunny days, and hence the drying rate depends mainly on climatic conditions and the season. Hybrid solar dryers are therefore, 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.

Design and Development of Dryer

The choice of the best type of dryer to use for a particular application is generally dictated by the following factors such as the nature of the product (physical and chemical); value of the product; scale of production; available heating mechanisms; product quality considerations; space requirements; nature of the vapor (toxicity and flammability); nature of the product; etc.

A mechanical dryer has different components. They are drying chamber, trays, direct or indirect air heating system, air inlet, air distribution systems (fan and blower), air outlet and a control system. There are many factors to be considered while designing a dryer. They are 1) dryer factors – size and shape of dryer, sample quantity, drying time, air flow pattern and air distribution system; 2) Air factors – velocity, air flow rate, temperature and RH, pressure of air, ambient conditions; 3) Sample factors – type, variety of sample, initial and final moisture content of sample, final usage of sample, latent heat of vaporization; 4) Heating system – type and rate of fuel, type of heat source, heat distribution system.

A mechanical dryer with electrical heating coil system was considered for the humid climatic conditions of Kerala, India. The dryer was designed to reduce the moisture content of the shrimp from 75% (w.b.) to 15% (w.b.) within 6 h. The drying conditions and assumptions reported in Table 1 were considered for the design and fabrication of the dryer.

Evaporation load calculation

Moisture content of sample was calculated by hot air oven method.

$$\text{Moisture content}(\%\text{w.b.}) = \frac{\text{Weight of moisture}}{\text{Weight of sample}} \times 100$$

The amount of water to be removed from shrimps during drying (M_w, kg) was estimated from the initial moisture content, final moisture content and total mass of the shrimps.

$$M_w = M \times \frac{M_i - M_f}{100 - M} \times 100$$

where,

M_i = Initial moisture content

M_f = Final moisture content

M = Dryer Capacity

Table 1 Design considerations and assumptions for fabrication of electrical dryer

Product	Shrimp
Dryer capacity	10 kg
Initial moisture content of shrimp	75% (w.b.)
Final moisture content of shrimp	15% (w.b.)
Drying air temperature	55±5℃
Ambient air temperature & relative humidity	30℃& 70%
Specific heat of air	1 KJ/kg K

Density of air	1.2 kg/m^3
Latent heat of vaporization	2260 KJ/kg
Drying time	6 h

Heat energy calculations

Energy required to dry the shrimp is calculated by taking into account of mass of material to be dried (kg), mass of water to be evaporated (kg), specific heat (KJ/kg), latent heat of evaporation (KJ/kg) and temperature difference between ambient and drying conditions.

$$Q = MC_p\Delta T + M_w\lambda$$

where,

Q = energy requirement

C_p = Specific heat of water

λ = Latent heat of evaporation

ΔT = Temperature difference between dryer and atmosphere

Power rating of coil

The power requirement of heating coil can be calculated from the energy required to dry the material in specific drying time.

$$P_C = \frac{Q}{D_t}$$

where, D_t = drying time

Drying air flow rate calculation

The total mass of air required to dry the material in 6 h,

$$m_a = \frac{Q}{\Delta T \times C_p}$$

The total volume of air required (kg) for completing the drying process,

$$V_a = \frac{m_a RT}{P}$$

The total volume of air required per hour (m^3/h),

$$V_{ra} = \frac{V_a}{D_t}$$

where,

m_a = mass flow rate of air

V_a = Volume of air

R = Universal gas constant

T = Temperature of air

P = Pressure of air

The blower capacity can be decided accordingly for the volumetric flow rate of the air.

Drying Chamber size

By doing preliminary experiments, the area required to spread 1 kg shrimp can be calculated. The drying area requirement for loading a definite weight of shrimp can be found out. The area of each tray and total no. of trays can be calculated from this data and accordingly the drying chamber dimensions can be worked out.

Fabrication of electrical dryer

The materials used for fabrication of electrical dryer are stainless steel (SS 304 for drying trays), mild steel (MS) angles for frame, galvanized iron (GI) for supporting structure, polyurethane foam (PUF) for insulation, glazing, blowers and exhaust fans. The dryer consists of drying chamber and heating coils. The base frame of the structure was fabricated using GI pipes and drying chamber supporting frames were made of stainless steel. Initially GI and SS base supporting frames are welded to drying chamber to enable loading and unloading of material. Drying chamber is insulated with PUF material. The drying chamber is designed to hold 5 trays for 10 kg fresh shrimp. The drying trays were made of perforated SS 304. There should be a spacing provided between each tray to provide efficient air movement between trays. A blower is placed on the top of the drying chamber of 0.5 hp capacity. That blower supplies air in to the heating coil, then the heated air comes inside the chamber from the vent at the bottom of drying chamber. Then the hot air went outside through two exhaust fans 0.03 hp on both top sides of the drying chamber. The cost of electrical dryer is estimated considering the cost of drying chamber, frames or stands, control panels, electrical heating coil.

Drying efficiency

The performance of a dryer was evaluated by calculating its drying efficiency. The drying efficiency is defined as the ratio of total heat energy required to the total heat energy supplied by the dryer to remove the moisture from the material.

$$Drying\ efficiency = \frac{Energy\ required}{Energy\ supplied\ by\ heating\ coil + Energy\ supplied\ by\ solar\ collector}$$

Shrinkage

Shrinkage is one of the key parameters to be checked during drying of shrimps. It was calculated by measuring the of dimensions of shrimps using vernier caliper before and after drying. Shrinkage of shrimp may be calculated from the geometric mean dimensions using the formula:

$$Shrinkage = \frac{Avg\ GMD\ before\ drying - Avg\ GMD\ after\ drying}{Avg\ GMD\ before\ drying}$$

Rehydration ratio

About five grams of dried shrimp samples were soaked in 200 mL of distilled water at ambient conditions. After 30 minutes of soaking, samples were removed, then weighed and immediately returned to the soaking water and process continued till the weight of samples become constant.

$$Rehydration\ ratio = \frac{weight\ of\ rehydrated\ sample}{weight\ of\ dried\ sample}$$

Advanced drying techniques for fish and fishery products

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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.

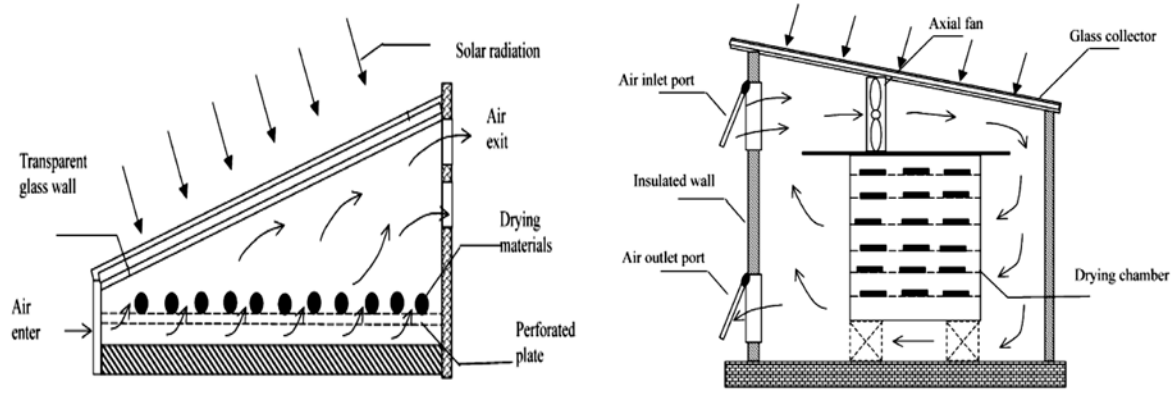
Advanced drying methods

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.

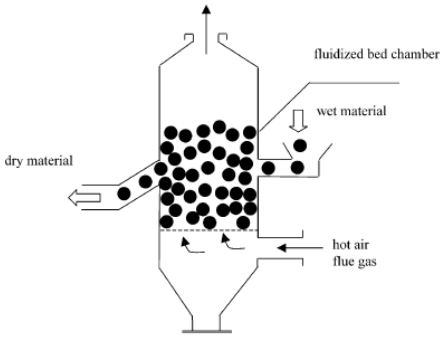
5. Using freely available solar energy instead of conventional fuels to dry products or using a cheap supplementary supply of solar heat, so reducing conventional fuel demand can result in significant cost savings.



(a) Passive solar dryer (Grabowski and Mujumdar, 2000) (b) Active solar convective dryer (Imre, 1995)

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.



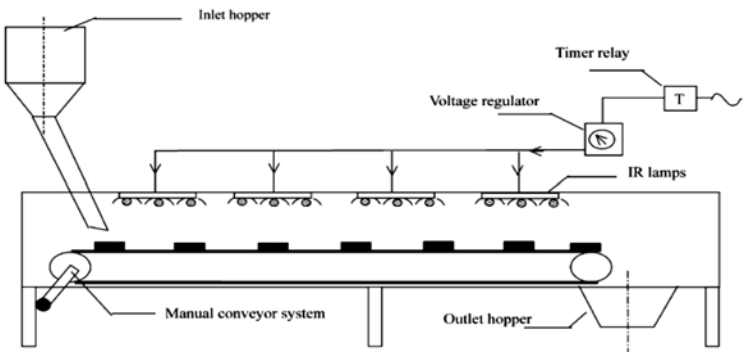
Simple fluidized bed system (Hovmand, 1995)

A simple FBD system can be easily constructed with simple steel frames, steel sheets, and wooden planks. Wet material enters from one side of the fluidized bed into the drying chamber. Hot air passes from the bottom through the perforated plate and interacts with wet feed in a cross-flow manner and causing the particles to fluidize and particles to be dried completely. Dried particles are discharged through the exit port of the FBD system. 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.

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.

IR drying can be considered to be an artificial sun drying method and it can sustain throughout the day. Advantages of using IR for drying include flexibility of operation, simplicity of the required 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, 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.



Conveyor type IR drying system (Ratti and Mujumdar, 1995)

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.

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.

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).

Supercritical CO_2 Drying

This drying is in the research and development stage for various heat-sensitive, high-value commodities in the food sector. The critical point of CO_2 gas is at 304.17K and 7.38Mpa. CO_2 can be made to dissolve in the available free moisture of food material. This combination of pressure and temperature as process parameters can cause sudden expansion of CO_2, resulting in moisture removal from the material (Kudra and Mujumdar, 2005).

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.

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.

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 has 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. Some combined drying methods are discussed below:

1. Radio frequency-assisted hot air drying: RF heats all parts of the material and evaporates the water at a low temperature. The limitation of heat transfer in convective drying with hot air alone can be overcome by combining RF heat with conventional convective drying.
2. Radiofrequency assisted heat pump drying: Combined RF energy with heat pump batch drier showed a reduction in discoloring of dried products and absence of cracking caused by stress due to uneven shrinkage during drying.
3. Microwave heating under vacuum

4. Microwave heating with freeze drying
5. Intermittent/pulsed microwave heating
6. Microwave-enhanced spouted bed drying: This can produce more uniform drying because uniform exposure of product to microwave energy is achieved by pneumatic agitation. Fluidization also facilitates heat and mass transfer due to a constant renewed boundary layer at particle surface. Combined fluidized or spouted bed is considered as an effective way to solve non-uniform drying problem of microwave heating.
7. Combination of infrared (IR) with convective heating: This combination could shorter the drying time, maintain nutritional properties, improve sensorial and functional properties of dried food.
8. Infrared radiation with freeze drying: helps in reducing drying time and energy consumption and produces high quality dried products.

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, vacuum drying) offer a wide scope for the production of best quality dried products. But usage of these methods for drying are 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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Engineering interventions in post harvest fisheries sector

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Introduction

Major areas of technological interventions in the field of fishery engineering cover design and development of fish processing equipment and machinery, energy-efficient and eco-friendly solar fish dryers, fuel-efficient fishing vessels and fiberglass canoes, indigenous electronic instruments for application in harvest and post-harvest technology of fish, quality improvement of Indian fishing fleet and energy and water optimization techniques for fish processing industries. Focused areas include the CO2 refrigeration system, development of cost-effective solar dryers, adavanced drying techniques and fish de-scaling machines, Fish freshness sensors, etc. Post-harvesting processing of fish is important to reduce wastage, increase shelf-life, add more value to the products and ensure higher returns. The major engineering interventions for fish post-harvest operations, processing, and value addition are given in subsequent sections.

1. Fish Descaling Machines

1.1.Fish descaling machine with variable drum speed

The fish de-scaling machine is designed and fabricated for removing the scales of fishes easily. This equipment can remove scales from almost all types/sizes/ species of fishes ranging from marine to freshwater species like Sardine, Tilapia to Rohu. The machine is made of SS 304 and has a 10 kg capacity (Fig. 5). 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 load. The drum is made of a perforated SS 304 sheet fitted in a strong SS Frame. A water inlet facility is provided in the drum for easy removal of the scales from the drum so that area of contact to the surface will be more for removal of scales. The water outlet is also provided to remove scales and water from the machine. An Electronic RPM meter was attached with the de-scaling machine which directly displays the RPM of the drum. The speed of the drum is a factor influencing the efficiency. The machine takes only 3-5 minutes to clean 10 kg fish depending on the size.

Fig. 5. Fish de-scaling machine with variable drum speed

1.2.Fish de-scaling machine with fixed drum speed- tabletop

The fish de-scaling machine is designed and fabricated for removing the scales of fishes easily. This equipment can remove scales from almost all types/sizes/ species of fishes ranging from marine to freshwater species like Sardine, Tilapia to Rohu. This machine is made of SS 304 and has a 5 kg capacity. It contains a 0.5 HP AC motor with a proper belt reduction mechanism to achieve the required drum speed of 20-30 rpm. The body is fabricated in dismantling type one-inch square SS tube with a suitable covering in the electrical parts (Fig. 6). The drum is made of a perforated SS sheet fitted in a strong SS Frame having suitable projections to remove the scale and provided with a leak-proof door with a suitable lock.

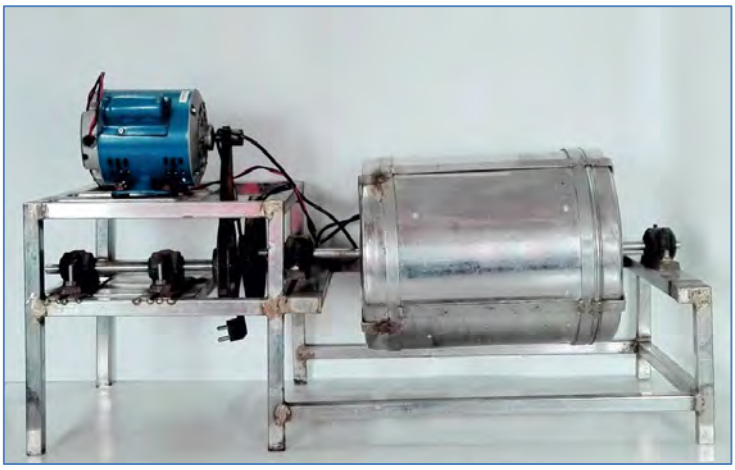

Fig. 6. Fish de-scaling machine with fixed drum speed

1.3. Hand operated Fish descaling machine

The fish descaling machine is designed and fabricated for removing the scales of fishes easily. This equipment can remove scales from almost all types/sizes/ species of fishes ranging from marine to freshwater species like Sardine, Tilapia to Rohu (Fig. 7). This machine is made of SS 304 and has a 5 kg capacity. The body is fabricated in dismantling a type 1-inch square SS tube. The drum of 255.5 mm diameter and 270 mm length is made of a perforated SS sheet fitted in a strong SS Frame having suitable projections to remove the scale and provided with a leak-proof door with a suitable lock. A pedal is fitted in the side to rotate the drum manually (Delfiya et al. 2019).

Fig. 7. Hand operated fish de-scaling machine

2. Fish meat bone separator

A Fish Meat Bone Separator with variable frequency drive (VFD) to separate pin bones from freshwater fishes was designed and developed (Fig. 8). This can be used at a range of 5-100 rpm. With a unique belt tighten system developed; the new machine can be easily adapted to any species and need not be customized for specimen during the design stage. In existing imported models, only two speeds are possible which restricts the yield efficiency in a single span operation and also limits easy switching of the system for utilizing specimens other than for which the yield has been originally customized. The meat yield of this machine was about 60% against 35% in imported models. The capacity of the machine is 100 kg/hour.

Fig. 8. Fish meat bone separator

3. Refrigerated mobile fish vending kiosk

ICAR-CIFT has designed and developed a mobile fish vending kiosk for selling fish in the closed chilled chamber under hygienic conditions at the consumer doorstep. The mobile unit is mounted on a frame with wheels at the bottom. The kiosk can carry 100kg fish with 20kg under chilled storage display in a glass chamber and remaining in an insulated icebox. The main components of the kiosk are fish storage & display facility, a hand-operated descaling machine, and a fish dressing deck with a washbasin, water tank, cutting tool, waste collection chamber, and working space. The vending unit has been fabricated using stainless steel (SS 304 Food Grade. The stored fish is covered with a transparent glass cover through which consumers can see the fish and select according to their choice of purchase. A kiosk is attached with a hand-operated descaling machine for the removal of scales. The fishes coming out of de-scaler is free of scales, dirt, or slime. It also reduces human drudgery and avoids cross-contamination, consumes lesser time. Fish dressing deck with washbasin is also designed conveniently to prepare fresh clean fish under hygienic conditions. The unit also extends the keeping quality of fish for 4- 5 days and increases the marginal benefit to fish vendors. It also helps change the practice of unhygienic handling and marketing of fish.

Fig. 8. Refrigerated mobile fish vending kiosk

4. Electronics and Instrumentation

ICAR-CIFT identified the vast scope of electronics and instrumentation for fisheries technological investigations and started research and development activities. This resulted in a series of instruments for systematic monitoring, analysis, and assessment of the marine environment including the performance of the machinery used for harvesting the resources and post-harvest technology. Basic technologies developed in ICAR-CIFT include more than five dozen electronic instruments with fully indigenous technology and more than 50 sensors with novel features and designs. The notable achievement is the development of indigenous sensors, which are rugged to withstand the hostile marine environment and enable us to monitor field data from remote areas. The total instrumentation is built up around these sensors, with required electronics, new signal processors, and other peripherals for solid-state data storing, compatibility to PC, wireless transmission to distant points, *etc*.

Some of the instruments, which has got great attention and acceptance are as follows: environmental data acquisition system, freezer temperature monitor, salinity temperature-depth meter, hydro-meteorological data acquisition system, warp load meter, solar radiation monitor and integrator, shipborne data acquisition system, water level recorder, ocean current meter, remote operated soil moisture meter, water activity meter, rheometer, and microalgae concentration monitor. Since the instruments are designed to be compatible with the computer and solid-state memory module, the information can be stored for a long duration and retrieved at our convenience.

By effective use of efficient and appropriate engineering technologies which are cost-effective, adaptable, and environment friendly, the fishermen community, as well as the seafood industry, can reduce the harvest and post-harvest expenses and losses, add more value to the products, ensure better fish value chain dynamics and thereby obtain more income. The use of green and clean technologies also ensures less carbon and water footprints.

5. Energy and Water Use Optimization in Seafood Processing Industry

In the seafood industry, the increasing importance to ensure effective usage of energy and water needs the implementation of sustainable technologies and cleaner production

practices. The review findings report that replacement of outdated technologies, use of renewable energy sources, and creation of awareness about energy consumption among manpower, and continuous energy auditing results in effective energy usage in the seafood processing sector. Similarly, adopting water optimization techniques such as automation of water flow lines, wastewater treatment, recycling and recirculation of water, continuous monitoring of water use patterns, and dry-cleaning process in the industry would result in water savings. The smart cloud-connected intelligent real-time energy and water use monitoring systems could be considered as suitable methods to optimize energy and water usage in the seafood industry. The application of software using the Internet of things (IoT) can help analyze the daily, weekly, monthly, or yearly consumption pattern. Mobile alert systems can be installed for giving warnings regarding peak specific energy consumption. Besides, developing new applications of byproducts and generating energy from wastes can reduce waste disposal and environmental pollution issues in the seafood sector. It is also important to understand the nexus between energy, water, and seafood from the environmental and sustainability perspective. Each of these three sectors has an impact on the security of others in a variety of ways. The authors observed that additional studies should be carried out on the entire seafood supply chain, starting from harvesting to consumption for the sustainability of the whole sector. The government authorities should provide tax benefits and other financial incentives for the individuals and seafood firms for being eco-friendly with the effective management of energy and water with the generation of minimum waste and GHG emissions. The government should also form a committee of assessors for the periodic evaluation of seafood processing firms to improve their competence while being sensitive to socio-economic and environmental implications.

6. Development of portable fish quality and freshness assessment sensor

A novel handheld, portable and non-destructive instrumental sensor was developed to detect the freshness of Indian Mackerel (*Rastrelliger kanagurta*) stored under ice. The freshness sensor consists of a webcam, raspberry-Pi (small single-board computer), LCD display and a power bank. The color change in fish eye as a result of spoilage during iced storage was measured as pixel count using image processing technique. Simultaneously, destructive fish quality and freshness results were obtained by estimating K-value and Psychrophilic count during the storage. Multiple linear regression analyses were performed to assess the relationship between pixel count and quality indicators. The analysis of results of K-value and Psychrophilic count revealed that fish quality and freshness limits can be established in storage days as extremely fresh up to 3rd day, fresh till 13th day and then spoiled. Further, these quality limits were correlated with pixel count against storage days to establish three different ranges in pixel count. These ranges were provided as input to the sensor for classification of fish samples into three indicative freshness levels i.e. extremely fresh, fresh and spoiled. The validation study of sensor was conducted by assessing fish samples collected from local markets and observed that sensor is accurately predicting the freshness of fish.

7. Commercialization of engineering technologies

A more pragmatic system for business incubation and promoting start-up companies concerning agricultural technologies have been evolved in recent times within the ICAR-CIFT.The Agri-Business Incubation (ABI) center along with Institute Technology

Management Unit (ITMU) seeks to provide business consulting services to agriculture-related businesses and helps to develop a strategic business plan. ABIs facilities for incubation of new business ideas based on new agricultural technologies by providing cheap space, facilities, and required information and research inputs. The Agribusiness Incubator Program also seeks to provide business consulting services to agriculture-related businesses and helps to develop a strategic business plan.

The Engineering Division of ICAR-CIFT has commercialized its technologies like solar fish dryers, fish descaling machines, refrigeration enabled fish vending machines, etc through the ABI.

Identification of unknown bacteria by 16s rRNA sequencing

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What is 16s rDNA?

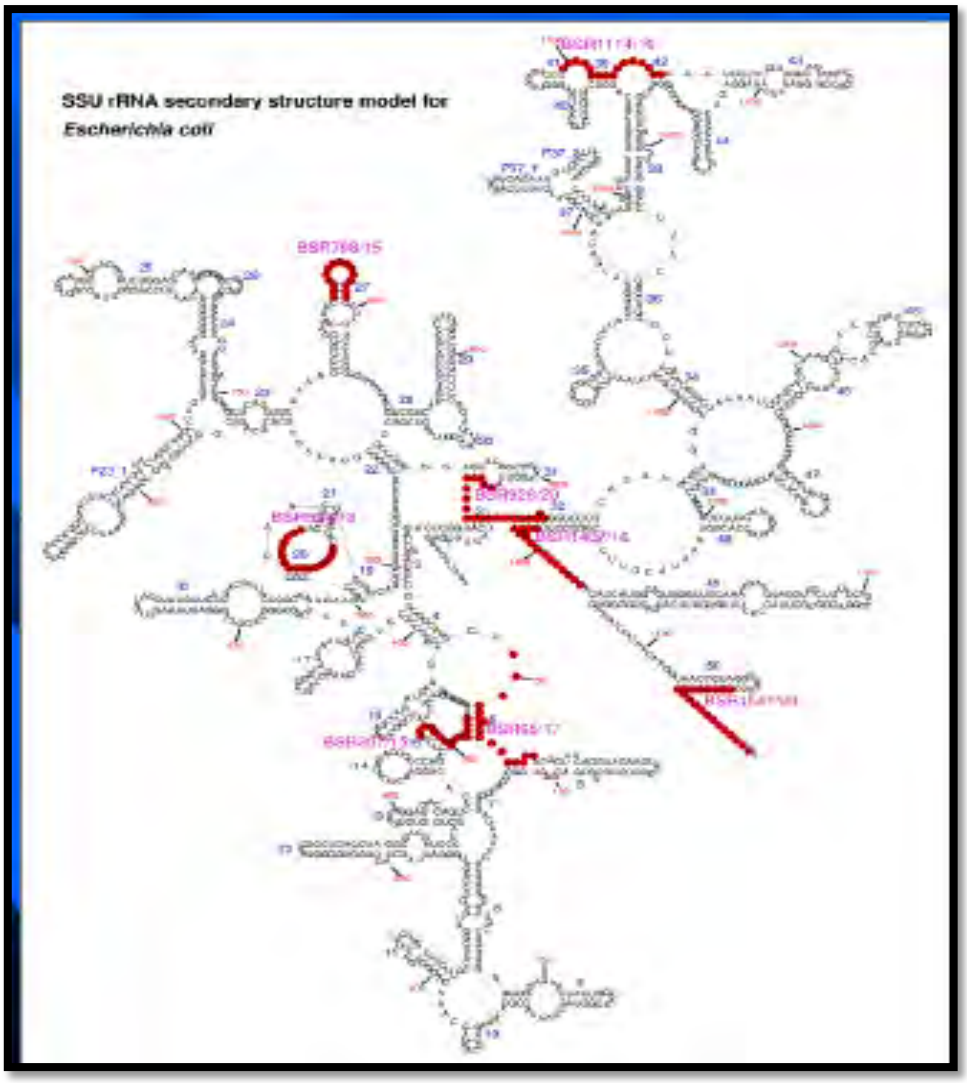
rRNA gene is the most conserved and used to determine taxonomy, phylogeny (evolutionary relationships). It is also used to infer relationships between organism that span the diversity of known life look. These genes are conserved through the billions of years of evolutionary divergence.

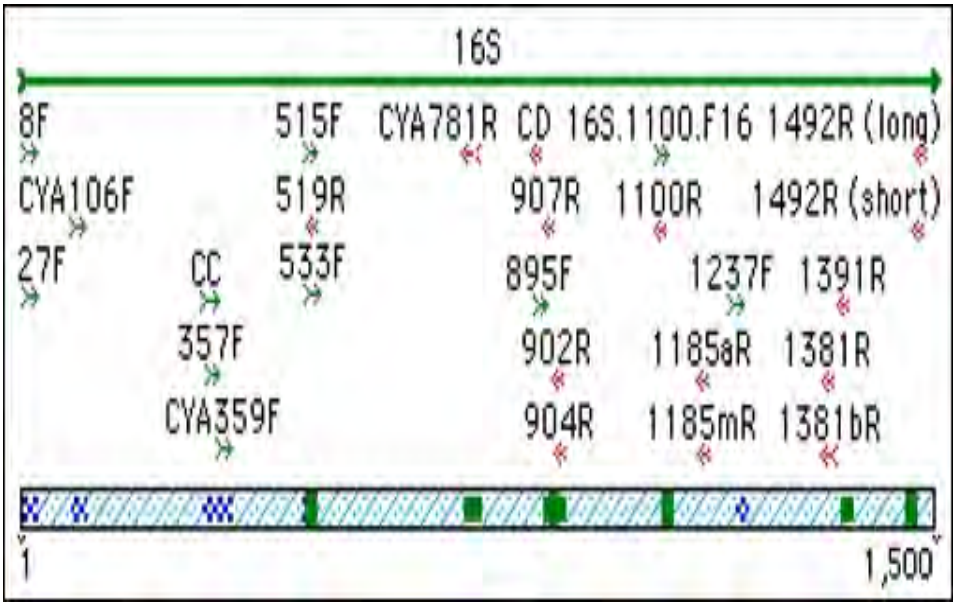
What is Microbial systematics?

Novel species is recognized using the polyphasic approach - multidimensional aspects of organisms (phenotypic, genotypic and chemotaxonomic traits). Biochemical methods of identification of bacteria are called as phenotypic methods, 16s rRNA sequencing and DNA-DNA hybridization are called as genotypic methods and other FAME analysis are chemotaxonomic methods. Phylogenetic analysis based on 16S rRNA gene sequences and determination of similarity between sequences - first step in identifying novel organisms - most widely used methodology in the world. DNA–DNA hybridization (DDH), which measures indirectly the degree of genetic similarity between two genomes, has been the 'gold standard'

What is 16s rRNA sequencing analysis?

One or more copies of the operon dispersed in the genome (mostly 3, *E. coli* 7). Ribosomal RNAs in Prokaryotes: 5S 120 Large subunit of ribosome; 16S 1500 Small subunit of ribosome; 23S 2900 Large subunit of ribosome. The 16s rDNA sequence has hypervariable regions, where sequences have diverged over evolutionary time. Strongly conserved regions often flank these hypervariable regions. Primers are designed to bind to conserved regions and amplify variable regions. Numbered primers are named for the approximate position on the *E. coli* 16S rRNA molecule. More details can be sought from National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) and the Ribosomal Database Project (http://rdp.cme.msu.edu/). Minimum: 500 to 525 bp sequenced; ideal: 1,300 to 1,500 bp sequenced 1% position ambiguities. minimum: 99% sequence similarity; ideal: 99.5% sequence similarity. Sequence match is to type strain or reference strain of species that has undergone DNA-relatedness studies. For matches with distance scores 0.5% to the next closest species, other properties, including phenotype, should be considered in final species identification.


SSU rRNA secondary structure model for
Escherichia coli


16S
8F
515F
CYA781R
CD
16S.1100.F16
1492R (long)
CYA106F
519R
907R
1100R
1492R (short)
27F
CC
533F
895F
1237F
1391R
357F
902R
1185aR
1381R
CYA359F
904R
1185mR
1381bR
1
1,500

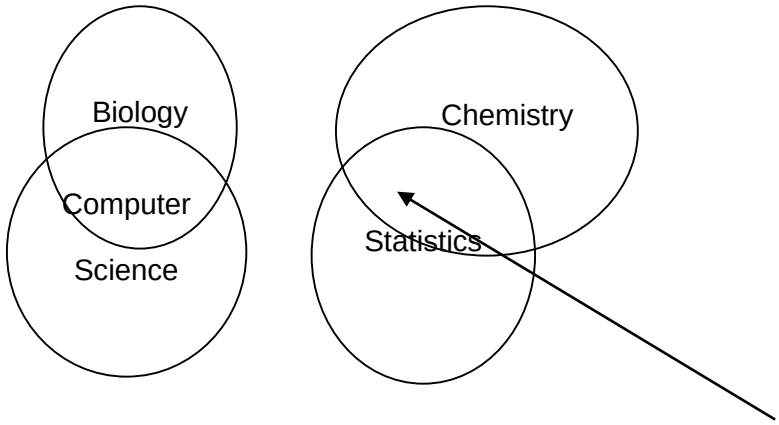
Primer		
Name	Sequence[a]	Amplified hypervariable region
V3F	5′ CCAgACTCCTACGGGAGGCAG 3′ (334–354)	V3 (334–537)[b]
V3R	5′ CGTATTACCGCGGCTGCTG 3′ (519–537)	
V6F	5′ TCGAtGCAACGCGAAGAA 3′ (961–78)	V6 (986–1043)
V6R	5′ ACATtTCACaACACGAGCTGACGA 3′ (1062–85)	
Molecular beacon probe[c]		
Name	Sequence	Target region
SEP-V6 probe	TxR-5′ tgcgcCTAGAGGGGTCAGAGGATgcgca 3′-BHQ2	1005–1022*

Other genes for identification or differentiation of bacteria?

- 23s
- 16s-23s ITS
- *rpoB*
- *gyrB*
- *Hsp*
- *recB*

What is bioinformatics?

Bioinformatics is a new science that uses computational approaches to answer biological questions. Bioinformatics is a new scientific discipline created from the interaction of biology and computer. Biological questions raised from the researchers will be investigated with the large & complex data sets available in public as well as generated by the own laboratory in private to arrive at a valid biological conclusion.



The National Center for Biotechnology Information (NCBI) defines bioinformatics as: "Bioinformatics is the field of science in which biology, computer science, and information technology merge into a single discipline"

Broad Areas in Bioinformatics

- Genomics

- Proteomics
- others

Some of the bioinformatics applicable are

Similarity search

- Sequence comparison: Alignment, multiple alignment, retrieval
- Sequence's analysis: Signal peptide, transmembrane domain,
- Protein folding: secondary structure from sequence
- Sequence evolution: phylogenetic trees

Important terms in Bioinformatics

Fasta sequences

The FASTA format is used in a variety of molecular biology software suites. In its simplest incarnation (as shown above) the ''greater than'' character (>) designates the beginning of a new file. An identifier (L04459 in the first of the preceding examples) is followed by the DNA sequence in lowercase or uppercase letters, usually with 60 characters per line. Users and databases can then, if they wish, add a certain degree of complexity to this format. For example, without breaking any of the rules just outlined, one could add more information to the FASTA definition line, making the simple format a little more informative, as follows:

>gi|171361|gb|L04459|YSCCYS3A Saccharomyces cerevisiae cystathionine gamma-lyase (CYS3) gene, complete cds.

