IN-PLANT TRAINING UNDER STUDENT READY PROGRAMME

7th August to 29th September 2023

Organized by

ICAR-Central Institute of Fisheries Technology

CIFT Jn., Willingdon Island, Matsyapuri P.O., Cochin-682023, Kerala

Ph: 0484-2412300; E-mail: akmu.cift@icar.gov.in

IN-PLANT TRAINING UNDER STUDENT READY PROGRAM

for

Students of College of Fisheries, Raha, Assam

(07 August, 2023 - 29 September, 2023)

Course Directors

Dr. Bindu J.

Dr. Nikita Gopal

Course Co-ordinators

Dr. Binsi P. K.
Dr. Jeyakumari A.
Dr. Sreelakshmi K.R.
Dr. Pe Jeyya Jeyanthi
Dr. Rejula K
Dr. Renjith R. K.
Dr. Pankaj Kishore
Shri. Ezhil Nilavan
Shri. Anas K. K.
Dr. Neethu K. C.

Organized by

ICAR - Central Institute of Fisheries Technology
(Indian Council of Agricultural Research)
Willingdon Island, Matsyapuri P.O.,
Cochin-682 029, Kerala, India

Published by:
Director, ICAR-CIFT, Cochin-29

Compiled and Edited:
Sreelakshmi K.R.
Binsi P. K.
Jeyakumari A.
Renjith R. K.
Ezhil Nilavan
Anas K. K.
Neethu K. C.
Rejula .
Pe Jeyya Jeyanthi
Pankaj Kishore
Bindu J.
Nikita Gopal

For citing the book:
Sreelakshmi K.R., Binsi P. K., Jeyakumari A., Renjith R. K., Ezhil Nilavan, Anas K. K., Neethu K. C., K Rejula, Pe Jeyya Jeyanthi, Pankaj Kishore, Bindu, J., Nikita Gopal (eds) (2023) Training manual on In-plant training under student ready program, Central Institute of Fisheries Technology, Cochin, India.

Example for citing a chapter in the book:
Bindu, J (2023). Packaging of Fishery Products *In:* Sreelakshmi K.R., Binsi P. K., Jeyakumari A., Renjith R. K., Ezhil Nilavan, Anas K. K., Neethu K. C., K Rejula, Pe Jeyya Jeyanthi, Pankaj Kishore, Bindu, J., Nikita Gopal (eds) (2023) Training manual on In-plant training under student ready program, Central Institute of Fisheries Technology, Cochin, India. pp. 166-180.

Cover page design: Vijith Shaji

FOREWORD

ICAR-Central Institute of Fisheries Technology (CIFT) has developed various technologies in harvest and post-harvest sector of fisheries and many have been commercialized.

The practical knowledge is far different from bookish knowledge. The in-plant training under student READY program for B. F. Sc. students from College of Fisheries, Assam, assumes a greater importance as the expertise developed over many decades by the Institute could be shared with students that can help in building their future in relevant field. Over duration of 56 days, seventeen participants were exposed to various technologies. The topics for the program were selected to give a comprehensive knowledge on pre-processing and processing of fish and shellfish, quality control and conversion of the waste generated to high value products. The training program also consisted of different harvest practices, gear fabrication, nanotechnological applications, marketing and entrepreneurship development in fisheries. This training manual consists of 60 chapters including the topics on green harvesting technologies, post-harvest handling practices, microbial quality and food safety, antimicrobial resistance issues, nutraceutical and biomedical products, green technologies for extraction of biomolecules, recent engineering interventions in processing machineries, and the role of extension in fisheries. I am sure that this training manual will be very useful for the students so that the purpose of the training can be fulfilled.

Dr. George Ninan

Director

ICAR-Central Institute of Fisheries Technology

PREFACE

The increasing fishery resources calls for the development and adoption of new protocols for better utilization of fish meat and byproducts. This manual, through its various chapters, discusses different technologies used in the harvest sector of fisheries, opportunities for upgrading technologies in fish processing as well as better handling techniques, innovations in value addition, advanced packaging solutions, safety and quality assurance and fishery waste management. Furthermore, an attempt is made to consolidate the research inputs, particularly in the areas of bioactive compounds, recovery of biomolecules, and development of health and biomedical compounds. Topics covering HACCP, AMR in fisheries sector, and related extension tools have been included in order to address safety and quality issues associated with the utilization of fish and processing discards. Furthermore, a chapter is dedicated on basic statistical methods for research and tools for data analysis. We hope this publication will serve as a guide for students in sharpening their skills and enriching the career.

CONTENTS

1

RECENT ADVANCEMENTS IN FISH HARVEST SECTOR BY ICAR-CIFT

Remesan M. P.

Fishing Technology Division

Email ID: ***mpremesan@gmail.com***

With about 171 million tonnes of fish production which peaked at in 2016 globally, aquaculture contributed around 80.3 million tonnes and 90.9 million tonnes through the total capture production (FAO, 2018). Worldwide 59.6 million people engaged fisheries and aquaculture in 2006, out of that 19.3 million people engaged in aquaculture and 40.3 million people engaged in fisheries. 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 20.2 kg in 2015. Preliminary estimates for 2016 and 2017 pointed to further growth to about 20.3 and 20.5 kg, respectively (FAO, 2018). 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. The marine fisheries wealth is estimated to have the annual harvestable potential of 4.412 million metric tonnes. In the year 2017-18 the marine fish landings of India was 3.83 million tonnes which is 5.6% more than the preceding year (CMFRI, 2018). There were 1,99,141 fishing vessels operates in marine fisheries sector of India out of which mechanised, motorised and traditional artisanal vessels contributes about 36.5%, 36.9% and 26.6% respectively. Among the mechanized crafts fully owned by fishermen, 29% were trawlers, 43% were gillneters and 19% were dolnetters. Where as in terms of total catch landed during year 2017- 18, mechanized, motorized and artisanal contributed around 75%, 23% and 2% respectively (CMFRI, 2018). Indian marine fisheries resource supports the livelihood of about 4 million people. The increased demand for fish has prompted the development of new harvesting techniques mainly fuel-efficient and resources specific craft and gear and responsible fishing techniques. The recent developments in fish harvesting techniques are briefly reviewed in this chapter. 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. This institute has also been in the forefront of recommending standards for netting, netting yarn and netting twine used for fishing net and standardization of fishing gear accessories.

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 and built by Goa Shipyard Limited (GSL). 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 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. The ship's super structure above deck level has been made from FRP using the latest 'resin infusion technology' thereby significantly enhancing the sea keeping performance.

F V Sagar Haritha (LOA 19.75m, fuel efficient multipurpose fishing vessel)

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.

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. Large mesh demersal trawls, have been extensively adopted by mechanized fishermen of north-west coast, Mangalore and Kerala, for resources like Ribbonfish, Squid, Horse Mackerel, Mackerel and Pomfrets, due to its low drag and fuel efficiency. Fuel cost alone constitute up to 75% of operational expenditure. Drag offered by trawl depends on factors like design and rigging of the net alone contributed 58% of the total drag offered by a trawl. Estimation of drag of commercial trawls in Kerala reveal that it ranges from 1.5 to 49.0 kN according to the design used. 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. Trials of 24 m UHMWPE low drag trawl developed by ICAR-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.

Double Slotted V form otterboards : Experiments onboard CIFT research vessel and commercial vessels revealed that depending on the sea conditions 2-3 liters of diesel can be saved per hour of trawling, compared to the existing boards of same dimensions. The new technology has been readily accepted by the trawl owners and several trawlers in Kerala, Tamil Nadu and Karnataka have already started using the new otter boards. About 20 million liters of diesel can be saved annually in India if all the medium and large category trawlers adopt this technology. Further it helps to improve the income of fishermen and also reduce the emission greenhouse gases, which are the two most important needs in the fisheries sector globally.

V Form Double Slotted otter boards

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.

Cambered steel otter boards

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.

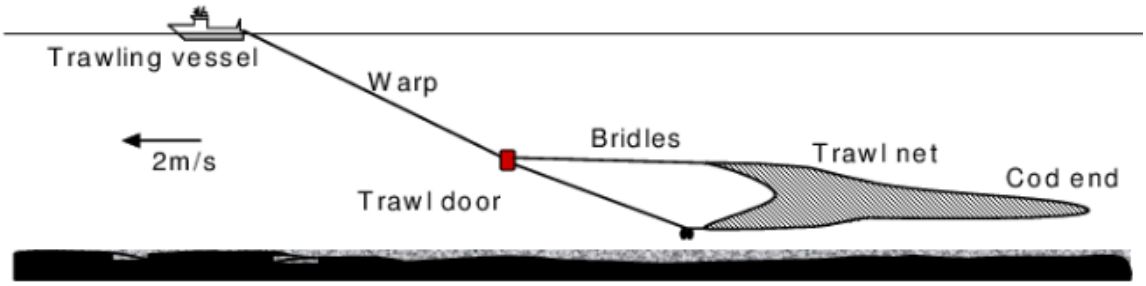
V Form otter boards

Low energy and eco-friendly harvest technologies for the inland fisheries and traditional marine sector

ICAR-CIFT Collapsible Fish Trap: ICAR-CIFT improved the design of traditional trap as collapsible fish trap (1 m×0.6 m×0.6m) with two rectangular and square frames with stainless steel. HDPE webbing of 80 mm mesh size is used as cover of the trap to allow fish to enter. Two entrance funnels made of plastic mesh are fixed on both sides. These traps were supplied to local fishermen and experimental trials were conducted along backwaters of Vypeen Island, Kumbalangi, Cheranellor and Varapuzha. Etroplus suratensis, Lutjanus argentimaculatus, Lates calcarifer, Epinephelus sp, Scylla serrata are the common target species and the trap can be set and hauled after 2-3 days of soaking. Average catch/haul is 1.5 kg. Design of the trap is simple and any fishermen can adopt the technology and is 40% lighter in weight and durability is 3- 4 times more than the conventional traps. Cost of ICAR-CIFT collapsible fish trap is only 50% of the conventional bamboo traps with same dimension. ICAR-CIFT collapsible fish trap will be a better option for the traditional fishermen to improve the livelihood.

Myctophid trawl (28.4 m & 45 m): Myctophids are the most abundant group of mesopelagic fishes in the Indian Ocean. About 137 species of myctophids are reported in the Indian Ocean. About 75% of total global catch of mesopelagic fishes is accounted by myctophids. Two mesopelagic trawls (28.4 m and 45m) with four equal panels were designed and operated from FORV Sagar Sampada and ICARCIFT research vessel R.V. Matsyakumari-II. Estimated trawl drag for 45 m trawl in terms of towing speeds of 2to 3kn range from 4.9 to 7.3 t. The new mid-water trawl system designed to attain largermouth area, smoothly tapering trawl body with small meshes in belly and codend, which can be towed at about 2.5 kn is adjudged to be appropriate, taking into consideration available information on biological characteristics and behavior of myctophids, fishing conditions and vessel characteristics.

ICAR-CIFT Off-bottom Trawl System (OBTS): Trawler fishermen in India cannot depend on shrimp and associated species alone for viable commercial operations any more, and there is need to adopt responsible alternate trawl systems for harvesting large demersal and semi-pelagic species. ICAR-CIFT developed as an alternative to shrimp trawling in the small-scale mechanized trawler sector, after extensive field-testing. It is capable of attaining catch rates beyond 200 kg h-1 in moderately productive grounds and selectively harvest fast swimming demersal and semi-pelagic finfishes and cephalopods, which are generally beyond the reach of conventional bottom trawls, currently used in commercial trawl fisheries in India. ICAR-CIFT OBTS has been developed and perfected after extensive field trials and observations, using acoustic gear monitoring instrumentation and inference from statistical evaluation of catch, over an extended period.



Representation of ICAR-CIFT Off-bottom Trawl System (OBTS)

Large mesh gillnet and monofilament long lines in Lakshadweep: 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 gillnets and monolines (monofilament long lines) in Lakshadweep waters, for targeted fishing of Tunas, Billfishes, Seerfishes, Carangids and Perches, in an effort to diversify fishing methods and improve catching efficiency.

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 ICARCIFT has led to the revival of small mechanized purse seine fishery in Kerala. The changeover of mesh size in the purse seine from the conventional 20 mm to 45 mm has shown good results and the purse seiners has been able to land larger size classes of high value species. The traditional fishermen and the purse seiners were targeting small pelagic like anchovies, sardines and small mackerels in the coastal waters. The purse seiners were also targeting the same resource in the coastal waters. There was severe competition and rifts between the tradition and mechanized purse seiners. 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 watersExperimental fishing operations carried out from the purse seiner Bharat Darshan during the period 2007-10 in the depth range of 50 to 220 m revealed that the catch mainly comprised of large sized mackerels (62%), followed by tunas (16%), carangids (14%), miscellaneous fishes (6%) and pomfrets (2%). All the mechanised purse seiners based at the Cochin Fisheries harbour, Kerala have changed over to 45 mm mesh size purse seines and started operations in the deeper waters targeting skipjack tuna, little tunnies, carangids, black pomfrets, horse mackerels, barracudas, seerfish and mackerel.

Bycatch Reduction Devices (BRDs) for responsible fishing and sustainable resources

BRDs for trawls: Among the different types of fishing, trawling accounts for the highest rate of bycatch along with the target species. Almost 70-90% of the trawl catch is bycatch, among which, about 40% is constituted by juveniles that are invariably discarded resulting in two serious consequences- depletion of the resources and pollution of the marine water and the consequential threat to the ecosystem. Further, higher the quantum of bycatch the less will be the economic benefit accruing from the fishing operation. Bycatch is unavoidable in any fishing operation; only its quantities vary according to the type of the gear and its operation. Therefore, one of the important research focuses of the Fishing Technology Division was development of bycatch reduction devices. Bycatch reduction device (BRD) is a device aimed at reducing the catch of non-targeted and unwanted species of fish in shrimp trawling. While BRD is a broad term used to describe any device that can be employed to eliminate or reduce the bycatch, turtle excluder device (TED), though in principle a BRD, is a specialized form of BRD designed to eliminate turtles, sharks and rays also from the trawl. These devices have been designed and developed taking into consideration the differential size and behaviour pattern of shrimp and fish inside the net. BRDs include Fisheye which is stainless steel escape chute attached in the codend for the escape of actively swimming finfishes and rigid grid devices; and soft BRDs such as square mesh windows, Bigeye, Sieve net and International Award winning design Juvenile Excluder cum Shrimp Sorting Device (JFE-SSD) which have been evaluated and recommended for use in Indian waters. Various protection measures have been adopted the world over, including India, for its protection. ICAR-CIFT has developed an indigenous design of the turtle excluder device which is appropriate for the Indian conditions. ICAR-CIFT-TED is a single grid hard TED with top opening of 1000x800 mm grid size for use by small and medium mechanized trawlers operating in Indian waters. In the TED developed by ICAR-CIFT, great care has been taken to ensure 100% escapement of the turtles while escapement of fish and shrimp at the minimum possible level

Low-cost substitutes for conventional craft materials

Traditionally, wood is used for construction of fishing vessels in India which has become scarce and costlier. Focused attention has been given in identifying alternate materials for fishing vessel construction, in order to reduce the dependence on traditional scarce wood species. Cheaper and readily available cultivated wood species with short life cycle such as rubber wood, fortified with dual preservative treatment using 7.5% ASCU and creosote, has been identified for construction of canoes operated in backwater and coastal fisheries. A number of preservative treated rubber wood canoes have been distributed for field operations by fishermen groups and cooperatives. The cost of the canoe is 35 – 40% less than a canoe of same size built of 'Anjili', the usually used wood. This saves the depleting forest wealth, helps the rubber farmer to get a better price for his underutilized wood and gives a durable, maintenance free boat at affordable cost to the poor fisherman especially of the South West

and North East where rubber trees are grown. Designs of fiberglass crafts have been developed for operation in inland waters. Fibreglass sheathing as protection against borer attack and biodeterioaration and as preventive against environmental pollution while using preservative treated wood in boat construction has been popularized, in traditional sector. Use of Aluminium alloy for construction of inland and coastal fishing craft has been demonstrated. Durability, light weight, corrosion resistance, toughness and resilience, low maintenance and high re-sale value make aluminum alloy a good material for construction of fishing craft.

Treated Rubber Wood Canoe: Central Institute of Fisheries Technology has evolved a simple technology for development of traditional fishing canoe from the rubber wood, which comes as a waste from rubber plantations. Though rubber wood is comparable to many structural timbers in terms of mechanical properties and working qualities, it is highly perishable under marine conditions. The study proved rubber wood as suitable for construction of canoe after upgrading by chemical preservative treatment. The conventional prime quality boat building timbers are very scarce and have become very costly. Traditional fishermen using wooden canoe find it extremely difficult to afford the cost. The new technology can reduce construction cost of small canoes by 35-40%.

FRP-Sheathed Rubber Wood Canoe: ICAR-Central Institute of Fisheries Technology has developed a fibre glass reinforced plastic

(FRP) sheathed rubber wood canoe for operation in marine and inland waters. The rubber wood, which comes as a waste from rubber plantations is upgraded through chemical preservative treatment and the canoe made using the treated wood is further given a sheathing of FRP. The technology has made possible the utilization of rubber wood and also provided additional dimensional stability through sheathing. The FRP sheathing provides water proofing, reduces maintenance, resistance to impact and abrasion and prevents attack of marine borers and other decay causing organisms besides giving an extended service life and better appearance for the wooden canoe. Canoe made of treated rubber wood and sheathed with FRP will give a maintenance free service life of 15-20 years.

Nano Cerium oxide, Titanium oxide & Iron oxide coating for corrosion resistance in boat building steel: BIS 2062 carbon steel is extensively used for fishing boat construction and is highly susceptible for corrosion in the hull, welding joints and coating failures under marine environments. This technology demonstrates the application of novel multifunctional nano metal oxide mixtures comprised of iron, titanium and cerium as marine coating to prevent corrosion. The electrochemical performance of nano metal oxide mixture coatings, applied over boat building steel, was evaluated inNaCl medium. The thin film surface coatings showed an efficient corrosion resistance with increased polarization resistance and low corrosion current density. The electrochemical impedance spectral data exhibited the improvement in the polarization resistance of outermost surface and internal layers. The coating responded

faster recovery to normal state when subjected to an induced stress over the coating. The nano material in the coating behaves as a semiconductor; this enhanced electronic activity over the surface of the steel. The photo oxidation behavior of Fe2O3 and TiO2, deter the microbial attack

Biofouling resistant polyethylene cage aquaculture nettings: A new approach using polyaniline and nano copper oxide: Biofouling in aquaculture cage nets causes occlusion of mesh openings, thereby increasing weight and drag, deformation of cages due to the ensuing stress, reduction of volume, thereby decreased stocking density per area, anoxic condition due to disruption of dissolved oxygen flow, blocking of food waste diffusion, restriction of water exchange, increased hydrodynamic force, all of which adversely impacted fish health. It has been reported that removal of fouling from a cage net costs 25% of the total project budget. Cages are fabricated mainly with high density netting whose non polar nature makes incorporation of antifouling biocides difficult. The surface of polyethylene needs to be modified to develop strategies against fouling. The novel approach employed by ICAR-CIFT was to synthesise a coating of polar or conducting molecule over non-polar polyethylene to incorporate antifouling biocides thereby rendering protection to protect the polyethylene aquaculture cage nets from biofouling.

Use of advanced fish finding and navigation techniques

Recent advances in technology have provided fishermen with equipment to reach the potential fishing ground accurately (Global Positioning Systems), detect the presence of fish acoustically (echosounder and sonar), thus saving the search time and fishing time and hence saving energy. These advances in technology have been popularized among fishermen, in collaboration with agencies like MPEDA and Department of Fisheries, for bringing down fuel use and environmental impact through fuel use. This, coupled with affordability and subsidy support, has resulted in significant penetration of GPS and Echosounder among small mechanized commercial fishermen, all along the coast.

Fishing craft and gear materials

Various cost effective protective measures against bio deterioration of wooden fishing vessels have been developed and are in use. Use of low cost timber like rubber and coconut have also been experimented successfully for small canoes. In India, ICAR-CIFT which plays a major role in the development of harvest technologies has also developed aluminium alloy sheathing for wooden fishing vessels, cathodic protection against marine corrosion in fishing boats, new substitutes for propeller material for cost savings, marine anti-corrosive paints, marine antifouling paints, chemical wood preservatives, indigenous resin based protective coatings for wooden crafts, ferrocement for boat building, rubber wood canoes, fibreglass reinforced plastic coated fishing canoes. Primarily, mechanized boats were using local gear. Major advances in fibre technology, along with the introduction of modern gear materials, have directly influenced

and brought about important changes in the design, dimensions and method of handling fishing gears. Extensive use of synthetic materials like PA, PE and PP have perceived in 1960s which created a revolution in fabrication of fishing gears. Today, the entire fisheries sector uses only synthetic fibers for gears. Twisted netting yarns and braided netting yarns of different sizes are available in the country. Combination rope of Polyethylene and Polypropylene (Danline) and Polyamide monofilament is being extensively used as an import substitute for tuna and shark longlines. The development of combination wire rope as an import substitute for deep-sea fishing is a recent innovation which has now been commercialised. ICAR-CIFT has standardised specifications for the use of polypropylene multifilament netting yarn with lower specific gravity and better tenacity than nylon (Silas, 2003; Meenakumari, 2011).

Conclusion

In recent years, the developments in harvest technologies in fisheries sector have taken place rapidly. ICAR-Central Institute of Fisheries Technology has contributed greatly to the revolution of fishing industry as well as technology diffusion programmes in a very significant way in the fisheries sector across India. While the fisheries sector is facing challenges in terms of excess capacity of fleet, diminution of fish resources and degradation of the fisheries environment in the coastal waters. The under-utilised and resources in the deeper waters hold potential along with rapid expansion envisaged. It's very imperative to have appropriate technology for application of resource conservation in the shelf waters under an appropriate management plan and diversification of fishing to unconventional resources such as mesopelagics, oceanic cephalopods and large pelagics in the deeper waters. Minimisation of harvest and post-harvest losses, development of technologies for reducing carbon and ecological footprints in the harvest sectors are areas which need focussed attention. Today fisheries sector is watched by many as a sunrise sector as it helps in alleviating food security as well as supports many auxiliary sectors. ICAR-CIFT has major contributions for this transformation. Over the years institute has carried out research on harvest and post-harvest aspects of fish extensively based on the sectors need and developed many ready-to-transfer technologies. Notable ones in harvest sector include design and construction of fishing vessels, eco-friendly fishing gear, satellite-based imaging systems to locate fishing grounds, and automated fish hauling systems. The research information generated is transferred to the end users by adopting suitable extension methodologies

References

FAO, IFAD, UNICEF, WFP and WHO. 2018. The State of Food Security and Nutrition in the World 2018. Building climate resilience for food security and nutrition. Rome, FAO.

CMFRI 2018 Annual Report. 302 p, Technical Report. ICAR-Central Marine Fisheries Research Institute, Kochi.

Gopal, N. and Leela Edwin, 2013, Technology Evaluation Model for Rural Innovations – Case Study of Rubberwood Fishing Craft for the Small-scale Fisheries Sector Fishery Technology. 50(4): 331p

****

2

INDIAN DEEPSEA FISHING: STATUS AND CHALLENGES

Renjith R. K.
Fishing Technology Division
Email ID: ***renjith.rk@icar.gov.in***

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

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.

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.

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.

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 nonconventional 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.

****

3

FISHING GEAR MATERIALS & IDENTIFICATION

Sandhya K. M.
Fishing Technology Division
Email ID: ***sandhyafrm@gmail.com***

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 thea 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

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

Reagent	Type of fibre			
	PA 6	PES	PE	PP
Hydrochloric acid/HCL (37%) 30 minutes at room temperature	+	o	o	o
Sulphuric acid/H2SO4 (97-98%) 30 minutes at room temperature	+	+	o	o
(1) Dimethylformamide/HCON (CH3)2 5 minutes boiling	+	+	o (2)	o (2)

Formic acid/HCOOH (96-100%) 30 minutes at room temperature	+	o	o	o
Glacial acetic acid/CH3-COOH 5 minutes boiling	+	o	o	o
Xylene/C6H4 (CH3)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

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

Reference

Hameed, M.S. and Boopendranath, M.R. (2000) Modern Fishing Gear Technology, DayaPublishing House, Delhi: 186 p.

Klust, G. (1982). Netting materials for fishing gear, FAO, Fishing News (Books) Ltd., England 75p.

Meenakumari, B. (2009). Fishing gear materials. (Meenakumari, B., Boopendranath, M.R., Pravin, P., Thomas, S.N. and Edwin, L. eds), Handbook of Fishing Technology , Central Institute of Fisheries Technology, Cochin: 372 p

Meenakumari, B. and Radhalekshmi. K (2003). Synthetic Fish Netting Yarns, CIFT Special bulletion No.11, CIFT Cochin:38p

Muhammed Sherief, P. S., Sreejith, P. T., Sayana, K. A., Dhiju Das P. H., Saly N. Thomas., Remesan, M. P. and Leela Edwin. (2015). Drift Gillnets made of Sapphire® and Polyamide in Gujarat, India, Fish. Technol. 51: 62-66

Tang H, Hu F, Xu L, Dong S, Zhou C, Wang X. (2019). Variations in hydrodynamic characteristics of netting panels with various twine materials, knot types, and weave patterns at small attack angles. Sci Rep. 9(1):1923.

Thomas,S. N. and Edwin, L. (2012) UHMWPE-The strongest fibre enters the fisheries sector, Fish Technol. Newsletter 23(4) : 3-7

****

4

DESIGN CONSIDERATIONS FOR FABRICATION OF BYCATCH REDUCTION DEVICES

Paras Nath Jha and Renjith R.K.
Fishing Technology Division
Email ID: ***paras.jhaa@icar.gov.in***

With the development and broader availability of synthetic gear materials, recent advances in vessel technology, navigational electronics, gear handling machinery, fish detection methods and fish behaviour studies, large-scale changes have taken place in the design, fabrication, operation and catching capacity of modern fishing gears such as trawls, purse seines and long lines. Widely used traditional fishing gears such as entangling nets, hooks and lines and traps have also benefited by way of design upgradation and efficiency improvement in the recent years. New innovative fishing systems such as electrical fishing, light-assisted fishing, FAD-assisted fishing and fish pumps have also been developed and accepted in different parts of the world. Design process for fishing gear has been greatly influenced in recent years by resource management and conservation, environmental safety and energy efficiency imperatives.

Mechanisms of fish capture

There are different systems of fish harvesting used in the world, ranging from primitive to highly sophisticated systems. Fishing gear varies with structure, materials used, principle of capture process and method of operation. The selection of fishing gear mostly dependent on fish species, environmental factors and fishing ground conditions. Fishing gear use five mechanisms to capture fishes

1. Gilling and Tangling
2. Trapping
3. Filtering
4. Hooking and spearing
5. Pumping

The most commercially used fishing gears are – Purse seine and Trawl net followed by gillnet, entangling nets and traps. Based on the usage of material of construction the fishing gears are grouped into – 1. Net fishing gear – Fishing with netting which is constructed with webbing – Gillnets, Trawl nets, Purse seines etc. 2. Tackles – fishing gear in which hooks are an

important part and catch fish individually – Hooks and lines 3. Miscellaneous gears – Traps, Grappling and wounding, stupefying methods and 3electrical fishing.

Factors affecting fishing gear design

Important factors which influence the design of fishing gears are (i) biology, behaviour and distribution of target species; (ii) fishing depth, current and visibility; (iii) sea bottom conditions; and (iv)other factors such as the scale of operations, size and engine power of fishing vessel, energy conservation objectives, selectivity and resource conservation objectives.

Design of fishing gear is greatly influenced by biological characteristics such as body size and shape, feeding habits and swimming speed; behavior in the vicinity of fishing gear and during capture process; spatial distribution and aggregation behavior of the target species. Body size and shape determine the mesh size required to enmesh and hold the fish in gill in nets and the mesh size to retain the target size groups of the species without gilling in the trawls, seines and traps. Body size is also related to the tensile strength requirements for the netting twine in gill nets and hook size and lines in hook and lines. Feeding habit of the target species is more important in passive fishing methods like hook and line and traps where the fish is attracted by the bait, and in the active fishing methods like troll line used for catching predatory fishes Consideration of the swimming speed of the target species is more important particularly in the active fishing methods like trawling, seining and trolling. Fishes are known to sustain a cruising speed of 3-4 body lengths per second for short duration. Catching efficiency is maximized when the vertical opening of the trawl mouth, vertical dimension in gill nets, and the catenary of the main line of the long line with branch of lines and hooks, coincide with the vertical range of the layer of maximum fish abundance. Hence knowledge of the vertical distribution of the target species could be used to optimize the horizontal and vertical dimensions of the netting panels in gill nets, main line catenary in long line and mouth configuration in trawls

Hydro-acoustic pressure increases approximately at the rate of one-unit atmospheric pressure (I bar) for every 10m depth. Buoyancy elements used in the deep sea fishing gear such as deep sea trawls, gill nets and bottom vertical lines have to be strong enough to withstand the high pressure at the fishing depth. Prevailing strong currents in the fishing grounds may restrict the choice of fishing gears to longlines and gillnets which are less affected by currents. Light levels at the fishing depth could influence the fishing success, as vision of fish is affected by light levels. In passive fishing gears such as gillnets, visibility of netting panel adversely affects fishing efficiency, visibility is again negatively indicated in hook and line operation while in light-assisted jigging-controlled light plays an important part.

Rough sea bottom conditions limit the operation of most of the fishing gears close to the ground except handlines, vertical longlines, bottom vertical longlines and traps. Trawling on

rough bottom requires special rigging such as bobbin rig on rock hopper rig, improvements in trawl design to minimize gear damage or loss and selection of appropriate otter boards. Design features of fishing gears will also be influenced by the scale of operations, size and engine power of fishing vessel, energy conservation objectives, selectivity and resource conservation objectives, catch volume requirements, operational and handling requirements of the gear, prevailing weather conditions, skill required for fabrication, maintenance and operation, material availability, local traditions and economic considerations.

Gear Based Technical Interventions – considerations

The appropriate match between MMS (Minimum Mesh Size) and MLS (Minimum Legal Size) is a particular problem in multispecies fisheries. The link between MLS, gear selectivity, and discarding rate is often poorly understood. Stress-induced behavioral deficits increase the risk of predation in the hours or days after the encounter (Behavioral impairment of fish escaping trawls). Behavioral impairment not measured in the field and survival studies, which traditionally use enclosures to measure mortality, do not have predation risk. Reflex action mortality predictors are often employed, but may not mimic the actual field conditions. There is a need to go beyond well-known areas of research and to define possible behavioral ecology frameworks. The crucial questions that need to be answered before implementation of any gear based technical measure:

How large a change in gear/mesh size is possible?

How long will it take to realize economic benefits and who will get them?

How easily can the fishers manipulate selective properties of gear, legally and illegally?

How to compensate for potential catch losses?

How much does it cost to improve gear selectivity and who will pay?

Are other unrelated measures, more efficient to improving stock status and future conditions?

Conclusion

Gear based technical devices can address juvenile bycatch to a great extent. The most practical method in juvenile exclusion in a multi species multi gear system like ours, is gear based modifications. Considering the devices and operation, we can see that the structure and size made them effective and easy to incorporate in the existing fishing gear. Most of the devices like fish eye and hooks have considerably less installation procedure. Since most of these devices, are based on interactions and escapement, mortality could be an issue. Devices that avoid direct contact such as aberration and funnelling are safe to operate whereas filtering type of excluding devices are harmful and increase mortality. The best option would be to avoid the interaction by spatial / temporal closures based on prior knowledge. Which is in fact leads to socio economic adversity as the lean periods coincides with restricted fishing season. Alternative livelihood is yet a hurdle in the under developed and developing countries. Economic status of fisherfolk should be considered while implementing such restrictions.

Studies to be taking up for accessing behavioural responses of fish, to avoid interaction of non-targets are novel approach yet taking pace gradually, could serve a better alternative option. Close association with fishermen community will help significantly in the outcome of the studies carried out by research institutions. Monitoring the practices at sea is the bigger task hardly affordable by legislative bodies. Development of MCS systems in the sector will play a better role.

****

5

FISHING VESSELS OF INDIA: AN UPDATE

M.V.Baiju

Fishing Technology Division

Email ID: ***vishnubaiju@yahoo.com***

According to CMFRI, 2016 the total number of fishing vessels in India is 1,66,333. Of this 30,772 are mechanized trawlers, 6,548 are gill netters 3,396 are Dolnetters. The total mechanized fishing vessels are 42,985. The total number of motorised fishing vessels is 97,659.

Types of fishing 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 and catamaran.bAndaman, coastal sates use artisanal boats as shown below.

Traditional fishing vessel: Traditional vessels use small engines for propulsion, but there is no insulated or cold storage for fish. The traditional gillnetters and liners used in Andaman is shown below:

These are vessels using traditional methods for fishing and use no deck equipment such as winch. No insulated/cold storage onboard these vessels. No wheel house and accommodation onboard. In general these boats are simple traditional fishing vessels only.

Motorised vessels: Vessels fitted with motors for propulsion, like the ring seiners- inboard engine fitted as shown below.

Mechanised vessels: Mechanised vessels use engine power for cruising and fishing. They use mechanical/hydraulic/electric power for fishing gear handling. These vessels are installed with insulated/cold storage/freezer storage onboard.

Accommodation, galley, toilet are also made in the modern for multiday commercial fishing vessels. Communication, life saving, fire control, light and sound signals, etc onboard The most common commercial fishing vessels are trawler, gillnetters, Liners, seiners and combination fishing vessels. Trawlers include stern trawlers, side trawlers, factory trawlers and pair trawlers. Liners consist of hand liners, long liners and pole and liners. Seiners are purse seiners and ring seiners.

Types of mechanised fishing vessels are:

Trawler

-Side trawler

-Stern trawler

Hydraulic trawl winch

Stern trawler

Seiner

Purse seiner: A commercial Purse seiner operating in Goa is shown below.

Ring Seiner: A traditional Ring seiner is shown below.

Gill netters: Boats and canoes use gill net in inland waters. The decked small gill netters fish in coastal waters and medium sized vessels operate gillnets in offshore. Small gillnetters have their wheelhouse either aft or forward. On medium sized vessels, using drifting gillnets and called drifters, the bridge is usually located aft.

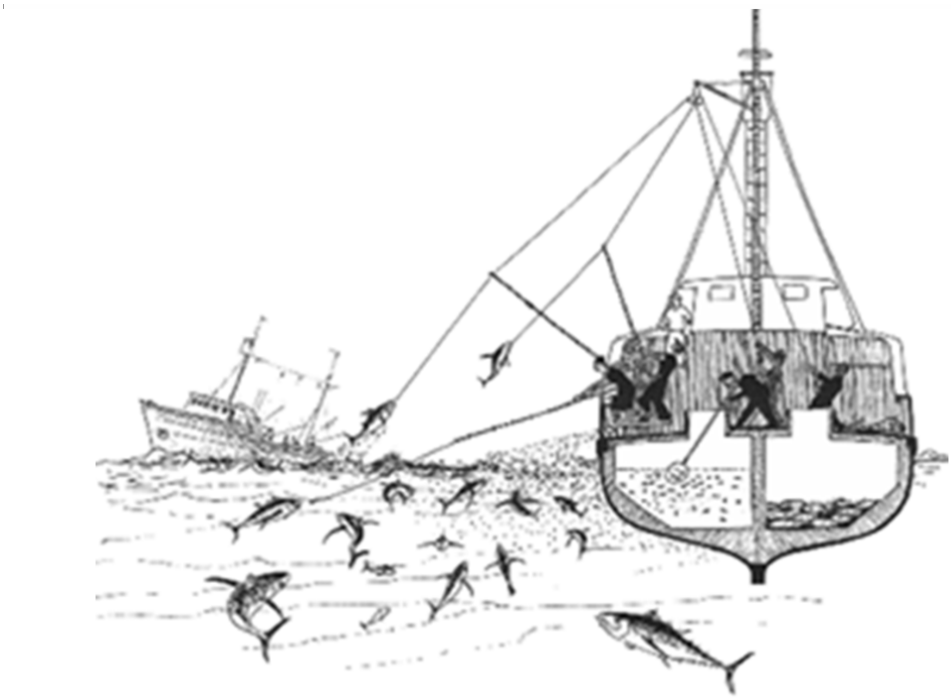
On small vessels setting and hauling operations are performed by hand. Larger vessels are often equipped with hydraulic net haulers or net drums.