GCAGCGCACGACAGCTGTGCTATCCCGGCGAGCCCGTGGCAGAGGACCTCGCTT
GCGAAAGCATCGAGTACCGCTACAGAGCCAACCCGGTGGACAAACTCGAAGTCA
TTGTGGACCGAATGAGGCTCAATAACGAGATTAGCG

Similarly, the protein record in fasta as follows

>P31373

MTLQESDKFATKAIHAGEHVDVHGSVIEPISLSTTFKQSSPANPIGTYEYSRSQNPNRE
NLERAVAALENAQYGLAFSSGSATTATILQSLPQGSHAVSIGDVYGGTHRYFTKVAN
AHGVETSFTNDLLNDLPQLIKENTKLVW

Majority of the procedure analysing either DNA or Protein sequences involves the use of fasta format

Practical on Blast analysis and identification of unknown bacteria from 16s rRNA gene sequence data

1. Check for the quality of sequence data with chromatogram file and pdf
2. Check the quality value of each sequence base call in the chromatogram file
3. Trim the sequence according to the sequence data quality value more than 20
4. Blast analysis of raw and trim sequence data in NCBI_Blast_nucleotide.
5. Perform merging with emboss merger after reverse complementing the reverse sequence data
6. Avoid the low-quality sequences in the analysis.

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Antimicrobial resistance in foodborne pathogens

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Introduction

Antimicrobial resistance (AMR) is the ability of the microorganisms (bacteria, fungi, viruses and parasites) to resist the action of treatments. So, it's very difficult to treat the common infections and could, which may cause severe illnesses, chance of spreading of infections and finally lead to death. If we fail to tackle AMR as a pandemic issue, it could lead to 10 million deaths per year by 2050 and may cost $100 trillion lost from global GDP (O'Neill 2014). AMR is a major cause of death globally, with a burden likely to be higher than that of HIV or malaria. A comprehensive systematic study estimated that globally AMR was associated with 4.95 million deaths, including the direct contribution to 1.27 million deaths, in 2019 and India had one of the highest burdens of AMR and maximal resistance trends in Asia [Murray et al., 2022]. Moreover, AMR threat has long been signaled from the Recommendation-Commission on antibiotic resistance in 2013 and the O'Neil report, Global Antimicrobial Resistance and Use Surveillance System (GLASS) by WHO in 2015, Fleming Fund, 2015 and G7 Finance Ministers issued statements to support antibiotic development in 2021. But action has been episodic and uneven, resulting in global inequities in AMR. However, surveillance on AMR, diagnostics, treatment, control, vaccines and discovery of new antibiotics are extremely in slow progress. Moreover, the recent SARS-COVID-19 pandemic could have been worsened emergence of AMR due to unexpected and unpredicted prescriptions of antibiotics (Hsu, 2020).

Aquaculture farming and use of antibiotics in aquaculture

Due to consumer's food habit and awareness on health, fish and fisheries products get more attention across the globe due to nutrient contents viz., essential protein, fatty acids (PUFA), micro, and macro-nutrients. The per capita consumption of fish in the world was 9.0 kg in the year 1961, which grew to 20.5 kg in 2017 [FAO, 2018]. Because of its higher demand and exponential population growth, the intensified aquaculture farming system is blooming on every year. The intensive aquaculture often demands the use of formulated feeds, antibiotics, disinfectants, water, soil treatment compounds, algaecides, pesticides, fertilizers, probiotics, prebiotics etc., (Subasinghe et al., 2000; Bondad- Reantaso et al., 2005 and Rico et al., 2013] which could cause severe stress on fishes that lead to disease outbreak [Rottmann et al., 1992]. So, the fish farmers are often bound to use antibiotics to control the diseases. Generally, the antibiotics are administered through feed or applied directly into the aquaticulturte ponds [Heuer et al., 2009, Pham et al., 2015, Okocha et al., 2018]. Moreover, the administered antibiotics are not metabolized completely by the fishes and almost 75 % of the consumed antibiotics are excreted in to the pond through feces and directly applied antibiotics in the ponds will remain for a certain period (varied days of withdrawal period for different antibiotics). As on now, there is no defined antibiotics are produced for the control of fish diseases and often veterinary antibiotics are being used in fish farming [Chi et al., 2017].

Trends of antibiotic consumption

Global antimicrobial consumption in aquaculture in 2017 was estimated at 10,259 tons and antimicrobial consumption in aquaculture is expected to increase 33 % between 2017 and 2030 and mainly due to its expansion of aquaculture farming. The four countries with the largest share of antimicrobial consumption in 2017 were all in the Asia–Pacifc region: China (57.9 %), India (11.3 %), Indonesia (8.6 %), and Vietnam (5 %) and they represented the largest share of aquatic animal production output in 2017: China (51.2 %); India (9.9 %); Indonesia (9.8 %) and Vietnam (5.7 %) [Schar et al., 2020]. India accounts for about 3 % of the global consumption of antimicrobials in food animals [Van Boeckel et al., 2015]. By 2030, global antimicrobial use in human, terrestrial and aquatic food producing animal sectors is projected to reach 236,757 tons annually. On an equivalent biomass basis, estimated antimicrobial consumption in 2017 in aquaculture (164.8 mg kg−1) is 79 % higher than human consumption (92.2 mg kg−1) and 18 % higher than terrestrial food producing animal consumption (140 mg kg−1), which is projected to change to 80 % higher than human (91.7 mg kg−1) consumption and 18 % higher than terrestrial food producing animal consumption in 2030 [Schar et al., 2020].

Antibiotics used in Aquaculture

Globally, the most commonly used classes of antimicrobials were quinolones (27 %), tetracyclines (20 %), amphenicols (18 %), and sulfonamides (14 %) [Lulijwa Ronald et al., 2020]. Most frequently reported antibiotic compounds in Asian aquaculture production were sulphonamides: sulphadiazine, sulfamethoxine; beta-lactam: amoxicillin and florfenicol [Rico et al., 2012]. Food and Drug Administration (USFDA) has approved oxytetracycline, florfenicol, and Sulfadimethoxine/ormetoprim antibiotics for use in aquaculture [Romero et al., 2012].

Factors influencing antimicrobial resistance (AMR)

AMR is poorly understood in this aquaculture sector. Often the waterbodies/ aquaculture system may act as the source of AMR pathogen. The aquaculture system either use the natural waterbodies (rivers, lakes, streams, marine backwater and sea cage) or human made systems (fin fishes and shell fish farming). This frequently gives a chance of contracting with the AMR pathogen, antibiotic residues and AMR contributing factors such as biocides, chemical residues (Copper, Selenium, Lead etc.), heavy metal contaminations, pesticides, global warming and water quality parameters (pH, salinity, DO, ammonia, nitrate, nitrites etc.) through domestic, industrial and hospital sewage and agricultural runoff. The existing potential normal microflora of the aquatic system would acquire these ARGs and develop resistance against these pollutants and influence the transfer of ARGs between them, which lead to the accumulation of AMR pathogens [Michael et al., 2013]. Antimicrobial resistant bacteria can be transferred from food animals to humans either through direct contact with animals, contaminated foods, or indirectly through contaminated environments [Sharma et al., 2018, Argudín et al., 2017, Muloi et al., 2018]. The important listed AMR pathogens by FAO/ WHO/ OIE tripartite are ESKAPE whereas, numerous publications are pouring in the recent years with non- pathogenic bacterial species are also harboring from a few to more than 10 numbers of antimicrobial resistance genes (ARGs) and also harbor virulence and toxigenic genes. These

non-pathogenic antibiotic resistant bacterial species in this aquatic system are either ignored or not monitored properly. A clear-cut understanding of the origin and environmental factors that accounts for the clinical appearance of ARGs is still lacking. Moreover, consistent study is warranted to prevent the extent of AMR amplification and its dissemination under the influence of environmental selection pressure/ factors and also to evaluate its risks (pathogenicity) to human, animal and aquatic animal health. So, spread of AMR infection has to be prevented through proper sanitation, hygiene, use of protective gears, proper disposal of waste and infection prevention measures, proper treatment of effluent from hospitals, manufacturing waste and impact of antibiotic discharges, reducing unnecessary use in aquaculture, promoting development of new rapid AMR diagnostics, promoting the development of vaccines, immune-modulators, antimicrobial peptides, digestible enzymes in feed, endolysins, hydrolases, and new drugs, enhancing the potential of existing antibiotics and finding alternatives to the antibiotics (bacteriophage therapy, pre and probiotics) and CRISPR- cas9 genome editing etc.

Regulation of antibiotics used in aquaculture

The use of antibiotics in aquaculture in India is regulated by government agencies: Coastal Aquaculture Authority of India (CAA), Marine Products Export Development Authority (MPEDA), Export Inspection Agency (EIA), Food Safety Standard Authority of India (FSSAI) and State Government. These agencies have aligned their antibiotics regulations to Maximum Residual Limits (MRLs) of the European Council (EC) and the FDA requirements, to meet export requirements. In India, government have listed antibiotic compounds as authorized and banned for use in aquaculture (CAA) and have adopted EC MRLs to meet export requirements of the importing countries.

Conclusion

It is imperative to identify and mitigate the source and spread of AMR as they are contributing to antimicrobial resistance, alterations of microbial community, health hazards to the stakeholders, food safety and quality issues and economic loss worldwide. It is well known that AMR is a one health approach. Thus, for eliminating the contamination of antibiotics and resistance genes in the aquaculture field, it is necessary to implement better management practices, effective biosecurity measures and employ other disease prevention measures instead of chemotherapy.

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Techniques in molecular detection of seafood borne pathogens

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Introduction

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 syages 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 synthesised 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 hydrolysed 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 10^9 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 labelled 16S or23S *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 analysed. 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 informations 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 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 audio radiography. 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 DHA attaches to the complementary sequences and bend over and attached to next oilgo forming a g 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.

Bacteria of public health importance in fish and fishery products

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What is public health?

As per Centre for Disease Control, Atlanta "Public health is the science of protecting and improving the health of people and their communities. This work is achieved by promoting healthy lifestyles, researching disease and injury prevention, and detecting, preventing and responding to infectious diseases. Overall, public health is concerned with protecting the health of entire populations. These populations can be as small as a local neighbourhood, or as big as an entire country or region of the world".

What is zoonoses?

As per World Health Organization, Geneva "A zoonosis is **an infectious disease that has jumped from a non-human animal to humans**. Zoonotic pathogens may be bacterial, viral or parasitic, or may involve unconventional agents and can spread to humans through direct contact or through food, water or the environment".

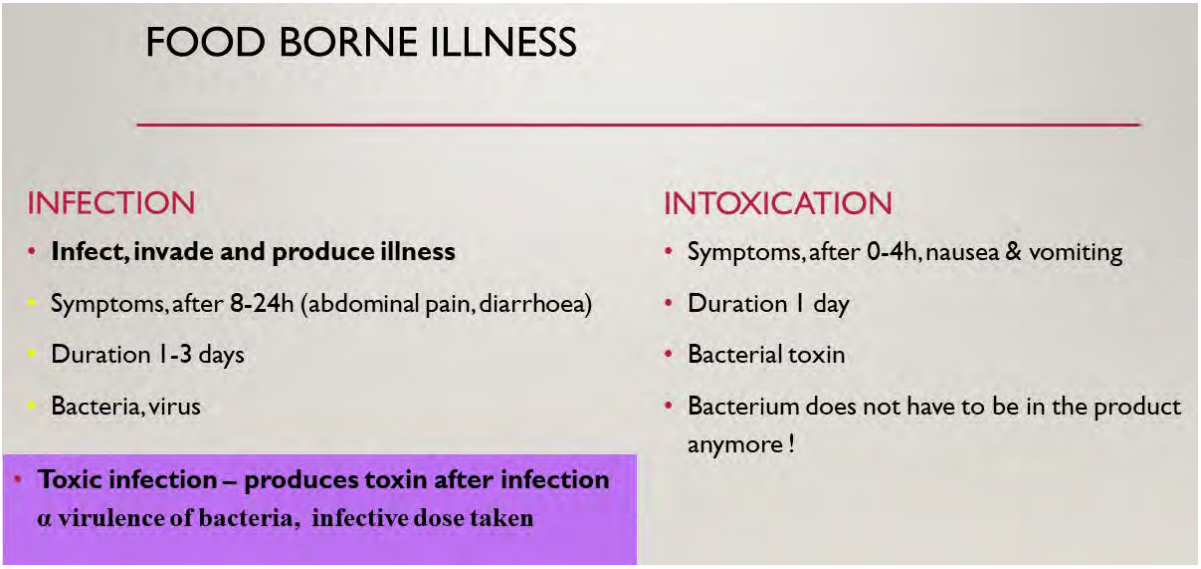
What is Zoonotic pathogen?

An infectious agent capable of spreading from animal to human or vice versa are called zoonotic pathogen. A zoonotic pathogen may be bacterial, viral or parasitic. It spreads from animals to human through direct contact or through food, water or the environment

What is foodborne pathogen and seafood borne pathogen?

Foodborne pathogens (viruses, bacteria, parasites) are **biological agents that can cause a foodborne illness event**. A foodborne disease outbreak is defined as the occurrence of two or more cases of similar illness resulting from the ingestion of a common food. Foodborne agents which are implicated through consumption of seafood (Fish and fishery products)

What is foodborne illness?



How the seafood borne pathogens enters the seafood production chain?

Living organisms that multiply frequently and spread rapidly and very tiny in nature that cannot be seen in naked eye are microorganisms or microbes. Majority of the organisms

are existing as beneficial flora in each and every niche and contributing to the basic biogeochemical cycle of the life. However, some of the microbes do exists as pathogenic to either human or animals including the fish/shellfish. Examples are Bacteria (e.g., *Staphylococcus aureus*, Streptococcus pneumoniae), viruses (e.g., Measles, Mumps), fungi (e.g., *Candida albicans*,), parasites (Coccidia etc) which are pathogenic to human.

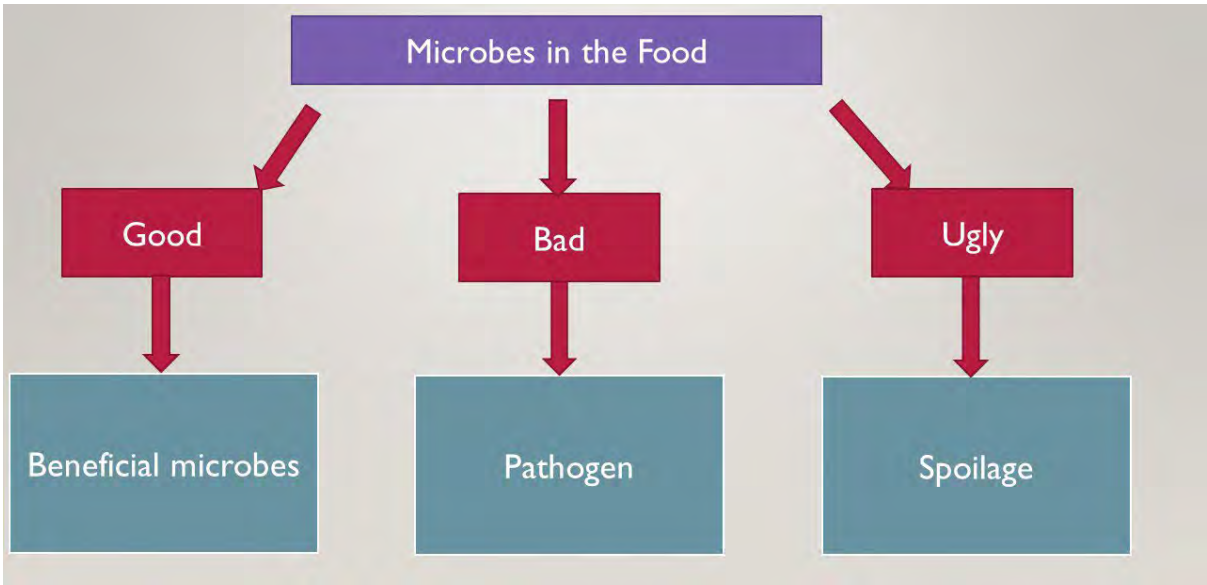


Figure. 1. Categorization of microbes associated with food

In general, to exception of commercially sterile and other pro, pre and synbiotics food products, food have the proximity of getting contaminated to various microbes during entire production and processing chain. The raw food in general has the highest culturable bacterial concentrations, followed by minimally and fully processed foods. Minimally or fully processed food including ready-to-eat food contamination depends on the level of sanitary hygiene followed during the processing and preservation steps.

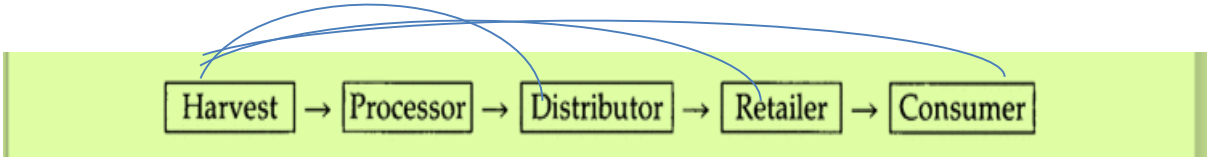


Fig.2. Steps contributes to the entry of microbes in the seafood chain

The food with acceptable microbiological quality range may also serve as the sink for the development of antibiotic resistances through bacteria, bacteriophages, bacterial DNA and mobile genetic elements, some of which may include AMR genes. Hence, the food chain ecosystem may be conducive niches for gene transfer, selection and persistence of AMR bacteria and this route cannot be generally disregarded. In the typical seafood production chain, the fish which are harvested has many distributions step viz., harvest to consumer, harvest to processor, harvest to retailers, harvest to distributors and retailers (Fig.2.). The more the number of handling steps, the more the probability of microbes being contaminated into the food production chain.

In the seafood production chain, the food fish gets harvested either from aquaculture farms or from capture fisheries activities. The harvested food fish gets transported to retail market, hypermarket, or unorganized retail vendors. The harvested food fish may be taken to the fish processing factories within their state or to the neighbouring state and get processed for domestic or export purposes. The major contributing factors which results in the contamination of pathogens in to the seafood are water and ice. In order to break the chain of contamination of these microbial pathogens into the seafood production and distribution channel, the places mentioned in the figure.2 has to be implemented.

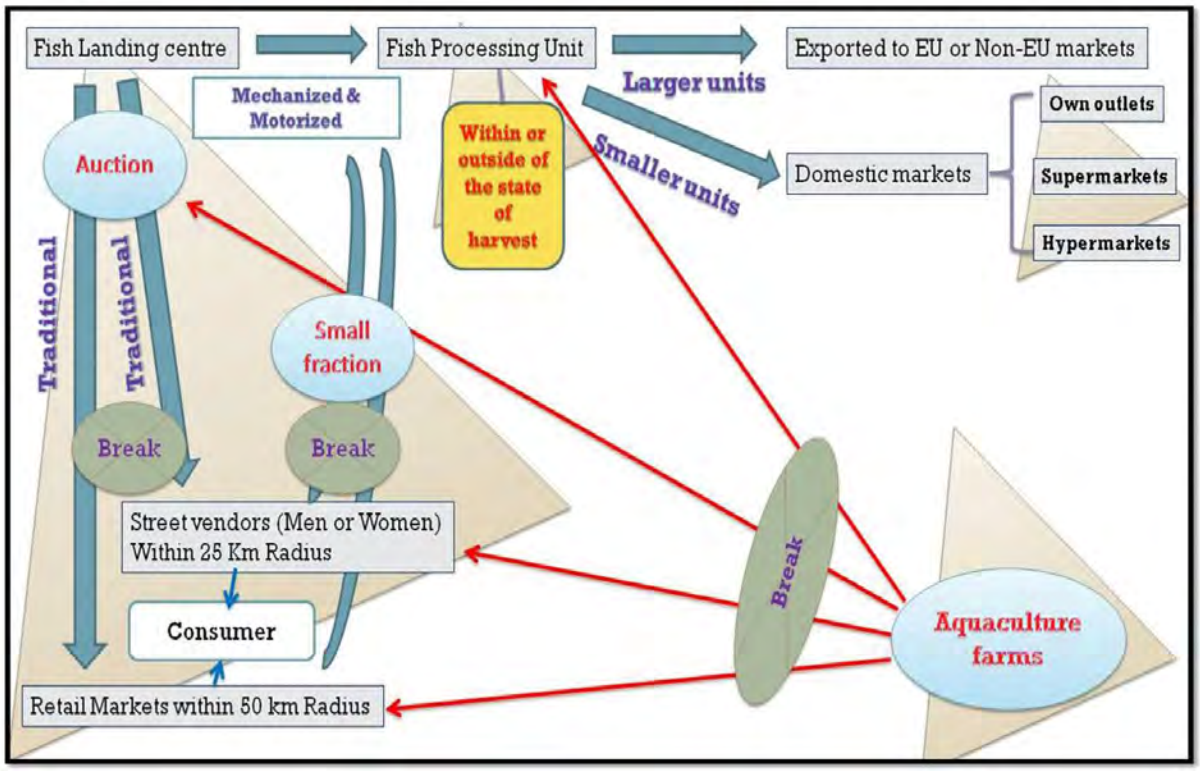


Fig. 3. Typical seafood production and distribution chain with major break point places for preventing contamination of microbial pathogens.

The seafood meant for human consumption either for domestic market or for exports has to be ascertained for predefined quality. In India, the seafood or fish/fishery products meant for domestic consumption is regulated by Food Safety and Standards Authority of India (FSSAI) and seafood meant for export purpose is handled by Export inspection council (EIC).

The end product (fish and fishery products) has to be examined for the absence of hazards. “Hazard in food is defined as anything that could contaminate food and cause illness or injury, or could otherwise violate established food safety program criteria if left uncontrolled”. Hazard in the food is classified into three categories viz., physical, chemical and biological. A physical hazard is any foreign matter unintentionally introduced to food or a naturally occurring object which could cause illness or injury to the person consuming the food item. Natural and manufactured chemicals can cause people to become sick if they have contaminated food at the source or during processing. Chemical hazards can be divided into

two categories: chemical agents and toxic metals. While physical and chemical hazards have potential to cause foodborne illness, the majority of foodborne illnesses result from biological hazards such as bacteria, viruses, and parasites (referred to collectively as pathogens). CDC has identified 31 different pathogens known to cause foodborne illness.

These hazardous microbes are classified once again as severe hazards, moderate hazardous with limited spread and moderately hazardous with extreme spread.

BACTERIAL PATHOGENS OF PUBLIC HEALTH IMPORTANCE – FISH AND FISHERY PRODUCTS

- *Aeromanas spp.*
- Bacterial toxins (*B. cereus*)
- Bacterial toxins (*S. aureus*)
- *Clostridium botulinum*
- Enteroaggerative *E. coli* (EAggEC)
- Entero-pathogenic *E. coli* (EPEC)
- Entero-toxigenic *E.coli* (ETEC)
- Listeria monocytogenes
- Non cholera Vibrios
- Salmonella (non-typhoidal) sp.
- Salmonella (typhoidal) sp.
- Shiga-toxin producing E. coli (STEC)
- Shigella sp.
- Vibrio cholerae 01/0139
- Yersinia sp.

Severe	Moderate hazard: extreme spread	Moderate hazard: Limited spread
• *Clostridium botulinum* types A, B, E, and F	• *Listeria monocytogenes*	• *Bacillus cereus*
• *Shigella dysenteriae*	• *Salmonella* spp.	• *Campylobacter jejuni*
• *Salmonella* Typhi; Paratyphi A, B	• *Shigella* spp.	• *Clostridium peifringens*
• Hepatitis A and E	• Diarrheagenic *Escherichia coli*	• *Staphylococcus aureus*
• *Brucella abortis; B. suis*	• *Streptococcus pyogenes*	• *Vibrio cholerae, non-O 1*
• *Vibrio cholerae* 01	• Rotavirus	• *Vibrio parahaemolyticus*
• *Vibrio vulnificus*	• Norwalk virus group	• *Yersinia enterocolitica*
• *Taenia solium*	• *Entamoeba histolytica*	• *Giardia lamblia*
• *Trichinella spiralis*	• *Diphyllobothrium latum*	• *Taenia saginata*
	• *Ascaris lumbricoides*	
	• *Cryptosporidium parvum*	

Seafood associated foodborne pathogens

- *Salmonella*
- *Yersinia* spp.
- *C. Botulinum*
- *S. aureus*
- *L. monocytogenes*
- ***Vibrio* spp. (*V. cholerae, V. vulnificus, and V. parahemolyticus*)**
- *Aeromonas* sp
- *Campylobacter* sp
- *Bacillus cereus*

Examples of severe hazard are *Clostridium botulinum* types A, B, E, and F, *Shigella dysenteriae, Salmonella* Typhi, *Salmonella* Paratyphi A, B, Hepatitis A and E, *Brucella abortis; B. suis, Vibrio cholerae* 01, *Vibrio vulnificus*, *Taenia solium* and *Trichinella spiralis*. Among these severe hazards, the *Clostridium botulinum* types A, B, E, and F, *Shigella dysenteriae, Salmonella* Typhi, *Salmonella* Paratyphi A, B, Hepatitis A and E, *Vibrio cholerae* 01, *Vibrio vulnificus* are relevant to seafood.

Examples of moderate hazards with extreme spread are *Listeria monocytogenes, Salmonella* spp., *Shigella* spp., Diarrheagenic *Escherichia coli, Streptococcus pyogenes,* Rotavirus, Norwalk virus group, *Entamoeba histolytica, Diphyllobothrium latum, Ascaris lumbricoides,* and *Cryptosporidium parvum*. Among these moderate hazards, *Listeria monocytogenes, Salmonella* spp., *Shigella* spp., Diarrheagenic *Escherichia coli, Diphyllobothrium latum* are very relevant to the seafood.

Examples of moderate hazards with limited spread are *Bacillus cereus, Campylobacter jejuni, Clostridium perfringens, Staphylococcus aureus, Vibrio cholerae, non-O 1, Vibrio parahaemolyticus, Yersinia enterocolitica, Giardia lamblia* and *Taenia saginata*. Among these, *Bacillus cereus, Campylobacter jejuni, Clostridium perfringens, Staphylococcus aureus, Vibrio cholerae, non-O 1, Vibrio parahaemolyticus,* and *Yersinia enterocolitica* are very relevant to the seafood industry.

For the seafood industry, the pathogens such as *Salmonella* sp. *Yersinia* spp., *C. Botulinum, S. aureus, L. monocytogenes, Vibrio* spp. (*V. cholerae, V. vulnificus, and V. parahemolyticus*), *Aeromonas* sp, *Campylobacter* sp and *Bacillus cereus* are very important. Few of the pathogens are emerging in nature and few are endemic to the seafood production system and others are reemerging in nature.

Examination of the biological hazards in the seafood

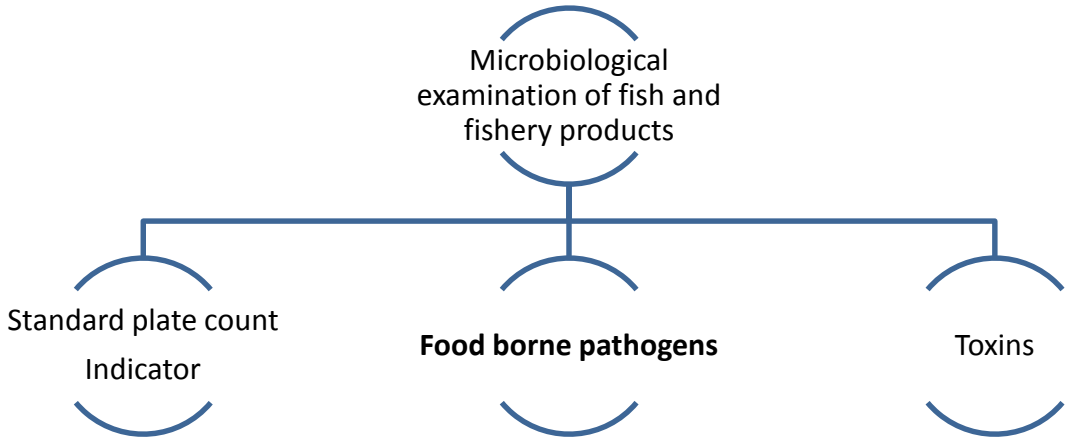


Fig. 4. Microbiological examination of seafood

Microbiological examination of seafood can be categorized into examination for indicator organisms, examination for the pathogens and or its toxins (Fig. 3).

Microbiological examination of seafood has few important steps (Fig. 4)

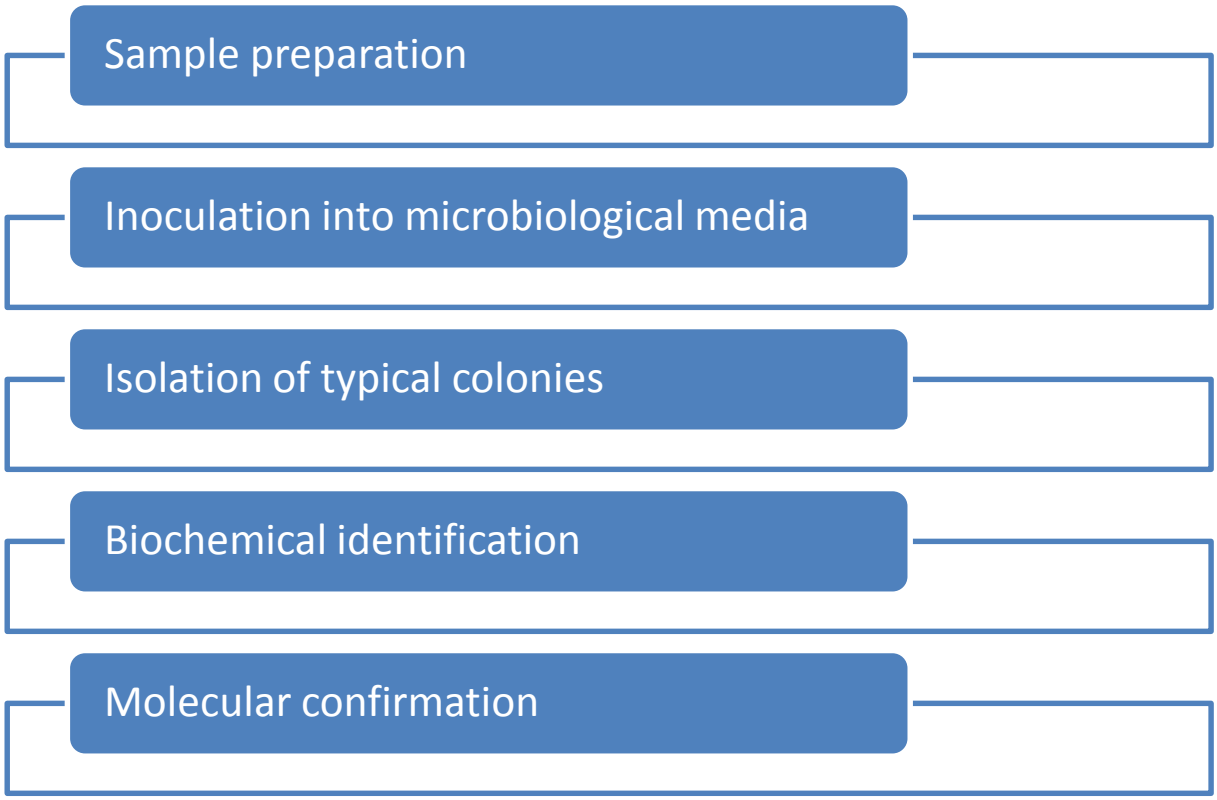


Fig. 5. Basic steps involved in the microbiological examination of seafood

For the microbiological examination of seafood, the laboratory should have these facilities. Sample receiving room, Sample processing room, Media preparation room, Media sterilization room, Inoculation room, Incubation room, Identification room, Decontamination and washing room.

Instrumentation required for setting up of microbiological testing facility for food includes Incubators / refrigerated / Co2/ BOD, Hot air oven, Autoclaves, Homogenizer / Stomacher / Mixer, Colony counter, Water bath, weighing balance, Thermal cycler including gradient, Gel electrophoresis system, Gel documentation system, Biosafety cabinet, Refrigerator centrifuges, Refrigerated shaker incubator and Microscope. A typical work flow in any standard microbiological laboratory is presented in Figure 5.