-Hand liner

-Long liner

-Pole and liner; In Lakshadweep, Pole and liner are used to catch tunas shown below.

Trollers: Use many lines with hook attached to the mast. The vessels move forward and fish tries to catch the baited hook and gets caught as shown below.



Multi purpose fishing vessels: A most common combination is Gill netter cum Long liner and Trawler cum Long liner is shown below

There are also fishery research, carriers, fishery training vessels and fishery survey vessels. A research vessel of CIFT is given below.

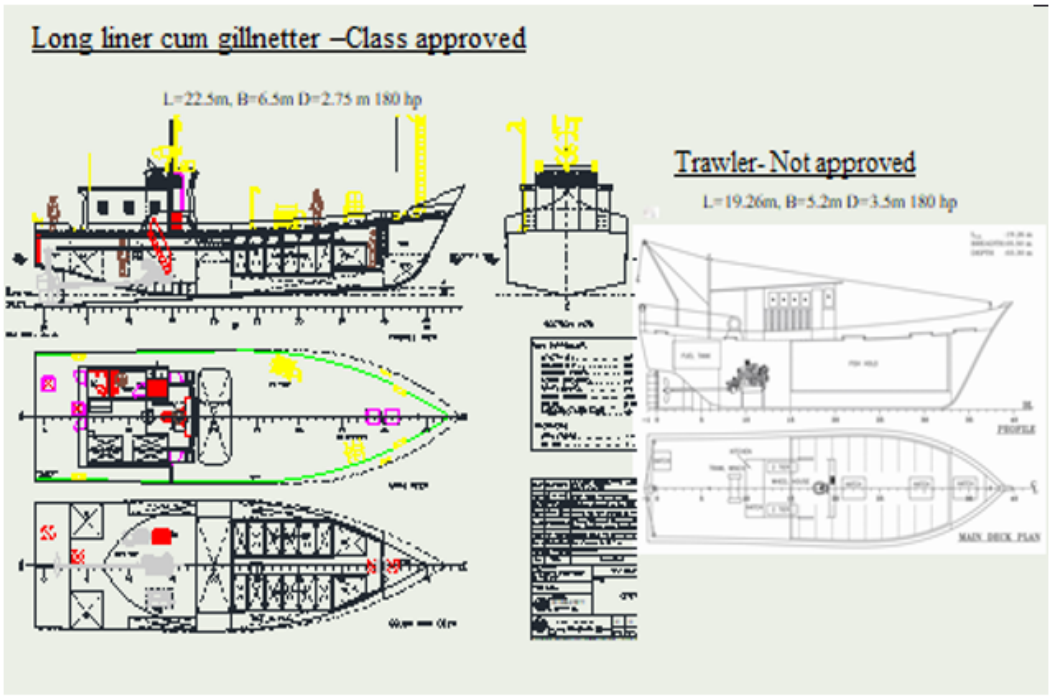
Design and construction of fishing vessels

The sea going boats and ships are designed and constructed based on the rules of the classification societies and the registering authorities of the flag nation. This ensures the structural and operational safety of the vessel as well as the crew, cargo and other items

onboard. Class or National Standard organisation approved raw materials only shall be used for the construction. Main engine, valves and other machinery ate to be approved type. Design of fishing vessel plays a vital role in fuel efficiency. Optimization of hull forms is the most

effective and logical way to reduce the drag force for increasing fuel efficiency and the result is minimal carbon emission and considerable saving in expenditure of fishing operations.

The design and construction of fishing vessel is to be carried out as per classification rules and according to the registration rules of the country. But generally this has not been followed till the last three years. Under the Blue Revolution and PMMSY schemes the boats are designed and constructed as per the rules as mentioned. The comparison of traditional design and class approved design of a commercial deep sea fishing vessel is given below.



Modern combination fishing vessel in India

Alternate energy application in fishing vessel propulsion

The commercial fishing vessels require an engine for propulsion. The trawler, gill netter and long liner uses main engine power to function the winch for hauling the fishing gear. Economic speed of fishing vessels depends on the speed and length of the vessel. For propulsion the following are used as fuel.

1. Petrol
2. Diesel
3. Kerosene+ Petrol – are the common type of fuel used for commercial fishing.

But recently ICAR-CIFT has started experiments with solar power and LNG. CIFT Solar fishing boat is shown in this picture. Solar panel mounted on top also act as a protection fron sun and rain.

There is no atmospheric pollution from solar boats since no fossil fuel is used in this. The sound pollution is also very low. Solar power for inland fisheries and LNG/LPG combination for marine fisheries as fusel for propulsion has been found to be successful after trials.

LNG powered vessel

LNG tank inside fishing vessel

Materials of vessel construction

The popular materials used in the construction of boats and ships are wood, steel, Aluminium, and Fiberglass reinforcement plastic. Among these wood utilizes least energy and is the most efficient material. But wood is costly now maintenance of wooden vessel is very expensive. Steel is the most popular material and has been used worldwide for ships and deep sea fishing vessels. This is corrosive in the marine environment and requires high care and maintenance. FRP is suitable for small vessels especially beach landing type fishing vessels due to its lightweight.

References

M.V. Baiju, P.H. Dhiju Das, Vipin Kumar V and K. Sivaprasad.(2019) Standardization of Design of a Commercial Combination Fishing Vessel in India, Journal of Offshore Structure and Technology ISSN: 2349-8986 (Online) Volume 6, Issue1, 49-61

M.V. Baiju, P.H. Dhiju Das, Vipin Kumar V., K. Sivaprasad Baiju, M.V and Boopendranath, (2014), Estimation of optimum engine power of fishing craft with reference to length, Fishery Technology, Vol.51.

****

6

ENERGY USE AND ITS OPTIMIZATION IN INDIAN FISHERIES

Paras Nath Jha and Leela Edwin
Fishing Technology Division
Email ID: *paras.jha@icar.gov.in*

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

produce carbon dioxide in atmosphere which leads to 'greenhouse effect' and other toxic pollutants which are harmful to the environment and human kind. Greenhouse effect leads to irreversible climatic and oceanographic changes.

Environmental burdens due to construction of fishing vessel

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

In fishing boat construction, the common materials used in India include wood, fibre reinforced plastic, aluminium, steel, plywood, ferrocement, etc. While selecting a material for boat construction some basic factors to be considered are type, size, speed, the shape of the vessel, availability and suitability of the material, and economic and environmental viability. The performance and efficiency of a boat are directly dependent on the choice of the boat-building material which also has a direct impact on the environment. By taking these facts into account, a boat designer can select the best possible alternative for building a boat of high efficiency and durability. A fishing boat is made up of different components and their construction is a complex process. Certain quantities of greenhouse gases (GHGs) are produced in the process of manufacture, transportation, and utilization of these components, which can be converted in terms of equivalent CO2. Every ocean has marine debris, and more than 60% of it is plastic that comes from the fishing industry, offshore platforms, recreational shipping etc. At present, the larger class of fishing vessels are made of steel while vessels belonging to the medium and lower categories mostly use wood for construction. Fiberglass, ferrocement, and aluminium are the new substitutes for conventional boat building materials as these can improve the lifespan of the boats. However, traditional fishing boats still play a vital role in this era. Despite

its obvious advantages, all boat-building materials are susceptible to the effects of the marine environment, for example, glass fibres are the most selected material for boat construction, which are vulnerable to the effects of sunlight in marine conditions. Fiberglass-reinforced plastic (FRP) is a polyester resin-based composite, reinforced with fine strands of glass filaments. Glass fibre is prone to osmosis, and gelcoat gets faded in sunlight resulting in the attack of UV radiation. FRP fragments have a higher density than seawater and will tend to concentrate nearshore. The polyester resins or epoxy resins in the FRP undergoing physical & chemical degradation lead to the release of microplastics which affects the environment. Marine organisms consume these plastic particles and end up in the human food chain causing severe health issues. Additionally, the deteriorating and peeling paint with high concentrations of tributyltin and lead from the abandoned boats may provide a long-term environmental issue

Fishing and Energy use

Commercial fishing operation mainly utilizes fossil fuels which result in the emission of greenhouse gases. The active cost of fishing is less understood and consequently receives less attention to GHG emissions than the direct impact on fishery stock and marine ecosystem. Similarly, in the harvest process, several reoccurring inputs are required for every fishing operation, viz. fuel, lubricant, ice, freshwater, etc. These inputs have their own carbon footprint value for construction/extraction/process, especially fuel contributes more than 95% out of all the components. Despite the fact that the prevailing pre-harvest phase of marine capture fisheries lacks general detail and standardization about LCA/carbon footprint studies; such studies and their findings can be useful in formulating constructional and operational recommendations to improve the environmental performance of fisheries, under the context of an ecosystem approach to fisheries along with future certification and different eco-labelling of fisheries. Studies related to pre-harvest, harvest, and post-harvest fisheries LCA/carbon footprint analysis would be more appreciated by policymakers for the regulation of fishing boat yards and other related fishing ventures. Based on behaviour and habitat, there are different methods of fish harvest and on the basis of their operation, the quantum of fuel and energy requirement also varies. As per the study by Parker et al., 2018, the world fishing fleet burned about 40 billion liters of fuel and emitted 179 million tonnes of CO2 equivalent and other GHGs to the atmosphere. Overcapacity and irresponsible use of fossil fuels leads to increased levels of fuel consumption in fishing contributing to climate change in the long run. India contributes 134 million metric tonnes (2.7%) of CO2 emission due to total marine capture fisheries, against 90 million metric tonnes (3.9% of global production) of fish production. The emissions due to fishing were not given importance as compared to other sectors for emission in India, however, the contribution of the fisheries sector is negligible which roughly may be <1% of global GHG emission. The other associated important environmental parameters by

which the health of the environment, humans, and resources can be evaluated due to the fishing process are; terrestrial acidification, formation of fine particulate matter, Water consumption, Ionizing radiation, ozone formation, human carcinogenic toxicity, fossil resource scarcity, mineral resource scarcity environment deterioration, human health, resource depletion, and stratospheric ozone depletion, etc.

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

Energy spent in different fishing operations

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

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

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

Purse seining: Purse seining is one of the most aggressive and efficient commercial fishing methods for capture of shoaling pelagic species. It is a fishing technique which targets pelagic

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

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

Energy intensive illegal fishing: There are several fish harvest practices which require more energy; light fishing is one of them. Fishing using lights has been practiced from historic times, a classic example is 200-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. Small scale fisheries require less capital investment and energy for operation. 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. Fishing operations requires scouting of

shoal/search of fishing ground which may be distantly located have relatively high gross energy requirement per t of fish landed.

Estimates of fuel use and cost

Annual fuel use is about 50 million m3, 1.2% of total global oil consumption. With marine fish and invertebrate landings at 80.4 million tonnes, global average fuel-use intensity was 620 litres (527 kg) per live weight tonne, or about 1.9 tonnes of catch per tonne of fuel. Fishing vessels released some 134 million kg of carbon dioxide (CO2) into the atmosphere at an average of 1.7 kg of CO2 per tonne of live-weight landings. They further noted that these were likely to be serious underestimates, as they did not account for freshwater fisheries or for substantial IUU catches. Global fisheries were estimated to use 12.5 times the amount of fuel energy as their edible-protein energy output, which, although significantly inefficient, compared well with a number of other animal-protein production systems. In context of Indian marine capture fisheries, the substantial increase in fossil fuel noticed due to increased fishing effort and efficiency during the last five decades. Which has resulted in, equivalent to CO2 emission of 0.30 million tonnes (mt) in the year 1961 to 3.60 mt in 2010. Roughly for every tonne of fish caught, the CO2 emission has increased from 0.50 to 1.02 t during above said period.

Conservation of fuel as a part of responsible way of fishing

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

Turbo-charged diesel engines are about 15% more fuel efficient than normally aspirated engines., which have a much better fuel economy and emission standards, are also being introduced in small-scale fisheries.

Summary

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

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

References

FAO (2015) Fuel and energy use in the fisheries sector – approaches, inventories and strategic implications, J.F. Muir. FAO Fisheries and Aquaculture Circular No. 1080. Rome, Italy.

FAO, The State of World Fisheries and Aquaculture 2020, Food and Agriculture Organization, Rome 2020 [Online], http://www.fao.org/documents/card/en/c/ca9229en/

ICAR-CMFRI (2018) Annual Report 2017-2018. 320 p.

Parker RWR, Blanchard JL, Gardner C, Green BS, Hartmann K, Tyedmers PH, Watson, RA (2018) Fuel use and greenhouse gas emissions of world fisheries. Nature climate change 8 (1): 333-337.

Tyedmers PH (2004) Fishing and energy use. In Encyclopedia of Energy (ed. Cleveland, C.), Elsevier, Amsterdam, pp. 683-693 (42)

****

7

MARINE MAMMAL INTERACTION IN PELAGIC FISHING GEARS AND ITS MITIGATION

Prajith K K
Fishing Technology Division
Email ID:*prajithkk@gmail.com*

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

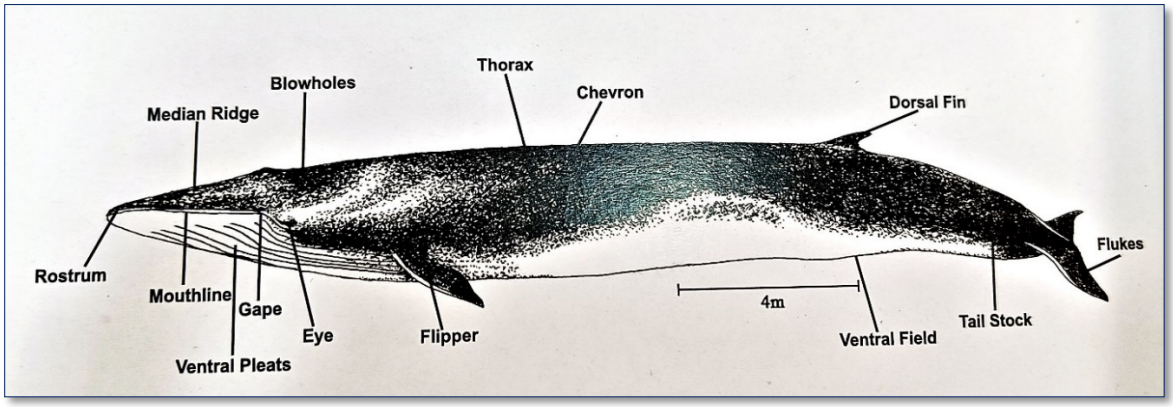
Biological features of marine mammals

Mammals are highly developed animal groups stand on the apex of the animal kingdom. They have a diverse distribution with suitable adaptation to live in the respective geographical

relams. Mammals lives in aquatic environment are Morphologically and anatomically adopted for the life in water. Hydrodynamic body, modified appendages for reducing drag and maximizing propulsion, efficient respiratory system with high oxygen retention, better thermoregulatory mechanisms, specialised sensory and communication mechanism etc. Make them unique from other group of mammals. 'Marine mammal' is a general term to address the members of 5 different groups Viz Cetaceans, sirenians, pinnipeds, sea otters, and the polar bears. A common feature for all the marine mammals is that they spent their entire life in ocean or nearby related ecosystems and derive all of their food from aquatic habitat (Jefferson et al 1993).