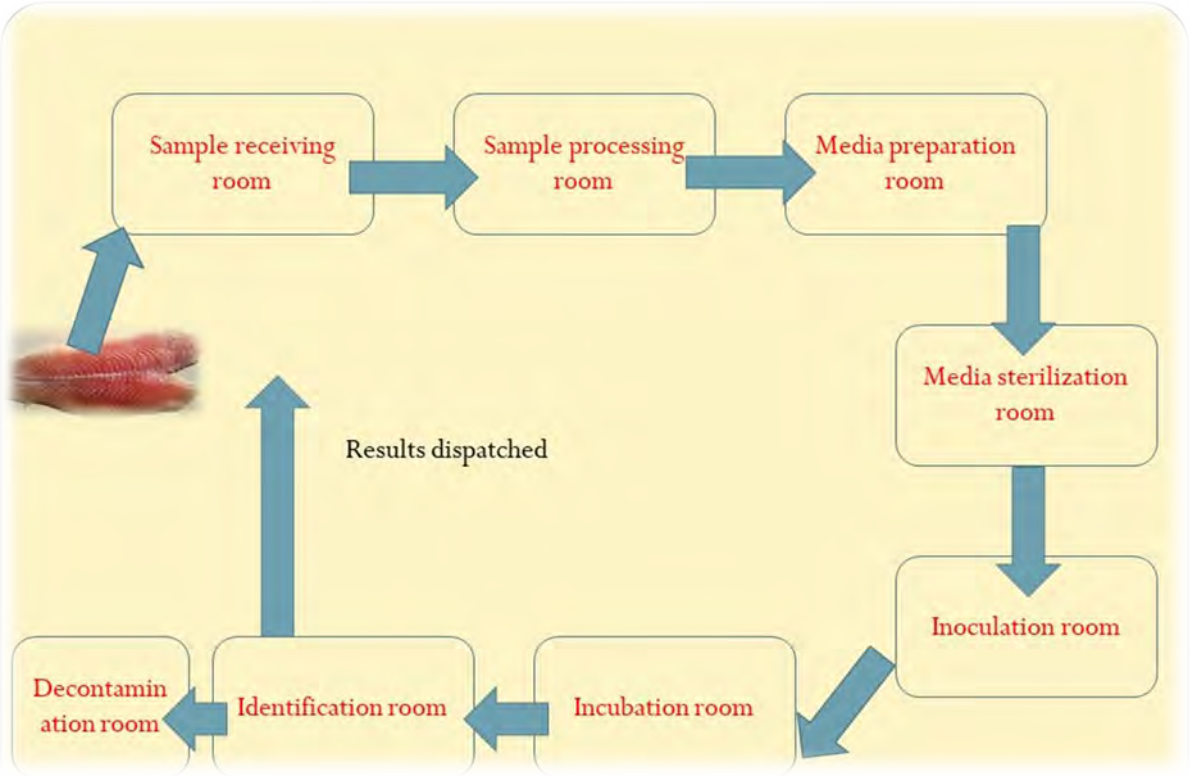
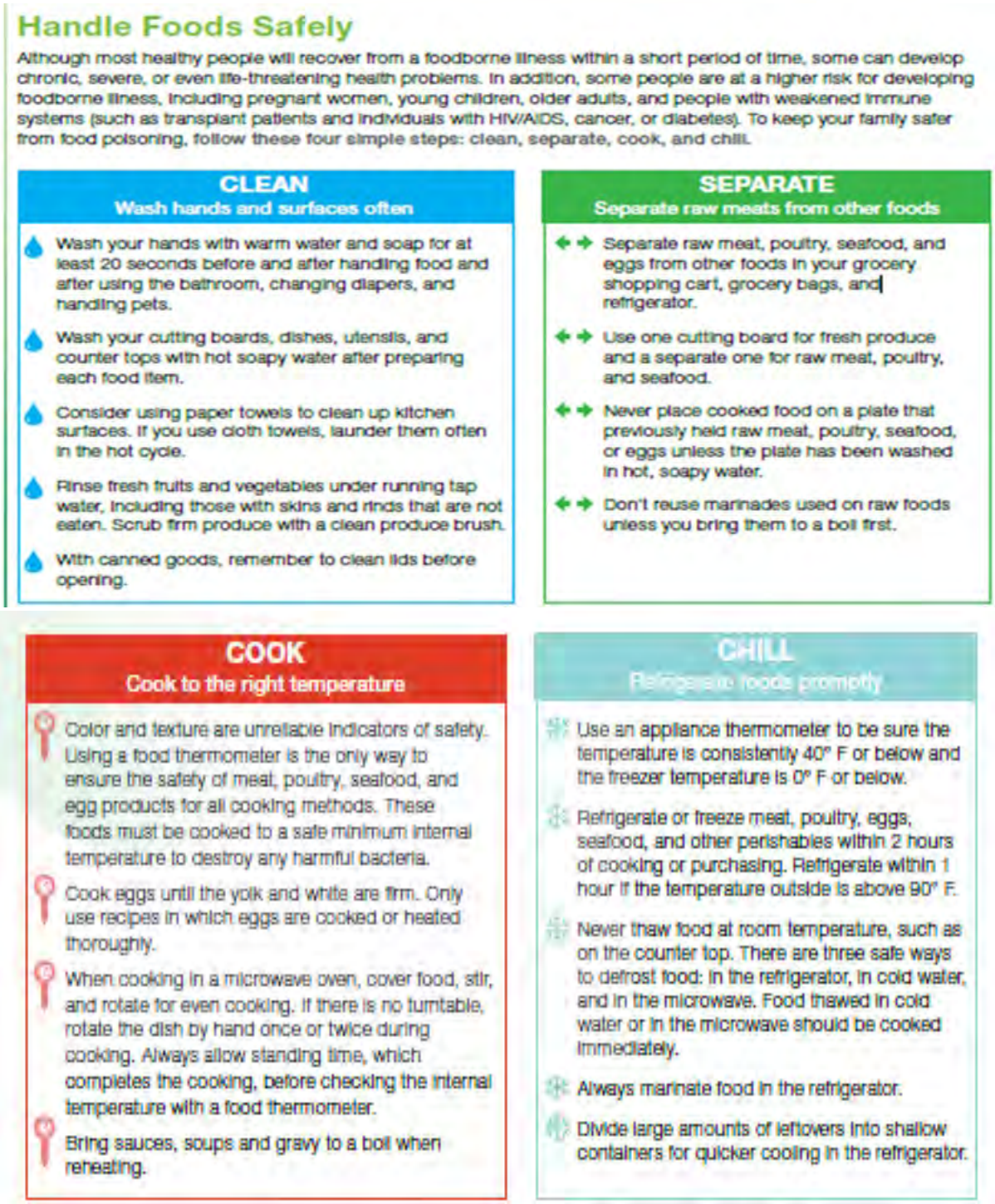


Fig. 6. Typical work flow and rooms involved in the microbiological testing.

For the microbiological examination of seafood (fish and fishery products) the laboratory should follow the exporting or importing countries guidelines viz., FSSAI – India, BAM – USA, ISO guidelines – EU countries and other based on the country's regulatory requirements.

To conclude, Fish and fishery products are most traded commodities across globe, For sustainability – Quality of food has to be maintained, Hazards – Biological hazards has to be controlled, Places where the biological hazard entry can be prevented should be defined in the seafood production system, Layout of microbiology laboratory and instrumentation involved in the testing varies based on the laboratory requirements, and the Guidelines sorting for each matrix testing is highly essential for the laboratory involved in the testing.

Basic control measures for the foodborne diseases?



Handle Foods Safely

Although most healthy people will recover from a foodborne illness within a short period of time, some can develop chronic, severe, or even life-threatening health problems. In addition, some people are at a higher risk for developing foodborne illness, including pregnant women, young children, older adults, and people with weakened immune systems (such as transplant patients and individuals with HIV/AIDS, cancer, or diabetes). To keep your family safer from food poisoning, follow these four simple steps: clean, separate, cook, and chill.

CLEAN
Wash hands and surfaces often

- Wash your hands with warm water and soap for at least 20 seconds before and after handling food and after using the bathroom, changing diapers, and handling pets.
- Wash your cutting boards, dishes, utensils, and counter tops with hot soapy water after preparing each food item.
- Consider using paper towels to clean up kitchen surfaces. If you use cloth towels, launder them often in the hot cycle.
- Rinse fresh fruits and vegetables under running tap water, including those with skins and rinds that are not eaten. Scrub firm produce with a clean produce brush.
- With canned goods, remember to clean lids before opening.

SEPARATE
Separate raw meats from other foods

- Separate raw meat, poultry, seafood, and eggs from other foods in your grocery shopping cart, grocery bags, and refrigerator.
- Use one cutting board for fresh produce and a separate one for raw meat, poultry, and seafood.
- Never place cooked food on a plate that previously held raw meat, poultry, seafood, or eggs unless the plate has been washed in hot, soapy water.
- Don't reuse marinades used on raw foods unless you bring them to a boil first.

COOK
Cook to the right temperature

- Color and texture are unreliable indicators of safety. Using a food thermometer is the only way to ensure the safety of meat, poultry, seafood, and egg products for all cooking methods. These foods must be cooked to a safe minimum internal temperature to destroy any harmful bacteria.
- Cook eggs until the yolk and white are firm. Only use recipes in which eggs are cooked or heated thoroughly.
- When cooking in a microwave oven, cover food, stir, and rotate for even cooking. If there is no turntable, rotate the dish by hand once or twice during cooking. Always allow standing time, which completes the cooking, before checking the internal temperature with a food thermometer.
- Bring sauces, soups and gravy to a boil when reheating.

CHILL
Refrigerate foods promptly

- Use an appliance thermometer to be sure the temperature is consistently 40° F or below and the freezer temperature is 0° F or below.
- Refrigerate or freeze meat, poultry, eggs, seafood, and other perishables within 2 hours of cooking or purchasing. Refrigerate within 1 hour if the temperature outside is above 90° F.
- Never thaw food at room temperature, such as on the counter top. There are three safe ways to defrost food: in the refrigerator, in cold water, and in the microwave. Food thawed in cold water or in the microwave should be cooked immediately.
- Always marinate food in the refrigerator.
- Divide large amounts of leftovers into shallow containers for quicker cooling in the refrigerator.

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Determination of AMR in bacteria

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Introduction

Presently, different methods of detection of antibiotic susceptibility are being available for both phenotypic and genotypic characterization of antimicrobial resistance (AMR) in different bacterial isolates. Some are regularly used in diagnostic laboratories, while others are still employed by academicians and professionals as research tools. The routine/ conventional testing of AMR involves plating of samples (fish, water, sediment & feed etc.) for the isolation and identification of a bacterial species of interest with pre-enrichment with selective and differential media. Disk diffusion, broth dilution, and gradient strip with respect to type of bacteria (Gram positive/ Gram negative) a panel of antibiotics is used to determine the AST as per minimum inhibitory concentration (MIC), and the breakpoint values set by the Clinical Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines and is known as phenotypic susceptibility tests. Recently, World Health Organization (WHO) has developed the software viz. WHONET for the analysis of antibiotic sensitive test (AST) to derive multiple interpretations with world unified protocol to support clear and error-free concept. The phenotypic method can be tested by conventional method (Diffusion and Dilution) or advanced method (Automated systems and Mass spectrometry). Bauer and Kirby's invented the disc diffusion method in 1956 and it is now the most used for determining the results of the phenotypic antibiotic sensitive test (AST). This conventional method is the gold standard and is very informative, but labor intensive and time consuming, sometime need several days to complete the AST with different classes of antibiotics. Recently, Automated bacterial characterization systems such as VIDAS, Vitek, BD Phoenix, MicroScan WalkAway, Micronaut and and Sensititre ARIS 2X based on turbidimetric, colorimetric, fluorometer or photometer or its combination are in used in large multispecialty hospitals, diagnostic centers and research institutions for the high throughput screening of more samples in a shorter time but is costlier, technical skill is required and it does not provide the mechanisms of resistance. However, PC based detection of antimicrobial resistance genes (ARGs) could able to detect the mechanisms of resistance. The possibility of quickly determining the ARGs in bacteria by the introduction of next-generation sequencing methods (Whole Genome Sequence). Although there are more and more phenotypic and genotypic characterization techniques for AMR detection now accessible, each of these techniques has some drawbacks. The adaptability of the bacterial genome should not be undervalued, either, given the potential future development of hitherto unimaginable new and unique resistance mechanisms. So, do both phenotypic and genotypic screening of AMR pathogens if possible as per the situations. Moreover, monitoring of AMR bacteria is a continuous process not only in clinical setting but also in healthy humans, animals and environment for proper understanding and to make effective combat strategies.

Disk diffusion assay: One phenotypic technique that can be used to assess the antibiotic resistance is disc diffusion testing i.e. in vitro susceptibility testing of antimicrobial resistance

(antibiogram). A standard inoculum of the bacteria (McFarland Standard 0.5 = ~1.5×108 CFU/mL) is used to inoculate agar plates, and then an antimicrobial disc is placed on the inoculated agar plate. Following the recommendations of the Clinical and Laboratory Standards Institute (CLSI), the plate is incubated under controlled circumstances. When in contact with the surface of the agar, the antimicrobial agent (set concentration, as per CLSI) contained in the discs used for a disc diffusion experiment diffuses into the agar. A "zone of inhibition" forms around the disc as a result of the antimicrobial drug diffusing into the agar during incubation and preventing bacterial growth. The diameter of this zone is measured and the findings are classified as resistant, moderate, or susceptible (CLSI M7, M31 and M100) and the inhibition zone's size reveals the level of resistance. This disk diffusion assay is extremely sensitive to changes in the following factors: bacterial concentration, media composition, pH, agar depth, diffusion rate of the antibiotics, growth rate of the bacteria, and incubation time. Internal quality control testing must be carried out on a regular basis as advised by CLSI (CLSI M2) to ensure the accuracy and repeatability of antimicrobial susceptibility test results.

Practical

Sample Preparation: The purified, single, and young culture (18-24 h) grown on non-selective agar must be used.

Media required

- Sterile saline solution (0.85 %) 3-4 mL each tube
- Mueller-Hinton agar plates (4 mm)
- Antimicrobial Disks (stored in -10 °C to -20 °C)
- Nutrient agar plates/ non-selective agar
- Quality control Strain

Equipment

- McFarland standard 0.5/ nephelometer
- Vortex
- Disk dispenser/ forceps
- Micropipette & tips (100 µl)
- Bunsen burner
- Small sterile cotton swabs/ spreader
- Ruler or caliper

Composition and preparation of culture media and reagents

- **Mueller Hinton Agar:** Mueller-Hinton Agar may be prepared from a commercially available base. Ensure that the Mueller-Hinton agar formulations have met the quality standards prescribed by CLSI document M6 *Protocols for Evaluating Dehydrated Mueller-Hinton Agar*.
- **Nutrient agar** (ISO 6579:2002)

  Meat extract 3.0 g
  Peptone 5.0 g
  Agar 12 g to 18 g
  Water 1000 mL

  Adjust pH to ~7.0 after sterilization
  Autoclave at 121 ^{O}C for 20 min.

- **Saline solution**

  Sodium chloride 8.5g

  Water 1000 mL

  Adjust pH to 7.0.

  Autoclave at 121 ^{o}C for 20 min

Procedure

- Check the bacteria and the quality control strains are pure and well isolated colonies on the grown agar plates and free of visible contamination
- Pick up at least 4 to 5 well isolated colonies with a loop or sterile swab and transfer to the tube of saline and emulsify the inoculum on the inside of the tube to avoid clumping of the cells.
- Prepare the inoculum standard to a 0.5 McFarland by compare turbidity to that in the 0.5 McFarland standards using a paper with black lines or nephelometer and adjust it.
- Dip a sterile cotton swab into the inoculum, rotate the swab several times and press firmly on the inside wall of the tube above the fluid level to remove excess inoculum.
- Streak the swab over the entire surface of the Mueller Hinton agar plate
- Keep the plates 3-5 minutes to allow the excess moisture to be absorbed
- Dispense the antibiotic disks on the agar surface with dispenser or sterile forceps (5 disks on a 10 cm plate)
- Incubate at 35+2^{o}C for 18-24hrs

Results: Measure the diameter of inhibition zones and measure the more obvious margin of the zone diameter. If no inhibition is present, the diameter of the disk should be recorded (6mm).

Interpretation and reporting of the results: Refer the CLSI Guideline M100 and report as sensitive (S), intermediate (I) or resistant ®.

Reference

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Probiotics in Aquaculture

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Background

Aquaculture is a major food-producing activity that helps to cater to the needs of an ever-increasing populace. Nevertheless, infectious diseases have become a serious issue in aquaculture, resulting in significant financial losses for the industry. Treatment with costly chemotherapy medications has a detrimental effect on the aquatic ecosystem. As a result, there is an increasing need for finding alternatives that are safe, non-antibiotic-based, and environmentally. Probiotics are a possible alternative to antibiotics for controlling infectious agents and treating disorders. Growth promotion, better metabolism, improved immunological response, and water quality maintenance are all advantages of probiotics. Probiotics help aquatic animals to fight diseases and promote well-being since they have antibacterial, antifungal, and antiviral capabilities. Probiotics are a unique concept in aquaculture, and their effectiveness in an aquatic setting is still to be well investigated. This article presents current information about using probiotics, including selection criteria, kinds of probiotics utilized in fish farming, the mechanisms underlying, and probiotics administration methods.

Definition of probiotic

Probiotic is originally a Greek term, where ‘Pro’ means benefit and ‘bios’ means life. In 1907, a Russian analyst named Elie Metchnikoff noted that Bulgarian laborers lived long lives because they ate fermented dairy products. Lilly and Stillwell (1965) used the term "probiotic" to describe unknown growth-promoting chemicals produced by a ciliated protozoan. In 1974, Parker defined probiotics as organisms and substances that add to intestinal balance.

In 1992, Fuller redefined the definition as a live microbial feed supplement that beneficially affects the host by improving the intestinal microbiological balance. According to a joint working group of the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (FAO/ WHO,2001). By releasing compounds such as bacteriocins and other inorganic compounds, probiotics defend the host body from harmful microorganisms. Aquaculture probiotics are live, dead or component of a microbial cell, which is administered via the feed or to the rearing water, benefiting the host by improving disease resistance, health status, growth performance, feed utilization, stress response, or general vigour, which is achieved via improving the hosts microbial balance or the microbial balance of the ambient environment (Merrifield et al. 2010a).

Probiotics have gained increasing prominence as an alternate to antibacterial drugs in the aquaculture sector for increasing productivity and preventing disease. Whenever probiotics are fed to the fish, they have a positive effect on the fish host. Dietary intake of probiotics aids in the modification of the intestinal tract's microbes balance, as well as the immune modulation and also offers several nutritional advantages (Kesarcodi-Watson et al., 2008). Probiotics have

a wide range of applications in aquaculture, in addition to their health and growth-promoting characteristics. Because of the complex link between an aquatic organism and its surroundings, the notion of probiotic use in fish culture has been developed to encompass water quality enhancement by directly introducing probiotics in ponds. For these reasons probiotics are defined as “water additives” (Moriarty, 1998). It is assumed that microorganisms that improve water quality also improve the health of aquatic animals, and various commercial products labeled as "probiotics" have attempted to capitalize on this theory. The research and potential application of probiotics in aquaculture have continued to grow during the last couple of decades. Representatives of roughly 20 bacterial genera have recently been recognized as prospective probiotic candidates, with *Bacillus* spp. and *Lactobacillus* spp. (LAB) representing the bulk of promising species (Knipe et al., 2020).

Characteristics of an ideal probiotic

The vital role of probiotics is to establish or maintain a healthy intestinal microbial flora in the fish (Thirumurugan and Vignesh, 2015). The following are the characteristics of an ideal probiotic.

- They should offer a beneficial effect on growth, maturation, and immunity against pathogens. Probiotics should have no negative consequences for the host.
- Antibiotic resistance should never be a feature of probiotics; instead, they must be able to maintain inherited features.

Probiotics should have the following characteristics in order to be used as an effective feed probiotic.

- ✓ Withstand acidic conditions
- ✓ Resistant to gastric secretions
- ✓ Attach to the epithelium of the digestive tract
- ✓ Antagonism towards pathogenic microorganisms
- ✓ Immune system stimulation
- ✓ Increase in gut movement
- ✓ Able to survive in mucus
- ✓ Probiotics should have fermentative activity, resistance to drying, and viability in food during transport and storage.

Organisms obtained from various sources are submitted to a series of tests in order to determine their suitability as ideal probiotic. The screening procedure includes Gram’s reaction, in-vitro assessment of antagonistic characters, tolerance to acids and bile and susceptibility to antimicrobial drugs. If all of these criteria are met, they are considered a promising probiotic for use in fish culture.

Sources of bacterial probiotics

Bacteria can be found in humans, animals, soil, sediment, aquatic environment and different numbers of bacteria (10^2 -10^{11} CFU/g) were observed in different environments (Liu et al., 2010). Bacteria from atmosphere, soil and anthropogenic activities can enter the aquatic system and alter the microbial load in the water, which further leads to the colonization of different bacteria in the gastrointestinal tract of aquatic organisms. The microbial load in the GIT of aquatic animals is normally 10^2 -10^9 CFU/g (Kim et al., 2007). Probiotic candidates’ potential has been evaluated in a variety of settings, including semi-intensive culture systems, intensive fish farms, and natural water bodies (Chantharasophon et al., 2011), where microbes

obtained from outside of the hosts are referred to as "allochthonous or exogenous," and the ones recovered from the host are referred to as "autochthonous or indigenous" (Ringo et al., 2016).

Selection of probiotics

The selection process of probiotics can be represented as follows.

Source organism

↓

Isolation of probiotic strain

↓

In-vitro examination of probiotic strain

↓

In-vivo assessment

↓

Challenge study, Performance study

↓

Approval from the government agency

↓

Commercialization of probiotics

Types of probiotics

Probiotics are grouped into two categories based on their mechanism of action. They are gut probiotics and water probiotics. Gut probiotics are normally administered through feed which helps to improve the gut Microflora. Water probiotics are administered in the aquatic environment which intakes all nutrients from the water and the harmful bacteria are eliminated from the system due to lack of nutrients.

Types	Description
Non-viable probiotics	Probiotics with dead microorganisms
Freeze-dried probiotics	These probiotics will rapidly die upon leaving refrigeration
Fermented probiotics	These are probiotics that are produced through fermentation
Viable probiotics	These are live microorganisms, have a protocol to be counting, and are very stable and efficacious

Probiotics in aquaculture

Fish raised in an aquaculture facility are highly influenced by the microorganisms in the surrounding water (Verschuere et al., 2010). Eukaryotes and commensal bacteria thrive in the aquaculture habitat, while opportunistic pathogens grow under favorable environmental conditions (Moraity 1998). Opportunistic pathogens such as *Vibrio* spp. invade the host through the gut and invade fish through the gills and skin (Weber et al., 2010). The Firmicutes phylum contains some of the most investigated probiotic candidates, such as LAB (lactic acid-producing bacteria) and *Bacillus* spp (Amoa et al., 2019, Azad et al., 2019, Balcazar et al., 2008, Venkat et al., 2004). Lactic acid bacteria can survive acidic pH and bile salts, allowing them to live in the gastrointestinal tract despite not being acclimated to the aquatic environment

(Merrifield et al., 2010b). These bacteria can colonize the intestinal mucus, whereupon they aid in the digestion and absorption of food, boosting the fish's growth and development.

Mode of administration of probiotics

Probiotics in aquaculture could be given in a variety of ways, including feed, injections, and direct exposure to water. Probiotics can be used alone or in combinations (Hai et al., 2015).

Feed additives, water additives and injection

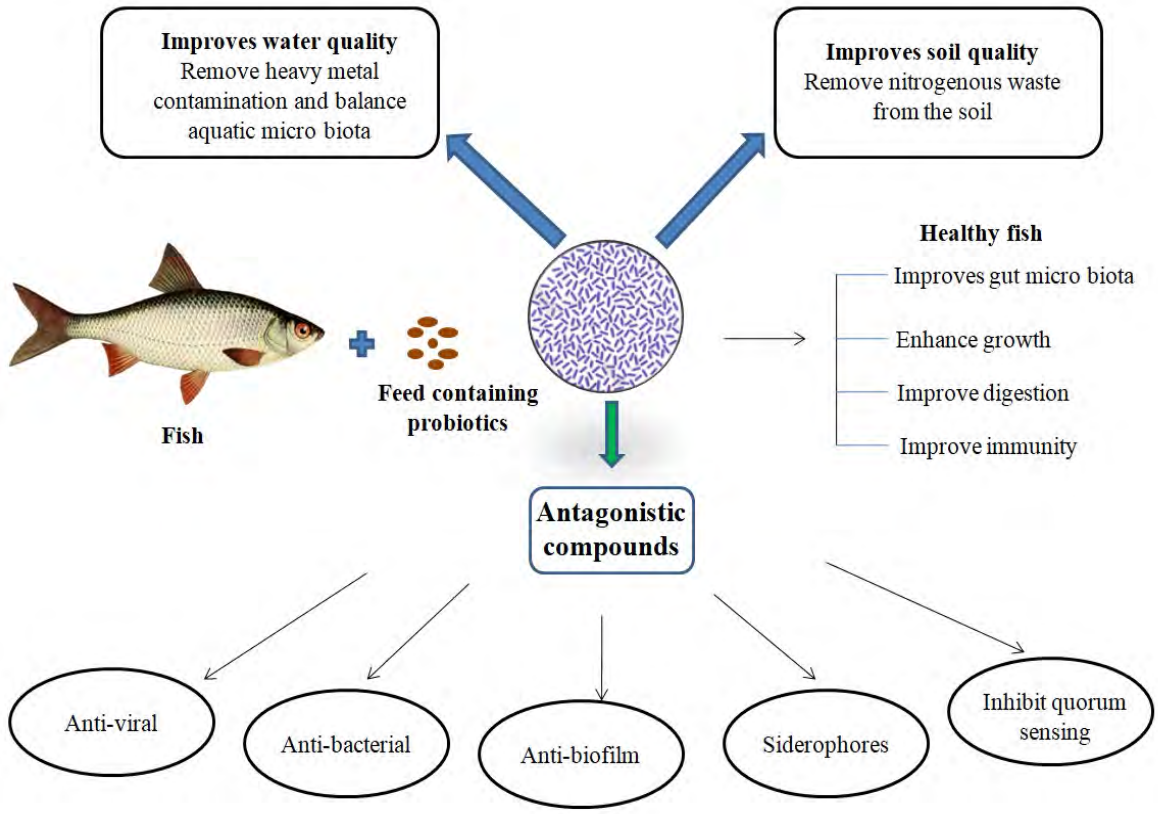
Incorporation of probiotic combinations into the feed is by far the most typical way of probiotic administration. Melo et al., 2021 reported that 92.8 % of probiotics are given as feed, followed by direct addition to water (4.8%) and in live food (1.8%) in fish culture systems. In aquaculture; probiotics such as bacterial strains, yeast, and extracted compounds are commonly used as food supplements. Dietary supplementation of probiotic strains of *Lactobacillus plantarum* has resulted in better growth and increased immunity in *Pangasius larnaudii* (Silarudee et al., 2019). Sahandi et al., 2019 reported that *Bifodobactrium* strains given as feed additive have improved the growth and nutrient utilization in rainbow trout fry. There are several reports suggesting that probiotics can also be administered through the water as an additive (Gopi et al., 2016, Gupta et al., 2016). In the sea bream, probiotic *Vibrio lentus* administered through water at a concentration of 10^6 CFU/ ml significantly altered gene expression, including immune response, cell proliferation adhesion, Reactive Oxygen Species, and iron transfer (Schaek et al., 2017). In addition to the above methods, probiotics can also be given as injection. Injection of *Enterobacter* spp. through intramuscular route enhanced the immunity in rainbow trout (Laptra et al., 2014).

Single and combinations of probiotics

Probiotics come in a variety of forms, including multi-strain probiotics, probiotics with bioactive compounds, and probiotics with fermented products. The majority of research on probiotics in aquaculture has concentrated on single probiotics, while probiotic combinations are more effective**.** Multi-strain probiotics have the benefit of being more sensitive to pathogenic organisms and active against a variety of hosts (Pannu et al., 2014). Multi-strain probiotic has a positive effect on the growth and survival of *Labeo rohita* fingerlings (Jha et al., 2014).

Beneficial effects and mode of action of probiotics in aquaculture: Figure 1

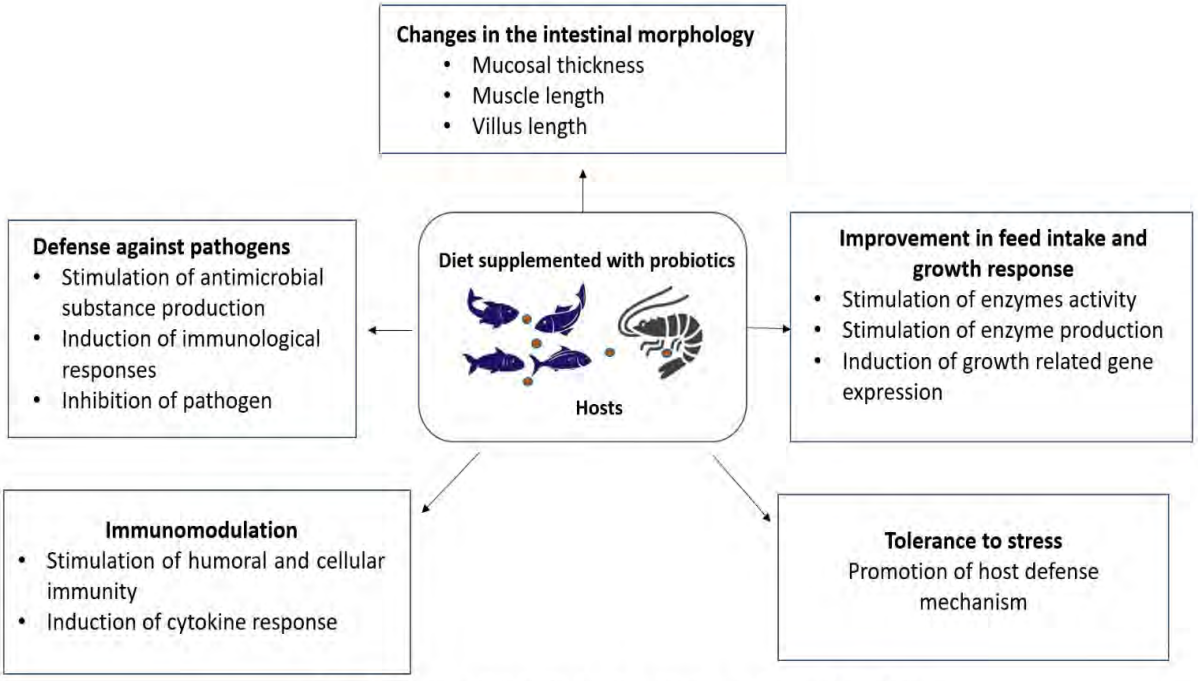
The threat of disease development inside the aquaculture sector stimulates probiotic research and analysis to build more sustainable aquaculture. With the increasing public awareness of the use of antibiotics, it’s not surprising that the probiotics for aquaculture are growing at a quick pace. Probiotics have now been recommended by the Food and Agricultural Organization (FAO) for improving aquatic environmental quality by reducing mortality (Subasinghe, 2005). *Bacillus*, *Lactobacillus*, and *Bifidobacterium* are by far the most often used probiotic bacteria. Different species of *Lactobacillus, Bifidobacterium* and *Streptococcus* are used as probiotics in aquaculture which include *L acidophilus, L. casei, L fermentum, L. plantarum, L. salivarius, B. bifidum, B.lactum, B. breve, S. boulardii, S. thermophiles* and *S. cremonis* (Reda et al.,2018).



Application of probiotics in Aquaculture: Figure 1

In the Indian aquaculture industry, intensive and semi-intensive farming practices have emerged as one of the most practical and viable choices for meeting the nutritional needs of a rapidly growing population. Furthermore, the use of new techniques, such as the administration of probiotics, has increased total production and quality (Bandyopadhyay et al., 2015). *Bacillus* spp., *Lactobacillus* spp., *Bifidobacterium* spp., *Enterococcus* spp., *Streptomyces* spp., *Carnobacterium* spp., and yeast are the most often employed probiotic bacteria in aquaculture today (Van Doan et al., 2020).

According to studies, Gram +ve bacteria (*Bacillus* species) are used as probiotics to improve water quality. Gram-positive bacteria, particularly *Bacillus* species, were shown to be highly efficient at converting organic materials to CO_2, slime, or microbial biomass. Gram-positive appears to be superior to Gram-negative in investigations. Producers can also manage the development of gaseous and particulate organic carbon during the growth period by ensuring a high standard of probiotics inside the production pond, according to the researchers (Mohapatra et al.,2013). Nitrifying probiotic bacteria are advantageous because they can substantially increase the microbial content in the water and improve the water quality by removing ammonia and nitrate toxicity (Zorriehzahra et al. 2016). Temperatures, acidity, dissolved oxygen, ammonia, and hydrogen sulphide in rearing water were also determined to be of higher quality after the administration of probiotics. Probiotics provide a favorable and healthy environment in aquatic systems for prawn and shrimp larval rearing (Banerjee et al. 2010).



Overview of beneficial effects of probiotics on fish and shellfish

Table 1. Probiotic species used in finfish aquaculture, source and beneficial effects to the host species

Probiotic species	Host	Beneficial effects	Reference
Bacillus amyloliquefaciens COFCAU-P1	*Labeo rohita*	Disease resistance against *A.hydrophila*	Khan et al., 2022
Bacillus amyloliquefaciens Bacillus subtilis Bacillus megaterium	*Labeo rohita*	Increased survival against *A. hydrophila* infection	Saravanan et al.,2021
Saccharomyces cerevisiae	*Labeo rohita*	Growth performance, hematological parameters, improved feed utilization	Jahan et al.,2021
Lactobacillus fermentum	*Cirrhinus mrigala*	Better growth, hematological parameters, improved feed utilization	Krishnaveni et al., 2021
Bacillus methyllotrophicus Bacillus licheniformes	*Labeo rohita*	Increased survival against *A. hydrophila* infection	Mukherjee et al., 2019
Bacillus amyloliquefaciens	*Labeo rohita*	Increased antibody concentration, stress reduction	Nandi et al.,2018
Bacillus subtilis Lactobacillus rhamnosus	*Labeo rohita*	Enhanced feed digestibility	Munirasu et al., 2017
Bacillus subtilis Terribacillus saccharophillus	*Labeo rohita*	Increased growth and immunity	Kalarani et al.,2016

Saccharomyces cerevisiae	*Labeo rohita*	Increased growth and immunity	Bandopadhyay et al., 2015
Bacillus subtilis FPTB13	*Catla catla*	Immunomodulation and disease resistance	Sangama et al., 2015
Bacillus subtilis *Pseudomonas aeruginosa* *Lactobacillus plantarum*	*Labeo rohita*	Highest survival rate against *A. hydrophila* infection	Giri et al., 2014
Bacillus subtilis *Lactobacillus lactis* *Saccharomyces cerevisiae*	*Labeo rohita*	Increased survival against *A. hydrophila* infection	Mohapatra et al., 2014
Bacillus cereus	*Penaeus monodon*	Growth promoter	Navinchandran et al., 2014
Lactobacillus plantarum VSG3	*Labeo rohita*	Improved growth, immunity and disease resistance	Giri et al.,2013
Bacillus amyloliquefaciens	*Catla catla*	Improved growth, immunity and disease resistance	Das et al., 2013
Lactobacillus rhamnosus	*Oncorhynchus mykiss*	Improved Blood parameters	Panigrahi et al., 2010
Bacillus NL 110 *Vibrio NE 17*	*Macrobrachium rosenbergii*	Increased growth and immunity	Mujeeb et al.,2010

Conclusion

Even though there are substantial research on the efficacy and actions of probiotic strains, many aspects remain unanswered. Additional and future research could focus on gut bacteria transcriptome and proteome profiling, host/microbe interactions, interactions among gut microorganisms, gut immune status, antioxidant status, antagonistic activity, and knowledge on the side effects of probiotics. Aquaculture is indeed one of the world's fastest-growing industries, accounting for more than 50% of world seafood production. Aquaculture offers a vital supply of nutritious food for human consumption; however, diseases in the fish farming have a negative impact on the nation's socioeconomic status and economic development. Because antimicrobial agents used in therapeutic strategies have side effects including residual toxic effects, emerging antimicrobial resistance, immune system suppression, and reduced customer desire for drug-treated fishery products available in the market, non-antibiotic-based, eco-friendly alternatives are in high demand for aquatic animal health management.

Probiotics are an excellent alternative sustainable option of beneficial microorganisms with strong antimicrobial activity, and immunostimulatory abilities to boost health and wellbeing to enhance growth and yield, strengthen the immune function, and mitigate the adverse effects of reactive oxygen species. In order to recommend potent therapeutic, bacteria-based approaches to enhance the health, production, and economic growth of the aquaculture sector, an interactive approach among academics, researchers, growers, and fish sector owners is needed to concentrate and start exploring the specific elements of bacteria host interactions bestowing the potential significant improvements in various immune function triggered by

different bacterial species. The synthesis of probiotics ought to be feasible on a broad scale with low operating costs. They ought not to be regarded as just a 'magic elixir,' but instead as a source of nourishment.

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Alternative to antibiotics in aquaculture practices

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Introduction

Antibiotic resistance has grown and spread as a result of the extensive and regular use of antibiotics in aquaculture. Antibiotics are being used by fish farmers to combat sickness in aquatic animals with the same antibiotics that humans use to treat illnesses, for therapeutic purposes. Afterwards, it was discovered that they could promote growth *i.e.*, they were shown to be capable of supporting growth. According to previous scientific data, antibiotic usage in food-producing animals can cause intestinal bacteria to become resistant to the drugs, which can subsequently be spread to the general population and result in diseases that are difficult to treat. These antibiotic applications can also lead to the development of antibiotic resistance in non-pathogenic bacteria, whose resistance genes can then be passed on to pathogenic bacteria, resulting in human illnesses that are resistant to antibiotic treatment. So, the application of antibiotics can be reduced by various alternative materials.

Probiotics

Probiotics are living microorganisms that are supposed to benefit the host's health by introducing beneficial bacteria into the stomach. Probiotic species studied in aquaculture include *Lactobacillus*, *Bacillus*, *Enterococcus*, *Carnobacterium*, *Saccharomyces*, and *Candida*. There are essentially two types of probiotics *i.e.* Water probiotics can grow in a water medium and exclude harmful bacteria by absorbing all available resources. Gut probiotics can be mixed with feed and administered orally to enhance the beneficial microbial flora of the gut, thus, malnutrition causes harmful bacteria to die (Sahu et al., 2008). Probiotics have been proven to protect rainbow trout from bacterial illnesses caused by *Vibrio, Aeromonas, Yersinia*, and *Ichthyophthirius*. In addition, *Lactobacillus* improved fish health, survival, and growth performance in African catfish. *Bacillus* bacteria were demonstrated to boost the survival of pond-raised catfish. These organisms are either directly put into the fish's aquatic habitat or delivered orally as feed. Probiotics are crucial in the degradation of organic waste, which considerably lowers the production of sludge and slime. By lowering the occurrences of diseases (including *Vibrio sp.*, *Aeromonas sp*., and viruses), the water quality will consequently improve the zooplankton populations, minimizing odours, and eventually increasing aquaculture output.

Prebiotics

A prebiotic is a substance considered to benefit the host by encouraging the development or activity of naturally occurring bacteria in the gastrointestinal system *E.g.*, fructooligosaccharides, galactooligosaccharides, and mannan-oligosaccharides, dextrins, inulin, lignin, waxes, beta-glucans. Many different types of prebiotics have been fed to various fish species, with diverse results E.g. Inulin has recently been found to modify the intestinal microbial populations of turbot, Arctic char, Atlantic salmon, and hybrid striped bass. Prebiotics such as glucans has been proven to protect channel catfish from enteric septicemia when injected but not when fed. Furthermore, a prebiotic made up of brewer's yeast, dairy

components, and dried fermentation products were discovered to greatly improve feed efficiency and minimize mortality in hybrid striped bass challenged with bacterial infections.

Synbiotics

Synbiotic is a combination of prebiotics with probiotics. Synbiotics may function by encouraging the growth of helpful bacteria in the host's gastrointestinal system. There are no studies in aquatic animals that have investigated the efficacy of these products, although synbiotics can manipulate gut microbiota and improve growth and disease resistance.

Bacteriophage

Bacteriophages are viruses that can infect, proliferate, and kill vulnerable bacteria. They are both pervasive and plentiful in the environment, particularly in saltwater, where the total number of viruses frequently surpasses the bacterial concentration by a factor of ten. Phages have been examined for their medicinal characteristics and capacity to control pathogenic germs since their discovery in 1915; however, these studies were eventually abandoned due to the emergence of cheap, broad-spectrum antibiotics. Following the rise of bacterial drug resistance, phage treatment has recently resurfaced as a viable alternative to the usage of antibiotics.

Bacteriocins

Bacteriocins are substances having an essential biological protein moiety that possesses a bactericidal mode of action against other bacteria. It is one of the immunity mechanisms of bacteria that protects from other bacteria *i.e.*, bacteriocins may serve as anti-competitor compounds to protect the own microbial community. The advantages of bacteriocin are nontoxic and non-antigenic to animals including humans; moreover, it is easily degraded by proteolytic enzymes of the gastrointestinal tract; hence, it can be incorporated into the feed. The role of bacteriocins in microbial communities hasn't been well-established yet. Since the use of prophylactic antibiotics is detrimental to aquatic and terrestrial environments, the application of bacteriocinogenic bacterial strains appears to be an excellent candidate for a friendly alternative.

Essential Oils

Compounds formed during plant secondary metabolism are found in essential oils, they are intricate combinations of low-molecular-weight compounds with a wide range of chemical characteristics. Some EOs can reduce oxidative stress when added to therapeutic baths (at doses lower than those that cause drowsiness). For instance, the essential oil of Melaleuca alternifolia might stop the effects of disease-induced splenic pyruvate kinase and creatine kinase inhibition. It is understood that several EOs control GABA, the primary inhibitory neurotransmitter in the CNS, to produce their anesthetic effects.

Organic Acids

The application of the organic acid would lower the pH (around 3.5) of the environment, which provides a favorable environment for the proliferation of beneficial bacteria. E.g. *Lactobacillus* can proliferate in acidic conditions. Most of the pathogenic bacteria such as *V. parahaemolyticus* and *V. cholera* would die in acidic condition. Various kinds of researches were carried out in the laboratory and found that the organic acids are highly efficient to control all pathogenic vibrio. The mechanism of action is mostly based on the lower pH. Since *Vibrio sp.* prefer to grow in alkaline condition, *Vibrio sp.* are highly susceptible to the short chain organic acid.

Antimicrobial Peptides

Antimicrobial peptides (AMP) are called host defence peptides and are responsible for the innate defined mechanism produced by the host cell to destroy the invading bacteria/viruses. It is well developed in fish and shellfish. Antimicrobial components are made up of short chain of amino acids *i.e.*, between 12 to 15 amino acids. Since these molecules are short-chain, they generally are thermostable. Recently, these antimicrobial peptides are considered as a novel substance as an alternative to antibiotics owing to their ability to kill the target organism with a broad range of bactericidal activity, e.g., Pleurocidin from winter flounder, cathelicidins from rainbow trout, defensins from zebrafish, piscidins from hybrid striped bass, dicentracin from sea bass, hepcidin from channel catfish and epinician from the groupers.

Plant compounds with antimicrobial activity

Numerous research has been carried out regarding the application of plant extract for the treatment of infection in humans as well as preservative materials for shelf-life extension of food materials. Most plant extracts are having excellent antimicrobial activity, but limited research has been carried out on the application of plant materials to aquaculture practices. Well-characterized plant materials can be tried in aquaculture practices. But the materials should be available regularly, capable of showing significant improvement for disease control, and should not have any negative effect on the fish/ shrimps as well as consumers.

Nanoparticles

Nanoparticles possess potential antibacterial activity; especially metal nanoparticles are highly active against a wide variety of microorganisms on multi-drug-resistant bacteria. The application of silver nanoparticles is able to control the MDR in aquaculture, but the application is highly restricted. Hence, a suitable substance needs to be investigated to reduce the toxicity of the nanoparticles for application in aquaculture.

Seaweed Extracts

Extract of seaweed *viz.*, *Ascophyllum nodosum* causes a better immune system to combat most threatening diseases. India is having a wide range of marine resources with various unexplored seaweed materials. So, it is a potential area to identify suitable seaweed material for aquaculture practices. FAO also suggested that research can be taken to explore the benefit of aquaculture species.

Competitive Exclusion (Nurmi Effect)

Competitive Exclusion is otherwise called as Nurmi Effect. In 1973, Nurmi and Rantala introduced a new technique in poultry to get rid of resistant and pathogenic bacteria. They collected intestinal gut microbes/faecal materials from the healthy birds and fed them to newly hatched chicks to establish a healthy bacterial population in the intestine. This technique was similar to the probiotics, but it will be applied to the newly hatched chicks. The Nurmi effect was tried in the Tilapia aquaculture form and found a greater effect on the pathogens.

Anti-Virulence Therapy

Anti-virulence therapy is either interfering with the control of virulence factor expression or particularly suppressing a particular virulence. Many Gram-negative bacteria produce i.e., N-acyl homoserine lactones (AHLs) as signal molecules, which favours biofilm formation. But, in the case of Vibrios species, various chemicals i.e., multichannel quorum

sensing mechanisms responsible for the biofilm formation. The substance exhibits quorum quenching properties and may be used as a replacement for antibiotics.

Vaccination

Although vaccination is the best way to avoid infectious illnesses, it is not a cure for already existing infections, and there are still relatively few commercially accessible vaccinations for the aquaculture industry. Autogenous immunization has advantages for animal welfare, transboundary biosecurity, local farmer and industry economics, and public health, which favour its use in aquaculture as a locally enabled response to the widespread issue of antimicrobial resistance. To produce 1,375,307 tonnes of fish in 2019, the Norwegian salmon industry utilized 222 kg of antimicrobials (160 mg antimicrobial per tonne). The development of vaccination against the main bacterial illnesses allowed for the shift from treating to preventing disease in farmed fish. Aquaculture also employs auto-vaccines, which have shown success against atypical *Aeromonas*, new *Yersinia ruckeri* biotypes, infections in salmonids, Streptococcal diseases in barramundi and stingrays, and others. Autogenous vaccinations against the intracellular pathogen Francisella noatuensis have been demonstrated to be efficacious in Tilapia. The variety of sizes of the sectors is reflected in Australia's usage of licensed and autogenous vaccines in the finfish aquaculture industry.

Immunologically-Active Compounds

A variety of immunologically active compounds are available in the market such as cytokines, freeze-dried eggs, spray-dried plasma, and antibodies. Research needs to be carried out on the effect of this product on aquaculture species. Most of the immunostimulants are obtained from either bacteria or red and brown algal groups. Since fish and shellfishes are devoid of acquired immunity, immunologically active components are needed in the aquaculture species to withstand the disease outbreak; which is indirectly useful to reduce the usage of antibiotics in the aquaculture system.

Hygienic Procedure

The use of antibiotics can be greatly reduced if proper health management practices are adopted. So, good aquaculture practices are a highly efficient way to produce an antibiotic-free environment. Office International des Epizooties (OIE) has given clear guidelines for good aquaculture practices.

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 organisms 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. Vaibrios 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 vibrios. Cholera causing vibrios can produce the cholera-toxin 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. In aquaculture, 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, 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 common inhabitants 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.

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 41.5 ^{o}C for fresh products, or 37 ^{o}C for frozen or salted products) is followed by a second enrichment in ASPW at 41.5 ^{o}C (for *V. cholerae* and *V. parahaemolyticus*) or 37 ^{o}C (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 37 ^{o}C 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 cholera* as 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 dehydrated 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 ingestion 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 are 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, out of which, 40 % are from *Vibrio parahaemolyticus* alone.

The *Vibrio parahaemolyticus* is a deadly pathogen for shrimp, which causes early mortality syndrome. It causes hepatopancreatic 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 these bacteria and it mostly follows 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 world-wide from temperature ranging from 9-35 °C 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 is necessary.

Vibrio vulnificus is known to cause Gastroenteritis, primary sepsis, and wound infection. Rare cases of spontaneous bacterial peritonitis, Pneumonia, Endometritis, Meningitis, Septic arthritis, Osteomyelitis, Endophthalmitis and 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 reasons behind shrimp aquaculture collapsed in South-Asian countries. It develops quickly, starting approximately 8 days post stocking and severe mortality (up to 100 %) occurs 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
- The handlers should wear gloves while handling diseased fishes
- Fish source should be disease free.
- Farm should have bio-security measures
- The affected farm water should be treated with bleaching powder before release
- Water quality parameters should be optimum
- Maintenance of personal hygiene including washing hands before and after handling fish.

Water quality parameters in aquatic animal health

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Aquatic Environment

The aquatic environment is classified into micro environment and macro environment. The microenvironment of fish is described as the environment directly surrounding it, the primary enclosures such as the tank, raceway, or pond. It contains many factors, including water quality, illumination, noise, humidity and temperature. The physical environment of the secondary enclosure, such as a room, constitutes the macro environment.

Water Quality

Water quality plays a vital role in the well being of experimental animals. It varies for different fish and also will differ with fish variety, age, weight and animal's use. The system's effectiveness and efficacy depend on its capacity to adapt to the experimental environment to the organisms' evolutionary biology. The important water quality parameters include pH, alkalinity, nitrogenous waste products, phosphorus, residual chlorine, redox potential, salinity, hardness, DO, total atmospheric pressure, minerals and the microorganisms present in the environment. Regular monitoring of environmental parameters is necessary for proper health management. Aquatic animal health lab analysts should be aware of measuring and managing different water quality parameters that affects fish health.

Dissolved oxygen

The recommended dissolved oxygen (DO) content of pond waters is in the range of 5 ppm saturation level. Aeration of pond water will increase DO availability. The use of paddle wheel aerators or air diffusers will help to improve the DO content of the pond water.

Temperature

Temperature sets the pace for metabolism and biochemical reaction rates. Operation of aerator helps in breaking thermal stratification while planting of trees gives shades.

Turbidity

Several factors like suspended soil particles, planktonic organisms and organic matter contributes to turbidity. Optimum turbidity visibility ranges from 40-60 cm. Turbidity can be measured by using Sechii Disc. Turbidity can be maintained by application of organic manure at 500-1000 kg/ha, gypsum @ 250-500 kg/ha or alum @25-50 kg/ha.

Ammonia

Fish are very sensitive to unionized ammonia (NH_3) and optimum range is 0.001-0.01 ppm in the pond water. The same is reduced in the case of high DO and high CO_2. Aeration, healthy phytoplankton population removes ammonia from water. Addition of salt @ 1200-1800 kg/ha reduces toxicity. Formalin is also used in certain cases. Biological filter may be used to treat water for converting ammonia to nitrate and then to harmless nitrate through nitrification process.

Hydrogen sulphide

Culture pond should be free from H_2S because at concentration of 0.01 ppm fish lose their equilibrium. Frequent exchange and increase of pH through liming can reduces its toxicity.

pH

Water pH affects fish metabolism, physiological process, toxicity of ammonia, hydrogen sulphides and solubility of nutrient thereby well-being and fertility. pH at the range of 7-9 is best for fish growth and can be increased by application of lime. Agriculture gypsum may be applied to correct alkaline pH.

Total Alkalinity

Ideal range from 60-200 ppm as $CaCO_3$ and it can be treated with lime. Lower levels lead to fluctuation and more than 200 ppm may become unproductive due to limitation of carbon dioxide availability.

Total hardness

It should be greater than 40 ppm because it helps to protect fish against harmful effect of pH and metal ions. Lime application can increase hardness.

Carbon dioxide

Pond water should contain low concentration of free CO_2.

Temperature, Humidity, and Ventilation

Fishes are poikilotherms, which depend, for the most part, on the temperature of their environment to maintain normal physiological activities. The temperature of the water may be controlled from the source by using proper biological filters and other treatment units. The relative humidity can be influenced by the amount of the water present in the room. The stability of the macro ambient temperature can also be affected by the thermal load generated by heating systems. Centralized air facilities have to be configured to help make up for these temperature and moisture difference.

Illumination

Fish are prone to environmental stress. Rapid changes in light intensity may cause stress and result in trauma. Hence, proper illumination is mandatory to facilitate adequate physiological function.

Noise and Vibration

Fish are subjected to sound and vibration, which are readily transmitted through water. They can be minimized by using insulation pads under aquarium tanks. Life supporting facilities such as biological filters, pumps can be placed away from the animal room to reduce sound and vibration.

Animal tank

The appropriate animal enclosure should,

- Facilitate normal physiological functions of the research animal.
- Support the fish spatial requirements.
- Provide a suitable environment for health monitoring
- Enable access to feed and removing nitrogenous products.
- Prevent injury or unintentional capture of fish or their body parts.
- Not cause injury to animals.
- Enable handling of fish with minimum stress.

- Be constructed of non-toxic materials
- Not possess any electrical issues.

Aquatic Environment Management

Behavioral management

External evaluations are typically used for monitoring the health of the experimental animals. Fish should be handled in a way to keep minimum stress. Fish handling types of equipment should be thoroughly disinfected before use and it should be restricted to use in a particular experimental setup to avoid cross-contamination.

Food

Food should be preserved adequately to prevent the nutritional loss, avoiding contamination, and preventing infestation of pests. Live food should be supplied in a healthy and disease-free condition. Experimental animals should be fed with a balanced diet to avoid nutritional deficiency diseases.

Sanitation

The cleanliness in the experimental area can be achieved through a properly built and well constructed supporting system, periodic waste removal, and regular water exchange.

De-contamination

It is usually accomplished through the treatment of water using biological filters, ozone, and ultraviolet light. The use of chlorine as a disinfectant in the aquatic system may be inappropriate because residual chlorine is toxic to fish. Hence, complete withdrawal of chorine is ensured if it is used as a disinfectant in the aquatic environment. The entire experimental area including fish tanks, supporting areas, storage facilities, washing rooms should be periodically disinfected with approved disinfectants. Care should be taken to avoid secondary contamination. Cleaning material should be made of corrosion-resistant materials.

Wastes disposal

Wastes including biomedical waste should be disposed off, according to the institute's biosafety management committee recommendations.

Emergency, Weekend, and Holiday Care

Experimental animals need regular care and maintenance from lab assistants, hence adequate emergency preparedness plans should be created to resolve major technical glitches.

Experimental animal record keeping

Proper recordkeeping is necessary for experiment system management. Details that may be regularly recorded include length, weight, age, sex, feeding, tank number, signs and symptoms of the disease, feeding regime, mortality, etc. Also, detailed recording of water quality testing is important for maintaining optimum water quality.

Duties and responsibilities of aquatic animal health lab assistants

The duties of aquatic animal health lab assistant typically include,

- Cleaning and disinfecting fish tanks
- Regular water exchange
- Monitoring fish behavior
- Feeding the fish with artificial or live feed
- Recording each fish length, weight and feeding behavior
- Maintaining records

- Collection and analysis of data
- Sterilization of equipments
- Taking inventory of supplies
- Report writing
- Submitting sample for analysis
- Also assisting researchers in the handling of aquatic animals.

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 hospitalization, 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 7.2º 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 α-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 β-toxin, ε-toxin, or ι-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, ear gum, 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 pathogens 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 be maintained 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 facultatively 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 °C to 50 °C. A recent study found that *E. coli* O157 strains possess inherent genetic mechanisms which enable growth at low temperatures (< 15 °C), 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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Pre-requisite programmes

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Introduction

HACCP IS NOT A STAND-ALONE SYSTEM. Effective HACCP system is built on a solid foundation of prerequisite programs. These are very much essential to the successful application and effective implementation of HACCP system. They provide basic environment and operating conditions that are necessary for the production of safe, wholesome food. Before applying HACCP system in an organization, it is necessary to ensure that the pre-requisite programmes are developed established and maintained effectively so as to provide a firm support to the HACCP system. Thus, a carefully developed and properly implemented pre-requisite programme can make HACCP implementation very simple. Thus, the following points are to be considered essentially for a successful HACCP plan:

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

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

STANDARD SANITATION OPERATING PROCEDURES

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

EIGHT KEY AREAS OF S S O P

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

8. Health of food handlers

1. Safety of process water

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

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

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

All food contact surfaces such as plant equipment and utensils, including equipments used for ice production and storage should be made of non-toxic materials. They should be so designed as to facilitate easy cleaning. They should be able to withstand the action of food, ingredients, and chemicals, cleaning compounds and the environmental conditions (like extremes of temperature, humidity, salinity etc.) under which they operate. Each factory should have a regular cleaning schedule to clean and disinfect the food contact surfaces. The Sanitation Supervisor should ensure that the contact surfaces are cleaned well and that there are no chances for contamination from these contact surfaces. The efficiency of cleaning should be verified once in 3 months by drawing swab samples from the contact surfaces. Gloves and outer garments that can come into contact with food should be made of water-proof material and should always be kept clean.

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

Employee's hands, gloves, outer garments, utensils and food contact surfaces of equipment that come into contact with unclean objects (like waste, and other insanitary objects) should not come into contact with food before they are cleaned and sanitized. Care should be taken to ensure that employee's hands, gloves, outer garments utensils and food contact surfaces of equipment that come into contact with raw products should not come in contact with cooked products. There should be physical separation for cooked, ready-to- eat products and raw food during refrigerated storage.

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

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

proper records to this effect are maintained. Adequate toilet facilities, maintained in sanitary conditions, should be provided.

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

Necessary control should be taken to protect food, food contact surfaces and food packaging materials from adulteration with fuel, lubricants, pesticides, cleaning compounds, sanitizing agents, metal fragments or other chemical or physical contaminants. Care should be taken to protect food and food contact surfaces from contaminants that may drip, drain or drawn into the food. Whenever compressed gases are used (such as in Modified Atmospheric Packaging) they should be filtered or treated to ensure that these gases do not contaminate the food with unapproved food additives or other physical, chemical or microbiological contaminants.

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

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

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

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

There should be physical separation of dry and wet chemicals.

7. **Control of employee health**

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

8. **Pest management**

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

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

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

Schedule for Monitoring and Documentation

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

CURRENT GOOD MANUFACTURING PRACTICES (cGMP)

These are measures of general hygiene as well as measures that prevent food from being adulterated due to unhygienic handling under insanitary conditions. Common cGMP activities include the following:

1. **Environmental hygiene**

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

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

Food processing factories should be selected in a locality where:

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

3. **Building exterior**

Premises should be devoid of vegetation, which can provide shelter to pests. The area immediate to the building should be either tarred or concreted to avoid windblown dusts. All

debris and garbage should be properly cleaned. Proper drainage is to be ensured. No branches of trees shall touch the building.

4. **Building interior**

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

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

5. **Ground level water tanks**

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

6. **Equipment**

All equipments should be designed and constructed so as to ensure proper cleaning and disinfection. Equipments and containers should be made of non-toxic materials. Only food grade plastic and food-grade steel are to be sued for food contact surfaces. Equipments used for cooking, cooling and freezing of food should attain the desired temperature as rapidly as possible. Such equipments should have temperature control and monitoring facilities. Containers used for collecting, holding and storing of waste products and inedible or dangerous substances should be made of impervious materials and should be specifically identifiable Containers for holding dangerous substances should be kept in locked room under strict vigil to prevent accidental contamination.

7. **Drainage and waste disposal**

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

8. **Personal hygiene facilities**

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

9. **Ventilation**

Adequate means of natural or mechanical ventilation should be provided. Air intakes should preferably be on the roof or at least six feet above the ground, the incoming air should

not take in dust, noxious odours or exhaust air from the plant. Ventilation system should be designed and constructed in such a way that air never flows from unclean areas to clean areas.

10. Lighting

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

11. Traffic flow pattern

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

12. Storage

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

13. Training

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

14. Calibration

A Schedule for calibration of equipments should be established. All CCP monitoring equipments like thermometer, pH meter, moisture meter, electronic clock, hygrometer etc. should be calibrated at regular frequencies usually once in a year. All weights, pressure gauges and temperature gauges of food processing equipment should also be calibrated and necessary documents generated.

15. Transport vehicle cleaning

All vehicles used for transporting raw materials, finished products, packaging material and water and ice (whenever sourced from outside) should be cleaned and sanitized prior to use. For perishable food articles like fishes, meat etc. use refrigerated trucks or reefer containers. When the same conveyance is used for transporting different food or non-food articles, proper cleaning should be done between loads and the cleaning should be documented.

16. Personnel hygiene and cleanliness

All employees who directly come in contact with food, food-contract surfaces and food packaging material should adhere to strict hygiene practices when on duty so as to prevent contamination. These hygiene practices mainly include the following:

a) Employees should wear proper outer garments suitable to the operation to prevent contamination of food, food-contract surfaces or food packaging materials
b) Utmost importance should be given to personnel cleanliness. Habits like biting nails, chewing, Scratching body parts etc should be discouraged.

c) Employees should be instructed to wash their hands thoroughly using sanitizers in a hand washing facility before commencement of work, after each absence from the workstation or whenever the hands become soiled or contaminated.
d) All food handlers should be directed to remove all unsecured jewellery and other objects that can fall into the food, equipment or container. They should also be instructed to remove jewellery like rings, bangles, hair pins, toe rings or anklets.
e) When gloves are used, they should be maintained clean and in sanitary condition. The gloves should be of an impermeable material and shall be replaced by fresh ones at the interval of 2 hrs.
f) Appropriate clothing like hairnet, cap and beard covers should be used to avoid contamination with hair.
g) Employee should not be allowed to eat, drink, smoke or chew gum in production areas

From the above, it is clear that the success of HACCP system depends greatly on the effective implementation of pre-requisite programmes like SSOP and CGMP. The HACCP Team. Therefore, should give due importance to these pre-requisite programmes while implementing the HACCP system.

Standard Operating Procedures (SOPs)

Approach: Standard operational procedures (SOPs) are written documents of the processor on the operating procedures to be followed in the unit. The processor should check what are the raw material to be used and the quality specification for the raw material.

Raw Material Sampling: In the case of non-branded items, quality is to be checked on each arrival. It is better to depend upon branded products and, in such cases, samples are drawn once in three months and tested for quality as per laid down specifications.

Responsibility: The Hygiene Officer/Sanitation Officer will be responsible to this and the records will be maintained.

Approved Vendors/Suppliers: Each Company shall effect purchase only through approved vendors/suppliers. All new suppliers are evaluated for their capability to supply products as per specification. The assessment of suppliers is done with on-site evaluation of their facilities, verification of track records and evaluation of samples. Records of assessment and a list of approved suppliers are maintained. Production Manager is usually responsible to approve the suppliers as well as to remove them from approved status in cases of poor performance. All suppliers are to be re-evaluated for their performance once in an year.

Visiting Premises of Vendors: SOP should specify whether any quality/safety guarantee is to be obtained from the Vendor. It should also spell out the company's schedule to inspect the premises of the Vendors. In cases, where the results of inspection indicate chances for hazards from these premises, the Vendor's name is to be removed from the approved list.

Receipt of Raw Material: Raw material shall preferably be received in air-conditioned receiving areas provided with air curtains and self-closing doors. All items are to be bought from well-reputed suppliers who maintain high standard of food, hygiene and requirement specification. Supplier's premises should also be inspected to know about the packaging and storage conditions. They have to be informed about standard and quality specifications of the product including the delivery temperature.

All materials received in are to be checked weighed and kept away from floor preferably on stainless steel platforms. The food shall be inspected for its freshness;

temperature, colour, odour, contamination infestation, satisfactory packing, expiry date and labelling. The external packing material such as cartons, gunny bags etc. are to be removed before the food item is taken to the store.

The temperature at which raw material is to be received is to be specified. Mode of storage and precautions to be taken are to be spelled out. SOP should explain in detail the various process step involved in the productionof food product including the time temperature conditions at various stages. The names of any preservatives, additives, chelating materials, antioxidants and colouring materials added have to be declared.

SOP should specify the end product quality specifications of the products produced and should specify the quality of tests to be performed, the testing frequency and the parameters to be tested is better to depend upon competent accredited laboratories, the unit may have to insist the source form where the packaging materials are to be purchased and the quality specifications. The unit may have to insist food grade certificate in case, the material comes direct contact with the packaging material. The mode, type and durationof cleaning and disinfection of process machinery, contact surfaces, water tank etc. may have to be specified in the SOP.SOPs should be written in a concise, step-by-step easy to read and easy to understand format. The information presented should be unambiguous and not complicated.

Prerequisite programs deal with the "Good housekeeping" concerns of the establishment, whereas, HACCP manages specific process hazards. Prerequisite programmes are outside the HACCP plan, but still within the HACCP system.

HACCP, Standards and Regulations

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Introduction

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

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

HACCP is a system that identifies, evaluates, and controls hazards that are significant for food safety. As described by Codex Alimentarius Commission, General Principles of Food Hygiene (CXC-1 1969) and its HACCP Annex in 2020, HACCP can be implemented by 12 logical steps that include five preliminary steps and seven principles.

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

Step 1: **Assemble an HACCP team**

HACCP team is a group of people who are assembled and given the responsibility of implementing HACCP system. People are chosen based upon their knowledge and experience in various aspects of food safety such as biological and chemical hazards, specific food production process, regulatory requirements, logistics and management. A member of the HACCP team serves as the leader and ensures that all requirements for institutionalizing HACCP system are addressed.

Example: For implementing HACCP in fish processing the HACCP team should comprise of persons having educational qualifications in fisheries science/fish processing/aquaculture, microbiology, biochemistry, engineering and management.

Step 2: **Describe the product**

Important characteristics of the product must be described in order to identify and evaluate hazards in subsequent steps. The product characteristics should include product nomenclature, composition, physical and chemical characteristics (a_w, pH, preservatives, allergenic potential), packaging, shelf life, storage conditions (temperature, humidity), labeling instructions for handling, storage and instructions prior to use by the consumer, distribution control, actual use and sale target.

Example: "Ready to Eat Fish Curry"

Product Description	
1. Product name(s):	Ready to Eat Rohu Curry
2. Important product characteristics	Retort Pouched
3. How it is to be used:	Ready to Eat
4. Packaging:	3 ply laminated (Polyester/Al/cast PP)
5. Shelf life:	3 years
6. Where it will be sold:	India, Nepal, Bangladesh, Sri Lanka
7. Labeling instructions: (i.e additives used)	Approved colour (E 160c), flavor enhancer (E621)
8. Special distribution control:	To be transported in shock-proof cartons

Step 3: **Identify the intended use of the product**

The normal or common use of the product must be identified. This step is especially designed taking into care vulnerability of target consumer against biological and chemical hazards. As YOPI (young, old, pregnant and immuno-compromised) populations are at higher risk, prior knowledge on the target consumer helps in stringent design of HACCP plan. Further, this step must take into account actual use of the product i.e. household use, institutional use or industrial use for further processing.

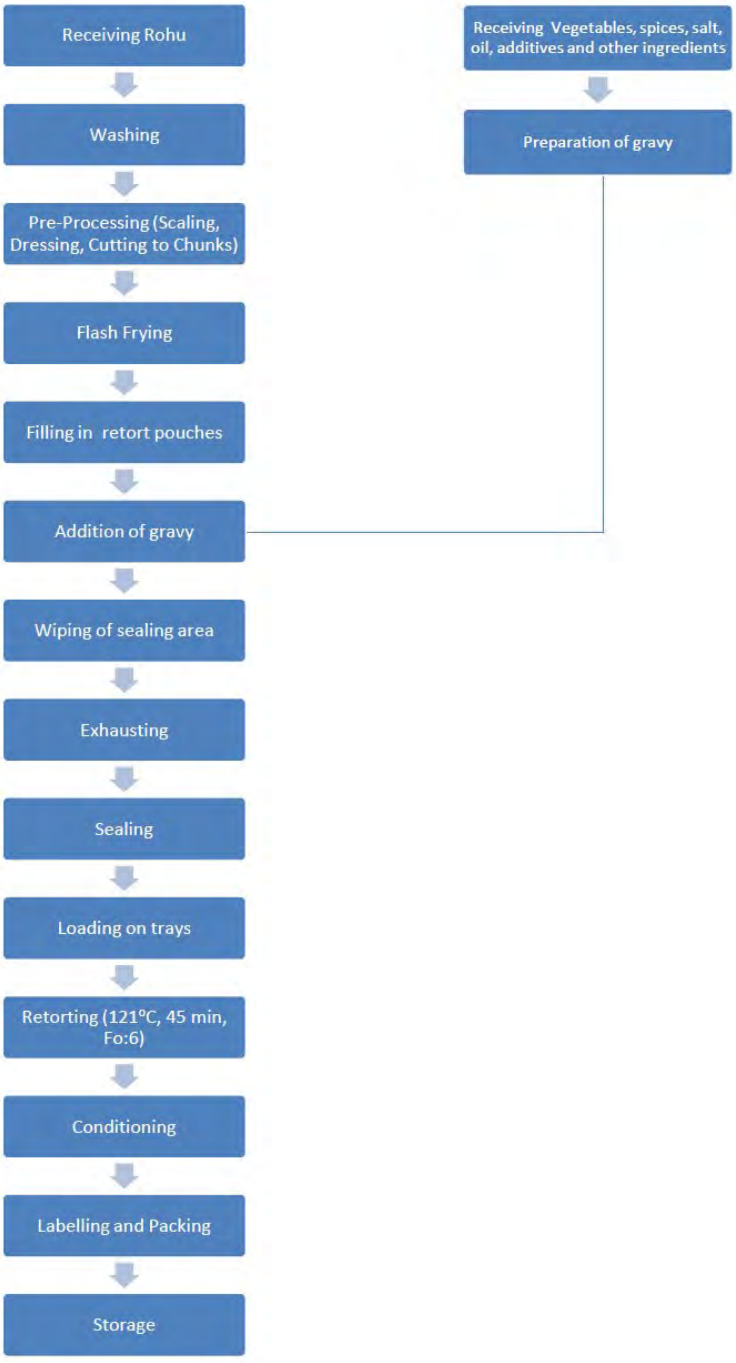
Example: Ready to Eat Fish curry is to be consumed by general public for house-hold consumption

Step 4: **Construct process flow diagram for the product**

The logical step-by-step process in the manufacture of the product should be represented with a flow diagram. All the processing steps starting from receiving till shipment should be included either in a single flow diagram or with modular diagrams when production is carried out in different sections.