Table: 1 Classification of Marine mammals

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



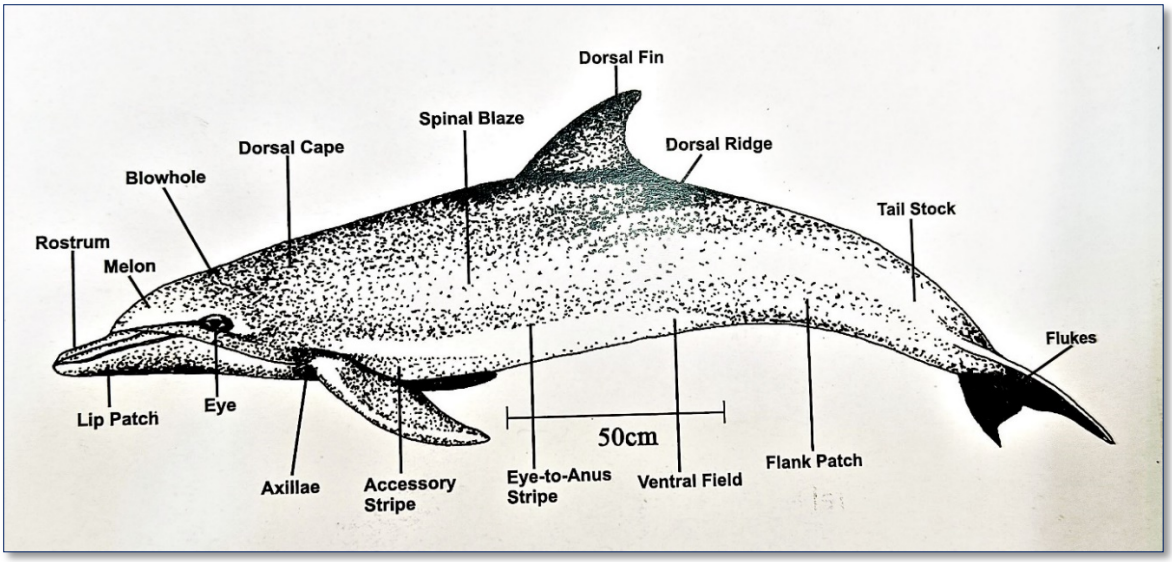


Fig: 1 Morphological features of a typical cetaceans (Vivekanandan and Jeyabaskaran, 2012)

Marine mammals of India

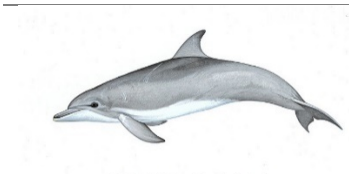
Worldwide 130 species of marine mammals are identified from various oceanic regions viz. tropical, subtropical, temperate and polar regions (Jefferson et al 2008). Indian seas accommodate verities of marine mammals belongs to two orders, cetaceans and sirenian. Which includes baleen whales, toothed whales,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

Interaction of marine mammals with fishing systems

Cetaceans coming under family Dephinidae shows more interaction with coastal fisheries in India. Dolphins are the members of this family. Active movement, overlapping with the feeding and activity zones of other commercially targeted nektonic groups are the some of the reasons for this higher interaction. While analysing the depth wise and zone wise distribution of marine

mammals of India, there are several species with active distribution in the coastal fishing zones. Four species of dolphis viz Stenella longirostris (Spinner dolphin) Tursiops aduncus (The Indo-Pacific bottlenose dolphin), Delphinus capensis (The long-beaked common dolphin) Sousa chinensis (The Indo-Pacific humpback dolphin) are the four major dolphin species abundant in the coastal waters (Jayapraksh et al., 1995). (Table: 2).

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

Species	
	Stenella longirostris
	Tursiops aduncus
	Delphinus capensis
	Sousa chinensis -

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

Major pelagic fishing systems with marine mammal interaction

Indian fisheries are multispecies-multi gear in nature and characterized by a heterogenous fishery management system (Najmudeen and Sathiadhas, 2008). Fishing is carried out with more than 20 gear and vessel combinations. Out of the various gear operated, gillnets and seine nets are more vulnerable to the marine mammal interaction (Cockcroft & Krohn, 1994; Perrin et al., 1994; Archer et al., 2001; Wise et al., 2001; Read et al., 2006, Joseph et al., 2021). Gillnets have either a single shot/unit of net or a number of units tied end to end to form a full fleet of length ranging from 600 to 16500 m with a hung depth of 3 to 20 m. Based on mesh size, Indian gillnets are classified into small meshed nets with 14 to 45 mm mesh size and large meshed nets with 45 to 500 mm mesh size which target verities of species viz. sardine, mackerel, anchovy, seer fish, shark, tuna, pomfret, hilsa, barracuda, billfish, carangid, perch, elasmobranch. etc. Fishing is normally conducted at a depth of 20-1000 m. study by ICAR-Central Institute of Fisheries Technology, Cochin from 20 major fishing harbours along Indian coast reports, coastal gillnets are more prone to cetacean interaction. As gill nets are stretched wall of net with very low visibility in water, the net will obstruct the movement of animals comes in the range of operation and lead to entanglement. Depredation may another reason for the interaction. There are many reports that marine mammals feed on fish caught by fishing gears (Gonener and Ozdemir, 2012). While examining the inshore and off shore gill net bycatch composition, finless porpoise, humpback dolphin and Indo-Pacific bottle nose dolphins are caught in the coastal gillnet targeting tuna and seer fishes. Whereas spinner dolphins, Risso's dolphin and dwarf sperm whale are the species reported from the offshore drift gillnet (Anderson et al. 2020, Yousuf et al. 2009)

Marine mammal interaction in gillnets

Almost 84% of the global cetacean by catch is reported from gill net fishery (Read et al., 2006) Most of the fishery-mammal interactions are reported during late 1980s (Northridge, 1984). due increase in incidental catch of protected species in gill nets, operation of gillnets in the high seas is banned by many countries by law (He, 2006). In India, technological advancement and modernization in the gillnet sector resulted in an increase in the quantum of gillnets taken for operation even in distant and oceanic waters (Thomas,2019). These escalation in the size of the gillnets increased the chances of marine mammal encounters. The modernisation also resulted in shifting of area operation of gillnets from coastal waters to deep sea so there have been a change in the species composition of cetacean bycatch in drift gillnets also (Anderson, 2014). Almost three decades of observation from Indian waters reports 98.8 % mammal mortality reported were due to entanglement in gillnets (Jeyabaskaran et al. 2016).Among the major fishing systems operated along Indian coast, cetacean interaction is reported maximum from gillnets (57.7%) particulary in the small meshed gillnets operated in the coastal waters. Joseph et al. (2021). In India finless porpoise, humpback dolphin and Indo-Pacific bottle nose

dolphins must have been caught in tuna/seer gillnets operated in inshore waters while spinner dolphins, Risso's dolphin and dwarf sperm whale among other species dominated the offshore drift gillnet bycatch (Anderson et al. 2020, Yousuf et al. 2009). The annual cetacean mortality caused by the Indian gillnet fishery is estimated in the range of 1000-10,000/year (Lal Mohan. 1994, Yousuf et al. 2009, Kumaran, 2002) and most of the moralities are associated with pelagic fishery of yellowfin tuna (Thunnus albacares), sharks, and seerfish (Scomberomorus commerson and S. guttatus).

Marine mammal interaction in seine nets

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

The dolphin species which has more access to coastal waters showed more interaction with fishing systems. 84.8% of the interaction with fishing systems was exhibited by four species Stenella longirostris, Tursiops aduncus, Delphinus capensis, Sousa chinensis, which are abundant in the coastal waters (Table.2). (Joseph et al. 2021, Raphael et al., 2017; Edwin et al., 2017; Koya et al., 2018). Larger herd size, active swimming behaviour, sharing of common ecological niche with the fishes which are targeted by seine fishing makes dolphins more susceptible to capture and entanglement in the fishing nets. There are reports of targeted capture and landing of dolphins from seine nets in India (Jayaprakash et al. 1995, Yousaf at al., 2009). During 1984, almost 42 common dolphin Delphinus delphis were landed in Kochi by 12.5m purse seiner and animals were sold to local market 27.5INR/specimen. Similarly, in 1995 and 2009, finless porpoise, Neophocaena phocaenoides were landed by purse seines from off Mangalore coast of Karnataka and Gulf of manner region. Dolphin fishery interaction in India is mainly associated with small pelagic fishery (especially oil sardine) in near shore shallow waters (Yousaf at al., 2009). Majority of the cetaceans- seine net interactions were reported from the states like Kerala, Karnataka, and Goa where the higher landing of small pelagic are reported. (Prathibha et al ., 2018, Yohannan and M. Sivadas, 2003, Joseph et al.2021, Yousaf at al., 2009, Edwin et al.2017, Raphael et al., 2017, Prajith et al 2014).

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

Mitigation

The mitigation measures to minimize the marine mammal fishery of active and passive type. Making alterations in the structural features, increasing the visibility of fishing gear by means of using thick twines, incorporation of add-on reflectors, colouring the netting panels are the major passive methods. Whereas mechanical sounds generation using crackers, explosives, gunshots etc. are come under active type (Jeffersons and Curry, 1996). Indian fishermen follow both active and passive mitigation measures to deter marine mammals from the fishing operation and to safeguard their catch and gear. which can be further classified as indigenous and modern methods. The major indigenous mitigation strategies adopted by the Indian fishermen to minimize cetacean bycatch/interaction are selection of suitable grounds, structural modifications in the gear, sound generation using crackers, vessel chasses, use of boat noises making loud noise, throwing bait to distract the mammals and jumping into water to scare them. While practising these indigenous methods, fishers are cautious to avoid injury to the animals. They even patrol in the fishing ground with small boats and alter the attention of mammals with the help of objects like tyres, boat anchors, stones covered in plastic bags etc. The major modern mitigation methods are the use of acoustic deterrent devices like pingers. Besides this several government agencies and research institutes of the country are engaged in various outreach programs to create awareness among fishers about the protected marine species and their importance in the ecosystems.

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

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

bunt as practical. The system is named after the Californian skipper who first used it. (FAO 2022)

Pingers: Sound has significant role in the lives of marine mammals and sound is the prime mode of information transformation used for communication. As an adaptation to live in a vast aquatic environment, the acoustic system of marine mammals is well developed. Understanding this advantage of communication mechanisms using sound, use of aquatics pingers is the most suitable and efficient mechanism to distract cetaceans from the fishing gears (Fig. 3). Pingers sometime referred as net alarms is one of the best options to reduce injury and resulted mortality of marine mammals. Dolphin pingers are the devices that produces ultrasound which alert and keep the dolphins and porpoises away from the nets. Pinger is designed to work by emitting a sound wave signal beyond 70 kHz that is known to be in the best hearing range of most dolphin species. The signal acts as an alarm and in some cases the pinger stimulates dolphins to use their echolocation which alerts them to the presence of the pingers and fishing nets. This sound wave is not audible to human beings, but it creates disturbance to dolphins and alert dolphins the presence of nets. Pingers are efficient to minimize cetacean interaction both in gillnets and seine nets.

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

Conclusion

Global survey report of United Nations in 2005 reports 70% of the dolphin species are at risk due to various human activities. Removal of the apex predators like cetaceans by incidental or purposeful killing may leads to imbalance in the ecosystem. Marine mammal fishery research in India is still in infancy stage. Most of the studies are based on the stranding events. Research to formulate suitable mechanism to reduce or avoid mammal interaction with fishing system is the need of the hour. Ecology, behaviour and biology of marine cetaceans need better understanding. To reduce marine mammal incidental catch and kill, there should be a management system or consortia which comprises of government agencies, academicians, researchers and fishermen. Understanding the fishermen perception is essential while formulating the research. A refinement in in the existing indigenous mitigation measures by the application of suitable scientific approach with the involvement of fishermen is needed. Besides this awareness about the importance of marine mammals and other mega fauna

should be created among fishers, general public, school students etc. through various outreach and extension programmes.

References

Anderson, R. C. (2014) Cetaceans and tuna fisheries in the western and central Indian Ocean. IPNLF Tech Rep 2. International Pole and Line Foundation, London.

Anderson, R. C., Herrera, M., Ilangakoon, A. D., Koya, K. M., Moazzam, M., Mustika, P. L. and Sutaria, D. N. (2020) Cetacean bycatch in Indian Ocean tuna gillnet fisheries. Endanger. Species. Res. 41: 39-53

Archer, F., Gerrodette, T., Dizon, A., Abella, K. & Southern. S., 2001. Unobserved kill of nursing dolphin calves in a tuna purse-seine fishery. Marine Mammal Science, 17, 540–554.

Ardill, D., D. Itano and R. Gillett 2011 A Review of Bycatch and Discard Issues in Indian Ocean Tuna Fisheries., SmartFish Working Papers No 00X, IOTC-2012-WPEB08-INF20

Barros, N. B. and R. S. Wells. 1998. Prey and feeding patterns of resident bottlenose dolphins (Tursiops truncatus) in Sarasota Bay, Florida. J. Mamm. 79: 1,045-1,059.

Cockcroft, V.G. & Krohn. R., 1994. Passive gear fisheries of the southwestern Indian and southeastern Atlantic Oceans. An Assessment of their possible impact on cetaceans. In Gillnets and cetaceans (ed. W.F. Perrin et al.). Report of International Whaling Commission, Special Issue, 15, 317–328.

Edwin, L., Joseph, R. and Raphael, L. (2017) Acoustic pingers: Prevention of fish catch depredation and dolphin entangling. Fishtech Reporter, Central Institute of Fisheries Technology 3(1):

FAO 2022. Medina panels. Text by V. Crespi. Fisheries and Aquaculture Division [online]. Rome. Updated 2008-11-28 [Cited Thursday, February 3rd 2022]. https://www.fao.org/fishery/en/equipment/medinapanels/en

FAO. 2011. The state of the world's land and water resources for food and agriculture (SOLAW) – Managing systems at risk. Food and Agriculture Organization of the United Nations, Rome and Earthscan, London.

Gordon R. Munro, Anthony D. Scott, Chapter 14 The economics of fisheries management, Handbook of Natural Resource and Energy Economics, Elsevier,Volume 2,1985,Pages 623-676,ISSN 1573-4439,ISBN 9780444876454,https://doi.org/10.1016/S1573-4439(85)80021-X.(https://www.sciencedirect.com/science/article/pii/S157344398580021X)

Jayaprakash, A.A., Nammalwar, P., Krishna Pillai, S., Elayathu, M.N.K., 1995. Incidental by-catch of dolphins at fisheries harbour, Cochin with a note on their conservation and management in India. J. Mar. Biol. Ass. India 37 (1-2), 126-133.

Jefferson, T.A., Curry, B.E., 1996. Acoustic methods of reducing or eliminating marine mammal fishery interactions: do they work? Ocean. Coast. Manag. 31 (1), 41-70

Jefferson, T.A., S. Leatherwood, and M.A. Webber., 1993. FAO species identification guide. Marine mammals of the world. Rome, FAO. 1993.320. p. 587 figs

Jefferson, T.A., Webber, M.A., Pitman, R.L., 2008. Marine Mammals of the World: a Comprehensive Guide to their Identification. Academic Press, PP 1-592 pp

Jeyabaskaran, R., J. Jayasankar, D. Prema and V. Kripa (2016) Enhancing the effectiveness of conservation potential of marine mammals in Indian seas. Final Report submitted to GIZ-CMPA, New Delhi, pp. 1-82, Central Marine Fisheries Research Institute, Kochi, India

Joseph, R, Das, P.H. and Leela. E (2021) Cetacean Fishery Interaction during Operation of Major Fishing Systems of India. Fish. Technol. 58:1-6

Koya, K. M., Prathibha, R., Vinay, K. V. and Abdul, A. P. (2018) Non-target species interactions in tuna fisheries and its implications in fisheries management: Case of large-mesh gillnet fisheries along the north-west coast of India. J. Mar. Biol. Ass. India, 60 (1): 18-26

Kumaran, P. L. (2002). Marine mammal research in India–a review and critique of the methods. Current Science, 1210-1220.

Lal Mohan, R. S. L. 1994. Review of gillnet fisheries and cetacean bycatches in the northeastern Indian Ocean. Rep.int. Whal Commn (special issue) 15: 329-43.

Najmudeen.T.M., Sathiadhas R. (2008) Economic impact of juvenile fishing in a tropical multi-gear multi-species fishery. Fisheries Research Volume 92, Issues 2–3, August 2008, Pages 322-332

Northridge, S.P., 1984 World review of interactions between marine mammals and fisheries. FAO Fish.Pap., (251):190 p.

Perrin, W.F., Donovan, G.P. & Barlow, J., 1994. Gillnets and cetaceans. Report of International Whaling Commission Special Issue, 15, 629 pp

Pingguo He. 2006. Gillnets: Gear Design, Fishing Performance and Conservation Challenges. Marine Technology Society Journal, 40 (3):12-19.

Prajith, K. K, Dhiju, D.P.H and Edwin, L (2014) Dolphin Wall Net (DWN)-An innovative management measure devised by ring seine fishermen of KeralaIndia to reducing or eliminating marine mammal fishery interactions. Ocean Coast. Manag. 102: 1-6

Prathibha Rohit, M. Sivadas, E. M. Abdussamad, A. Margaret Muthu Rethinam, K. P. Said Koya, U. Ganga, Shubhadeep Ghosh, K. M. Rajesh, K. Mohammed Koya, Anulekshmi Chellappan, K. G. Mini, Grinson George, Subal Kumar Roul, S. Surya., Sandhya Sukumaran, E. Vivekanandan, T. B. Retheesh, D. Prakasan, M. Satish Kumar, S. Mohan, R. Vasu and V. Suprabha. (2018). The Enigmatic Indian Oil Sardine: An Insight. CMFRI Special publication No. 130 p. 156

Raphael, L., Joseph, R. and Edwin, L. (2017) Depredation and catch loss due to the interaction of aquatic organisms with ring seines off Cochin region. Fish Tech. 54: 162-169

Read AJ, Drinker P, Northridge S (2006) Bycatch of marine mammals in US and global fisheries. Conserv Biol 20:163–169. doi:10.1111/j.1523-1739.2006.00338.x

Sedat Gönener1,*, Süleyman Özdemir Investigation of the Interaction Between Bottom Gillnet Fishery (Sinop, Black Sea) and Bottlenose Dolphins (Tursiops truncatus) in Terms of Economy. Turkish Journal of Fisheries and Aquatic Sciences 12: 115-126 (2012)

Srinivasan, U. T., Cheung, W. W., Watson, R., & Sumaila, U. R. (2010). Food security implications of global marine catch losses due to overfishing. Journal of Bioeconomics, 12(3), 183-200.

Sutaria, D., Panicker, D., Jog, K., Sule, M., Muralidharan, R., &Bopardikar, I. (2015). Humpback dolphins (Genus Sousa) in India: an overview of status and conservation issues. In Advances in marine biology (Vol. 72, pp. 229-256). Academic Press

Thomas S. N., 2019. Sustainable gillnet fishing. In: ICAR-CIFT Winterschool manual on Responsible Fishing: Recent advances in Resource & energy conservation, 21 November-11 December 2019, 239-249.

Vivekanandan, E. and Jeyabaskaran.2012. Marine mammal species of India, Central Marine Fisheries Research Institute, Kochi, 228p.