Step 5: **Verification of process flow diagram**

The flow diagram constructed should be verified on-site by actually observing each step of the process starting from receiving of raw material to shipment of finished product. Based on this the process flow should be modified or amended. Successful development of HACCP plan depends on accuracy of flow diagram.



Step 6: **List all potential hazards that are likely to occur and associated with each step, conduct a hazard analysis to identify the significant hazards, and consider any measures to control identified hazards**

Three categories of hazards are identified viz. physical, chemical and biological by an elaborate two-step process *hazard identification* and *hazard evaluation*.

Hazard Identification:

Hazard is defined as *a biological, chemical, or physical agent in, or condition of food with the potential to cause an adverse health effect* (Codex Alimentarius, 1997). As per NACMCF (1997), hazard is also defined as *a biological, chemical, or physical agent that is reasonably likely to cause illness or injury in the absence of its control*. At this step all potential hazards coming from ingredients, raw materials, packaging materials and environment used for the preparation of the product are identified based upon the following information:

- Nature of the product and the product characteristics (pH, composition, a_w, and additives)
- Safety record of the product
- Normal microbial characteristics of the product and changes during storage and handling
- Raw materials, ingredients, and packaging materials used
- Activities, operations, equipment and personnel involved at each of the steps listed in the process
- Environmental conditions at the time the product is produced and stored
- intended use of the product

Hazard Evaluation:

Only significant hazards are identified based upon severity and likelihood of occurrence. Severity should be assessed based on the consequences of exposure to the hazard, whereas, the likelihood of occurrence is based on the epidemiological records, scientific evidence and susceptibility of the target consumers.

Identification of control measures:

The control measure for each significant hazard is identified and HACCP team determines in which step of the process flow such measure can be implemented. If there is no step where the control measure for the identified hazard can be exercised then process is modified or alternate hazard control measure is suggested.

Hazard	Control Measure
Salmonella	Thermal processing (Heating, cooking, pasteurization, retorting)
Pesticides	Testing for presence of residues; source control
Metal pieces	Detection and removal of metal pieces by using strong magnet and online metal detector

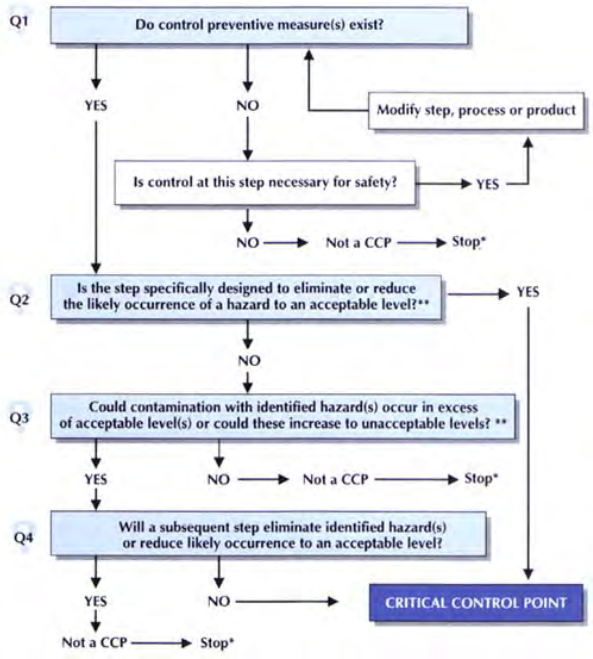
Step 7: **Determination of Critical Control Point (CCPs)**

The HACCP team determines the step at which there will be control of the hazards that present unacceptable risks. This step is called as critical control point. Various definitions of CCP are as follows:

- *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* (Codex Alimentarius, 1997; NACMCF, 1997)
- *The processing factors whose loss of control would result in an unacceptable food safety risk* (ASQ, 1998)

CCPs are determined by using a decision tree (a sequence of questions to assist in determining whether a control point is a CCP or not) developed by Codex Alimentarius or NACMCF.

Example: Retorting is a CCP for bacterial pathogens and receiving as a CCP for pesticide residues



Step 8: **Establish validated critical limits for each CCP**

The HACCP team must establish critical limits for accepting or rejecting a raw material, ingredient or a semi-finished or finished product that is obtained at a process step designated as CCP. Critical limit is defined as *a criterion that separates acceptability from unacceptability* (Codex Alimentarius, 1997). It is also defined as *a maximum or minimum value to which a biological, chemical, or physical parameter must be controlled at a CCP to prevent, eliminate, or reduce the occurrence of a food safety hazard to an acceptable level* (NACMCF, 1997). The

identified control measures can be validated as per Codex Alimentarius Commission CAC/GL-69-2008 and based upon those critical limits are determined.

Example: Validated time and temperature cooking regime; cooking at 72^{o}C core temperature for 1 minute for 6D reduction of *Listeria monocytogenes*

Step 9: **Establish monitoring procedures for each CCP**

At each CCP the HACCP team establishes monitoring procedures to determine whether the prior specified critical limits are respected or not. Monitoring reveals loss of control at CCP so that appropriate action can be taken. Visual inspection and chemical testing methods are generally employed. Microbiological tests are time consuming and rarely used. Monitoring (continuous or periodic) must be reliable and calibrated equipment should only be used. All monitoring records must be maintained.

CCP	Hazard	Monitoring Procedure
Retorting	Bacterial Pathogens	Monitoring time and temperature
Receiving raw material	Pesticides	Examination of certificate of analysis Examination of supplier guarantee/declaration
Metal detection	Metal pieces	Monitoring of product by metal detector

Step 10. **Establish corrective action procedures for each CCP**

The HACCP team must establish procedures to be followed if and when any deviation is observed during monitoring of a CCP. A product that is obtained at a process step where the CCPs are not respected is a nonconforming product and is unsafe for consumption. Corrective action procedures are established to prevent unsafe product from reaching the consumer. Corrective action procedures must include the following points:

- Identification of the cause of deviation
- Action to be taken to prevent recurrence of the deviation
- Time of occurrence of deviation
- Quantum of non-conforming products generated
- Action to be taken to prevent distribution of the product
- Rectification of the anomaly

Step 11: **Validation of the HACCP Plan and Verification Procedures**

HACCP team must establish verification procedures for each identified CCP and as well as for entire HACCP plan. The term verification is defined in a number of ways; some of them are as follows:

- *Confirmation, through the provision of objective evidence, that specified requirements have been fulfilled* (ISO 9000:2000).
- *The application of methods, procedures, tests, and other evaluations, in addition to monitoring to determine compliance with the HACCP plan* (Codex Alimentarius, 1997).
- *Those activities, other than monitoring, that determine the validity of the HACCP plan and that the system is operating according to the plan* (NACMCF, 1997).
- *The act of determining whether products and services conform to specific requirements* (QP, 2002).

CCP	Hazard	Verification Procedure
Retorting	Bacterial Pathogens	Review of retorting records, microbiological testing of end-product Review of Calibration records of temperature and pressure sensors of retort
Receiving raw material	Pesticides	Review of certificate of analysis, review of pesticide usage record; periodic sampling of water and fish for pesticide analysis; Pesticide level in end product

Initial HACCP plan (prior to implementation) is verified by validating the critical limits, monitoring procedure and corrective action procedure. Ongoing HACCP programmmes are verified by periodic review of monitoring and corrective action records, periodic sampling and analysis, product testing, system audit and periodic independent review of entire HACCP plan.

CCP	Hazard	Records
Retorting	Bacterial Pathogens	Retorting monitoring records Calibration records of temperature and pressure sensors of retort

Receiving raw material	Pesticides	Monitoring records of receipt of certificate of analysis; Farm visit and sampling records; pesticide usage record;

Step 12: **Establish record-keeping and documentation procedures**

HACCP team must identify the records that need to be maintained as per the monitoring, corrective action and verification procedures. The following documents related to HACCP plan must be maintained by the organization:

- Composition of HACCP team
- Description of food product and intended use
- Verified process flow diagram
- Summary of hazard evaluation and list of significant hazards
- Summary of CCP determination and justification
- HACCP Plan form indicating CCP, corresponding hazards, critical limit, monitoring procedure (what, how, frequency and personnel responsible), corrective action procedure, verification procedure and records maintained
- CCP validation records
- Records of monitoring, corrective action and verification

Standards and Regulations for Fish and Fish Products

National Standards

In India, standards related to fish and fish products are formulated by Bureau of Indian Standards (BIS) and Food Safety and Standards Authority of India (FSSAI). The standards of BIS are voluntary in nature and mostly used for certification purposed, whereas the standards by FSSAI are regulatory and mandatory for domestic trade and imported fish and fish products.

The BIS standards for fish and fish products are as follows:

1	IS 2168 : 1971 Reaffirmed In : 2016–Specification for pomfret canned in oil (First Revision)
2	IS 2236 : 1968 Reaffirmed In : 2016–Specification for prawns/shrimp canned in brine (First Revision)
3	IS 2237 : 1997 Reaffirmed In : 2018–Prawns (Shrimps) - Frozen - Specification (Third Revision)
4	IS 3336 : 1965 Reaffirmed In : 2016–Specification for shark liver oil for veterinary use
5	IS 3892 : 1975 Reaffirmed In : 2016–Specification for frozen lobster tails (First Revision)
6	IS 4303 (Part 1) : 1975 Reaffirmed In : 2018–Code of hygienic conditions for fish industry: Part 1 pre-processing stage (First Revision)
7	IS 4303 (Part 2) : 1975 Reaffirmed In : 2018–Code of hygienic conditions for fish industry: Part 2 canning stage (First Revision)
8	IS 4304 : 1976 Reaffirmed In : 2016–Specification for tuna canned in oil (First Revision)
9	IS 4780 : 1978 Reaffirmed In : 2016–Specification for pomfret, fresh (first revision)

10	IS 4793 : 1997 Reaffirmed In : 2016–Whole pomfret - Frozen - Specification (Second Revision)
11	IS 5734 : 1970 Reaffirmed In : 2016–Specification for sardine oil
12	IS 6122 : 1997 Reaffirmed In : 2018–Seer fish (Scomberomorus Sp.) - Frozen - Specification (First Revision)
13	IS 6123 : 1971 Reaffirmed In : 2016–Seer fish (Scomberomorus Spp.), fresh
14	IS 7143 : 1973 Reaffirmed In : 2016–Specification for crab meat canned in brine
15	IS 7313 : 1974 Reaffirmed In : 2016–Glossary of important fish species of india
16	IS 7582 : 1975 Reaffirmed In : 2016–Specification for crab meat, solid packed
17	IS 8076 : 2000 Reaffirmed In : 2016–Frozen cuttle fish and squid - Specification (First Revision)
18	IS 9808 : 1981 Reaffirmed In : 2016–Specification for fish protein concentrate
19	IS 10059 : 1981 Reaffirmed In : 2016–Specification for edible fish powder
20	IS 10449 : 1983 Reaffirmed In : 2016–Code for transport of live fish seeds for inland pisciculture purposes
21	IS 10450 : 1983 Reaffirmed In : 2016–Code for transport of fresh water aquarium fish
22	IS 10760 : 1983 Reaffirmed In : 2016–Specification for mussels canned in oil
23	IS 10762 : 1983 Reaffirmed In : 2016–Specification for mussels canned in oil
24	IS 10763 : 1983 Reaffirmed In : 2016–Specification for frozen minced fish meat
25	IS 11427 : 2001 Reaffirmed In : 2018–Fish and fishery products - Sampling (First Revision)
26	IS 14514 : 1998 Reaffirmed In : 2018–Clammeat - Frozen - Specification
27	IS 14515 : 1998 Reaffirmed In : 2018–Fish pickles - Specification
28	IS 14516 : 1998 Reaffirmed In : 2018–Cured fish andfishery products - Processing and storage - Code of practice
29	IS 14520 : 2018–Fish industry - Operational cleanliness and layout of market - Guidelines (First Revision)
30	IS 14890 : 2001 Reaffirmed In : 2018–Sardines - Fresh, frozen and canned - Specification
31	IS 14891 : 2001 Reaffirmed In : 2018–Mackerel - Fresh, frozen and canned - Specification
32	IS 14892 : 2000 Reaffirmed In : 2018–Threadfin - Fresh and frozen - Specification
33	IS 14949 : 2001 Reaffirmed In : 2018–Accelerated freeze dried prawns (Shrimps) - Specification
34	IS 14950 : 2001 Reaffirmed In : 2018–Fish - Dried and Dry - Salted - Specification
35	IS 16150 (Part 1) : 2014 Reaffirmed In : 2019–Fish feed - Specification: Part 1 carp feed
36	IS 16150 (Part 2) : 2014 Reaffirmed In : 2019–Fish feed - Specification: Part 2 catfish feed
37	IS 16150 (Part 3) : 2014 Reaffirmed In : 2019–Fish feed - Specification: Part 3 marine shrimp feed
38	IS 16150 (Part 4) : 2014 Reaffirmed In : 2019–Fish feed - Specification: Part 4 freshwater prawn (*Macrobrachium rosenbergii*) feed
39	IS 16292 : 2014/ISO 12877 : 2011Reaffirmed In : 2019–Traceability of finfish products - Specification on the information to be recorded in farmed finfish distribution chains

40	IS 16293 : 2014/ISO 12875: 2011 Reaffirmed In : 2019–Traceability of finfish products - Specification on the information to be recorded in captured finfish distribution chains
41	IS 17186 : 2019/ISO 16741 : 2015–Traceability of crustacean products - Specifications on the information to be recorded in farmed crustacean distribution chains
42	IS 17187 : 2019/ISO 18537 : 2015–Traceability of crustacean products - Specifications on the information to be recorded in captured crustacean distribution chains
43	IS 17188 : 2019/ISO 18538 : 2015–Traceability of molluscan products - Specifications on the information to be recorded in farmed molluscan distribution chains
44	IS 17189 : 2019/ISO 18539:2015–Traceability of Crustacean Products -Specifications on the Information to be Recorded in Captured Crustacean Distribution Chains
45	IS 17281 : 2019–Requirements for Good Aquaculture Practices-India GAqP Shrimp Hatchery and Grow Out Farms
46	IS 17282 : 2019–Requirements for Good Aquaculture Practices-India GAqP Striped Catfish (*Pangasianodon hypophthalmus*)
47	IS 17283 : 2019–Requirements for Good Aquaculture Practices -India GAqP CARPS
48	IS 17284 : 2019–Requirements for Good Aquaculture Practices-India GAqP for Freshwater Prawn Culture
49	IS 17285 : 2019–Good Aquaculture Practices for Cage Culture in Fresh Water

The regulatory standards of FSSAI related to fish and fish products are given in the following regulations:

1. Food Safety and Standards (Licensing and Registration of Food Businesses) Regulation, 2011
2. Food Safety and Standards (Food Products Standards and Food Additives) Regulation, 2011
3. Food Safety and Standards (Prohibition and Restriction of Sales) Regulation, 2011
4. Food Safety and Standards (Contaminants, Toxins and Residues) Regulation, 2011
5. Food Safety and Standards (Laboratory and Sampling Analysis) Regulation, 2011
6. Food Safety and Standards (Import) Regulation, 2017
7. Food Safety and Standards (Approval for Non-Specific Food and Food Ingredients) Regulation, 2017
8. Food Safety and Standards (Advertising and Claims) Regulation, 2018
9. Food Safety and Standards (Packaging) Regulation, 2018
10. Food Safety and Standards (Labelling and Display) Regulations, 2020

International Standards

The major international standards related to fish and fish products are formulated by the following agencies:

- Codex Alimentarius Commission
  - Codex Standards, Code of Practices and Guidelines
- European Union
  - Hygiene Regulations; Microbiological criteria; Official control; Maximum levels for certain contaminants in foodstuffs; Pharmacologically active substances; Food Additives

- USA
  - United States Department of Agriculture (USDA)
  - USFWS
  - NOAA (National Oceanic and Atmospheric Administration)
  - United States Food and Drug Administration (USFDA)
    - Seafood HACCP Regulation (21 CFR 123)
    - Food Safety and Modernization Act (FSMA)
- China
  - National Food Safety System Standards
    - Food Safety Law (2015)
    - Law on Farm Product Quality and Safety (2006)
- Japan
  - Ministry of Health, Labour and Welfare
  - Food Sanitation Act
  - Food Safety Basic Act
  - Agricultural Chemicals Regulation Law
- Australia & New Zealand
  - Australia New Zealand Food Standards Code
- Canada
  - Health Canada
- South Africa
  - The Department of Agriculture, Forestry and Fisheries (DAFF)
  - The National Department of Health
  - The Department of Trade and Industry
- Russia and Customs Union
  - Hygienic requirements for safety and nutrition value of food products. Sanitary and epidemiological rules and regulations, sanpin 2.3.2.1078-01

Analysis of pesticide residue in fish

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Introduction

Pesticides are chemical compounds that are used to kill pests, including insects, rodents, fungi, and unwanted plants (weeds). Over 1000 different pesticides are used around the world. Pesticides are used in public health to kill vectors of disease, such as mosquitoes, and in agriculture to kill pests that damage crops.

Pesticide can be defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest such as insect, rodent, nematode, fungus, weed, other forms of terrestrial or aquatic plant or animal life or viruses, bacteria, or other microorganisms on or in living man or other animals, which declares to be a pest, and any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant.

Pesticides can be classified in many ways on the basis of use, toxicity, mode of entry, mode of action, chemistry, and formulations. The major chemical types of pesticides include

(i) **Organochlorine pesticides (OC)** – This group consists of the polychlorinated derivatives of cyclohexane (Lindane), polychlorinated biphenyls (DDT, dicofol), and polychlorinated cyclodiene (Endosulfan).

Properties of OCs

Physical property: OCs are solids that possess low volatility, low solubility in water, high solubility in oils, fats, lipids, *etc*., and they are not prone to environmental degradation.

Chemical property: Organochlorine pesticides shows isomerism

Toxicity: These compounds possess high acute toxicity as well as chronic toxicity

Biological stability: OCs are not rapidly degraded by enzymes, not rapidly exerted, but get stored in the fatty tissues.

A number of organochlorine pesticides have been banned globally and they are controlled via the Stockholm convention on persistent organic pollutants (POP's). These include: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex, and toxaphene.

(ii) **Carbamates**: Carbamates are esters of either carbamic acids or thiocarbamic acids. Carbamates may be further subdivided into three sub-groups.

Group	**Example**
Aryl N methyl carbamate	Carbaryl, Propoxur
Hetero cyclic mono or dimethyl Carbamates	carbofuran
carbamoylated oximes	methomyl
Thiocarbamates	cartap hydrochloride (neriestoxin group of insecticide)

Properties:

Physical property: The organo-carbamates are available as nonvolatile solids. Carbaryl, and carbofuran are having very low water solubility (40-6000 ppm) whereas Cartap hydrochloride is hygroscopic in nature. And these compounds undergo degradation by environmental factors.

Chemical property: These compounds are unstable in an alkaline medium.

Toxicity: The organo-carbamate compounds exhibit moderate to extreme toxicity, and they do not display chronic toxicity

Biological stability: The organo-carbamate compounds undergo enzymatic degradation and are rapidly metabolized and excreted. Biomagnification is almost absent in this group of pesticides and chronic toxicity is insignificant.

(iii) **Organophosphates (OP):** These are the esters of derivatized phosphoric acid, thiophosphoric acid and dithio phosphoric acids, which are called phosphates, thiophosphates and dithiophosphates respectively. Some of the examples of each class of pesticides are as follows:

Group	**Example**
Phosphates	monocrotophos,phosphamidon, 2,2-dichlorovinyl dimethyl phosphate (DDVP) or Dichlorvos
Thiophosphates	methyl parathion, fenitrothion, Phosphorothiates oxy demeton methyl
Dithiophosphates	phosporodithioates dimethoate, pphosphorothioates

Based on the organic moiety attached to the phosphoric acid these can also be classified into aliphatic, phenyl, and heterocyclic derivatives.

Properties

Physical property: These compounds are available as liquids or semi-solids and possess significant vapour pressure and are comparatively volatile in nature. Some of these compounds are slightly soluble in water (example: Phosphamidon).

Chemical property: These compounds which are esters of phosphoric acid are not stable in alkaline pH, but stable over a narrow range of pH. Thiophosphates and dithiophosphates undergo molecular rearrangements, forms isomers with increased toxicity and undergo oxidation to give oxo compounds with increased toxicity. The organo phosphorous pesticides undergo the conversion of one pesticide into another pesticide. The following are some examples.

Trichlorfon → dichlorvos

Formothion → dimethoate

Acephate → methamidophos

Toxicity: These compounds exhibit acute extreme toxicity to slight toxicity. LD50 values may change with the purity of the compound. These compounds are having low chronic toxicity. They undergo rapid conversion into low fat-soluble metabolites which are excreted.

Biological stability: The OP compounds undergo enzymatic degradation and the metabolites are fat-soluble and easily get excreted. Biomagnification is almost absent and chronic toxicity is insignificant.

(iv) **Pyrethroids** – Living organisms do contain naturally a large number of chemicals some of which give them protection from foreign invasive substances. Many such chemicals have been isolated, identified, and evaluated for their biological activity. The flowers of chrysanthemum contain compounds called pyrethrins which are found to have possessed very good pesticidal activity but are found to be less stable in the environment. The pyrethrins are chemically the esters of chrysanthemic acid and pyrethric acid (which contains dimethyl cyclopropane group) with alcohols, namely pyrethrolone, cinerolone and jasmolone.

Synthetic Pyrethroids: Allethrin was the first synthetic pyrethroid developed in 1949, followed by resemethrin. However, they have failed to contain the desired properties and proved to be highly photolabile. The first photo sable pyrethroid developed was permethrin. This was followed by cypermethrin, deltamethrin, and fenvalerate. The synthetic Pyrethroids contain a halogenated derivative of dimethyl cyclopropane carboxylic acid and cyano phenoxy benzyl alcohol. Fenvalerate is an exception with the acid portion being p-chlorophenyl isopropyl acetic acid instead of cyclopropane carboxylic acid. In the case of permethrin, the alcohol portion does not have cyano - group, but it is simply phenoxy benzyl alcohol.

Water bodies around the world are threatened by various anthropogenic activities, resulting in poor water quality. The pesticide contamination in fish mainly comes through agricultural runoff and municipal sewage effluent. Later, intensive aquaculture practices with insecticides such as trichlorfon and dichlorvos to kill unwanted organisms or as algaecides to control water quality in fish/shrimp farms of different regions of the world also lead to pesticide contamination in fish. Several studies have reported the presence of organochlorine pesticides in fish harvested from Indian waters also.

Procedure for Pesticide residue analysis in fish by GC-MS/MS

- Take a 2 kg fish sample, and homogenize the muscle tissue. Weigh 5 g representative sample in 50 ml centrifuge tube
- *Extraction*: Add 5 ml distilled water and Vortex for 1 minute. Then add 10 mL of acetonitrile containing 100 μL of acetic acid and vortexed for 1 minute. Add 6 g of magnesium sulphate ($MgSO_4$) and 1.5 g of sodium acetate (CH_3COONa) and vortex for 2 minutes. Centrifuge the content at 4000 rpm for 5 minutes. Keep the supernatant at -20°C for 20 minutes n to avoid loss of thermo-labile analytes due to heat generated during dSPE clean-up
- *Clean up*: Transfer the extract to dispersive-SPE (dSPE) tubes containing 50 mg PSA sorbent + 150 mg MgSO4 per mL as per AOAC- QuEchERS 2007.01. Vortex the mixture for 1 minute. Centrifuge at 4000 rpm for 5 minutes
- Filter 2ml extract through 0.2µ PTFE membrane filter; Inject and analyse in GC-MS

References:

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Analysis of heavy metals in fish

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Introduction

Heavy metals are toxic metals and above a normal level can affect the quality, safety and marketability of seafood. They have atomic weight higher than 40.04 and specific density > 5g/ cm. Heavy metal contamination in fish and other aquatic organisms are highly depending upon geographic location, species and fish size, feeding pattern, solubility of chemical and their persistence in the environment. The major toxic heavy metals causing significant importance in seafood safety are Arsenic, Cadmium, Mercury and Lead.

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. 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 has elicited the most concern among consumers, affecting the nervous system. Arsenic is a widely distributed metalloid and major contaminant in case of ground water. IARC has classified inorganic arsenic as a human carcinogen.

Being denizen of aquatic ecosystem, fish and other aquatic species (molluscs, crustaceans, etc.) carry the natural burden of heavy metal concentration. Heavy metals in fish and other aquatic organisms come from both natural and anthropogenic sources. Presence of toxic heavy metals such as lead, cadmium, mercury, arsenic, nickel and chromium are of significant importance in seafood safety. Due to coastal pollution, in some areas of Indian coast the enrichment factor for metals is very high (>100). In aquatic environment cadmium is also extensively distributed and bioaccumulation of cadmium by aquatic organisms is a well-recognized fact. The cephalopods (Squid, Cuttlefish and Octopus) naturally bio-accumulate cadmium to toxic levels.

Similarly, predatory finfishes like Tuna, Marlin, Swordfish, Barracuda, which contribute significantly to India's fish production are associated with high mercury levels. Mercury is present in fish primarily in its organic form as methyl mercury and accumulates with age. Methylmercury accumulates rapidly, but depurates very slowly. Because of this reason most mercury in fish muscle is present as methylmercury.

Although more than 90 % of the mercury in fish is found as methylmercury, contents of methylmercury can vary considerably between species. Predatory species that are at the top of the food chain and having long life span accumulate higher levels of methylmercury. Methyl mercury is known to cross blood-brain barrier and placenta and causing irreversible prenatal and post-natal damage in the ingested population. Tuna and swordfish are found to be the main source of high MeHg exposure, followed by cod, haddock and octopus.

Although Codex prescribes limit for methyl mercury, many country regulations are based on total mercury content. Estimation of methyl mercury requires use of cost-prohibitive

hyphenated equipment's like HPLC-ICP-MS or IC-ICP-MS. Similarly, high Arsenic content is reported in seafood, but major chemical forms are organic (arsenobetaine and arsenosugars), which are non-toxic.

Determination of heavy metals in seafood

Principle

Plasma is a stream of highly ionized gas containing an equal number of electrons and positive ions. Plasma is electrically conductive. It is affected by a magnetic field. When plasma energy is given to an analysis sample from outside, the component elements (atoms) is excited. When the excited atoms return to low energy position, emission rays (spectrum rays) are released and the emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays and the content of each element is determined based on the ray's intensity.

To generate plasma, first argon gas is supplied to torch coil, and high frequency electric current is supplied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas is ionized and plasma is generated. This plasma has high electron density and temperature (10000K) and this energy is used in the excitation-emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the centre of torch tube. The steps leading to the emission are desolvation, vaporization, atomization and ionization.

Sample digestion

Sample should be homogenous, representative of bulk, free of suspended particles and free flowing. Samples are digested in a microwave digestion unit. Take 0.25 to 0.5 g of sample to pre-cleaned digestion vessel. Add 8 ml nitric acid and slowly add 2 ml H_2O_2 to it. Keep it for 10 minutes. Close the vessel and keep in microwave digestion chamber for digestion. After digestion the samples are made up to 100 ml. digested sample is introduced to ICP-OES for analysis.

Hydride generation kit is used for analysing elements like Hg, As, Bi and Se.

Sample analysis – Inductively Coupled Plasma (ICP) Spectrometer ICAP 6300 Duo view

The detector is solid state CID detector, which can simultaneously analyse a sample for multiple elements. ICAP 6300 has a high-performance optical system. The design has been optimized to offer resolution over the entire spectrum from 166 nm to 847 nm enabling access to all wavelengths and minimizing spectral interference.

Analysis of antibiotic residues in fish

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Introduction

Aquaculture is one of the worldwide strategic development fields and its importance is evident in its significant worldwide growth in the last decades. It exhibits a faster growth than any other animal production sector. It plays a significant goal in any country's economic development plan because of its worldwide growth. The rapid transition from a capture species model to a culture and production model was a necessary response to the market needs. Aquaculture has the possibility of producing larger quantities of products in reduced space than the wild capture of species. The fast growth of these productions has resulted in concerns over fish quality and safety. Similar to other sectors of animal production, fish production adopts intensive and semi-intensive practices. These practices lead to a higher concentration of animals in small spaces and substantially increase the risk of disease. This growth is associated with the implementation of intensive and semi-intensive production methods, with the use of antibiotics in order to prevent the emergence and spread of infectious diseases in fish.

Fluoroquinolones, tetracyclines, and sulphonamides among others, are widely used for this purpose. This practice constitutes a real public health concern, not only due to the presence of antimicrobial residues in edible tissues, which can cause allergic reactions in hypersensitive individuals, but also due to the emergence of bacterial resistance. Food safety, as well as its consequences on human health, has become an extremely important topic for consumers and for public health authorities. In particular, there have been numerous events involving large-scale contamination of foods of animal origin. As of now, there are no antibiotics specifically designed for aquaculture; therefore, authorized products developed for other areas of veterinary medicine are used.

Two major concerns arise from these practices related to their effect on consumers' health: a) The presence of antimicrobial residues in edible tissues of treated animals. In persistent low doses, they become part of the consumers' diet and b) The emergence of antimicrobial resistance, which represents a huge threat to public health worldwide according to health professionals, governments, WHO and other non-governmental international agencies. The inappropriate, and frequently abusive, use of antibiotics affects human health. It is also evident that the public health hazards related to antimicrobial use in aquaculture include the development and spread of antimicrobial resistant bacteria and resistance genes. 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, and in aquatic environments. These genes can be disseminated by horizontal gene transfer to other bacteria and ultimately reach human pathogens.

Antimicrobials are chemical substances that either destroy (bactericidal) or inhibit the growth of microorganisms (bacteriostatic). Although the term “antibiotic” refers to the group of these substances that are produced by microorganisms. 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. For each group of antibiotics, the analytical determination depends on the extraction and purification step for determination using different methods.

a) Aminoglycosides: Determination of AG can be performed either directly, e.g., by spectrophotometric, immunochemical, or microbiological methods, or after liquid chromatography (LC) separation. Regarding the LC-based methods, there is an important challenge to be considered, related with the molecular structures of AG.

b) Amphenicols: The most representative amphenicol is chloramphenicol (CAP). Gas chromatography (GC) was the analytical tool previously used to determine CAP, florfenicol, and thiamphenicol levels in fish and shrimp samples. Currently, LC-MS/MS without derivatization is the technique of choice to determine antibiotic residues. This hyphenation of liquid chromatography and mass spectrometry enables the detection and quantification, without derivatization, of polar non-volatile analytes, such as CAP.

c) Beta-lactam antibiotics: β-lactam antibiotics are antibiotic agents that contain a β-lactam ring in their molecular structure and include penicillin derivatives, cephalosporins, monobactams, carbapenems and β-lactamase inhibitor. The β-lactam family can be divided into two main groups: penicillins and cephalosporins. LC has become the analytical method of choice for the identification and quantification of these drugs. Recent advances in LC and LC-MS/MS analysis enables easy detection of penicillin residues in food products. The use of LC-MS/MS allowed for the characterization of amoxicillin's degradation products at trace levels.

d) Macrolides: Macrolides are highly potent antimicrobials used in veterinary practices against a wide variety of Gram-positive and Gram-negative bacteria. They consist of macrocyclic lactone rings with 14 (erythromycin, roxithromycin and clarithromycin), 15 (azithromycin) or 16 (spiramycin, tylosin and tilmicosin) carbons linked to the carbohydrate molecules. The molecular structure of macrolides contains chromophores, which allows them to be analysed by UV and fluorometric detection. However, the improved sensitivity and specificity of MS has replaced UV and fluorometric methods in detection and quantification of macrolides in different biological matrices.

e) Nitrofurans: Nitrofurans (furazolidone, furaltadone, nitrofurazone, nifursol, nifurpirinol and nitrofurantoin) are a group of synthetic antibacterial agents that were widely used in food-producing animals. Nifurpirinol and nitrofurazone are effective against many fish pathogens. However, they are carcinogenic and mutagenic, and it is illegal to use them in fish intended for consumption in many countries. LC-MS/MS is the current tool for the detection of nitrofuran tissue-bound side-chain metabolites. It is used throughout the world in animal tissue and other matrices.

f) Quinolones: Quinolones represent a group of synthetic antibiotics used in both human and veterinary medicine. They are used in the treatment of septicaemia or skin diseases in fish. The introduction of the fluorinated quinolones provided important therapeutic advantages because this antibiotic group has higher antibacterial activity than the parent compounds and is highly active against both Gram-positive and Gram-negative strains. HPLC is the most widely used analytical method for these compounds with UV or fluorescence detection. LC coupled with MS detection has become the preferred analytical method for quantification.

g) Sulfonamides: The sulfonamide family includes sulfadiazine, sulfamethizole, sulfamethoxazole, sulfasalazine, sulfisoxazole and various high-strength combinations of three

sulfonamides. GC-MS methods are considered to be an inappropriate option as they require a previous derivatization step, because of the high polarity and low volatility of these compounds. Several methods for SA determination, based on HPLC, have been reported but, nowadays, these methods are being replaced by MS/MS methods with the advantage of achieving more sensibility and specificity.

h) Tetracyclines: Tetracycline antibiotics (TC) are intensively used in therapy and prophylactic control of bacterial infections in human and veterinary medicine. They are also used as food additives for growth promotion in the farming industry. Their widespread use has caused antibiotic resistance among bacterial species, including resistance against TC. There are several different analytical methods that determine TC in products of animal origin including immunoassays and capillary electrophoresis. Liquid chromatography is the preferred method. Recent LC-MS/MS methods detect the epimers along with the tetracycline molecule.

i) Multi-residue and multi-class techniques: There is a trend toward the development of cost-effective methodologies that detect drug residues in food and maintain efficient screening technologies that prevent false positive and negative results. Multi-residue and multi-class techniques are important because they simultaneously detect numerous analytes of the identical family and different chemical classes in a single run. The desired efficiency is being achieved by multi-detection methods based on liquid chromatography technology coupled with tandem mass spectrometry and time of flight mass spectrometry. UHPLC also offers short running times and higher resolution and sensitivity.

New trends on the development of analytical methodologies concerning antimicrobial residues in aquaculture

The recent and recurrent episodes, involving large scale contamination of food products, especially with antimicrobial drug residues, has grown the consumer's awareness and the need to develop simpler, faster and, still, very sensitive and selective techniques for residues monitoring and control. On the other hand, the cost-effectiveness of analytical procedures is becoming a major issue for all laboratories involved in residue analysis, as the reagents and equipment are very expensive. The multiresidue and multi-class UHPLC–MS/MS methodology is the most powerful measurement tool, mainly with ToF. However, matrix effects could be observed when mass spectrometry is used. Ion suppression or increase of signal detection is frequently achieved. These phenomena need to be studied in order to know the real impact on final results. Thus, and if the final detection could be considered up-to-date to current knowledge, the different chemical structures of the different antibiotics, as well as their different physicochemical properties, implies that substantial improvements are still needed in the sample pre-treatment step. Last but not least, it is important to consider the concerns of antibiotic residues in causing adverse effects in the environment. In fact, antibiotics are “designed” to change specific biochemical pathways in target species but, when they are released into the environment they still have the potential to induce the same effects in non-target organisms or to promote other different and unknown actions, even in trace concentrations. Due to the need of monitoring natural ecosystems, it also becomes important to develop analytical methodologies that can be applied to environmental matrices, i.e., matrices not intended directly for human consumption but that can influence the presence of antibiotic residues in the food chain. The development, optimization and validation of

UHPLC–MS/MS multiresidue and multi-class antibiotic residue methods applied to multi-matrices could be a priority in a nearly future.

Public health concern of antibiotic use

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.

Biochemical indices of seafood quality & determination of adulterants in seafood

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Introduction

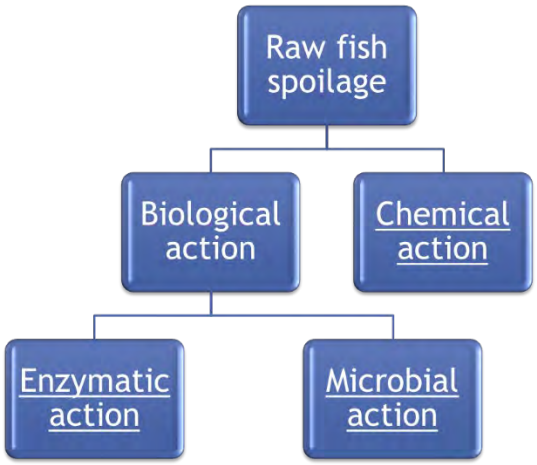
Fish and other seafood are highly important as they cover a part of protein demand for humans. The nutrient composition of fish is rich in health beneficial polyunsaturated fatty acids, vitamins and minerals. Fresh fish spoilage can be very rapid after it is caught. Freshness makes a major contribution to the quality of fish and fishery products. Nutritional values, color, texture, and edibility of foods are susceptible to spoilage. Improper pre and post-harvest handling conditions can enhance exacerbation of indigenous bacteria that could cause spoilage of fish.

Freshness is the most important attribute when assessing the quality of seafood and is of great concern. The quality of seafood degrades after death due to the chemical reactions [changes in protein and lipid fractions, the formation of biogenic amines and hypoxanthine (Hx)] and microbiological spoilage. This leads to the deterioration of sensory quality of seafood during inadequate storage. The factors contributing to spoilage of fish are

- High fat content
- High protein content
- High moisture content
- Weak muscle tissue
- Extent of bacterial contamination
- Unhygienic handling etc.

Spoilage of fish

Seafood is highly perishable food commodity and spoilage of fish involves three separate processes such as enzymatic spoilage, bacterial spoilage and chemical decomposition. "Spoilage refers to any change in the condition of food in which the food becomes less palatable, or even toxic; these changes may be accompanied by alterations in taste, smell, appearance or texture."



Spoilage of fish is also called “Putrefaction”. It refers to the contamination of fish, resulting in an undesirable change in the colour, texture, flavour, odour, appearance, etc.

Enzymatic spoilage

Shortly after capture of fish chemical and biological changes take place in dead fish due to enzymatic breakdown of major fish molecules. The changes textural quality during early stages of deterioration but did not produce the characteristic spoilage off-odors and off-flavors. The digestive enzymes cause extensive autolysis which results in meat softening, rupture of the belly wall and drain out of the blood water which contains both protein and oil. During improper storage of whole fish, proteolysis is responsible for degradation of proteins and is followed by a process of solubilization. Belly bursting is caused by leakage of proteolytic enzymes from pyloric caeca and intestine to the ventral muscle.

Table 1. Enzymes involved in spoilage of fish

Enzyme(s)	Substrate	Effect	Prevention
Glycolytic enzymes	Glycogen	Lactic acid production resulting in pH drop.	Avoid pre-rigor stress
Autolytic enzymes involved in nucleotide breakdown	ATO, ADP, AMP, IMP	Gradual production of Hypoxanthine	Avoid pre-rigor stress and improved handling.
Cathepsins	Proteins, peptides	Softening of tissue	Avoid rough handling during storage
Chymotrypsin, trypsin, carboxy-peptidases	Proteins, peptides	Belly-bursting	Problem increased with freezing/ thawing or long-term chill storage
Calpain	Myofibrillar proteins	Softening	Removal of calcium
Collagenases	Connective tissue	Softening and gaping of tissue	Time and temperature of chilled storage
Trimethylamine Oxide (TMAO) demethylase	TMAO	Formaldehyde	Storage temperature less than -30°C, physical abuse, freeze/thawing

*FAO 2005

Oxidative spoilage

Lipid oxidation is a major cause of deterioration and spoilage for the pelagic fish species such as mackerel and herring with high oil/fat content stored fat in their flesh. Fish lipids which consist of polyunsaturated fatty acids are highly susceptible to oxidation. Lipid oxidation involves a three-stage free radical mechanism: initiation, propagation and termination. Initiation involves the formation of lipid free radicals through catalysts such as heat, metal ions and irradiation. This free radical which reacts with oxygen to form peroxyl radical. During propagation, the peroxyl radicals reacting with other lipid molecules to form hydroperoxides and a new free radical. Termination occurs when a buildup of these free radicals interacts to form non-radical products. In fish, lipid oxidation can occur enzymatically or non-enzymatically. Enzymatic hydrolysis by lipases is called as lipolysis (fat deterioration) in which lipases split the glycerides forming free fatty acids resulting off flavor. Non-enzymatic oxidation is caused by hematin compounds (hemoglobin, myoglobin and cytochrome).

Microbial spoilage

Composition of the micro flora on newly caught fish depends on the microbial contents of the water in which the fish live. Fish micro flora includes bacterial species such as *Pseudomonas*, *Alcaligenes*, *Vibrio*, *Serratia* and *Micrococcus*. Microbial growth and metabolism are a major cause of fish spoilage which produce amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable off-flavors. For unpreserved fish, spoilage is a result of Gramnegative, fermentative bacteria (such as *Vibrionaceae*), whereas psychrotolerant Gram-negative bacteria (such as *Pseudomonas* spp. And *Shewanella* spp.) tend to spoil chilled fish.

Methods of Assessing Freshness Quality

Sensory methods

Sensory evaluation is the most important method in freshness assessments. Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyze, and interpret reactions to characteristics of food as perceived through the senses of sight, smell, taste, touch, and hearing. Sensory evaluation provides rapid measurements of freshness of seafood.

Freshness makes a major contribution to the overall quality of fish and fishery products and is greatly influence by both pre-harvest conditions and post-harvest handling practices. There are sensory and Non-sensory or instrumental methods are available. Non-sensory methods include chemical, physical and microbiological methods. Non-sensory assessment is based mainly on measuring major physical or chemical alterations from the original condition of the fish.

Sensory responses can be variously measured and can be assigned to sensory impression in different ways: nominal data, ordinal data, interval data and ratio data. In sensory evaluation of seafood, grading, ranking and scaling methods are the most frequently used methods. However, difference tests can be relevant to use in selected cases. Grading is a useful method of evaluation and is often used in commerce. It depends on one or two product experts. Graders usually learn the scale from other graders. The EU-scheme is an example of a grading scheme. In ranking, three or more samples are arranged in order of intensity or degree of some specific attribute. A category scaling is a method where the panellists are asked to rate the intensity of a particular stimulus by assigning a value on a limited numerical scale.

Torry scale

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

European Union Scheme

In EU scheme there are three quality levels in which E (Extra) is the highest quality; A is acceptable quality; and B is the level beyond which fish are not admitted for human consumption. The EU scheme is criticized for its limitations in that it does not consider the differences between species (uses only general parameters) and mixes both subjective and objective sensory methods in the assessment scheme.

Quality Index Method

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

Biochemical indices

Measuring the concentration of indicator compounds within the sample, which are closely related to the level of a specific sensory attribute of the fish (primarily odor or flavor). These

compounds are produced by autolytic enzymes, putrefactive microorganisms or by chemical reactions like lipid oxidation. These compounds accumulate gradually in the flesh and are found most useful quality indices and they include

- **Volatile bases** – Ammonia, Trimethylamine oxide (TMAO), trimethylamine (TMA), Dimethylamine (DMA) etc
- **Nucleotides** – Degradation products of ATP
- **Lipid oxidation** – Peroxides, hydroperoxides, aldehydes etc.

Total volatile base nitrogen (TVBN)

TVBN is a useful index of spoilage in different fresh and lightly preserved seafood. Most widely used method for assessing fish quality. TVB contains ammonia, trimethylamine (TMA) and dimethylamine (DMA). TVBN along with TMA is the most common index of spoilage of fish. In case of very fresh fish TVBN is < 20 mg %. A range of 35 – 40 mg TVB-N / 100 g of fish muscle is usually considered as limit of acceptability, beyond which the fish can be regarded as spoiled. TVB-N values identify the latter stages of spoilage as limited significant changes during the early stages of spoilage. Conway microdiffusion method and steam distillation method are commonly used method for estimation TVBN.

Trimethylamine (TMA)

TMA is a microbial metabolite and it can only be used as an index of spoilage and not as an index of freshness. Marine fish is characterized by the presence of an odourless compound called trimethylamine oxide (TMAO). TMAO appears to be part of the system used for osmoregulation. Th e TMAO content of seafood varies with species, age, fi sh size, time of year, and environmental factors [152]. Seawater fish have 1–100 mg TMAO in every 100 g muscular tissue, whereas freshwater fish generally contain only 5–20 mg %. TMA is produced by reduction of spoilage bacteria from Trimethylamine oxide (TMAO). Limit of acceptability in case of TMA is 10 – 15 mg%. Fresh fi sh has a very low amount of TMA with values less than 1.5 mg TMA/100 g TMA values increases with storage temperature. With different initial levels of TMAO, trimethylamine accumulates at different rates in different species. Trimethylamine usually does not indicate a change in quality until the fish have been stored in ice for approximately 6-10 days. This indicator is primarily suitable for evaluating samples of medium to poor freshness quality. Trimethylamine (TMA) levels are used universally to determine microbial deterioration leading to fish spoilage.

Dimethylamine (DMA)

TMAO is degraded to form dimethylamine (DMA) and formaldehyde by the enzyme TMAO demethylase in the absence of oxygen. DMA increase at a constant rate, even during the first few days of iced storage and, therefore, is a superior chemical indicator of freshness quality and is restricted to cod-like species and hakes, which contain TMAOases in their muscle tissue. There is no effect on the flavor or texture of the fish. It gives an indirect indication of formaldehyde-induced toughening of the muscle during frozen storage.

Ammonia

Ammonia is formed by the bacterial degradation/deamination of proteins, peptides and amino-acids. Significant increase in ammonia content occurs only after spoilage. Urea present in sharks & rays is degraded to ammonia by bacterial action. Thus, high level of ammonia in these species is an indication of spoilage.

Nucleotide degradation

One of the most extensively investigated methods of measuring odor and flavor aspects of the freshness quality of fish. ATP (Adenosine tri phosphate) is degraded into ADP (adenosine diphosphate), AMP (adenosine monophosphate), IMP (Inosine monophosphate), Ino (Inosine) and Hx (Hypoxanthine) during processing and storage of fresh and lightly preserved seafood. IMP is formed by autolytic enzymes. Spoilage bacteria contribute to Ino and Hx formation. Hx has a bitter taste which may be part of the off-flavor in stale fish. K value is an excellent index of freshness. In most fish, K-values increase linearly during the first days of chilled storage.

$$\text{K value (\%)} = \frac{(HxR+Hx)}{(ATP+ADP+AMP+IMP+HxR+Hx)} \times 100$$

K value is usually expressed as %. K value of Very fresh fish is 20 – 25 %. At rejection, the value will be above 50-60 %. ATP, ADP and AMP are almost completely converted to IMP within 24 hours post-mortem.

k1 value = [Ino + Hx]/ [IMP + Ino + Hx]

K value increase varies considerably between fish presumably due to differences in the species-dependent optimum pH of IMP-degrading enzymes. In general, K value increases more rapidly in cold-water fish. Increase has been observed to be five times faster in dark than in white muscles It may depend upon the location of fillet and is strongly influenced by the physiological effects of fish harvest and death struggle.

Hypoxanthine content

Hypoxanthine content is used for evaluating fish quality and the value increases with spoilage. In case of spoiled fish, the value will be > 2.5 micromoles/g.

Histamine

Many seafood spoilage bacteria produce one or more of the biogenic amines agmatine, cadaverine, histamine, putrescine, spermidine, spermine, and tyramine. Production of biogenic amines in seafood depends on concentrations of the free amino acid substrates and is, therefore, strongly species dependent. In fishes like mackerel, tuna, bonito, herring sardine etc histamine formation is an indication of spoilage. Dark fleshed fish will have high histidine content and will be converted to histamine by bacteria namely *Morganella morganii*, *Klebsiella pneumonia*, *Hafnia alvei*. Histamine is heat stable biogenic amine and cadaverine & putrescine act as potentiators of histamine formation. 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.

Indole

Indole is a useful freshness index of non-frozen shrimp which is produced by degradation of tryptophan by microbial enzymes. High level of indole indicates decomposed shrimp and temperature abuse is not toxic at high level. As per USFDA the acceptable limit is <25 microgram/100g.

Lipid oxidation indices

Compounds derived from the oxidation of the highly unsaturated fatty acid moieties in fish lipids have been used to quantify the extent of oxidative rancidity. The major chemical indices of oxidative rancidity are peroxide value (PV) and thiobarbituric acid-reactive substances (TBA-RS).

Peroxide value

The primary oxidation products (peroxides and hydroperoxides) are estimated by peroxide value and is a good guide to quality of fat. PV is a measure of first stage of oxidative rancidity. When peroxide value is > 10 – 20 milliequivalent Oxygen/Kg it smells and tastes rancid.

TBARS

The secondary oxidation products comprise carbonyl compounds yielding the fishy and rancid character associated with oxidized fish lipid. It measures malonaldehyde produced during fat oxidation. TBA react with malonaldehyde and gives a red chromogen and is measured spectrophotometrically. TBA above 1-2 mg malonaldehyde /Kg fat indicates rancidity.

Free fatty acid value

Free fatty acid value is a measure of hydrolytic rancidity. Prior to the appearance of oxidative rancidity in lean fish, there is rise in lipid hydrolysis that leads to build up of FFA. It is non-esterified fatty acids in “free” form. It is more readily oxidized than esterified fats. FFA can act as pro-oxidants in oils by speed up the rate of hydroperoxide decomposition. Thus, high FFA content in the oil may cause further oxidation and lead to development of offensive taste and flavor in the Fish.

Total volatile acids

Total volatile acids include formic acid and acetic acid formed during spoilage. It is formed only after putrefaction and can be used as a quality index. In case of fresh muscle, the content is low and it increases rapidly after a few days in ice. TVA content cannot increase or decrease during canning process hence can be used for checking quality of canned raw material.

pH

Natural pH of live fish is above 7 and (typically 7.3). pH Falls after death as it goes through rigor and glycogen is converted to lactic acid. Post mortem pH is 6-6.8 in case of most species. In Tuna, it is below 6, due to high initial glycogen level. pH increases as the spoilage progresses.

Determination of adulterants in seafood

There are so many components of food safety that still remain unattended, and make a major concern to consumer health. Economically motivated adulteration is such an activity. For thousands of years, fish is considered a healthy human diet due to its rich protein content, vitamins, minerals, and highly unsaturated fatty acids. Since fresh fish is highly perishable in nature, there is an emerging risk of economically motivated adulteration to enhance the keeping quality of fish. India’s domestic fish market is reported to be selling formaldehyde adulterated fishes, especially in markets located far away from landing centers or production sites. According to Indian and International regulations, fresh fish and shellfish should be preserved only by means of ice made out of potable quality water. The use of substances other than ice to extend the keeping quality is a fraudulent practice. Apart from direct application of

adulterants, even adding ammonia like substance during ice manufacture to slow down the melting of ice or to cut down the cost of ice, or even adding approved preservatives of the processed commodity such as benzoate, to fresh fish to control the microbial activity are illicit in nature and have potential to cause health problems to consumers.

Formaldehyde:

Formaldehyde is a colourless, flammable, strong-smelling chemical, well known for its preservative and anti-bacterial effects. This chemical is generally used in building materials and to produce household products, in pressed-wood products, glues, and adhesives, paper product coatings, as an industrial fungicide, germicide, and disinfectant, and as a preservative in mortuaries and medical laboratories. Formaldehyde also occurs naturally in the environment. It is produced in small amounts by most living organisms as part of normal metabolic processes. Short-term health effects of formaldehyde exposure include watery eyes, burning sensations in the eyes, nose, and throat, coughing, wheezing, nausea, and skin irritation. In 1987, the U.S. En vironmental Protection Agency (EPA) classified formaldehyde as a probable human carcinogen under conditions of unusually high or prolonged exposure. The International Agency for Research on Cancer (IARC) also classified formaldehyde as a human carcinogen.

Ammonia:

Ammonia is a naturally occurring chemical in the atmosphere, as well as a synthetically made chemical. At room temperature, ammonia is a colourless, pungent-smelling gas and is lighter than air. Ammonia is an essential element for the plant, animal, and human life. It is found in water, soil, and air, and is a source of much-needed nitrogen for plants and animals. Ammonia is also present in fertilizers, power plants, mobile sources, and other manufacturing emissions. Ammonia levels in the air at 5 ppm can be recognized by odor. An average person detects ammonia by odor at around 17 ppm. Continuous ingestion of ammonia can lead to many health issues like the mucous membrane of the mouth, throat, esophagus, and stomach. Ammonia readily dissolves in water and forms ammonium hydroxide, and the ingestion of ammonium hydroxide can result in corrosive damage to the mouth, throat, and stomach.

Benzoate:

Benzoate is a white solid that is slightly soluble in water. Benzoic acid and sodium benzoate are used as food preservatives and are most suitable for foods, fruit juices, and soft drinks that are naturally in an acidic pH range. Their use as preservatives in food, beverages, toothpaste, mouthwashes, dentifrices, cosmetics, and pharmaceuticals is regulated. Sodium benzoate is a permitted additive used in processed fish and fishery products to inhibit the growth of mold, yeast, and many bacteria. The acceptable daily intake (ADI) fixed by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) for sodium benzoate is 0-5 mg/Kg body weight and the maximum allowable limit is 0.1% as per EU regulations. Benzoic acid is slightly irritating to the skin and irritating to the eye, while sodium benzoate is not irritating to the skin and is only a slight eye irritant. In humans, the acute toxicity of benzoic acid and sodium benzoate is low. However, both substances are known to cause non-immunological contact reactions.

"CIFTest"- Rapid detection kits for detection of adulteration of formaldehyde and ammonia in fresh fish

The ICAR-CIFT developed two different kits containing chemically treated paper strips that can react with the adulterant – Formaldehyde/Ammonia present in the tissue of the fish. Adding one drop of reagent solution to the swabbed paper strip can result in colour development within one minute to a maximum of 2 minutes. The development of blue colour indicates the adulteration of fish with formaldehyde/ammonia. With the use of CIFTest kits, the illicit use of chemical substances like Formaldehyde and ammonia in fresh fish can be effectively controlled being the consumers are empowered to check the commodity they are purchasing. ICAR-CIFT had transferred the CIFTest technology to M/s HIMEDIA Laboratories Pvt. Ltd., Mumbai and it is commercially available in India under the trade names- HiRapid Formalin test kit (for fish) and HiRapid Ammonia test kit (for fish).

Advanced automated systems for microbiological analysis

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Introduction

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

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

b) Specimen Preparation (Plating/Inoculation/Streaking): Plates Most fully automated inoculation and streaking systems require liquid transport swabs or liquid samples. Specimens can be loaded into racks and then loaded onto the instrument; alternatively, samples can be added to a turntable for continuous loading. The sample is scanned, and the system will know how to process the specimen and what plates are required. After vortexing the required plates arrive ready barcoded so that they can be tracked and traced throughout the process. Plates are then planted/inoculated or streaked depending on what was specified for that particular

specimen. A HEPA environment ensures no cross-contamination. Specific streaking patterns can be pre-programmed and achieved by robotic loop. This results in a consistent, reproducible inoculation and streaking pattern and produces single colonies more often than by a manual process. Systems will include a monitoring step to ensure that some sample has indeed been taken up by the pipette or loop. Inoculated plates can then be sorted according to required atmospheric conditions and temperature and transported by conveyor belt to the appropriate incubators. Any non-liquid or other specialized samples can be done in a semi-automated fashion whereby the technician prepares the plate, which then goes back into the system with the bulk of samples.

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

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

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

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

Systems Available

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

A) API (Analytical Profile Index) KIT

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

The test kit enables the following tests:

ONPG: test for β-galactosidase enzyme by hydrolysis of the substrate o-nitrophenyl-b-D-galactopyranoside
ADH: decarboxylation of the amino acid arginine by arginine dihydrolase
LDC: decarboxylation of the amino acid lysine by lysine decarboxylase
ODC: decarboxylation of the amino acid ornithine by ornithine decarboxylase
CIT: utilization of citrate as only carbon source
H2S: production of hydrogen sulfide
URE: test for the enzyme urease
TDA (Tryptophan deaminase): detection of the enzyme tryptophan deaminase: Reagent- Ferric Chloride.
IND: Indole Test-production of indole from tryptophan by the enzyme tryptophanase. Reagent-Indole is detected by addition of Kovac's reagent.
VP: the Voges-Proskauer test for the detection of acetoin (acetyl methylcarbinol) produced by fermentation of glucose by bacteria utilizing the butylene glycol pathway
GEL: test for the production of the enzyme gelatinase, which liquefies gelatine
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

Confirming 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 37 ^oC for 18 to 24 hours.

Result interpretation

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

Add following reagents to these specific compartments:

TDA: Put one drop of Ferric Chloride

IND: Put one drop of Kovacs reagent

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

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

B. VITEK® 2 COMPACT

The VITEK® 2 Compact system offers quality control testing solutions for fast and accurate microbial identification. The efficiency of the VITEK® 2 COMPACT instrument and VITEK® 2 PC software have the capacity to help improve therapeutic success and patient outcomes through reliable microbial identification (ID) and antibiotic susceptibility testing (AST). The instrument also lets you enhance laboratory efficiencies with reduced hands-on time and rapid reporting capabilities. All this, in a cost-effective, space-saving design. With technology that includes an extensive and robust identification database, rapid results, and minimal training time, it will streamline laboratory workflow for increased productivity. The system identifies the majority of microorganisms that contaminate production areas and finished products in a minimal amount of time. Identification cards presently available for product safety include: Gram-negative bacilli (time to result: 2 – 10 h); Gram-positive cocci (time to result: 2 – 8 hours); Yeast-like organisms (time to result: 18 hours); Anaerobic bacteria (time to result: 6 hours); Gram-positive spore forming bacilli (time to result: 14 hours) Coryneform bacteria (Time to result: 8 hours).

Testing using VITEK 2 can be performed as follows:

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

b. Aseptically transfer at least 3 mL of sterile saline into a clear polystyrene 12×75 mm test tube. Using sterile cotton swabs, prepare a homogenous organism suspension by transferring several isolated colonies from the plates to the saline tube. Adjust the suspension to the

McFarland standard required by the ID reagent. The required inoculum concentrations card McF range for differnent bacteria are as follows: GN 0.5-0.63; GP 0.5-0.63; ANC 2.7-3.3; BCL 1.8-2.2.

c. Place the prepared suspensions in the cassette

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

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

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

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

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

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

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

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

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

C. VIDAS

VIDAS® is a multiparameter, automated immunoanalyser. It includes an analytical module, a computer and a printer. The analytical module automatically performs all stages of the analysis. The VIDAS® system contains five independent 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 generate 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.

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.

Advances in extension techniques for the development of fisheries sector

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Status and trends in aquaculture and fisheries

In the context of current challenges in food production, nutritional security, social transitions and growing climate uncertainties, fish and aquatic animals play important role to maintain the *status quo*. Global fish production has attained a target of 179 million tonnes in 2018 with an average annual growth of around 6 % in aquaculture and is expected to be increased to the extent of 186 million tonnes by the end of 2030. On the contrary, the trend of Indian fisheries has achieved a big leap in fish production during last seven decades witnessing a quantum leap in production i.e. from 0.75 million tonnes (1950-51) to 14.2 million tonnes (2019-20). Today, it shares about 7.7 % of the total global fish production and has established its dominance in global fisheries scenario as the 3rd largest in total fish production and 2nd in aquaculture production with an average annual growth rate of 14.8%. Out of total global production, around 87 % (156 million tonnes) accounted for human consumption covering more than 3.1 billion people in world (FAO, 2016). Mostly the developing countries account for over 60% of global fish catch, about 50% of global fishery exports in value terms and more than 60% in quantity.

In the livelihood sector, at global level about 59.6 million people are directly employed in fisheries and aquaculture at global level and more than 200 million engaged along the value chain in various upstream and downstream activities from production to distribution (FAO, 2016). In India, it provides livelihood security to more than 25 million (2018-19) of fishers and fish farmers at the primary level and almost twice along the fisheries value chain. Besides, about 84 percent of the globally engaged population in fisheries and aquaculture sector are in Asia, followed by Africa (almost 10 percent), and Latin America and the Caribbean countries (4 percent).

Despite the significant contributions of this 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 fisheries sector as a potential driver of local and national economic development.

Problems in small scale fisheries

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, cost-sharing; which can bring a change in the performance of the sector. 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. Keeping eye upon this, some of the advanced extension techniques have been suggested for an accelerated fishery development with focus on poverty alleviation of poor fishers.

Revamping extension systems for sustainable fisheries

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.

Advanced extension techniques for technology dissemination in fisheries

With the advent of global competitiveness and market liberalization, our prevailing extension system has become defunct, which needs to be strengthened with innovative extension techniques to tackle the interwoven challenges in fisheries *viz.*, enhancing production, climate change, weather aberrations, dwindling resources, quality and safety of products, growing market demand, entrepreneurial opportunities in fisheries, conservation of environment and international trade promotion etc.; so that fishers can adjust their production portfolio keeping eye upon the emerging trends in food consumerism in domestic as well as global markets. In India, in the course of development, many different models for transfer of

technology have been tested and some robust extension approaches have been tested and validated. Furthermore, the frontline extension system of the country has been revisited and sharpened through fishers oriented approaches for technology adaptation and dissemination. As a result, the extension system in India has been designed to move beyond technology and beyond commodity through reciprocal fishers-research-extension linkages for sustainable growth and livelihood security of the farmers. In order to streamline this mechanism, a conceptual framework has to be developed in response to recognizing and considering different livelihood assets viz., *human, social, physical, natural and financial resources*. In general, fish farmers suffer from lack of access to appropriate services like credit, inputs, market, extension, technologies etc. 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. Some of the following innovative and advanced extension techniques validated through research systems must be adopted on trial basis to make fisheries more lucrative and sustainable.

a. Asset Based Community Development (ABCD) approach

Conventionally, poor people consider themselves as the impoverished population with certain needs for development that can only be resolved by various 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 improving their livelihoods by developing their technical, organizational and management skills and practices (GFRAS, 2011; FAO, 2010). RAS must be designed to provide the information related to farm, organization, business management etc. recognizing the diversified actors involved in extension and fields advisory works (public, private, civil society); knowing the need of fishers, fish farmers' producer organizations (FFPOs), fishermen cooperatives and rural communities beyond technology related information and explaining them 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 technology information for the fishers. In this direction, innovative extension 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 FFPOs 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 FFPOs and also collaborate with FFPOs 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. This can act as a platform to articulate concerns, exchange knowledge, influence policies and engage in collective action so that their livelihood remains sustainable and profitable. Effective formation of Rural Resource Centres (RRCs), Fishermen Cooperative Society, Fish Farmers' Producers

Organisations (FFPOs) 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 multiple 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 experiments, 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 methods. Farmer Field School (FFS) model has been accepted as a good extension technique because of its exclusively participatory nature. 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 basket 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 agro-enterprises. 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 ICT application in agriculture and allied sectors 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. 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:

Recently, a new extension technique 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 with a pre-conceived idea about the field level problems. It is an entrepreneurial oriented sustainable extension system that can be able to transform every link in the food chain, from farm to fork, pond to plate and deck to door. It is a combination of different innovative extension techniques like ABCD, CRE (cost-recovery extension), MVSE, CBVD etc. blended with suitable conventional approaches, 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.

Fisheries embraces diverse actors in its endeavour to support their livelihood system giving an impact in food and nutritional security. At the same time, 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 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 advanced techniques of extension should be based on capacity building, skill development, people's participation along with government initiative to provide policy support in line 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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Application of quantitative statistics in fisheries research

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Introduction

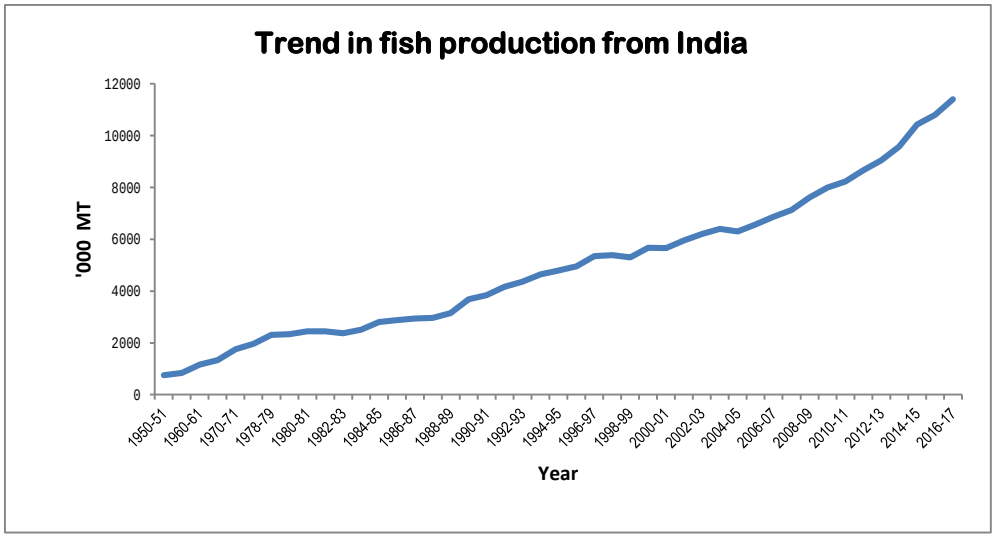
In every walk of life, there is a need for statistical data. After the discovery of fire and wheel, man's quest for knowledge started. He explored the nature and gathered information and learnt to utilize the facts and figures for taking decisions. No field of study is complete without quantitative data and its analysis to put forth the theories or conclusions evolved. For example, in planning the economy of the country, good statistical data on the available resources, returns is needed for fixing the production targets, allocating funds and laying out the policies. While the words "statistics" and "data" are often used interchangeably by the public, statistics actually goes far beyond the mere accumulation of data.

Data means a series of measurements or observations—usually in numerical form, of some phenomenon. On the other hand, the word "statistics" refers to an academic discipline and a set of best practices to convert data into meaningful, actionable information about the real world, particularly in the presence of uncertainty. The word "statistic" is also used in the specialist literature to mean "a numerical summary of data."

Statistics is extensively employed in many real-world measuring processes. It has wide applications in any branch of science or research viz., agriculture, meteorology, oceanography, forestry, fisheries, animal husbandry, geology, epidemiology, medicine, communication, visualization, education, politics, psychology, atomic physics, space research, climate change studies, economics and governance.

Indian fisheries

Indian fisheries sector has come a long way since independence with annual production levels of over 11.78 million tonnes of fish and shellfish from capture fisheries and aquaculture.



Data generation in fisheries

Fisheries sector undergoes continuous changes with the introduction of new technologies evolved through R & D institutions. Validation of these technologies and providing inputs for needs of the sector is one of the important mandates of Statisticians. Statistics per se deals with generation of data, data management, data analysis and information generation from data. The data needs in fisheries will vary according to the type of research. A biologist who works on species behavior, growth, abundance, etc. will require information on the spatial distribution and catch. Likewise, an economist who wishes to predict next year's profit should understand the effect of population size on producer's costs.