Wickens, P. (1994). Interactions between South African fur seals and the purse-seine fishery. Marine Mammal Science, 10(4), 442-457.

Wise, L., Silva, A., Ferreira, M., Silva, M. A. & Sequeira, M., 2001. Interactions between small cetaceans and the purse-seine fishery in western Portuguese waters. Scientia Marina, 71, 405–412.

Yohannan, T.M and Sivadas, M Indian Mackerel in Mohan Joseph, M and Jayaprakash, A A, eds. (2003) Status of Exploited Marine Fishery Resources of India. Central Marine Fisheries Research Institute, Kochi.

Yousuf, K. S. S. M., A. K. Anoop, B. Anoop, V. V. Afsal, E. Vivekanandan, R. P. Kumarran, and P. Jayasankar. 2009. Observations on incidental catch of cetaceans in three landing centres along the Indian coast. Marine Biodiversity Records, 2, e64.

****

NANOTECHNOLOGY AND ITS APPLICATIONS IN FISHERIES

Muhamed Ashraf P.
Fishing Technology Division
Email ID: ***ashrafp2008@gmail.com***

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, slowrelease nutraceuticals, material protection from degradation and sensor.

The term nanotechnology was coined by Prof Taniguchi, Japan in 1974 conference of the Japanese Society of Precision Engineering. 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. Prof R. Feynman 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. 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 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.

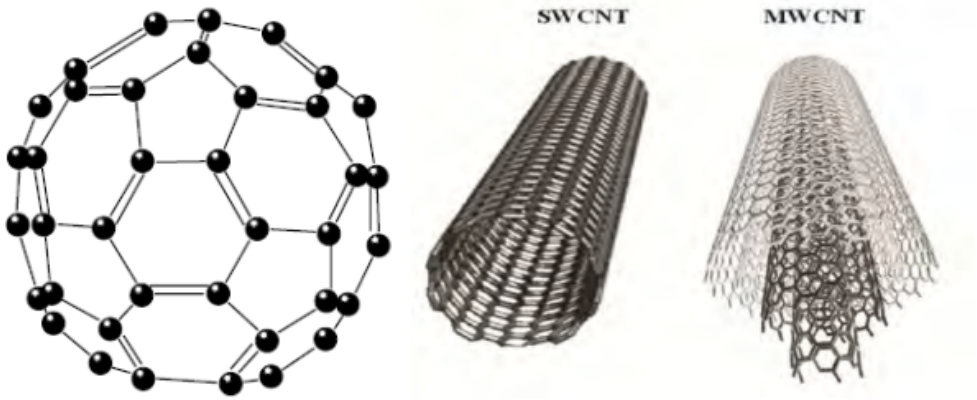


Fig 1. A) Fullerene C60 molecule B) SWCNT and C) MWCNT.

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. 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°C. CNTs are formed on the catalysts. 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°C 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:

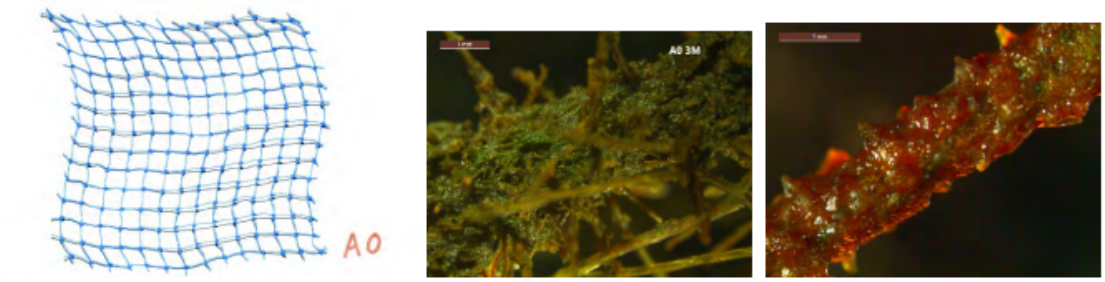


Fig 1. A) PE cage net b) PE cagenet after 3 months c) PE cagenet treated with PANI+nano CuO after three months exposure in the estuary.

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. 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. Applications of nano materials in medicine are like mucosal lining treatment and inflammatory bowel treatment using nano pharmaceuticals.

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 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. 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 described about the determination of adhesive strength using a calibrated flow channel. Diatom assays were generally carried out using Navicula perminuta 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. 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.

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, health environment and safety issued. 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.

References

Akesso, L., Pettitt, M.E., Callow, J.A., Callow, M.E., Stallard, J., Teer, D., Liu, C., Wang, S., Zhao, Q., D'Souza, F. and Willemsen, P.R., 2009. The potential of nano-structured silicon oxide type coatings deposited by PACVD for control of aquatic biofouling. Biofouling, 25(1), pp.55-67.

Andriyevsky R. A. and Khachoyan A. V. (2002) The Epilogue to the Book Nanotechnology in the Next Decade (Mir, Moscow).

Arseneau KO, Pizarro TT, Cominelli F. (2000) Curr Opin Gastroenterol.16: 310.

Ashraf PM, K.G. Sasikala, Saly N. Thomas, Leela Edwin (2020) Biofouling resistant polyethylene cage aquaculture nettings: A new approach using polyaniline and nano copper oxide. Arabian Journal of Chemistry.

Bement AL. (2007) Testimony before the House Committee on Science hearing on fundamental nanotechnology research: The key to finding the promise and minimizing the peril. Available at: http://www.nfs.gov/ about/congress/109/alb_nanotech_092106.jsp.

Bhardwaj, U., Papadimitrakopoulos, F., Burgess, D.J.(2008). J. Diabetes Sci. Technol. 2(6), 1016.

Callow, M.E., Jennings, A.R., Brennan, A.B., Seegert, C.E., Gibson, A., Wilson, L., Feinberg, A., Baney, R. and Callow, J.A., 2002. Microtopographic cues for settlement of zoospores of the green fouling alga Enteromorpha. Biofouling, 18(3), pp.229-236.

Chechenin N. G. (2006) Magnetic Nanostructures and their Application, (Grant Viktoriya TK, Moscow).

Grungberg, P., Burgler, D. E., Dassow, H. (2007) Acta Mater. 55 (4), 1171.

Heuschkel RB. (2000) New immunologic treatments for inflammatory bowel disease. Curr Opin Gastroenterol. 16: 565.

Ista, L.K., Fan, H., Baca, O. and López, G.P., 1996. Attachment of bacteria to model solid surfaces: oligo (ethylene glycol) surfaces inhibit bacterial attachment. FEMS Microbiology Letters, 142(1), pp.59-63.

Kobayasi N. (2005) An Introduction to Nanotechnology (BINOM, Moscow).

Likharev K. K. (2003) in Nano and Giga Challenges in Microelectronics, Ed. by J. Geer et al. (Elsevier, Amsterdam), p. 27.

Onuki, Y., Bhardwaj, U., Papadimitrakopoulos, F., Burgess, D.J.(2008) J. Diabetes Sci. Technol. 2 (6), 1003.

Peppas N A, Kavimandan N J. (2006) Eur J Pharm Sci. 29:183.

Pettitt, M.E., Henry, S.L., Callow, M.E., Callow, J.A. and Clare, A.S., 2004. Activity of commercial enzymes on settlement and adhesion of cypris larvae of the barnacle Balanus amphitrite, spores of the green alga Ulva linza, and the diatom Navicula perminuta. Biofouling, 20(6), pp.299-311.

Powell M C, Kanarek M S. (2006) Nanomaterial health effects—Part 2: Uncertainties and recommendations for the future. WMJ. 105:18.

Rao CNR, Muller A, Cheetham A K (ed) (2005) Chemistry of nanomaterials Vol 1 and 2. Wiley –VCH, Germany.

Roco M. C. (2003) J Nanoparticle Res. 5:181-189.

Schultz, M.P., Finlay, J.A., Callow, M.E. and Callow, J.A., 2000. A turbulent channel flow apparatus for the determination of the adhesion strength of microfouling organisms. Biofouling, 15(4), pp.243-251.

Sysoev, N.N, Osipov A.I and Uvarov A.V (2009) Nanotechnology and molecular physics. Moscow University Physics Bulletin. 64(1): 1.

Vaddirajua S, Tomazosb, I., Burgessc, D. J., Jain F. C., Papadimitrakopoulosa F, (2010) Biosensors and Bioelectronics 25 (2010) 1553–1565

Wamer W G, Yin J J, Wei R R. (1997) Free Radic Biol Med. 23: 851.

Wisniewski, N., Reichert, M. (2000). Colloids Surf. B: Biointerfaces 18 (3–4), 197–219.

Yu. D. T, (2007) Vestnik Rossiiskoi Akademii Nauk 77 (1), 3.

****

9

MICRO AND NANOPLASTICS POLLUTION IN MARINE ENVIRONMENT

Manju Lekshmi N
Fishing Technology Division
Email ID: ***Manjuarem@gmail.com***

UNEP defined "Marine litter consists of items that have been made or used by people and deliberately discarded into the sea or rivers or on beaches; brought indirectly to the sea with rivers, sewage, storm water or winds; or accidentally lost, including material lost at sea in bad weather". Marine litter is a pressing global concern, with plastics constitutes over 80% of all litter. Plastics encompass a wide range of synthetic or partially synthetic materials that use polymers as their main building blocks with intrinsic flexibility makes it possible to shape solid things with a variety of shapes by molding, extruding, or pressing. This property, along with numerous of other qualities including light weight, durability, adaptability, and cost-effective manufacture, has driven their widespread acceptance. Chemicals made from fossil fuels, particularly natural gas and petroleum, are a major component in modern plastic manufacture. However, recent advancements in industrial methodologies have introduced alternatives manufactured from renewable resources, including derivatives sourced natural materials.

Plastics have become an integral part of modern life and are used in various industries, including packaging, construction, electronics, automotive, healthcare, fishing and more. The production of plastic experienced a remarkable surge, escalating from 2 million tons in 1950 to an astonishing 200 million tons by the year 2020. Notably, 40% of the global plastic output finds application in packaging purposes. Most of the packaging purposes use single-use plastics. Single-use plastics do pose significant environmental challenges and have been widely recognized as a major contributor to plastic pollution. Single-use plastics are described as plastic products that are intended to be used just once before being discarded. Due to their affordability, toughness, and adaptability, these polymers are frequently utilised for convenience and packaging. Plastic straws, water bottles, plastic bags, plastic cutlery, and other food packaging materials are all examples of single-use plastics.

In the marine litter, approximately 35% plastic waste materials are denser than seawater which results the sinking of these materials to the seafloor and infiltrating the depths of our oceans. The remaining 65% remains buoyant on the ocean's surface, capable of traversing extensive distances through wind-driven currents. Plastic production and consumption persist

at current levels, projections from the UNEP suggest that by 2050, the oceans will contain more plastic (in terms of weight, measured in thousands of tonnes) than fish. Furthermore, UNEP estimates that approximately 99% of seabirds have ingested plastic, underscoring the widespread and concerning impact of plastic pollution on wildlife and marine life.

Different types of plastics in the marine environment

Polyethylene (PE): This is one of the most widely used plastics. It comes in different forms, such as Low-Density Polyethylene (LDPE) and High-Density Polyethylene (HDPE). LDPE is flexible and used in items like plastic bags and squeeze bottles, while HDPE is stiffer and used for containers, pipes, and toys. HDPE is also used as a webbing material in fishing industry.

Polypropylene (PP): PP is renowned for withstanding heat and chemicals. It is utilized for packaging, automotive components, laboratory equipment, and ropes in the fishing industry.

Polyvinyl Chloride (PVC): PVC is versatile and used in pipes, cables, flooring, and a variety of products. It can be rigid or flexible, depending on additives used during production.

Polystyrene (PS): PS can be found in two main forms: expanded (EPS) and solid. EPS is used in packaging and insulation, while solid PS is used for items like disposable cutlery and CD cases.

Polyethylene Terephthalate (PET): PET is commonly used for beverage bottles and food packaging due to its clarity and resistance to gas and moisture. PET bottles are the most widely recycled plastic in the world.

Polycarbonate (PC): PC is known for its impact resistance and transparency. It's used in items like eyeglass lenses, medical devices, and electronic components.

Acrylonitrile Butadiene Styrene (ABS): ABS is a strong and rigid plastic used in products like toys, automotive parts, and consumer electronics.

Polyamide (PA) or Nylon: Nylon is known for its strength, abrasion resistance, and heat tolerance. It's used in textiles, industrial components, mechanical parts and as webbing material in fishing industry.

Polyurethane (PU): PU is highly flexible and can range from soft foam to rigid plastics. It's used in furniture, footwear, insulation, and more.

Bioplastics: These are derived from renewable resources like corn and cotton.Compared with fossil-based plastics, bio-based plastics can have a lower carbon footprint and exhibit advantageous materials properties

Sources of plastic debris

There are many different sources of plastic debris, including both human and natural processes. These sources all contribute to the buildup of plastic trash in the environment. One of the main causes of plastic debris is improperly managing the disposal of plastic waste. A significant portion of plastics derived from land and sea based sources like fishing industry, offshore platforms, recreational shipping, household and industrial wastes. Inadequate waste

management systems, littering, and a lack of recycling infrastructure led to plastics getting into the environment and polluting rivers, oceans, and even natural landscapes. The widespread usage of single-use plastics is an equally important source of plastic waste. The weight of plastic trash is further increased by items like plastic bottles, bags, straws, and other kinds of packaging that make up a sizeable amount of plastic garbage and have a limited useful life before being discarded. Transport and shipping activities also contribute to the environmental pollution of plastic garbage. Plastic packaging, wrapping, and containers are especially vulnerable to accidental loss or deliberate disposal during transit in marine commerce. Rain, in the form of stormwater runoff and urban runoff, aggravates the problem by removing plastic waste from streets and urban areas, transporting it down storm drains, and ultimately disposing of it in rivers and oceans. Even coastal areas are not immune to plastic debris, often due to the practices of beachgoers, tourists, and recreational activities that leave behind litter. This debris can then be transported into the ocean by tides and currents, posing a direct threat to marine ecosystems. Fishing and maritime activities also play a role in the formation of marine litter which results from abandoned, lost, or discarded fishing gear and abandoned end-of-life boats adding to the accumulation of marine plastic debris. These discarded materials pose serious hazards to marine life. About 20% of the plastics in the marine environment is contributed by the fishing sector.

Plastics from fishing sector

Plastics from fishing gears: The fishing gear materials made of cotton is very popular before the introduction of synthetic materials like nylon. The cotton materials decompose relatively quickly within 2-3 months and its impacts on marine life is negligible while materials like nylon persist for an astonishingly long time. This advantage of synthetic materials make more popular and acceptable but same time it adversely affected the marine life. Synthetic materials like nylon may take 500 to 600 years to break down. The damaged and discarded synthetic material causes pollution. A significant portion of this pollution is attributed to ALDFG, which encompasses nets, ropes, traps, and other fishing equipment that has been abandoned, lost, or discarded in marine environments. ALDFG is defined by the UNEP as a collection of fishing equipment that has been abandoned or thrown into the water and that continues to trap both targeted marine species and undesired ones, resulting in ghost fishing. Ghost fishing refers to a phenomenon where abandoned, lost, or discarded fishing gear continues to actively capture and entangle marine life in a seemingly never-ending cycle. The discarded fishing gear includes nets, lines, traps, and other equipment, becomes a lethal hazard for marine organisms long after it has been left in the ocean. Ghost fishing poses a grave threat to various marine species, as the ensnared animals can suffer injury, suffocation, or death. This process not only harms the targeted fish but also affects unintended species, disrupting ecosystems and perpetuating a destructive cycle.

Plastics from fishing vessels: Fiberglass Reinforced Plastic (FRP) is a boat building composite material which became popular in boat construction since late 1940s as an alternative to traditional materials due to scarcity and cost constraints. FRP boats offer benefits such as corrosion resistance, durability, light weight, and high strength-to-weight ratio, making them suitable for small fishing vessels. FRP is made by binding glass fibers with a thermosetting plastic resin. Glass fibers are used in the form of glass mat and woven roving to create thick layers, which are bonded together using resin, catalyst, and accelerator. Polyester resins, including biphenolic, ortho and isophthalic resins, make up around 75% of the FRP matrix.

FRP is maintenance-free and has many benefits over typical wood materials. Its sleek finish and light weight help the fishermen to navigate quickly. Earlier FRP was used as a sheathing material for fishing vessels constructed with plywood and wood. But presently many of fishing vessels are constructed with FRP as the primary material. The life span of sheathed vessels is only a life of less than 10 years while boats constructed only with FRP having a life more than 30 years.

As the numbers of boats are increasing disposal became an issue for the ELB (End of Life Boats) FRP fishing boats. Due to lack of recyclability, it became a burden to the owners when it comes to an end of its service life. Because there is no simple way to dispose of plastic ELBs and existing options are quite expensive, it may seem tempting to get rid of the problem by dumping them some place in nature or in the sea. Abandoned or derelict vessels (ADVs) are a sort of maritime debris that are aground, broken apart, submerged, exhibit no signs of maintenance or usage, or are generally deteriorated. Abandoned boats are commonly observed on the foreshores, intertidal flats and reefs, throughout the coast. There is currently no financially viable solution for recycling FRP materials used in the hull of ships and boats that are manufactured with thermoset resins. Such composite hull components cannot be formed by melting, rolling, thermal forming, or molding into other usable physical forms. In 2016, London convention and protocol discussed and identified abandoned FRP boats is an environmental threat and to be regulated.

Environmental interactions of plastics

Weathering of plastics: Formation of micro and nano plastics: Weathering is a process that entails the transformation of plastic materials when subjected to various environmental factors, including sunlight, temperature fluctuations, and mechanical forces. This prolonged exposure leads to the gradual breakdown of larger plastic items into smaller fragments. Based on size, these breakdown fragments are classified into Mega (>100mm),Macro (21-100 mm),Meso (5-20 mm) and Micro (<5 mm) plastics and nanoplastics (1 to 1000 nm). Nano & microplastics, produced through weathering, encompass a wide range of sizes and are more prone to ingestion by various organisms, potentially entering the food chain and accumulating up the ecological hierarchy. The adverse effects extend to human health as microplastics and

associated contaminants can infiltrate the food chain through seafood consumption. The IUCN (International Union for Conservation of Nature) has documented that South Asia, including India, is discharging 274,000 metric tonnes of primary microplastics into the ocean. On a global scale, the yearly average release of primary microplastics into the ocean is estimated to be 1.5 million metric tonnes. Notably, research conducted by IIT Mumbai has revealed the presence of microplastics even in sea salt sourced from Indian waters.

Microplastic can further undergo weathering to form nano plastics. Nanoplastics refer to extremely small plastic particles that have dimensions in the nanometer range, typically ranging from 1 to 1000 nanometers in size. These particles are even smaller than microplastics and are a subset of the broader category of plastic pollution.Because of their tiny size, nanoplastics have unique properties and behaviors that differentiate them from larger plastic particles. They have a higher surface area relative to their volume, which can lead to increased interactions with other substances in the environment, including chemicals and pollutants. This characteristic makes nanoplastics potentially more chemically reactive and capable of adsorbing or carrying pollutants from the surrounding environment.

They may spread out quickly in a variety of habitats, including soil, water, and the air thanks to their tiny size. Nanoplastics may take on a variety of shapes, from spherical to asymmetrical, which impacts how they interact with the environment and living things. They demonstrate higher mobility and bioavailability due to their large surface area compared to volume, which might cause them to enter the food chain and have an impact on diverse creatures. Their potential toxicity, ecological effects, and function as carriers of pollutants are still being studied. Regulations and more research are essential to address the possible dangers of nanoplastics and reduce their prevalence in the environment since they are a growing problem.

Leaching of plastics: *Leaching* refers to the release of chemical additives present in plastics into the surroundings, often triggered by interactions with water or other solvents. Plastic products, including single-use items and larger plastic structures, often contain various chemical additives to enhance their properties, such as flexibility, flame resistance, or color stability. These additives can include plasticizers, stabilizers, flame retardants, and pigments. When plastic items degrade or interact with their environment, either through weathering, mechanical stress, or exposure to different temperatures, these additives can gradually leach out into the surrounding environment.

In aquatic environments, leaching can occur when plastic items like bottles, packaging, or microplastics come into contact with water. As water interacts with the plastic surface, it can dissolve and carry away the additives, potentially releasing them into the water. This process can lead to the contamination of water bodies with these chemical compounds, raising concerns about their impact on aquatic life and ecosystems.Leaching can also be relevant in

the context of landfill sites where plastic waste is disposed of. Rainwater or other liquids can percolate through landfills, causing the leaching of chemicals from the decomposing plastics and potentially contaminating groundwater.

Plastics may survive for decades or even centuries because of their strength and resistance to degradation. This persistence can lead to various ecological and environmental issues.

Impacts on Flora and fauna

Animals can mistake plastic items for food or become entangled in plastic debris. Ingesting plastics can lead to choking, internal injuries, and even death. This is a significant concern for marine life, birds, and other animals.

Ecotoxicity: Plastics can contain additives and chemicals that are toxic to both wildlife and humans. These toxins can leach into the environment, posing a threat to aquatic ecosystems and the organisms living in them.

Habitat Degradation: Accumulations of plastic waste can alter natural habitats, disrupt ecosystems, and damage fragile environments like coral reefs and coastal areas.

Aesthetic Impacts: Plastic pollution can tarnish the beauty of landscapes and water bodies, affecting tourism and recreational activities. Cleanup efforts also incur significant costs.

Social and livelihood impacts: Plastic pollution raises the issue by encroaching upon the spaces traditionally used for fish landing and various related activities. As plastic waste accumulates along coastlines, beaches, and water bodies, it diminishes the available area for fishing operations, processing, and other essential tasks. This not only disrupts the livelihoods of fishing communities but also hampers the overall efficiency of the fisheries industry.

Mitigation initiatives for fishing plastics

Addressing environmental concerns related to fishing gear and boat disposal requires a different approach.These include the implementation of stringent gear regulations, marking for easy tracking and identification, to enhance responsible fishing practices. Encouraging the adoption of biodegradable materials for fishing material construction contributes to reducing environmental impacts. Additionally, the proper disposal of fishing materials including end-of-life Fiberglass Reinforced Plastic (FRP) fishing boats necessitates the establishment of clear guidelines. Ensuring the construction of FRP fishing boats adheres to set standards is essential for long-term sustainability. Creating awareness within the fishing community can be achieved through seminars, symposiums, and field demonstrations. Incentive-based programs for litter collection by fishers, as well as promoting recycling options, provide practical ways to combat pollution. Initiatives like the "SuchitwaSagaram", a Kerala government project which aimed for the eradication of plastics from the sea, further contribute to effective waste management in coastal areas, collectively driving the pursuit of a more environmentally conscious fishing industry.

The 6Rs represent a set of principles aimed at promoting sustainable and responsible consumption and waste management in the case of plastics. Each "R" stands for a different action that individuals and communities can take to minimize their environmental impact.

Rethink: Reevaluating our consumption habits and considering the environmental and social consequences of our choices.

Refuse: The "refuse" principle encourages saying no to products or items that are unnecessary or harmful to the environment. This can include refusing single-use plastics, excessive packaging, and other items that contribute to waste.

Reduce: This principle promotes the idea of consuming less and minimizing our overall consumption. By using resources more efficiently and avoiding overconsumption, we can reduce our ecological footprint.

Reuse: Reusing involves finding ways to use items again instead of throwing them away after a single use. This can include using durable containers, repairing and repurposing items, and participating in activities like thrift shopping.

Recycle: Recycling involves the proper sorting and processing of materials to create new products from old ones.

Repair: Repairing items instead of discarding them helps extend their lifespan and reduces the demand for new products. This contributes to a more circular economy where items are used for as long as possible before being recycled or disposed of.

It's important to note that addressing plastic pollution requires global cooperation and individual actions to reduce plastic consumption and improve waste management practices.

References

Andrady, A. L. (Ed.). (2003). Plastics and the Environment.John Wiley & Sons.

Hammer, J., Kraak, M. H., & Parsons, J. R. (2012). Plastics in the marine environment: the dark side of a modern gift. Reviews of environmental contamination and toxicology, 1-44.

https://www.plasticsforchange.org/blog/different-types-of-plastic

Macfadyen, G., Huntington, T., & Cappell, R. (2009). Abandoned, lost or otherwise discarded fishing gear (No. 523). Food and Agriculture Organization of the United Nations (FAO).

Miljö, A. (2001). Marine Litter--Trash that Kills.Swedish Environmental Protection Agency.

UNEP Regional Seas Programme, et al. "Marine litter: an analytical overview." (2005).

United Nations Environment Programme. (2009). Marine litter: a global challenge. UNEP.

****

10

IMPORTANCE OF FISH IN HUMAN NUTRITION

Elavarasan, K.
Fish Processing division
Email ID: *elafishes@gmail.com*

The fisheries and aquaculture sector is crucial for improving food security and human nutrition. The quantity of fish consumed and demand is increasing continuously. Aquaculture is considered as the world's fastest growing food production industry. Aquaculture has provided more fish for human consumption than capture fisheries, and by 2030 it is estimated that 60 % of the fish consumed by human will be from aquaculture. Increasingly intensive aquaculture production methods, with greater use of crop-based feedstuffs and lower fishmeal and fish oil inclusion rates, are likely to influence the nutrient content of farmed aquatic products. A focus on the nutrient content of farmed aquatic foods is especially important where they have a key role in food-based approaches to food security and nutrition. The awareness about the fish as a part of healthy diet is well accepted by the majority of the population. In addition to providing essential nutrients at affordable price, fish also contributes to the food and nutritional security of poor households in developing countries. Fish can be considered as a treasure store of nutrients. It provides more than 20 % of the average per capita animal protein intake for 3 billion people, and more than 50 % in some less developed countries. Fish and fish products are excellent sources of high-quality protein; bioavailability of protein from fish is approximately 5-15 % higher than that from plant sources. Fish contains all the amino acids essential for human health.

Many fish (especially fatty fish) are a source of long-chain omega-3 fatty acids, which contribute to visual and cognitive human development, especially during the first 1 000 days of a child's life. The fat content and fatty acid profiles of farm raised fishes affected by the feed used in culture practice. Though the fish consumption has increased, people are obtaining smaller amounts of omega-3 fatty acids from aquatic foods, because these fats are more prevalent in marine fishes than in freshwater fish. Fish also provides essential minerals such as calcium, phosphorus, zinc, iron, selenium and iodine as well as vitamins A, D and B, thus helping to reduce the risks of both malnutrition and noncommunicable diseases which may co-occur when high energy intake is combined with a lack of balanced nutrition. Nutritional content is especially high in small fish species consumed whole and in fish parts that are not usually consumed (such as heads, bones and skin) which are having lower economic value. It

is desirable to increase the production and consumption of small fish and to find ways of transforming the non-consumed parts into nutritionally rich products.

There remains considerable scope to increase the amount of fish or nutrients derived from fish for human consumption by reducing post-harvest losses, especially from capture fisheries; by more efficient use of fishmeal and fish oil and in animal (especially aquaculture) feeds; and by improved feed formulations for farmed fish and crustaceans. The fish industry often only extracts fillets for human consumption consigning nutritious co-products to be used for animal feeds instead of exploring their use in tackling micronutrient deficiencies. Fish processing co-products, such as fish carcasses, which are increasingly used to produce fishmeal and fish oil, represent an underutilized source of nutrients and micronutrients for human consumption. The fishmeal and fish oil content of aquaculture feeds can be reduced without compromising the nutrient content of farmed aquatic products. Improvements in feed formulations and in feed manufacture, combined with better on-farm feed management, can hugely reduce the quantities of feed (and thus fishmeal and fish oil) used per kilogram of farmed aquatic food produced.

The FAO/INFOODS Global Food Composition Database for Fish and Shellfish (uFiSH) includes a complete nutrient profile (minerals, vitamins, amino acids and fatty acids) for 78 species in raw, cooked and processed forms. The data were extracted from 2 630 food records from 250 data sources and compiled following international FAO/INFOODS (International Network of Food Data Systems) standards. Such information is much usefull to have better understanding the nutritional value of fish.

Nutritional Value of Fish and Shellfish

Fish Proteins

Fish and shellfish are excellent sources of protein. A 100 g cooked serving of most types of fish and shellfish provides approximately 18–20 g of protein, or about a third of the average daily recommended protein intake. The recommended dietary allowance (RDA) of protein for human male and female adults is in the range of 45–65 g day. In accordance with this, an intake of 100 g of fish would contribute 15–25% of the total daily protein require-ment of healthy adults and 70% of that of children. The fish protein is of high quality, containing an abundance of essential amino acids, and is very digestible by people of all ages. Both finfish and shellfish are highly valuable sources of pro-teins in human nutrition, supplying approximately 7.9% of the world's protein requirements and 15.3% of the total ani-mal protein.

The protein content of fish flesh, in contrast to the fat content, is highly constant, independent of seasonal variations caused by the feeding and reproductive cycles, and shows only small differences among species. The approximate protein contents of the various finfish and shellfish groups are given in the following table.

Fish group	Percentage
White finfish	16–19
Fatty finfish	18–21
Crustaceans	18–22
Bivalves	10–12
Cephalopods	16–18

Fatty finfish and crustaceans have slightly higher than average protein concentrations. Bivalves have the lowest values if the whole body mass is considered (most of them are usually eaten whole), whereas values are roughly average if specific muscular parts alone are consumed; this is the case with the scallop, in which only the adductor muscle is usually eaten. Fish proteins, with only slight differences among groups, possess a high nutritive value, similar to that of meat proteins and slightly lower than that of egg. It is worth pointing out the elevated supply, relative to meat, of essential amino acids such as lysine, methionine, and threonine. In addition, owing in part to the low collagen content, fish proteins are easily digestible, giving rise to a digestibility co-efficient of nearly 100.

Essential amino acids in fish and shellfish (g/100g)

Fish group	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine
Finfish	5.3	8.5	9.8	2.9	4.2	4.8	1.1	5.8
Crustaceans	4.6	8.6	7.8	2.9	4.0	4.6	1.1	4.8
Molluscs	4.8	7.7	8.0	2.7	4.2	4.6	1.3	6.2

Fish lipids

In fish, depot fat is liquid at room temperature (oil) and is seldom visible to the consumer; an exception is the belly flaps of certain fishes mainly farm arose. Many species of finfish and almost all shellfish contain less than 2.5% total fat, and less than 20% of the total calories come from fat. Almost all fish has less than 10% total fat, and even the fattiest fish, such as herring, mackerel, and salmon, contains no more than 20% fat. In order to obtain a good general idea of the fat contents of most finfish species, flesh color might be considered. The leanest species, such as cod and flounder, have a white or lighter color, whereas fattier fishes, such as salmon, herring, and mackerel, have a much darker color.

The triacylglycerol depot fat in edible fish muscle is subject to seasonal variation in all marine and freshwater fishes from all over the world. Fat levels tend to be higher during times of the year when fishes are feeding heavily (usually during the warmer months) and in older and healthier individual fishes. Fat levels tend to be lower during spawning or reproduction. When comparing fat contents between farmed and wild-caught food fish, it should be remembered

that farmed species have a tendency to show a higher proportion of muscle fat than their wild counterparts. Also, the fatty acid composition of farmed fish depends on the type of dietary fat used in raising the fish.

Cholesterol in Fish

Cholesterol is independent of fat content and is similar in wild and cultivated fishes. The fish and shellfish contain well under 100 mg of cholesterol per 100 g, and many of the leaner types of fish typically have 40–60 mg of cholesterol in each 100 g of edible muscle. It is known that most shellfish also contain less than 100 mg of cholesterol per 100 g. Shrimp contain somewhat higher amounts of cholesterol, over 150 mg per 100 g, and squid is the only fish product with a significantly elevated cholesterol content, which averages 300 mg per 100 g portion. Fish roe, caviar, internal organs of fishes (such as livers), the tomalley of lobsters, and the hepatopancreas of crabs can contain high amounts of cholesterol.

A note on Omega-3 PUFA in Fish and Shellfish

The PUFA of many fish lipids are dominated by two members of the omega-3 (n-3) family, C20:5 n-3 (EPA), and C22:6 n-3 (DHA). They are so named because the first of several double bonds occurs three carbon atoms away from the terminal end of the carbon chain.

All fish and shellfish contain some omega-3, but the amount can vary, as their relative concentrations are species specific. Generally, the fattier fishes contain more omega-3 fatty acids than the leaner fishes. The amount of omega-3 fatty acids in farm-raised products can also vary greatly, depending on the diet of the fishes or shellfish. Many companies now recognize this fact and provide a source of omega-3 fatty acids in their fish diets. Omega-3 fatty acids can be destroyed by heat, air, and light, so the less processing, heat, air exposure, and storage time the better for preserving omega-3 in fish. Freezing and normal cooking cause minimal omega-3 losses, whereas deep frying and conditions leading to oxidation (rancidity) can destroy some omega-3 fatty acids.

Vitamins

Water-soluble vitamins are well represented in all kinds of fish, with the sole exception of vitamin C (ascorbic acid), which is almost absent in all of them. The concentrations of the rest are highly variable; however, with few exceptions, they constitute a medium-to-good source of such vitamins, com-parable with, or even better than, meat. The contents of vita-mins B_2 (riboflavin), B_6 (pyridoxine), niacin, biotin, and B_{12} (cobalamin) are relatively high. Indeed, 100 g of fish can contribute up to 38, 60, 50, 33, and 100%, respectively, of the total daily requirements of those vitamins. Fatty fish also provides a higher supply of many of the water-soluble vitamins (namely pyridoxine, niacin, pantothenic acid, and cobalamin) than does white fish or shellfish. Crustaceans also possess a relatively higher content of pantothenic acid, whereas bivalve molluscs have much higher concentrations of folate and cobalamine.

Fish Minerals

Seafood is also loaded with minerals such as phosphorus, magnesium, iron, zinc, and iodine in marine fish. The first point to note is that all kinds of finfish and shellfish present a well-balanced content of most minerals, either macrominerals or trace elements, with only a few exceptions. Sodium content is low, as in other muscle and animal origin foods. However, it must be remembered that sodium is usually added to fish in most cooking practices in the form of common salt; also, surimi-based and other manufactured foods contain high amounts of added sodium.

Calorific value

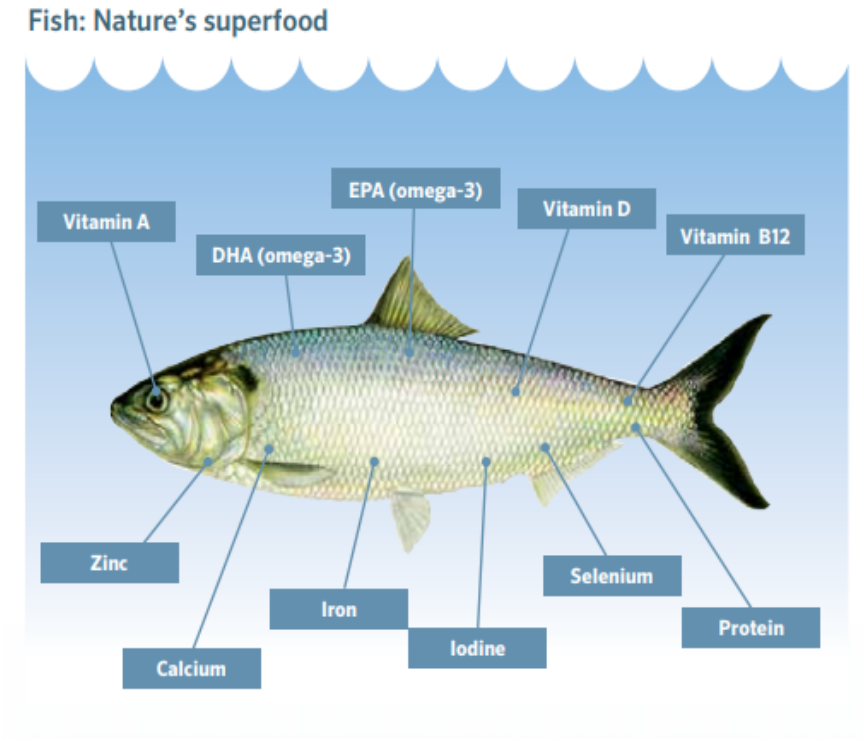
The caloric value of fish is related to the fat and protein content. The fat varies with species, size, diet, and season. Seafood is generally lower in fat and calories than beef, poultry, or pork. Most lean or low-fat species of fish, such as cod, hake, flounder, and sole, contain less than 100 kcal (418 kJ) per 100 g portion, and even fatty fish, like mackerel contain approximately 250 kcal (1045 kJ) or less in a 100 g serving.