Policy makers may need macro level data on infrastructure, employment, earnings, investment etc. to formulate management measures. Data on marine fisheries gets generated from the operation of commercial fishing vessels and research vessels. In 'Fishery technology' large volumes of data generated in a wide range of applied scientific areas of fishing technology, fish processing, quality control, fishery economics, marketing and management. Apart from statistical data collected in technological research, data also collected on production, export, socio-economics etc. for administrative and management decision making. Major areas of data generation:

- fishing vessel and gear designs
- fishing methods
- craft and gear materials
- craft and gear preservation methods
- fishing efficiency studies
- fishing accessories
- emerging areas include use of GIS and remote sensing

Data on various aspects of fishing gets collected for administrative purposes and policy making. For administrative purposes, voluminous data gets generated through fisheries departments of states. Each district has officials entrusted with the work of collection of data which are coordinated at the state level. State level figures are compiled at the National level by Department of Animal Husbandry and Dairying, Ministry of Agriculture, New Delhi. Information is also compiled on macro-economic variables like GSDP from fishing by the respective Directorates of Economics & Statistics.

Fish production statistics

Indian fisheries has seen tremendous development over the past six decades owing to technology changes in fishing like mechanization of propulsion, gear and handling, introduction of synthetic gear materials, development of acoustic fish finding devices, satellite based fish detection techniques, advances in electronic navigation and communication equipment. The increase in fish production can be said as exponential with a mere 75000 MT in 1950-51 to 11.42 million MT in the current year. Both marine fisheries and aquaculture have contributed to the present level of production with share from culture fisheries more than the capture fisheries. It is important task to collect macro level data from state and country on fish production and details of the species caught in the sea.

The data on fish catch and effort (a measure of fishing activity of vessels at sea), from all the coastal states, Union territories, Islands is being done by ICAR-Central Marine Fisheries Research institute and maintained as database. Based on standard sampling methodology

developed by CMFRI, daily data on commercial landings from selected centres/zones all over the coast is collected, compiled and published. Detailed time series data has been generated on species wise, region wise, gear wise fish landings are collected and compiled for the use of researchers and policy makers. The beach price of fish (species wise) is also collected periodically.

Data on fish farms, production and area under aquaculture is maintained by the respective State Fisheries departments and compiled at the National level. Apart from capture fisheries (marine) and culture fisheries (aquaculture) the fish production from inland water bodies like lake, ponds, reservoirs, etc. is collected and compiled at State level. For developing the sector, various programmes and projects have to be formulated and implemented. To achieve the objectives of such developmental programmes, the current status of production of fish from various regions has to be made known. The need for fish production data maintained by these agencies from marine sources, aquaculture and inland water bodies arises while formulating various research studies and development projects at district, state and National level.

Quantitative statistics on fish exports

Fresh fish after harvest is iced and distributed through various channels into the domestic markets and overseas markets. Around 80% of the fish is marketed fresh, 12% of fish gets processed for the export sector, 5% is sent for drying/curing and the rest is utilized for other purposes.

Marine Products Export Development Authority (MPEDA) maintains the database on export of fish and fishery products from India to various country. The weekly prices realized by Indian seafood products in the various overseas markets are also collected and compiled by the agency. Marine Products Export Development Authority (MPEDA) established in 1972 under the Ministry of Commerce responsible for collecting data regarding production and exports, apart from formulating and implementing export promotion strategies. Prior to the establishment of MPEDA, Export Promotion Council of India was undertaking this task.

Fish processing factories established all over the country generate data on daily production, procurement of raw material and movement of price structure etc. which is generally kept confidential. Data on quality aspects maintained by Export Inspection Council of India through Export Inspection Agency (EIA) in each region, under Ministry of Commerce and Industry. The EIA is the agency approving the suitability of the products for export.

- bacteriological organisms present in the products
- rejections in terms of quantity
- reason for rejection etc.

Quantitative statistics on fish quality control

Other types of data generated by CIFT in fishing and fish processing technology are quality control data on fish and fishery products, ice, water, etc. Offshoot of processing technology is Quality Control of which Statistical Quality Control forms an integral part. Due to the stringent quality control measures imposed by importing countries, especially the EU and USFDA standards samples of fish and related products like raw materials, ice and water samples and swabs from fish processing factories are tested at the quality control labs. Another area where statistics gets generated is in product development: consumer acceptability and

preference studies mainly for value-added products. Using statistical sensory evaluation methods this data gets analysed.

At Central Institute of Fisheries Technology (CIFT) we are periodically collecting data on the following aspects which is used for policy decisions.

- Techno-economic data on various technologies developed
- Data on Economics of operation of mechanized, motorized and traditional crafts
- Data for the estimation of fuel utilization by the fishing industry
- Year wise data on Installed capacity utilization in the Indian seafood processing industry
- Demand – supply and forecast studies on the fishing webs
- Harvest and post-harvest losses in fisheries
- Transportation of fresh fish and utilization of trash fish
- Impact of major trade policies like impact of anti-dumping, trend analysis of price movement of marine products in the export markets
- Study on impact of technology and study on socio-economic aspects

Quantitative estimation of Fish losses in harvest and post-harvest sector

Loss per se is defined as the quantity of marine fish which is not fit for human consumption due to physical loss or spoilage of some other reason. Losses at the time of harvesting and onboard the fishing craft are called harvest losses and losses occurring after harvesting *i.e.* from the landing centre up to the consumer at different stages are called post-harvest losses.

Post-harvest losses occur due to improper handling and lack of infrastructure at different points starting from the landing centre to the consumer. Apart from these, there are latent losses such as realization of low value due to glut, multi-day fishing etc.

Discarding takes place because, in the course of fishing, many species other than the target species are often caught. This by-catch is usually discarded at sea unless it is worth keeping. Discarding by-catch consisting of a small proportion of mature specimens from healthy stocks causes relatively little damage, but when it consists of juveniles of commercial species it will disturb the balance of the system. Catching large numbers of juveniles is likely to reduce the future number of mature fish. This will have a direct impact on the fishery taking the by-catch, or on other fisheries if the juveniles belong to their target species.

A recent study completed at CIFT, Cochin attempted to estimate harvest and post-harvest losses in marine fisheries. Ernakulam and Alleppey districts were covered for the study. The estimation was carried out at the two stages harvest and post-harvest stages using stratified random sampling design. The channels of fish production namely mechanised, motorised and traditional formed the various strata at the harvest stage. In the post-harvest stage, losses occurring at landing centre, processing, marketing and transportation sectors were observed. The study was conducted for a full fishing season to observe loss pattern during monsoon, pre-monsoon and post-monsoon seasons.

Harvest losses in marine fisheries was estimated from Ernakulam district by stratifying fishing crafts into mechanized, motorized and traditional. Primary data on fish catch and losses was collected for 12 months from fishing crafts operating in six selected fish landing centres at Ernakulam. Loss estimates were computed analyzing the season wise data and pooled data. The sector wise harvest loss estimates are as under:

Harvest losses

Sector	Pre-monsoon (%)	Post-monsoon (%)	Monsoon (%)	Overall (%)
Traditional	1.93 (0.43)	0.98 (0.37)	0.83 (0.28)	1.14 (0.28)
Motorised	3.45 (0.54)	2.76 (0.13)	4.38 (0.53)	3.65 (0.17)
Mechanised (upto 7 days fishing duration)	12.74 (1.23)	11.09 (0.11)	9.11 (0.05)	14.15 (2.10)
Mechanised (More than 7 days)	13.78 (1.24)	14.98 (1.35)	13.35 (1.32)	18.73 (2.22)

Multiday fishing by the mechanized trawlers reported maximum loss due to capture of juveniles and their discards. Around 1500 to 2750 kg of fish gets discarded at sea by trawlers during fishing trips for more than 7 days' duration. The no. of hauls during fishing and loss was positively correlated (0.69) at 5% level of significance. The estimate of loss due to mechanized fishing was computed by utilizing information on no. of hauls which was more precise than the traditional estimator. The losses due to motorized fishing crafts was very less in comparison with trawlers. The traditional fisheries sector reported minimal or no loss during the period.

Post-harvest losses

The post-harvest losses in marine fisheries (at the landing centre level) were estimated as below:

Sector	Loss % (SE)
Traditional	0.09 (0.0004)
Motorised	1.19 (0.07)
Mechanised	4.79 (1.09)

Losses in the marketing sector was due to damage during transportation, spoilage when delay in transport and weather. Two wholesale markets for fresh fish and one wholesale market for dry fish were covered fortnightly for recording losses due to marketing. Similarly, four retail markets were surveyed fortnightly of reporting loss in retailing fish. The estimates for post-harvest losses due in processing and marketing are given below:

Post-harvest losses in marine fisheries

Sector	Loss % (SE)
Pre-processing	0.38 (0.04)
Processing	1.19 (0.07)
Dry fish production	36.97 (12.88)
Wholesale market (fresh)	3.79 (1.09)
Wholesale market (Dry)	7.56 (2.12)

Retail market (fresh)	3.13 (0.02)
Retail market (Dry)	8.23 (0.13)
Roadside market (fresh)	2.54 (0.11)
Roadside market (dry)	5.43 (1.19)

The reasons for post-harvest losses in fisheries are summarised below:

Type of Post-harvest loss	Reasons
Loss in nutritional value (1. Unfit for human consumption 2. Product is unattractive and rejected by consumer)	High temperature, washing in polluted water, poor handling, poor storage
Physical loss (1. Thrown away 2.Loss of material due to damage)	It may be a bycatch not intended for capture, not worth marketing due to low price realization, poor packaging, rough handling
Quality loss (Deterioration in quality)	Most common of PH loss- Must have undergone changes due to spoilage or mishandling, marketed several hours after catching without proper icing
Loss due to market forces (Economic loss)	Inadequacy between demand and supply - Bulk landing of same species by subsequent boats in the same day
Losses due to traditional processing	Sun-dried, processed smoked fish, salting prone to quality losses
Losses at Transportation, storage	Improper packaging which triggers spoilage, spillage, insufficient icing, rough handling
Loss due to insect infestation	Infestation in sun dried products

Quantitative techniques for evaluating consumer preferences

The emerging fast-food culture among the young and affordable has brought focus on processed food and its demand in the domestic food market in India. Domestically, spending on food and food products constitutes the largest portion of the Indian consumer's spending – more than a 31% share of wallet. Evaluation of consumer preferences before introducing a new product will help the marketer to refine the product for better reach. Conjoint analysis is a popular technique used in marketing research to study the features a product should possess to have a wide consumer reach. Conjoint analysis was initially conceptualized by Luce and Tukey (1964) and further developed by Green and Rao (1971) for marketing research. It employs a decompositional method to estimate the structure of consumer preferences and consumer utility values of different attributes of a product or service. It is a decompositional method that

disaggregates the structure of consumer preferences into utility values. The relative importance of a product can also be estimated using this method.

Conjoint analysis assumes that consumers make purchases by simultaneously considering several attributes of a product. The ability to analyze several attributes at once distinguishes conjoint analysis from traditional market research methods where each attribute is studied separately. Usually, conjoint analysis consists of a main-effects analysis of variance with ordinally scaled dependent variables. Consumer preferences are the dependent variables, and product attributes are the independent variables. The following are some of the questions that can be answered with a conjoint analysis.

- How important is each product attribute to consumers?
- Which existing products do consumers prefer?
- What combination of product attributes do consumers prefer most?
- How well will my product do in the current market?

Subjects provide data about their preferences for hypothetical products defined by attribute combinations. Conjoint analysis decomposes the judgment data into components, based on qualitative attributes of the products. A numerical part-worth utility value is computed for each level of each attribute. Large part-worth utilities are assigned to the most preferred levels, and small part-worth utilities are assigned to the least preferred levels. The attributes with the largest part-worth utility range are considered the most important in predicting preference.

Big data

Big data and analytics can play a major role in Enterprise Information Management. Globally, the volume of available data in all the sectors has continued to double every three years as information pours in from transactions, social media, sensors in the physical world, and billions of mobile phones. Data storage capacity has increased, while its cost has plummeted. Data scientists now have unprecedented computing power at their disposal, and they are devising ever more sophisticated algorithms that can instantly sift through troves of data to find patterns and reveal insights. The upshot of all this innovation is that decisions no longer have to be based on gut instinct, or subject to human error. Algorithms can make them instantly and consistently, drawing on a mountain of evidence. Systems enabled by machine learning can provide customer service, manage logistics, analyse medical records, or even write news stories.

Determinants of Fish Consumption: A consumer behaviour perspective

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Introduction

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.

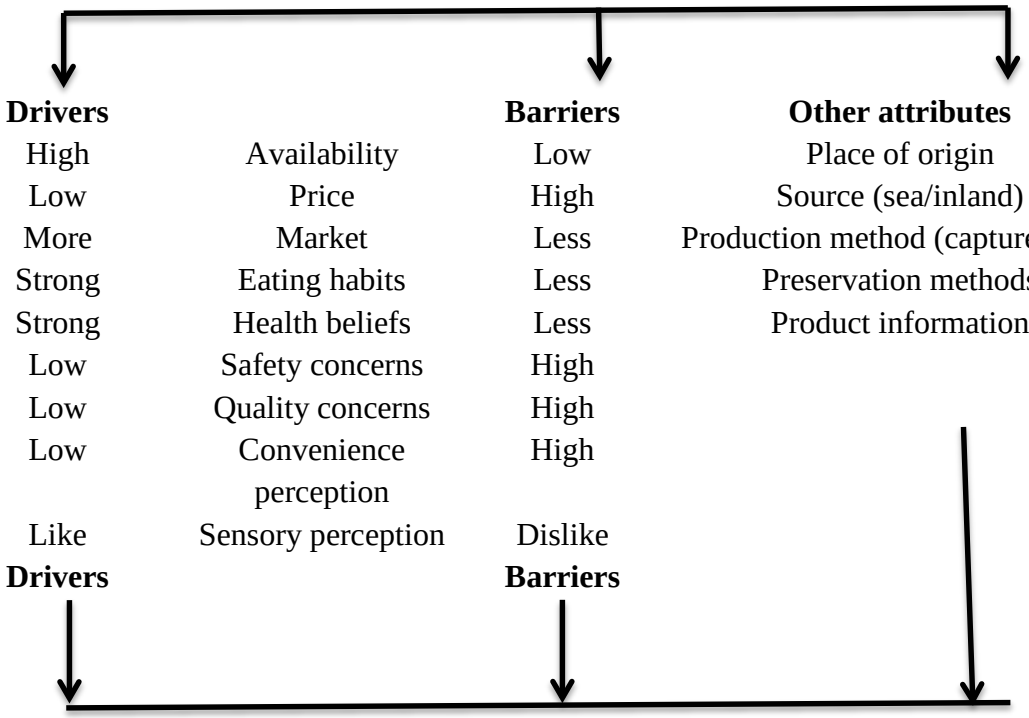
In another study conducted among urban consumers of Kerala, Conjoint analysis revealed that the factors like 'place of origin of fish', '24x7 accessibility' and 'sensory perception' were the most contributing drivers while 'price of fish' and 'availability of favourite fish' were the most important barriers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

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.

Drivers and barriers to fish consumption

Personal factors (values, beliefs, attitudes, demographics),
Situational factors and Environmental factors



Quantity, frequency and characteristic of fish consumed

Sajeev *et al.*, 2018

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Input and service delivery system in fisheries

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Introduction

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.

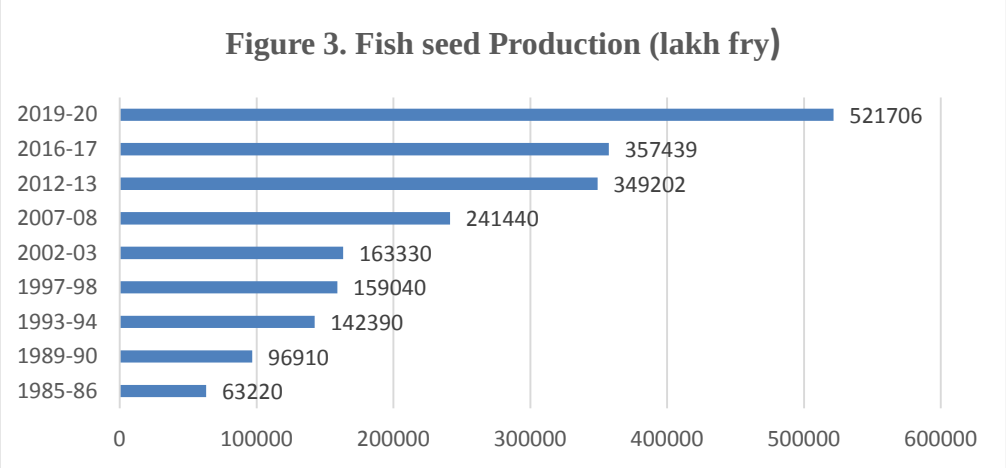
Table 1: Number of fishermen engaged in fishery activities

Particulars	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.



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	Numbers
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

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.

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.

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.

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.

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	Tunnies	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

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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Training need assessment and problem analysis

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Training needs

The assessment of training needs is pre-requisite to any type of training for successful implementation of the training objectives. In Agricultural extension, it is the most important activity undertaken for human resource development and capacity building of different stakeholders like farmers, fishers, extension personnel, researchers, department officials' students etc. The gap between what is going on now with regard to the work/job performance of the trainee and what should go on now (or) in the future indicates the future need for training Johnson (1967). The gap if any, needs to be addressed by the trainer's organization.

Dimensions of need

There are four dimensions of need, as identified by David Deshler (1979) such as felt need, expressed need, normative need, comparative need and inequity in the availability of services, all other things being equal. A training need exists when an individual lacks the knowledge and skills to perform an assigned task satisfactorily (Dugan Laird,1978).

Training Need Assessment (TNA)

Training need identification is a tool used to identify the required educational courses or activities to be implemented for the employees for enhancing work productivity (Singh *et al*., 2011). TNA help to recognise current problems and future challenges which can be solved through training. TNA also help to enhance professional competency for performing assigned job in an organization. There are different methods and techniques used by the researchers to study the training needs of people intending for knowledge or skill enhancement. The training needs may be determined in terms of analysis of intended organisational change, existing work problems and man power wastage data. Training needs could be in the areas of skill, knowledge and change in attitudes.

Scales for measuring TNA

Scales are developed on a context specific method. The steps involved are collection of need items, scoring techniques and ranking (Ramulu 1992).

A. Knowledge test

Here training need may be defined as the gap between the existing knowledge and desirable knowledge of the trainees regarding any subject matter. In this method TN is studied by administering a structured knowledge test. Knowledge test consist of items like multiple choice questions and open-ended questions regarding various aspects of the subject under consideration. Score will be given to right answer as defined by the researcher and total score for a respondent will be calculated. A training need quotient value is calculated by identifying the gap between the required knowledge (what out to be) and the existing knowledge (what is).

B. Training Need Index

In this method researcher need to identify the dimensions of training need first. Based on the dimensions need items are identified with help of experts in the field and also through literature review (scales can be developed by self or existing scales can be used based on the

context). Response categories are prepared and then data is collected from the respondents. Training need index is then calculated by dividing total score by maximum obtainable score, and by multiplying with 100.it is more accurate method than direct questioning method.

C. Direct questioning

Identify areas of training. Fix a response continuum based on people's perception and assign score to each category

D. Matrix Ranking- An important PRA tool to assess preferences

Direct matrix ranking refers to placing different challenges in the field in the order of importance like I, II, III etc. according to their severity with regard to a reason. 3-5 key informants are required for data collection. Interview schedules has to be prepared having matrices to enable a range of different items to be assessed against selected criteria. Separate matrices have to be prepared for each technology and the key informants should indicate the reasons for their behavior. The pooled matrix table has to be prepared for each technology and scores are added up for each column. The final rank will be used to infer which technology got the maximum score for a particular criterion as perceived by the farmers.

E. Problem tree

The aim of the problem tree analysis is to create a structural analysis of the causes and effects of an issue or problem. A focal problem, will be identified first and then in-depth analysis of causes and the consequences are done.

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Application of ICT tools in fisheries

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Introduction

Information has played an important role in adding value to all sections of society since the dawn of civilization. For communicating information requires various digital technology and tools to reach the ultimate beneficiaries of the respective field. In the initial years use of ICT was limited to academic and research institutes along with costly access. However, over time its reach has touched every stratum of society as it has become the most popular and cost-effective way of sharing knowledge and information. As we thought that what do fish and fishing have to do with computers, the internet, and communications? Information technology is playing a significant role in the modernization and growth of the fishing industry. This traditional industry is facing economic and environmental pressures, as well as ever-changing regulations. Such pressures have led the fishing industry to invest in information technology to maintain sustainability and streamline its operations and be more effective and efficient. The world is undergoing an Information Communication Technology (ICT) revolution, a revolution that has enormous socio-economic implications for developed and developing countries. ICTs play a vital role by adding value to the entire Indian fisheries at each stage of the fisheries supply chain starting from fish catch to reaching the ultimate consumer. The latest ICT application will transform the fishermen's lifestyle as well as their livelihood activities mainly for-profit motive by reducing the labor and also reduce the vulnerability by timely getting of information which paving the way for social equity and ultimately uplifting fishermen to the mainstream. Nowadays clearly seen that there is fast expansion and development in the fisheries sector through ICTs apart from GPS, Navigation devices, sonar, fish finders, and wireless communication at very high frequency (VHF), etc were significantly contributed to the field of the marine fisheries sector. Different initiatives in ICTs have been taken up which would also help in expanding and developing the fisheries technologies for the fisher communities.

It is vividly believed that ICT is a basic resource for development, several ICT tools used in fishing such as mobile phones, television, radio, GPS, and fish finder, can bring significant changes in the fishermen’s livelihood and reduction in the level of poverty of different fishing communities (Kularatne, 1997). ICT plays an important role in linking the knowledge among all stakeholders such as researchers, fisheries officials, etc by improving the linkages between the researcher and clients. This will mainly save the cost, time, and energy of the fishermen especially through mobile used by the fishermen will provide the best price for their catch before being brought into the landing center. With the help of this technology, fishermen were moving farther into the deep sea to get better catch high-value fish. This will be highly helpful for the fishermen to decide on the various constraints such as higher operational costs, more investment, the decline in the fish catch rate, fewer infrastructure facilities, and low profitability. All these factors are affecting the overall performance or fishing efficiency. Using ICT applications in fisheries will be an advantage for the fishermen to reduce

their operational costs as well as increase their quantity of catch. But the rural communities in developing countries like India still lack basic communication infrastructure was seen.

Definition

Information technology (IT) is the use of any computers, storage, networking, and other physical devices, infrastructure, and processes to create, process, store, secure, and exchange all forms of electronic data. e.g., letter, Photograph, Digital sensor, GPS, or satellite.

Communication: It acts as a medium to transfer information from one to another eg: the internet, mobile network, local and wide area network.

Information communication technologies (ICT). As per the definition of UNESCO "Diverse set of technological tools and resources used to transmit, store, create, share or exchange information". ICT is a set of tools that assist in capturing, storing, processing, transmission, and display of information by electronic means of technologies. ICT play important role in the sustainable development of the fisheries sector by a timely collection of essential information, processing them, and distributing among various organizations

ICT Technologies applied in the fisheries sector

There are various ICT tools were used by marine fishermen to communicate and increase the fish catch such as What's up, Television, Radio, Mobile, Global Positioning System (GPS), GPRS, Echo sounder, Sound Navigation and Ranging (SONAR), Search and Rescue Transponder (SART), Automatic Identification System (AIS), Distress Alert Transponder (DAT), Internet-enabled PC, Radio Deduction and Ranging (RADAR), Community Radio, portal, Very High-frequency wireless sets (VHF).

Identity technologies used in the fisheries value chain

- **Barcoding:** A barcode is a method of representing data in a visual, machine-readable form. Initially, barcodes represented data by varying the widths and spacings of parallel lines. These barcodes, now commonly referred to as linear or one-dimensional (1D), can be scanned by special optical scanners, called barcode readers. 2D barcodes, although they do not use bars as such 2D barcodes can be read or deconstructed using application software on mobile devices with inbuilt cameras, such as smartphones. These barcodes were used in the seafood products to ensure the authenticity and the origin of fish and other information such as price, product packed date etc.
- **Vessel tracking devices** - Vessel tracking devices such as the Pelagic Data Systems (PDS) tracker can be used to establish locations in which fish are caught and landed. These data can serve as part of a digital record of seafood provenance.
- **Supply chain tracking software** - Several software systems are now available for tracking fish through the supply chain to reduce fish fraud and reliably transmit information about the seafood to buyers. First, the fish must be labelled with a unique identifier. For high-value products, a QR code, barcode or NFC-enabled labels (small passive electronic disks that encode information and are activated by the magnetic fields produced by smartphones) might be required to ensure sufficient security. For other products, text messages or app input fields that include information on where the fish was caught, how it was caught, how it was handled, where it was landed and other information can be validated by trusted entities.
- **Sensors:** It is highly used in many equipment's along the fisheries value chain and it is majorly used in the aquaculture farm and fish processing industries. Monitoring the various water

quality parameters and weather parameters of aquaculture farms using both wired as well as wireless sensor technology, embedded computing technology, MEMS technology (Micro-Electro-Mechanical Systems), distributing information processing technology and wireless communication technology to build the wireless network sensor network system. This system is a digital, networked, intelligent real-time dynamic for monitoring the aquaculture water quality. The system not only can deal with the normal detection of the aquaculture environment indicators (temperature, PH, dissolved oxygen, turbidity, ammonia, etc.) monitor in real-time.

- **Image processing:** Image processing-based technique used to find the freshness of the fish by capturing the segmentation of gill tissues from fish images. The segmented image of gills tissue is used for the assessment of fish freshness, which is the most required property from the consumers because of its strong relationship to taste and health. A number of sensorial inspection procedures have been introduced to point to the state of freshness. These procedures involve the use of the sight (to evaluate the skin appearance and the colour and the global aspect of the eyes). Eg: The FishAPP mobile application software enables smartphones and tablets to capture a photo of a fish, or to select one from the local device photo library, and connect with the FishAPP remote server. FishAPP mobile software has been developed with PhoneGap, a free and open-source framework that allows the creation of mobile apps using a set of standardized web APIs for the desired platforms. The photo must include the full fish and it needs to respect the following guidelines: The fish must be photographed sideway; The caudal fin must be arranged in a relaxed anatomical way; Other fins should be set in a close-fitting manner. Since lifeless fishes cannot keep the fins completely visible, we opted to consider only the caudal fin as an anatomical discriminative feature.
- **Data management:** Web-based Seafood export management software system that simplifies and helps you in a smarter way to increase your business productivity and profitability for data storing and easy access at anywhere and any point of time. Along the fisheries supply chain, inventory could operate in multiple warehouse locations. It calculates the true yield and margin on everything you cut and meets the unique challenges of weight, products where yields, collection hub, product accounting, settlement processing, catch weight, multiple freezer/warehouses, and Shipment.
- **Server Side:** Web server, Search Engines
- **Clients side:** Browsers, Apps
- **Cloud:** Google Drive, iCloud, Dropbox, SkyDrive
- **Access Devices:** Desktop, Laptop, Tablet, smartphone.

1. Fisheries repository management:

a. Fish Base

Fish Base is a global biodiversity information system on finfish. Its initial goal to provide key facts on population dynamics for 200 major commercial species has now grown to have a wide range of information on all species currently known in the world: taxonomy, biology, trophic ecology, life history, and uses, as well as historical data reaching back to 250 years. At present, Fish Base covers >33,000 fish species compiled from >52,000 references in partnership with >2,000 collaborators: >300,000 common names and >55,000 pictures. https://www.fishbase.de/home.html.

2. **Identity management:**

AIS (Automatic Identification System)

The Shipborne Automatic Identification System (AIS) is a vessel tracking system capable of communicating navigation information automatically between AIS-equipped vessels and coastal authorities. It is a collision-avoidance system that gives information on all the ships in your area, their speed and courses and how to contact them (name, callsign, MMSI). This information is publicly broadcast on VHF radio which can be picked up either by other ships or by shore-based receivers. The main purpose is to improve the safety of navigation by assisting in the efficient navigation of the ship, protection of the environment, and operation of Vessel Traffic Services (VTS), by satisfying the following functional requirements In a ship-to-ship mode for collision avoidance, As a means for littoral States to obtain information about a ship and its cargo and As a VTS tool, i.e. ship-to-shore (traffic management).

Location recognition:

a. **GPS (Global Positioning System)**

A network of satellites that continuously transmit coded information, which makes it possible to precisely identify locations on earth by measuring the distance from the satellites. As stated in the definition above, the satellites transmit very low-power radio signals allowing anyone with a GPS receiver to determine their location on Earth. The advantage is that the global positioning system (GPS) enables the fishermen to plot a course to the potential fishing area. A fisherman can plot his course from any location by using stand-alone GPS, which can work without a mobile network.

b. Fish Finder:

It provides valuable information to help you locate rich fishing grounds and boost your catch the Bottom Discrimination Function - Analyse bottom structure Configurable Alarm function (depth, fish echoes, etc.) Post-processing Gain Control applied to all echoes displayed on the screen Share and display information on a chart plotter

c. Very High-frequency wireless sets (VHF)

VHF has been retained for short-distance communications but the range is limited under normal circumstances to less than 20 nm. VHF channels at sea especially the distress, safety and calling Channels 16 (156.8 MHz) and 70 (156.525 MHz).

Application of ICT solutions in the fisheries

Advisories

Indian Marine Fishery Advisory System: Dissemination of PFZ Advisories:

SMS, IVRS, Helplines, Voice Messages, Information Kiosks, etc. through Location-Based, New Generation E.D. Boards, Door darshan, E.D. Boards, News Papers, Emails, Website with Web GIS Facility, Phones & Faxes.

Web-based Dissemination

Unique website for multi-lingual advisories. Provides information in eight local languages (Gujarati, Marathi, Kannada, Malayalam, Tamil, Telugu, Oriya, Bengali) as well as in Hindi and English. Web GIS Facility without any commercial package installation. Retrieve PFZ information about any area in the Indian EEZ of their interest by doing simple GIS operations.

Mobile phone

Using mobile phones, fishermen can keep themselves up to date about prices and quality of fish in surrounding markets which ultimately enhances their income (Jensen, 2007). In addition, mobile phones have provided easy access to fishermen to search for the best prices for their catches in different markets (Evoh, 2009). Mobile phone penetration in rural India has revolutionized information access as also connectivity between people, the mobile phones not only have provided information concerning market information to the fishermen but also have facilitated weather. Mobile phones allow fishermen to avoid potential losses to boats and nets as well as risks to personal safety. Emergency and safety benefits were consistently described as the most important impacts on their life (Mittal, & Tripathi, 2009). It has been also observed that coastal fishermen used to get information about weather conditions through SMS about before entering the sea.

Mobile applications are used to get alerts if fishermen cross the border respectively, fisheries inspectors used mobile applications for reporting cases of illegal, unregulated, or unreported (IUU) fishing. However, the use of mobile apps for fisheries catch landings is scarce and freely available, modifiable, fisheries apps were not available at the start of this trial. Instead of only consultancies offering their services apps, liaising with the following service providers for disseminating the PFZ, OSF, and Tsunami warnings through their Mobile Networks.

PFZ Advisory mobile application

Potential Fishing Zone (PFZ) advisories are useful to fishermen along the coastal areas. It also provides daily advisories to fisherfolk about the presence of chlorophyll, sea temperature, and water clarity and helps them easily locate areas of abundant fish in the ocean while saving on both fuel and time used to search for the same.

mKRISHI mobile application

mKRISHI® Fisheries is a mobile app developed by Tata Consultancy Services (TCS) Innovation Lab – Mumbai, in collaboration with ICAR- Central Marine Fisheries Research Institute and Indian National Centre for Ocean Information Services (INCOIS) Hyderabad. This app is a result of multi-dimensional research and fieldwork involving the best of the expertise of all the partner organizations. INCOIS generates Potential Fishing Zone (PFZ), a fish shoals' prediction information based on the remote sensing data received from NOAA satellites, sea surface temperature and the presence of phytoplankton which form the food of several fish species. mKRISHI® Fisheries app consolidates this information and presents advisories in a local language.

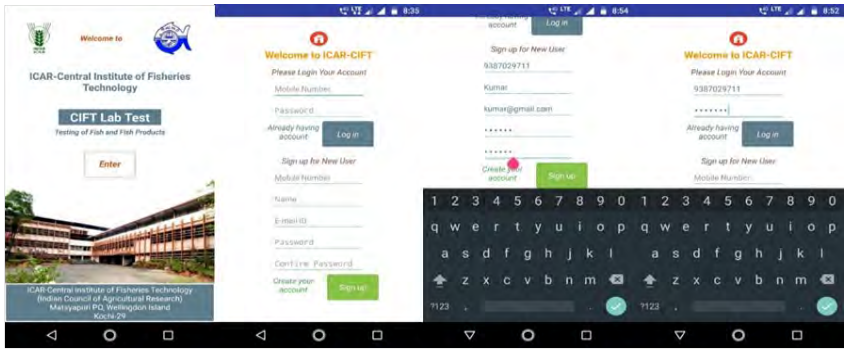
CIFT Lab Test mobile application

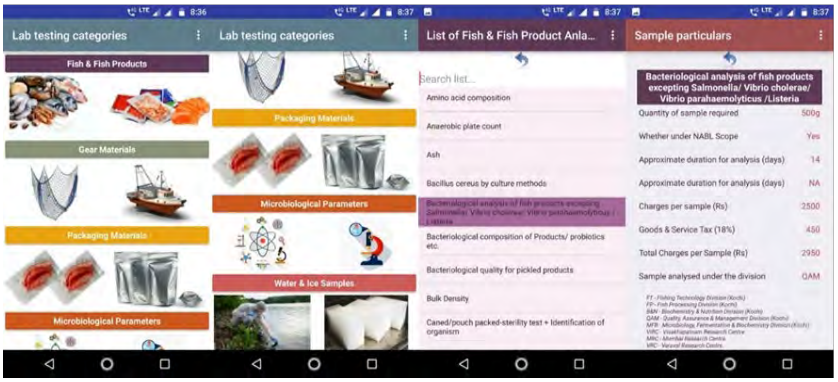
ICAR- Central Institute of Fisheries Technology, Cochin, an ISO 9001: 2008 certified organization has been recognized as a National Referral Laboratory for Fish and Fishery Products by the Food Safety and Standards Authority of India (FSSAI) under the Ministry of Health and Family Welfare, Government of India.