Vitamin content of the different groups of fish and shellfish (mg or mg per 100 g), and relation to DRIs

	A (mg)	D (mg)	E (mg)	B_1 (mg)	B_2 (mg)	B_6 (mg)	Niacin (mg)	Biotin (mg)	Pantothenic acid (mg)	Folate (mg)	B_{12} (mg)	C (mg)
White finfish	Trace	Trace	0.3–1.0	0.02–0.2	0.05–0.5	0.15–0.5	1.0–5.0	1.0–10	0.1–0.5	5.0–15	1.0–5.0	Trace
Fatty finfish	20–60	5–20	0.2–3.0	0.01–0.1	0.1–0.5	0.2–0.8	3.0–8.0	1.0–10	0.4–1.0	5.0–15	5.0–20	Trace
Crustaceans	Trace	Trace	0.5–2.0	0.01–0.1	0.02–0.3	0.1–0.3	0.5–3.0	1.0–10	0.5–1.0	1.0–10	1.0–10	Trace
Molluscs	10–100	Trace	0.5–1.0	0.03–0.1	0.05–0.3	0.05–0.2	0.2–2.0	1.0–10	0.1–0.5	20–50	2.0–30	Trace
Cephalopods	Trace	Trace	0.2–1.0	0.02–0.1	0.05–0.5	0.3–0.1	1.0–5.0	1.0–10	0.5–1.0	10–20	1.0–5.0	Trace
RDA	700/900	5	15	1.1/1.2	1.1/1.3	1.3	14/16	30	5.0	400	2.4	75/90
% RDA per 100 g	0–11	0–100	2–20	1–20	2–38	5–60	1–50	3–33	2–20	0.3–12	40–100	0
% RDAMd	2	50	7	5	15	25	18	5	8	2	100	0

Selected mineral content of the different groups of fish and shellfish (mg per 100 g), and relation to DRIs

	Na	K	Ca	Mg	P	Fe	Zn	Mn	Cu	Se	Cr	Mo	I
White finfish	50–150	200–500	10–50	15–30	100–300	0.2–0.6	0.2–1.0	0.01–0.05	0.01–0.05	0.02–0.1	0.005–0.02	0.005–0.02	0.01–0.5
Fatty finfish	50–200	200–500	10–200	20–50	200–500	1.0–5.0	0.2–1.0	0.01–0.05	0.01–0.05	0.02–0.1	0.005–0.02	0.005–0.02	0.01–0.5
Crustaceans	100–500	100–500	20–200	20–200	100–700	0.2–2.0	1.0–5.0	0.02–0.2	0.1–2.0	0.05–0.1	0.005–0.02	0.01–0.05	0.01–0.2
Molluscs	50–300	100–500	50–200	20–200	100–300	0.5–10	2.0–10	0.02–0.2	0.02–10	0.05–0.1	0.005–0.02	0.01–0.2	0.05––0.5
Cephalopods	100–200	200–300	10–100	20–100	100–300	0.2–1.0	1.0––5.0	0.01–0.1	0.02–0.1	0.02–0.1	0.005–0.02	0.01–0.2	0.01–0.1
RDA	1500	4700	1000	320/420	700	18/8	8/11	1.8/2.3	0.9	0.025/0.05	0.035	0.045	0.15
% RDA per 100 g	3–33	2–10	1–20	4–50	15–100	2–50	1–90	0–10	1–100	25–100	15–60	10–100	8-100
% RDA/Md			6	5	30	18	2		2	100			100



Source: FAO - Fish and human nutrition

Further reading

- Lands, W.E., 1986. *Fish and human health*. Academic Press, Inc..
- Ruxton, C., Reed, S., Simpson, M. and Millington, K., 2007. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *Journal of human nutrition and dietetics: the official journal of the British Dietetic Association*, *20*(3), pp.275-285

****

11

HYGIENIC HANDLING AND LOW TEMPERATURE PRESERVATION

Sreelakshmi K. R.
Fish Processing division
Email ID: ***sreecift@gmail.com***

The most important factors to be considered in the initial handling and transport are the temperature, duration of storage/transport and the hygiene in all respects including that of the handlers. The important requirements are cleaning the fish from dirt and debris, chilling it immediately to prevent its temperature from rising and maintaining high standards of cleanliness at all stages. Fish, which has struggled for long in the net or onboard, is likely to spoil more quickly than a fish, which dies instantaneously or is killed quickly. Similarly, fish with its stomach full while catching, will spoil more quickly and fish, which is bruised while catching or handling, will spoil more quickly than a physically sound fish. The harvested fish should be washed well with potable water to free it from dirt and other extraneous matter. Water chlorinated at 10 ppm level is ideal for initial cleaning. Most of the surface bacterial load is cleared by washing. After washing the catch should be sorted species – wise and size – wise. Bruised, damaged and decomposed fish shall be separated from the catch during sorting. The dressing operations of the catch include heading, bleeding and gutting have to be carried out as fast as possible without significant bacterial contamination. Gills and viscera harbour several spoilage bacteria in large numbers. Partially digested food in the viscera may become sour or putrid due to bacterial action. The powerful digestive enzymes in the viscera can bring about accelerated spoilage of fish. Therefore, wherever possible, it is advisable to remove the gills and viscera before the fish is preserved and stored. Gutting or evisceration should not cause any bruise on the exposed belly portion. Retention of any visceral parts can easily contaminate the soft belly and bruises can cause accelerated spoilage by permitting easy penetration of bacteria. The fish should be washed thoroughly after each operation. The larger fish are gutted by hand, washed and iced. Gutting helps to remove digestive enzymes and foul smelling compounds associated with gut. It also prevents accumulation of bloodstains and control haemoglobin catalyzed lipid oxidation in the fillets. The blood in the fish can clot and turn black or brown in colour adversely affecting the colour and appearance of the meat. Therefore, bleeding is done to preserve the quality of the meat. Bleeding and evisceration can be done only to fish of reasonably large size. Slitting the throat followed by hanging the fish by tail or slitting the throat and immersing in cold water are the methods for bleeding.

Chilling of fish

Chilling is an effective way of reducing spoilage in fish if it is done quickly and if the fish are kept chilled and handled carefully and hygienically. Immediate chilling of fish ensures high quality products. For every 10 ^{0}C reduction in temperature, the rate of deterioration decreases by a factor of 2-3. The objective of chilling is to cool the fish as quickly as possible to as low a temperature as possible without freezing. Chilling cannot prevent the spoilage together but in general, the colder the fish, the greater the reduction in bacterial and enzyme activity.

The important chilling methods of fish and fish products at non-freezing temperature are:

- Icing
- Chilled seawater (CSW) storage.
- Chilled freshwater (CFW) storage.
- Mechanically Refrigerated seawater (RSW) storage.
- Cold air storage.

The most common means of chilling is by the use of ice. Although ice can preserve fish for some time, it is still a relatively short-term means of preservation when compared to freezing, canning, salting or drying, for instance. When used properly it can keep fish fresh so that it is attractive in the market place.Ice is available in several forms such as blocks, plates, tubes, shells, soft and flakes. Of these, flake ice is the most popular form 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. It also causes minimum damage to the flesh To ensure maximum contact of ice with the fish, proper selection of the size of ice particles and good stowage practices are needed. The rate of chilling is governed by:

- The size, shape and thickness of fish;
- The method of stowage;
- Adequate mixing of ice, water and fish (in ice slurries);
- Adequate contact of ice with the fish;
- The size of the ice particles.

Icing is widely employed for chilled storage of freshwater fish in the country. The dressed and cleaned fish is kept in a chill store in insulated boxes with proper icing prior to preprocessing. The major advantage of using ice for chilling the fish is that it has a high latent heat of fusion so that it is capable of removing large amount of heat as it melts without changing the temperature at 0 ^{O}C. In tropical conditions a 1: 1 fish to ice ratio is ideal for ice storage. Fish of the same size and species are placed in the same boxes. It is always recommended to add about 12-20% extra ice to the fish in order to compensate for water loss from melting and bad handling. The effectiveness of chilling by temperature exchange depends on the thickness of the layers of fish and the distribution of ice.

Disadvantages of icing

Icing in the conventional method using crushed ice can bruise the flesh which results in leaching of flavour compounds and water soluble proteins. Prolonged ice storage can cause changes in the texture of the muscle, particularly the reduction in breaking strength and hardness of fillets. Muscle proteases including cathepsin D and cathepsin L, calcium activated proteases (calpains), trypsin, chymotrypsin, alkaline proteases and collaginases are involved in softening of fish tissue during storage. Ice storage has been found to adversely influence protein stability and water holding capacity in salmon and cod fillets*. Icing cannot completely arrest the activities of psychrotrophic organisms in fish, which is a quality problem in refrigerated food.

Development in fish preservation and transportation has significantly increased the proportion of fish products that enters International trade. Experience and observation are the best teachers when learning how to avoid stressing fish during handling and transport. Since the outcome of poor handling is generally sick or dying fish it does not make sense to take chances. Establish and follow a set of procedures, which minimize stress, and risk of injury to fish when they are handled so that the quality is maintained irrespective of the way in which fish and shellfish are traded.

Low temperature preservation is the best method to retain the quality and freshness of fish and fish products for a long time. Among them, chill storage i.e., keeping the fish in the unfrozen condition has only limited shelf life and it will vary between 4 and 20 days depending on the condition and species of fish. In frozen storage also the shelf life is restricted but it varies from few weeks to years. The various factors that affect the frozen storage shelf lives are condition of fish at the time of catch, handling, processing and product development, packaging and glazing of the product, freezing method adopted, frozen storage temperature, stacking methods and transportation techniques. These factors can be put together and can be termed as 'Product, Processing and Packaging' (PPP) and 'Time Temperature Tolerance' factors (TTT).

The shelf life of a fish vary with species, size, environment, life cycle stage and condition of fish in addition to handling factors. Fatty fish like have shorter shelf life while lean fish such as rohu etc. have longer shelf life under same conditions of freezing and frozen storage. Seasonal and biological variations in fish contribute towards the quality characteristics and nature of fish muscle. The factors mainly responsible for these changes are environmental conditions like temperature and availability of food, type and nature of food and spawning. Immediately after spawning the quality of fish is poor, though it recovers rapidly. Even the catching methods influence the quality. Belly bursting, muddy flavour, jelly like appearance etc. are examples of changes in fish quality because of the product variation.

Freezing of fish

It is already established that the rate of freezing has effect on tissue damage and hence on texture and cook drip. Though quick freezing is necessary to retain the quality during storage, extra rapid freezing does not have a proportional effect. Commercial quick freezing practices adopted have been found sufficient to maintain product quality. Freezing of whole fish onboard is practiced in large fishing vessels. The effect of thawing, processing into various products and refreezing of onboard frozen fish is a matter of concern for seafood industry. There are varying reports on the stability of the product. It is generally agreed that the effect is species specific. Certain species of fish like perch have little damage by double freezing while fatty species like sardines are sensitive.

Freezing Characteristics

The water present in fish products are converted to ice during freezing i.e., a change from the liquid phase to the solid phase. The change of water from liquid to solid phase results in increase in volume and a consequent decrease in density, increase in thermal conductivity and thermal diffusivity, and decrease in heat capacity. The volume increase on freezing of water is by about 9%, thermal conductivity 4 times and thermal diffusivity 11 times. Heat capacity is found to reduce from one cal/g to 0.5 cal/g. A proportional change in these properties may also observed in food products. Since water is the major component undergoing changes during freezing, knowledge of the effect of pressure and temperature on the phase diagram of water is necessary. Various combinations of temperature and pressure on two or three states of water in equilibrium are given in Fig. 1. The normal atmospheric pressure lowers the freezing point of water by 0.0075°C. The dissolved air at one atmospheric pressure depresses the freezing point of water by 0.0024°C. Thus at 4.578 mm Hg pressure the freezing point of water is 0.0099°C greater than that at one atmosphere. This point is called triple point where all three phases of water exists in equilibrium.

Freezing Curve for Pure Water

The freezing curve of pure water is given in Fig. 2. During the early stages of cooling i.e. cooling

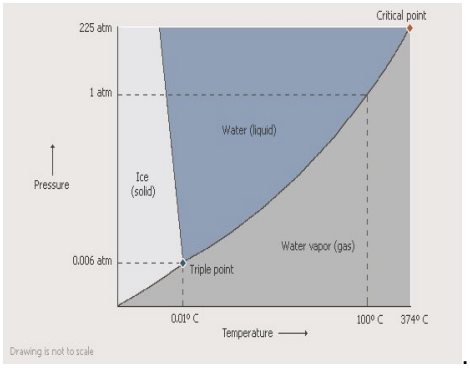


Fig. 1. Pressure temperature phase diagram of water

from ambient temperature to 0°C (T1 to T2) the sensible heat amounting to 1cal/g° C is removed. Point S represents super cooling. Super cooling is needed to remove sufficient quantity of heat so as to get stable ice nuclei for crystal growth. On crystallization of ice at S the heat of crystallization is released and the temperature of the system rises to 0°C (T2) from S. The temperature remains at 0°C until all the water is converted to ice. This period is called thermal arrest period. 79.8 cal of heat must be removed for each gram of ice formed. The water-ice transformation usually involves a long period. On completion of solidification further heat removal is faster and about 0.5cal/g of heat is removed for the decrease by every 1°C.

The freezing point of water in fish is not at 0°C, but is around -1°C depending on the concentration of solutes in fish muscle. Table 1 gives the approximate amount of water frozen at various freezing temperatures. It can be seen that at a temperature of -30°C about 8% of water remains unfrozen.

Table 1. Percentage of water frozen at various temperatures in a typical fish fillets with 80% water

Temperature (°C)	Frozen water (%)
0	0
-1	10
-2	55
-3	69
-4	76
-5	80
-10	81
-20	91
-30	92

Crystallization

Crystallization during freezing is the formation of a systematically organized solid phase from a solution or liquid. It consists of two phases viz. nucleation and crystallization. During removal of heat, water molecules tend to combine into an ordered manner in the form of a particle. When the size of the particles is sufficient to survive and serve as a site for crystal growth, it is called nucleus. The process of formation of nucleus is called nucleation. When the size is not sufficient for the growth it is called embryo. During crystal growth an enlargement of the nucleus takes place by an orderly addition of water molecules.

Crystal Growth

The second phase of crystallization consists of crystal growth. It requires some super cooling usually less than 0.1°C. The crystal growth occurs by the systematic addition of molecules to the crystal surface. Crystallization of water from a solution containing different solutes is limited by mass transfer and heat transfer. Water molecules move from the liquid phase and attach to this sites where the energy is sufficiently low to provide stability. At the same time the solute must diffuse away from the vicinity of the interface towards the interior of the liquid phase. The latent heat of crystallization must be removed to sustain crystallization.

Crystal Size

Rapidly frozen food contain ice crystals which are small and numerous. But similar specimens, which have been frozen slowly, contain few ice crystals of large size. The sizes of the completed crystal vary inversely with the number of nuclei formed. Even though freezing conditions are held constant, large differences in crystal size are sometimes noticed among different substances, different samples of the same substance and even within the same sample. Fish frozen in pre-rigor contain smaller and more numerous ice crystals than that in post rigor under similar conditions. This is attributed to the greater amount of bound water in the pre rigor samples than post rigor samples. These bindings retard the migration of free water in the pre-rigor muscle and thereby encourage formation of more nucleuses.

Location of Ice Crystals in Tissue

The location of ice crystals in tissue is a function of freezing rate, specimen temperature and the nature of the cells. Slow freezing of fish muscle generally causes ice crystals to form exclusively in extracellular areas. Although uncommon, intracellular ice crystals have been observed in some slowly frozen specimens e.g. pre-rigor cod and tissue frozen for a second time. Conditions leading to preferential extra-cellular ice crystals result in large ice crystals, maximum dislocation of water and a shrunken appearance of cells in the frozen state.

All kinds of tissues exhibit a uniform distribution of ice crystals both intracellurlarly and extracellurlarly when frozen very rapidly. In tissue, uniform crystallization is essentially synonymous with intracellular crystallization. The rate of freezing needed to produce uniform crystallization generally increases as cell size decreases. Conditions which produce intracellular crystallization result in numerous small ice crystals with minimum dislocation of water and a frozen appearance similar to the original unfrozen appearance. The food quality is usually superior than that obtain by slow freezing.

Cells contain a greater concentration of non-diffusible ions like protein than the surrounding fluids. Diffusible ions exist in unequal concentration on opposite sides of the cell membrane and the concentration of the ionic particles is greater inside the cell than outside. On this basis a lower freezing point is expected for the cell contents than the surrounding fluids. Regardless of freezing rate, crystallization is initiated primarily in the extra cellular fluid. However,

crystallization of systems containing limited quantity of extracellular fluid such as pre rigor cod begins in intracellular areas.