ICAR-CIFT has developed an innovative Mobile Application christened "CIFT Lab Test" intended for providing information related to different types of sample testing and analysis of various fish and fish-based products, fishing gear materials, packaging materials, microbiological parameters, quality parameters of ice and water samples, etc. This Mobile App may be useful for the aquaculture farmers, processing industries, and other stakeholders in the

sector to access the contents of different lab tests as per their interest online and get the desired information on the number of samples required, the time required for test report and cost particulars, etc. available at 24X7 times.

CIFT Lab Test

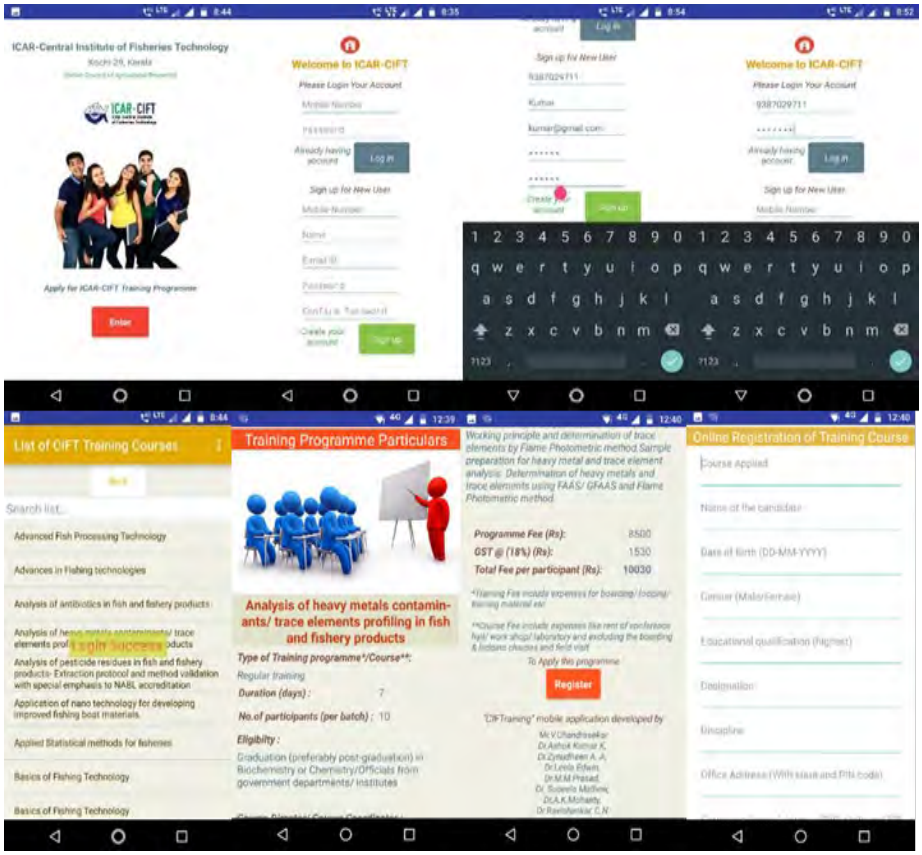




CIFTraining mobile application

ICAR-Central Institute of Fisheries Technology, Cochin has developed an innovative Mobile Application christened as "CIFTraining" that provides a complete package of information on ICAR-CIFT Training programs. This App is highly useful for the fisheries students, researchers, industry personnel, state extension personnel, fisheries-based entrepreneurs, fishers and other stakeholders in the sector to access the online information 24X7 times regarding different types of training programs in the field of Fishing Technology, Fish Processing, Biochemistry & Nutrition, Microbiology, Quality control, Engineering and Extension & Economics.

The "CIFTraining" Mobile App has embedded a total list of 68 types of clientele-based training programs available in ICAR-CIFT, which contain 60 regular training courses along with 2 comprehensives, 3 specialized and 3 certified courses covering the themes of seven divisions. The "CIFTraining" mobile app will help the stakeholders to search for the training of their interest and see the training program details like course contents, course fee, duration, eligibility, and other facilities at their fingertips so that the right stakeholder can opt for the right training program for improving the technical knowledge and skill in the concerned field. Finally applying for the training program through online registration mode.



Fisher Friend Mobile Application

Developed on Android mobile platform which supports English, Tamil, Telugu, Odia and Malayalam languages.

FFMA provides following facilities to fisher folks:

Potential Fishing Zone	Weather Forecast
GPS facility	Government Schemes
International Border Line Alert	Market Information
Ocean State Forecast	News
Disaster Alert	Important Contacts

E-Commerce in fishery

www.marinefishsales.com is developed under the NICRA project of ICAR-CMFRI as innovative multi-vendor e-commerce. The platform is made available as an android application for mobile phones to facilitate direct sales between fisherfolk and the customers. The app envisions reasonable prices as a direct sale between fishermen/farmer to consumer is facilitated.

Daily fish: The voyage of your 'Daily Fish' from 'catch' to 'kitchen' has never been so world-class. Daily Fish, the online seafood store serves you ready-to-cook seafood that is 'As good as Live' with all the goodness of nutrients stored in it. This is in step with the vision of Baby Marine; promoters of Daily Fish and one of the leading exporters of marine products from India

to Europe, the US, South America, Japan, South East Asia, Gulf, South Africa and Australia for over four decades.

Decision support system

A decision support system (DSS) is a computer-based application that collects, organizes and analyzes business data to facilitate quality business decision-making for management, operations and planning along the fisheries value chain. A well-designed DSS aids decision-makers in compiling a variety of data from many sources: raw data, documents, personal knowledge from employees, management, executives, and business models. DSS analysis helps companies to identify and solve problems and farm-level make decisions.

Types of Decision Support Systems (DSS)

These can be categorized into five types: Communication-driven, data-driven DSS, document-driven DSS, knowledge-driven DSS, and model-driven DSS

Example: Aqua manager is a comprehensive, integrated software solution for improved efficiency in aquaculture industries. It is a complete fish farming software that supports all stages of fish production, from hatchery to harvest.

Supply chain

Integrating technology into a supply chain can be a challenge, and the seafood industry is no exception with the advent of traceability technology that monitors the catch from water to plate. As more consumers demand to know where the fish they eat comes from, companies have started developing high-tech solutions to capture, receive and transmit data across every component of the seafood supply chain, from fishermen to processors, transporters, distributors, and retailers.

Traceability

Traceability is linked to the validity of seafood labels that boast about a product's sustainability, authenticity, location and other factors important to consumers. Providing a socially responsible product can translate to higher profit margins, enhanced customer loyalty, and improved brand reputation. Suppliers are under increased pressure from consumers and retailers to provide traceability for their products. Traceability is seen as a way to soothe such worries. Traceability technology can mitigate risks and limit the impact of public health incidents.

A unique ID code for fisheries and its application in traceability and data-sharing. The unique codes for fisheries maintained as part of the Global Record for Stocks and Fisheries (GRSF) will save time and money for the seafood supply chain, traceability/technology companies, governments, and non-governmental organizations (NGOs).

The GRSF, the Global Record of Stocks and Fisheries, integrates data from three authoritative sources: FIRMS (Fisheries and Resources Monitoring System), RAM (RAM Legacy Stock Assessment Database) and Fish Source (Program of the Sustainable Fisheries Partnership).

Expert Systems

Expert systems are computer applications developed to solve complex problems in a particular domain, at the level of extraordinary intelligence and expertise. Development of Expert System for Shrimp Aquaculture (ESSHA) involved five steps viz., problem selection, knowledge acquisition, knowledge representation, system design, and development as well as system validation (Zetian et al., 2005).

Expert Systems in Fisheries Sector

Expert systems are rapidly becoming an integral part of applications in several domains ranging from traditional manufacturing processes to applications in outer space. Expert systems have been shown to improve traditional approaches by as much as an order of magnitude. There are several areas, including fisheries and aquaculture, in which the return on investment in an expert system can be tremendous.

Social networking

The penetration of the internet and subsequent usage of social media, especially among the youth is increasing day by day. In this context, a study was conducted to identify the internet and social media usage by students as well as their mode of accessing professional (fisheries) information through social media. social media has been classified into two types, namely social networking sites, and Instant messaging applications based on both form and content of the media

Social Networking Sites	**Instant Messaging Applications**
Instagram	WhatsApp
Twitter	FB Messenger
Pinterest	Yahoo Messenger
Google plus	Skype
Google groups	Google Hangouts
Research Gate	IMO
Google Scholar	Snap Chat
Wikipedia	Viber
Facebook	Hike
YouTube	Telegram
LinkedIn	We Chat
Bharat Student	

The Department of Fisheries through the following agencies serves this sector.

Information source exposure: Seminar, workshop, Training programme, scientific books/ Literature, Fisheries related magazine and other publications, radio programme, Television programme, Exhibition, Newsletter, Mobile help line communication, Newspaper, NGOs and others.

Fisheries related government organisation:

a. Fisheries Department

- Kerala State Cooperative Federation for Fisheries development Ltd (Matsyafed), http://www.matsyafed.in/
- Agency for Development of Aquaculture, Kerala (ADAK),
- Kerala Fishermen's Welfare Fund (KFWEB),

- State Fisheries Resource Management Society (FIRMA),
- Fish Farmers Development Agency (FFDA),
- Kerala State Coastal Area Development Corporation (KSCADC),
- National Institute of Fisheries Administration and Management (NIFAM),
- Society for Assistance to fisherwomen (SAF)
- Kerala Aqua ventures international limited (KAVIL)

b. MPEDA, Fisheries College, Research institute, CMFRI,

c. KVK, ATIC, AFCA, CIFNET, CIFT, NGO.

Mass media

Newspaper, Magazine, Newsletter, Farm Journals, Periodicals, Exhibitions, TV, Radio, Internet, Video lessons.

Social organization

Village panchayat, Co-operative credit, Co-operative group, Fisheries co-operative society, Fishermen Association, Community organization, Harbour mechanized boat association.

Initiatives in Fisheries Sector and aquaculture in India (CIBA 2012)

Aquaculture is a technology-driven farming enterprise and aqua farmers are looking for quality information in time at an affordable cost. ICT aided tools like e-learning courses, publications, compact discs, short films, mobile telephony, Phone in a program, information kiosks, expert systems and decision support systems have been developed and implemented on a limited scale as projects or programs. Some of the initiatives are detailed below.

- E-learning courses on aquaculture
- The 'Phone-in Programme (PiP)
- Technology dissemination through mobile phones
- Village/ Rural Knowledge Centre
- Kisan Call Centre
- e-Sagu Aqua
- Aqua-Choupal
- e-TSA
- Decision Support Systems
- Farmer-friendly touch screen information kiosk on BMPs in shrimp culture
- One stop aqua shop
- Helpline

Latest technology used in the fisheries

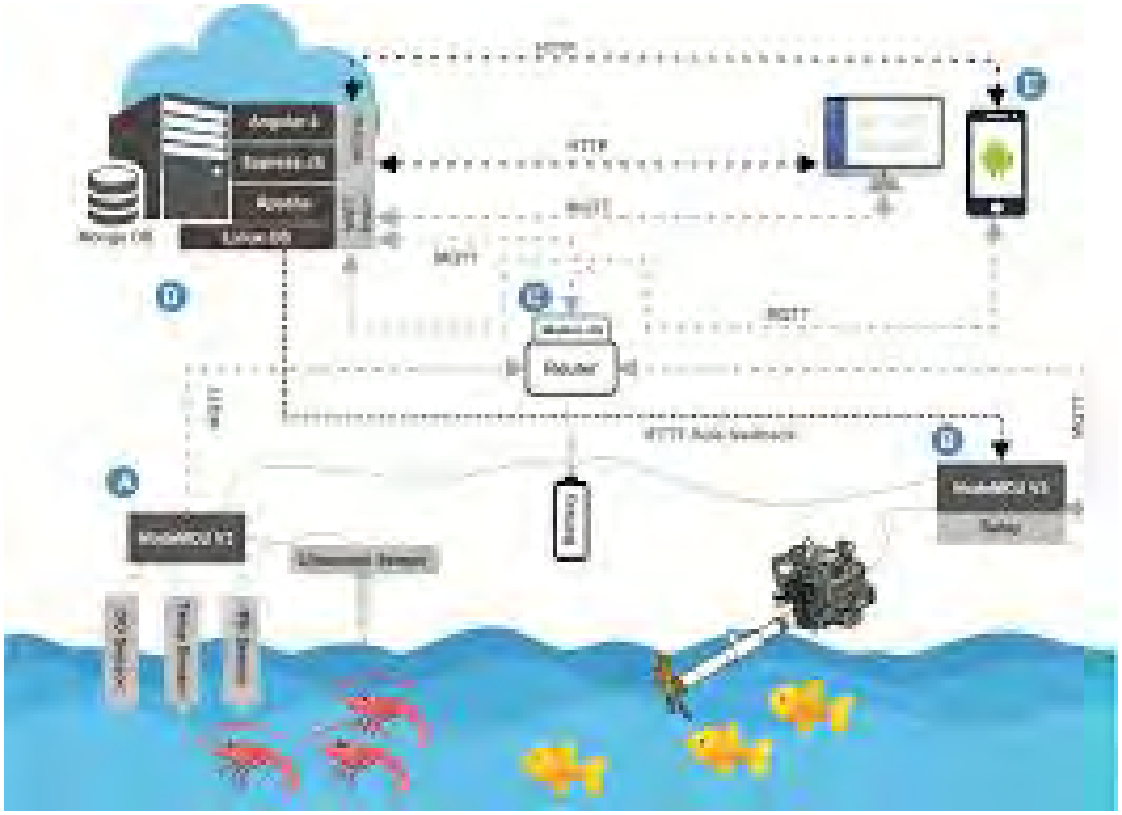
1. **Blockchain technology in fisheries**

It is mainly used to addressing the traceability issue in seafood industries by integrate fish farmers with blockchain solutions and gathering specific data on the environmental impact, feed, growth and fish health as these contribute as key factors when raising fish sustainably this traceability technology monitors the fish catch from water to plate.

- Transparent resourcing for marine conservation,
- Reducing pollution from plastics,
- Reducing slavery at sea
- Sustainable fisheries management.

IoT: Smart aquaculture farming enhance the value chain.

IoT make a tremendous change in both monitoring and automation of highly helpful to the aquaculture sector to operate remotely anywhere in the world. useful to know the real-time water parameter of the pond such as dissolved oxygen (DO), Temperature, pH, and water level. microcontroller development kits such as Arduino, Raspberry Pi, ESP etc. It will generate big data consciously in frequent intervals which will be sent to the cloud storage, which will be processed and accessed through the web portal or mobile application.



Artificial Intelligence in Fisheries

Artificial Intelligence (AI) by definition means 'the future made from the pieces of past'. These are programs that learn new solutions through experience. AI has been implemented in a variety of fields starting from agriculture to complete automation in industries. Through AI, fisheries sector can develop rapidly and production can be quadrupled within a short period as it makes aquaculture a less labor-intensive field. It can take the form of any labourers at work for example feeders, water quality control, harvesting, processing etc. In aquaculture feed costs itself nearly 60% of the total operation expenditure so reduce feed wastage increase profitability and also maintain water quality, hence AI feed dispenser releases right amount of feed at the right time, which will be remote control. Further AI read the fishes through vibration-based sensor and acoustic signals. Reduce cost of feed by about 21% measures and tracks the feeding pattern of stocks. AI programmed drones equipped with sensors can collect and analyze water quality data such as turbidity, temperature, dissolved oxygen.

AI in Fish Processing industries: Cutting, filleting, or cleaning the products can be done through programmed AI robots with much accuracy towards size, shape, and hygiene. Quality control and grading can be done through AI programs equipped with visual image sensors and

cameras. After grading, processed foods can even be packed and transported through AI robots. This makes zero labor cost and needs no human supervision.

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Post-Harvest Losses in Fish: An Economic Approach

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Introduction

Fish is a highly perishable commodity among the food commodities. Fish has evidence of serious loss from harvest to consumption but there was little documentation on the overall proportion of losses from fish. Assessment of post-harvest losses (PHL) in fish is a crucial challenge in developing countries. The fish supply chain involves many functionaries through them the fish is passed on from one stage to another stage. According to FAO, 1984, it has been estimated that almost 10 per cent of world fish catch in terms of weight is lost by poor handling and processing. In general, one-third of all the food produced for human consumption in the world is lost or wasted (1.3 billion tons.)

The objective of the post-harvest loss assessment in fish is mainly on determining the type of losses and measurement of the amount and extent of losses. PHL in fisheries is important as fish is considered the cheapest animal protein for the consumers which put restrictions in terms of food security and income loss on the actors of the fish supply chain. Post-harvest loss of fish is high than chicken and meat. Reduction of post-harvest losses is a vital development goal in the view of sustainable fisheries development. Under the Sustainable Development Goals (SDGs) it was recommended to 'by 2030, halve per capita global food waste at the retail and consumer levels and reduce food losses along production and supply chains, including post-harvest losses (Target 12.3).

Post-harvest loss in fish

Loss is defined as a reduction in the weight of edible products available for consumption. It is a measurable reduction in foodstuffs and may affect either quantity or quality" (Tyler and Gilman, 1979). The major proportion of loss is due to quality and economic losses than quantitative losses. Post-harvest fish losses are often caused by biochemical and microbiological spoilage changes that occur in fish after death. PHL refers to measurable quantitative and qualitative food loss in the post-harvest system. It is defined as the loss from various stages of harvesting to the stage of consumption resulting from qualitative loss, quantitative loss and the food waste. FAO has estimated that post-harvest losses in developing countries vary up to 50% of domestic fish production. Globally, 35% of fish and seafood losses occurred every year which includes 8% of fish harvested being thrown back into the sea.

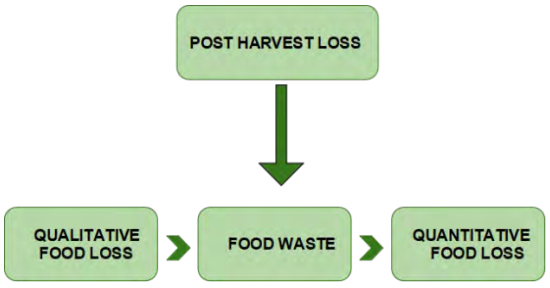


Fig. 1 Components of post-harvest loss

Types of post-harvest losses in fish

Post-harvest losses may occur in quantitative or qualitative terms, otherwise, it may be direct or indirect losses. Quality losses include those that affect the nutrient/caloric composition, the acceptability, and the edibility of a given product. These losses are generally more common in developed countries (Kader, 2002). Quantity losses refer to those that result in the loss of the amount of a product. Loss of quantity is more common in developing countries (Kitinoja and Gorny, 2010). Post-harvest losses are associated with loss of income, loss of quality, quantity of fish loss, loss of food, food insecurity and loss of nutritional value. According to Ward and Jefferies (2000), losses can be assessed by physical, quality and market force.

a. **Physical losses:** Physical loss is defined as fishes that are thrown away or eaten by insects, birds or animals. It is expressed in terms of losses in weight and/or monetary value.
b. **Quality losses:** Quality losses are associated with changes due to spoilage or physical damage but the fish is still sold, often for a low price. It is usually expressed in monetary terms.
c. **Market force losses:** Market force losses are the loss induced by market changes, in which fishermen are forced to sell their products at a price below their expectations. At later times, apart from three losses, nutritional loss was also included under the post-harvest loss assessment.
d. **Nutritional loss:** Nutritional loss refers to specific changes in the nutritional content or properties of fish as a result of spoilage or processing. Besides food wastage, all types of losses have certain financial implications in terms of resource sustainability and economic development.

Approaches to fish loss assessment

The estimate of post-harvest loss follows either a micro or macro approach depending on the objective and scope of the assessment (FAO, 2016).

a. ***Micro approach:***
   Micro approach estimates the fish loss for a particular single value chain usually located in limited geographical areas, based on direct physical measurements, observations or questionnaires to collect information directly from the actors of the fish value chain.
b. ***Macro approach:***
   Macro approach provides an estimate of the physical loss of the whole fishery sector at the national, regional, or global level using generally of secondary data from various sources.

Causes of post-harvest losses in fish

The post-harvest losses may occur both in terms of quantity and quality due to discards at sea, improper handling, storage and icing, lack of cold chain facilities and delay in transportation. The post-harvest losses at various stages of fish supply chain is presented in table1.

Table. 1. Causes of post-harvest fish losses

Stages	Causes	Type of loss
During fishing	Destructive/harmful methods of fishing resulted in inferior quality of fish	Physical, Quality
	Falling from the net and discarded as by-catch	Physical
	Setting fishing gear for long periods	Physical, quality
Handling fish onboard	Delay returning to landing centre after fishing and high temperature at sea	Physical, Quality
	Failure to wash and chill the fish onboard	Quality
During unloading	Poor hygiene practices causing contamination of fish	Quality
	Fish falling from basket/ crate to the floor	Physical
	Delayed bargaining at the first point of sale	Quality
	Theft at landing site during offloading of fish	Physical
Fresh fish marketing	Inadequate application of ice and no insulated container is used	Physical, Quality
	Limited preservation capacity during bumper catches	Physical, Quality
	Lack of marketing information	Physical, quality, market
	Delay in purchasing fish by traders	Quality
During processing and packaging	Processing of already spoiled / poor quality fish	Physical, quality
	Processing fish under unhygienic conditions	Physical, quality
	Inadequate control of heat intensity during smoking leads to over smoking of fish and possible burning	Physical, quality
	Drying fish under unsupervised places – on ground, rocks or herbs	Physical, quality
	Damage due to inadequate packaging method and materials	Physical, quality
	Oxidation of fatty acids leading to rancidity	Quality
During storage	Microbial growth causes spoilage	Quality
	Insect infestation	Physical, quality
	Discoloration due to chemical changes	Quality
	Inadequate storage facilities	Physical, quality
During Distribution	Delays due to problems with transport vehicles and inaccessible to production areas	Physical, quality
	Fish damage during transportation	Physical
During Marketing	Delay in selling	Quality
	Inadequate cold storage facilities and lack of ice	Physical, quality
	Delay in supplying to markets	Market
	Poor purchasing power of consumers	Market

Source: Torell et al. (2020)

Fish loss assessment methods (FLAMs)

In general, fish loss assessment methods are carried out by FAO methodology which includes the Informal Fish Loss Assessment Method (IFLAM), Load Tracking (LT) and the Questionnaire Loss Assessment Method (QLAM).

a. Informal Fish Loss Assessment Method (IFLAM)

IFLAM is also known as the exploratory loss assessment method. It is a rapid method used for loss assessment based on the Rapid and Participatory Rural Appraisal approach

including checklists and group discussions to identify the hotspots. It provides qualitative and indicative quantitative data on various issues related to losses.

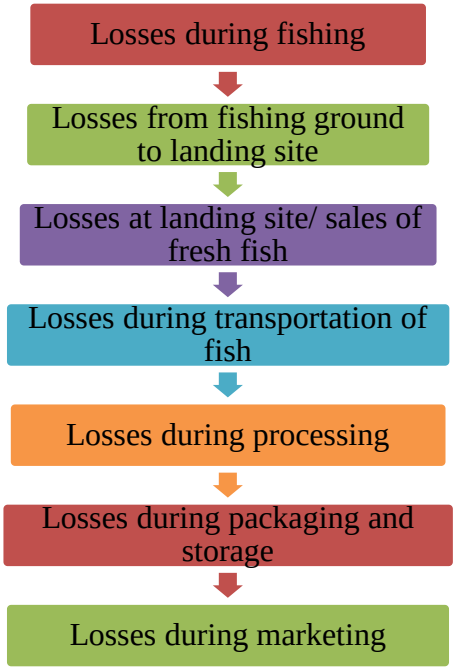
b. Load Tracking (LT)

LT is an experimental method that produces statistically valid results for the calculation of loss between stages in a distribution chain involving the loads at different stages. This method is most robust because of the experimental and replicable nature of the procedure.

c. Questionnaire Loss Assessment Method (QLAM)

QLAM is a formal survey-based method that provides quantitative data on issues such as types of loss, reasons for the loss, frequency of loss, and variables that affect the loss. This analysis of survey data provides quantitative information, which can be used to validate the IFLAM and LT methods.

The methodology uses both qualitative and quantitative survey methods which is the basis of the economic method/ approach. Loss assessment can be carried out at various stages and /or nodes of fish supply chain.



Source: FAO, 2011

Fig. 2 Stages in post-harvest loss in the fish supply chain

Strategies to reduce the post-harvest losses

Post-harvest losses can be effectively reduced by providing the proper infrastructure needed at various stages of fishing activities and regional-specific interventions are required to tackle the problem. The safety, quality assurance, and value addition need to be strengthened to reduce the PHFL. A sustainable loss reduction methodology incorporating capacity building of functionaries involved in the fish value chain is essential for an inclusive fisheries development.

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Career opportunities for Fisheries Graduates

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Introduction

Career opportunities for fisheries and aquaculture graduates are available in range of areas including state and central government agencies, academic institutions and private agencies manning hatcheries, fish farms and input supply. Employment in fisheries and aquaculture has grown substantially since 1980, with an annual average rate of 3.6 percent increase. The estimated demand of fisheries professionals and para-professionals will be in the range of 26,900 a year by 2022 (The Hindu,2018). With the existing intake capacity of 30 professional colleges in the country (ie. 1,079, 417 and 181 respectively for undergraduate, post-graduate and PhD programmes) there exists a huge demand-supply gap. There is also a need of skilled field workers. Opportunities for fisheries graduates exist in a wide range of avenues in the public and private sectors. Entry-level positions require undergraduate degrees or diplomas while higher-level positions often require master's or doctorate degrees.

State government jobs

Departments of fisheries at state level are the major agency dealing with the fisheries development in each state. Department and associated agencies offer employment opportunities for fisheries graduates like Fisheries Extension Officer, Assistant Fisheries Extension Officer, Fisheries Officer, Inspector of Fisheries etc. Recruitment to the posts is conducted Public Service Commission/ Service Selection Boards.

Central government

Career opportunities also exist in central agencies as technical officers and assistant directors in Maine Product Export Development Authority (MPEDA), National Fisheries Development Board(NFDB), Export Inspection Agency (EIA), Coastal Aquaculture Authority of India (CAA), Food Safety and Standards Authority of India (FSSI) and as Technical Officers and Scientists in Fisheries Survey of India (FSI), National Institute of Oceanography (NIO), Indian National Centre for Ocean and Information Services (INCOIS), Hyderabad, etc.

Academic Institutes

Positions in academic and research institutions demand master's or doctorate degrees, especially for the positions of Assistant professors and above. Recruitment to the position of scientists in various institutes (Agricultural Research Service) requires similar qualifications, where selection is conducted by Agricultural Scientist Recruitment Board (ASRB) through a national-level competitive examination.

Administrative jobs

The graduates can apply for various positions advertised by UPSC and State PSCs for various administrative jobs.

Financial institutions

In recent years, graduates from agriculture and allied sectors have opted for employment opportunities in public sector and private banks as probationary officers,

agricultural/rural development officers etc. National Bank for Agriculture and Rural Development also offer many positions for agriculture and allied sector graduates.

Private Sector

Jobs in private sector exist mainly with feed companies (manager/marketing manager), Hatcheries (Consultants), seafood processing firms (Quality consultants, EIC approved technologists etc).

Entrepreneurship

Fisheries and aquaculture sector has vast potential for entrepreneurship development. Fisheries graduates can initiate their own venture in the areas like ornamental fish culture, hatchery management, Agri-clinic and agribusiness centres, testing and analytical facilities, input supply etc. Technology incubation facility in fisheries research institutes and universities offer initial hand holding for entrepreneurship development.

Overseas employment opportunities

Fisheries graduates have lots of opportunities Middle East and African countries as seafood auditors, consultants in processing firms and hatcheries.

The above-mentioned opportunities are few among the wide range of opportunities available for fisheries graduates. There exists huge number of opportunities in research, extension, education, management and entrepreneurial fronts. Many such positions require further specialization.

Women in Fish-preneurship

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Introduction

Entrepreneurship has been universally recognized as the mainspring behind economic development of nations. (Baumol, 2002; Wennekers & Thurik, 1999). The factors contributing to entrepreneurship is still a debate and within that the role of personal and environmental conditions facilitate or hinder initiation, growth and sustainability of entrepreneurship. Growth of entrepreneurship in any country is primarily indicated by the number of potential entrepreneurs, who play a crucial role in the economy. Because of the same reason, they are considered as a national asset. By providing employment opportunities and giving more income to those involved and ultimately to the nation, entreprenuers help progress of the nations and this is more significant to the developing nations. Women also have a major role in developing entrepreneurship. In countries like India, where women comprise almost half the population,

Women are involved in many fisheries activities, although their degree and type of participation is variable depending on local cultural conditions. In small scale aquaculture, rural women's involvement could augment fish production, uplift their social and economic conditions and promote gender equality. This will enable them to participate productively and independently to improve their family's nutritional and living standards. The contributions of women to fisheries are often invisible, ignored, and unrecognized even though they represent 47% of the global fisheries workforce, especially in pre- and post-production activities. In some cases, they may even be the main source of family income as urban male migration and other social problems have led to an increased number of permanently or temporarily women headed households. Women outweighed men in fishing allied activities accounting about 67%. Among the major fishing allied activities, women dominated in peeling (96%), curing/processing (84%) and marketing (79%).

As international development agency USAID explained, *The Hidden Half*, "Without [women], boats would remain unprepared on shore, fish would not be processed for market and communities would be left uncared for.

Fisheries Sector- The existing issues

The catch from marine sector declines and at the maximum, it remains stagnant due to the following reasons

Entrepreneurship in Production Sector (Mainly men)

- Overfishing from sea
- Ocean Acidification
- Ghost Fishing
- Plastic pollution
- Habitat Destruction
- Declining catch /unit effort
- Class conflicts
- Occupational migration

- Job displacement(women)
- Low income to primary producers

MARINE

- Fishing- M
- Craft making- M
- Ancillary equipment making (winch, propeller, etc)-M
- Gear making- M…F
- Maintenance of fishing equipment -M
- Cage culture of marine spp etc-M..F
- Feed manufacturing -M…F
- Culture of Sea weed, Pearl etc- M…F
- Ornamental Fish rearing- M…F

INLAND

- Fishing- M
- Hatchery- M…F
- Culture of shrimp, fish, Crab, Mussel/Clam etc- M…F
- Feed manufacturing -M…F
- Manufacture of aquaculture equipment- M

Entrepreneurship in Processing Sector (Mainly women)

- Fish sorting - F
- Transportation- M
- Trading –M…F
- Ice manufacturing –M
- Preprocessing- F
- Processing –M…F
- (Making frozen/chilled fresh fish/ dry fish/value added product making etc)
- Fish value addition-based business (novel)- M…F
- (M- Male dominant, M- Male non-dominant. F- Female dominant and f- female non-dominant)

VALUE ADDITION- a special focus

Why to invest in food processing sector?

According to Ministry of Food Processing, with a huge population of 1.08 billion and population growth of about **1.6 % per annum**, India is a large and growing market for food products.

Its 350 million strong urban middle class with its changing food habits poses a huge market for agricultural products and processed food. Food processing industry will show the annual growth of **40-60 % in next five years**

Women as entrepreneurs

Female entrepreneurs represent the fastest growing category of entrepreneurship worldwide Maria et al (2020)

Earlier definitions frequently related entrepreneurship with creating new business (Yalcin and Kapu 2008) or maintaining existing business (Jones and Butler 1992; Lazear

2005); or both (Hebert and Link 1989; Lumpkin and Dess 1996; Sharma and Chrisman 1999; Bolton and Thompson 2004)

But when woman turn out to be entrepreneur, either as solo or group, the essential entrepreneurial qualities and skill sets, the pre-requisites for establishing the enterprise, the nature of support required from the growth of entrepreneurship eco system etc changes considerably.

The distinguishing Features of the Women Initiated Enterprises in Fisheries (WIFE) were identified and compared with those of well-defined entrepreneurship features

- Objective: **Passio**n vs profit
- Growth- **Rapid** Vs slow
- Leadership Traits: **Change** Vs no change
- Team Traits: care about **wins or profit** Vs care more about recurring duties and obligations.
- Management Strategy: **High risk& meticulous plan** throughout Vs **Comfortable with routines in long run**
- Idea- **Innovation** Vs **Proven**
- Market share- impact on a large number of people& their market share is usually quite **high**. Vs Smaller share of the market & provide service to a small number of people.

So, if there is such a perceptible difference, when can a woman be called an entrepreneur?

A woman can be called as an entrepreneur when she is a *confident, creative and innovative* woman desiring economic independence individually and simultaneously creating employment opportunities for others.

Thus, the women, mostly in group, when attempt to start a business, they are usually necessity entrepreneurs or venturing a small business or livelihood.. It's not out of passion, out of absolute necessity. Hence, women from coastal areas, when venturing into an already problematic sector, for making a livelihood, the outside environment, comprising institutions like financial, infra structural, market, technology, social support and information should provide a customising hand holding environment for them to sustain and grow. Such a supportive "Entrepreneurial eco system' is highly essential, especially in a developing country like India, to foster women entrepreneurship in fisheries.

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