Freezing Techniques

There are a number of methods by which fish can be frozen. It may be either sharp (slow) freezing or quick freezing. Slow freezing is accomplished by placing the product at a low temperature and allowing it to freeze slowly usually in still air. Quick freezing is accomplished in any one or in any combination of the following four methods:

- Immersion freezing
- Indirect contact freezing
- Air blast freezing and
- Cryogenic freezing:

Air freezing

Sharp freezing

Packaged or unpackaged marine products can be frozen in air at temperature from --18 to -40°C. If "sharp" freezing is employed, air is circulated slowly or not at all and the rate of freezing is very slow. It ranges from 3-72 hour or more depending on the conditions and size of the product. Sharp freezing is not common in modern freezing operations.

Tunnel freezing

Circulating cold air at high speed enables freezing to proceed at a moderately rapid rate and this method is referred to as air-blast freezing. Air-blast freezing is usually accomplished by placing the products on a mesh belt and passing it slowly through an insulated tunnel containing air at-18 to -34°C or lower, moving counter current to the product at a speed of l to 20 meter/sec. Air at -29°C and at a speed of 10-12 meter/sec, is often satisfactory, although lower temperatures are preferred.

Spiral Belt Freezer

Modern designs of belt freezers are mostly based in the spiral belt freezer concept. In these freezers a product belt that can be bent laterally is used. The present design consists of a self-staking and self-enclosing belt for compactness and improved air flow control. The number of tiers in the belt stack can be varied to accommodate different capanlies and line layouts. The belt is continuous. The products are placed on the belt outside the freezer where it can be supervised. As the belt is continuous it is easy for proper cleaning. Both unpacked and packed 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 used. Vertical airflow is more efficient.

Carton freezer

This freezer consists of a number of carrier shelves which are automatically moved through the section of the unit. The operations are carried out hydraulic power with mechanical linkage

to coordinate different movements. The boxes are fed automatically into the freezer on a feeding conveyor.

Fluidized-Bed Freezing

Marine products of small size like prawns can be fluidized by forming a bed of prawns on a mesh belt and then forcing air upward through the bed at a rate sufficient to partially lift or suspend the particles. If the air used for fluidization is sufficiently cooled, freezing can be achieved at a rapid rate. An air velocity of at least 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. In this instance since fluidization is not involved a more proper name is "through-flow air freezing. It will take about 30-35min to bring down temperature from 30°C to −18°C for fish fillets up to 3 cm thick. Fluidized bed freezing has proven successful for many kinds and sizes of 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 as compared to air- blast freezing are (1) more efficient heat transfer and more rapid rates of freezing and (2) 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 disadvantages of fluidized-bed freezing are that large or non uniform products cannot be fluidized at reasonable air velocities.

Plate Freezing

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

Liquid Immersion Freezing

Liquid immersion freezing or direct immersion freezing is accomplished when a product is frozen by immersion in or by spraying with a freezant that remains liquid throughout the process. This technique is occasionally used for fish and prawns. Liquid immersion freezing can result in moderately rapid freezing. Freezants used for liquid immersion freezing should be non-toxic, inexpensive, stable, reasonably inert, and should have a low viscosity, low values of vapour pressure and freezing point and reasonably high values for thermal conductivity. Freezants should have a low tendency to penetrate the product, little or no undesirable effects on organoleptic properties and require little effort to maintain desired standards for sanitation and composition. Aqueous solutions of propylene glycol, glycerol, sodium chloride, calcium chloride and mixtures of sugars and salt have been used as freezant.

Cryogenic Freezing

Cryogenic freezing refers to very rapid freezing by exposing food products to an extremely cold freezant undergoing change of state. The fact that heat removal is accomplished during a change of state by the freezant is used to distinguish cryogenic freezing from liquid immersion freezing. The most common food grade cryogenic freezants are boiling nitrogen and boiling or subliming carbondioxide. Boiling nitrous oxide also has been considered, but at present it is not being used commercially. Boiling CCl2F2 (freon-12) does not have sufficiently low boiling point to qualify as a true cryogenic fluid, but it is included in this category since it can provide, by the change of state principle, rates of freezing comparable to those obtained commercially with true cryogenic freezants. 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. For example, shrimp freeze in about 9 min in a commercial liquid nitrogen freezer and in about 12 min in a fluidized bed freezer.

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. None of the current commercial liquid nitrogen freezers employ the technique of direct immersion. It should be noted that the final product temperature is usually not different from that obtained during conventional methods of freezing. The following are some of the advantages of liquid nitrogen freezing.

- Dehydration loss from the product is less than 1%.
- Oxygen is excluded during freezing.
- The individually frozen products undergo minimal freezing damage. Fish/prawns frozen cryogenically exhibit minimum thaw exudate and minimum damage to texture. These quality advantages are retained if the frozen storage is minimised and / or the temperature is -23°C or lower.

- The equipment is simple, suitable for continuous flow operations, adaptable to various production rates and product sizes, or relatively low initial cost, and capable of high production rates in a minimal space.

The only disadvantage is the high operating cost and this is attributable nearly entire to the cost of liquid nitrogen. Freezing with carbon dioxide usually involves tumbling the product in the presence of powdered or liquid carbon dioxide. This method provides most of the advantages cited for liquid nitrogen freezing. Carbon dioxide is absorbed or entrained by the product in this method. This entrapped CO2 should be removed before it is packaged in an impervious material. In freon 12 as cryogenic freezant the material is placed on stainless steel mesh belt and conveyed through an insulated freezing chamber. Freezing is accomplished either by spraying the product with food grade CCl2F2 or by a combination of initial immersion of the product followed by spraying. In both procedures vapours are collected for reuse. Advantages for freezing in liquid freon-12 are essentially the same as those cited for liquid nitrogen. This method has an advantage of lower operating costs.

Crusto Freezer

This 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 fludized bed freezer without deformation or breakage.

Physical Changes during Frozen Storage

The major physical changes during frozen storage of fish are freezer burn and recrystallization. Freezer burn is a surface phenomenon which occurs in improperly packed products. Freezer burn appears as an opaque dehydrated surface. It is caused by the sublimation of ice on the surface of the muscle. The sublimation takes place when the vapour pressure of ice on the surface of fish muscle is higher than the vapour pressure of the cold store. Other factors contributing to freezer burn are air velocity in the cold store, cold storage temperature and post mortem condition of the muscle. It can be prevented or reduced by glazing the product in chilled water and air tight packaging with water impermeable packaging materials.

The ice crystals in the frozen muscle undergo transformations during frozen storage causing changes in number, size and shape. This phenomenon is called re-crystallization. During frozen storage, the ice crystals in rapidly frozen samples are found to grow slowly. The sizes of the ice crystals between rapidly frozen and slow frozen samples have almost the same size after a long storage. There are many reasons for the changes in size and shape. During storage, the reorientation of the ice crystals takes place to give a stable shape with a compact structure having smaller surface to volume ratio and lower surface energy. In frozen products, the large ice crystals may grow at the expense of small crystals. This may be caused by melting-diffusion-refreezing or sublimation-diffusion - refreezing. The net result is an increase

in average crystal size, decrease in the number of crystals and decrease in surface energy of the crystalline phase. Fluctuating temperature and associated vapour pressure gradients enhance this type of re-crystallization. Each frozen product exhibits a critical temperature below which re-crystallization does not occur at a significant rate. Low and uniform temperature of frozen storage can minimize re-crystallization.

Drip

Drip is the exudates coming out from a frozen product on thawing. Fish after freezing, frozen storage and thawing often exudates a considerable amount of drip. Drip may amount to 1 to 5% or much more. Drip loss may cause sizable financial loss. On thawing, if the drip loss is high, the frozen products appear somewhat dry and stringy. However, the relationship between texture and drip loss need not be linear upto moderate drip loss, but at high drip loss, the loss of texture is directly related. Though factors like internal pressure developed during freezing, freezing rate, size and location of ice crystals may influence thaw drip, the major factors are the quality of the raw material, abuse of frozen storage and the extent of resultant denaturation. When the quality is poor and the frozen product is stored especially at a higher frozen storage temperature for a long duration the amount of drip is found high and is almost proportional to the storage period. Very slow freezing and the development of large extracellular ice crystals also have some influence. In quick freezing the cell dehydration during freezing is minimum due to the formation of uniform intracellular and extracellular ice crystals. This causes minimum damage to the cell and consequently expects a low drip.

Quality Changes

Most of the quality changes normally attributed to the freezing process are indeed unrelated to that process. In fact, except for cases where texture is adversely affected by freezing, the frozen product is often practically indistinguishable from the fresh product when thawed immediately. However, after few months of storage, depending on product, process, packaging and storage temperature, changes are noticed. These changes are due to changes during frozen storage. The drip is very much increased by warm freezer storage temperatures. The explanation generally offered is that the high ionic strength of the solution causes rapid denaturation of proteins with poor binding of water as a consequence. This effect is not pronounced at colder freezer storage temperature because of reduced reaction rates. The most important adverse effect on freezing and frozen storage on nutritive value may be a loss of vitamins, mostly the more labile ones such as ascorbic acid, thiamin and riboflavin vitamins are water soluble and hence some losses occur in the drip.

Time Temperature Tolerance

Longer keeping times are recorded at colder temperatures in frozen storage shelf life studies. Many chemical reactions such as lipid oxidation, lipid hydrolysis and protein denaturation and the resultant sensory changes in texture and flavour are temperature dependent. Time

temperature tolerance studies for quality changes during frozen storage showed a logarithmic relationship of storage time vs. temperature of the storage. Various studies indicated that the frozen storage temperature has pronounced influence on quality and shelf life. In general, the retention of the qualities will be better at lower temperatures and an inversely proportional shelf life.

Freeze/Thaw Stability

Most frozen food will suffer some physical deterioration if they are subjected to thawing and refreezing. There are often textural changes brought about by the formation and reformation of ice crystals. Fish and meat both suffer under these circumstances and cause protein denaturation. It is possible to give some protection against damage from freeze/thaw cycles by using certain stabilizers. Polysaccharides such as sucrose, sorbitol, carrageenan and modified starches exhibit such cryoprotective properties.

Selecting a method for Freezing Fish / Prawns

The selection of a method for freezing fish / prawns should be based on cost and quality considerations. It is quite possible that a product with the lowest retail price may be rejected by the consumer for a product of better quality which has been achieved by using a superior but costlier processing method. On the other hand, it is possible that some products processed by the most economical methods may have qualities which are only slightly inferior to products processed by more expensive methods.

To properly assess the effect of freezing methods on product quality, the product must be evaluated following a treatment similar to that which it will receive commercially. To assess the quality of frozen fish products, the product must be evaluated after the following sequence of events:

- Pre-freezing treatments such as chilling, addition of chemicals etc.
- Freezing
- Frozen storage for a commercially realistic time and temperature.
- Thawing and also cooking. Quality information can be combined with data on freezing cost to determine which method of freezing is best for the product under consideration.

Pre-freezing and Freezing Consideration

The quality of frozen-thawed cooked fish is influenced by a number off actors including species, composition, size, how and where caught, 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. Fish are subject to deterioration by microorganisms and by autolysis when it is unfrozen. So great care must be taken to follow sanitary practices, to promptly cool to near 0°C for preprocessing operations

and to freeze without undue delay. Effective prefreezing and freezing techniques are available for controlling many of these problems except toughening and loss of Juiciness. Reasonable control of toughening and loss of juiciness can be accomplished only by storing fish for a minimal time and / or at temperatures at -18°C or lower. Undesirable oxidative changes in fish can be minimised 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.

Packaging

Adequate packaging is needed to prevent loss of moisture from the surface of the fish products and penetration of oxygen into the product. If water and oxygen impermeable packaging materials are not used, dehydration and oxidation respectively may take place in fish. Dehydration accelerates protein denaturation. The presence of oxygen leads to peroxidation of highly unsaturated fatty acids of fish. These peroxides are highly unstable and undergo decomposition causing flavour and texture changes. It is better to have impermeable or high barrier packaging materials which fit tightly to the product. If the packaging material is not fit tightly to the product, moisture will migrate to the inside surface of the package from the products forming ice crystals. Fatty fish benefit considerably by vacuum packaging. Before packaging many fish products are glazed in chilled water, which gives a layer of ice on the surface of the fish. It is done on individually frozen product and also block frozen product. Significant enhancement of frozen shelf life is noticed by glazing. Some chemicals are also added to the glaze water to improve certain specific properties. Generally, a water glaze renewed periodically gives sufficient protection. Low density polyethylene, polyester laminated with LD-HD co-extruded films are found ideal for packing frozen foods.

****

12

VALUE ADDITION IN SEAFOOD SECTOR

Parvathy U.
Fish Processing Division
Email ID: ***p.pillai2012@gmail.com***

Seafood is valued globally for its availability, accessibility, nutritional content, quality and safety aspects. These valuable commodities are simultaneously convenient due to its potentiality for high product diversification. It is rich in quality protein, omega-3 fatty acids, essential vitamins, and minerals. Though an excellent source of nutrients, it is highly perishable too, which demands its effective processing and preservation. Effective approaches in this regard is crucial to maintain its inherent quality characteristics and extended stability for better returns, to abate post-harvest losses as well as for consumer safety and satisfaction. Preservation aims to delay, lessen or hinder spoilage on account of chemical, enzymatic or microbial causes which can be achieved by controlling the storage temperature, maintaining proper water activity, proper pH, use of preservatives, alone or in combination. 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 if properly processed and stored. Traditional preservation techniques, collectively known as curing techniques viz., drying, salting, smoking, pickling etc. are commonly adopted methods for seafood preservation. The demand for fishery products with minimal processing has drawn the attention of innovative processing and preservation technologies like retort processing, high pressure processing, irradiation, pulse light technology, cold plasma processing, ohmic heating etc. to preserve and extend the quality and stability of these valuable commodities without compromising its nutritional, functional, and sensory characteristics.

Value addition approaches

Being one of the fastest growing economies and the second largest consumer market in the world, India offers a strong platform for processed seafood industry. As far as fish processing industry is concerned value addition is one of the possible approaches to raise profitability on account of the highly competitive and increasingly expensive nature of the industry. In foods, value is a combination of functional attributes as well as emotional benefits arising on account of nutritional as well as sensory facets at superior quality as well as affordable price. In

addition, it promises utilization of the under-exploited nutrient rich resources in the most effective manner.

Value can be added to fish and fishery products according to the requirements of different markets. Value added fish products are presented in a preparatory and convenient form such as dressed/trimmed, minced etc. or those that have added ingredients such as a coating, bioactive/functional constituents in it. A number of such diverse products have already invaded the industry, globally ranging from live fish and shellfish to ready to serve convenience products. Value added fishery products primarily fall under the categories viz., mince/mince-based products, surimi/surimi-based products, enrobed or coated products, ready to serve retorted products, cold/hot extruded products, speciality products, ethnic products like marinated, dried products etc.

Fish mince can be defined as deboned and unwashed fish flesh from fillets or frames and is produced at the initial step of surimi manufacturing. When compared to surimi, fish mince can be obtained at a significantly higher yield with much less capital investment. Fish mince also offers nutritional advantages, economic benefits as well as functional advantages compared to the other intermediate materials. Fish mince can also be successfully used directly in various food systems and in a physically or chemically altered form to produce an array of nutritional and functional products. It finds application in processing several convenience foods like fish finger, cutlet, burger, fish momos and also in some low cost salted and dried products. For preparation of fish finger, stick, etc., the mince stripped from the bone frame is incorporated to increase the yield. Surimi is a Japanese term for mechanically deboned fish flesh that has been washed with water and mixed with cryoprotectants for good frozen shelf life. Washing not only removes fat and undesirable matters such as blood, pigments and odoriferous substances but also increases the concentration of myofibrillar protein, the content of which improves the gel strength and elasticity of the product. This property can be made use in developing a variety of products like fish sausage, balls, burgers as well as fabricated products like shellfish analogues which fetches good demand in both domestic and export markets. Low cost fishes can also be conveniently used for the preparation of surimi. Block frozen surimi and surimi-based products are popular, especially in South east Asian countries.

Coated/battered and breaded commodities are highly appreciated form of value-added products on account of their convenience, sensory appealness and nutritional attributes. In view of the increasing consumer demand, the technology has made several advancements. The most important advantage of coating is value addition as it increases the bulk of the product. This technology also paves way for better utilization of underutilized seafood resources. A wide array of seafood products can be categorized in it, with the first commercially launched coated product being fish finger/fish stick followed by commodities in similar line viz., coated fish fillet, fish portions, fish cakes, fish medallions, fish nuggets, breaded

oysters and scallops, crab balls, fish balls, coated shrimp products, coated squid rings etc. The most popular battered and battered products in India include fish nuggets, cutlet, balls, finger, patties etc. Various ready to eat novel battered and breaded snack products have good scope in value added markets.

Ready to serve fish products are gaining popularity in both domestic and export market. A wide array of products are categorized under this including retorted fish curries, rice-fish combos, seafood biriyanis etc. These products have a shelf life of more than one year at room temperature. The most common retortable pouch consists of a 3-ply laminated material consisting of polyester/aluminium/cast polypropylene. As there is increasing demand in domestic and International market for ready to serve products, proper exploration of this technology can provide a lively market for these commodities. The technology for retort pouch processing of several varieties of ready to serve fish and fish products including curries from mackerel, rohu, sardine, tuna, pomfret, prawn, seer fish molly, pearl spot molly, fried mussel, fish sausage, prawn kurma, prawn manchurian, fried mussel masala etc. has been standardized at ICAR-CIFT and this technology has been transferred successfully to entrepreneurs.

Food extrusion provides a great versatility for the development of low-cost, high-nutritive and convenient food products such as cereal-based snacks and food products. Extruded products are gaining importance nowadays on account of their unique flavour, texture and convenience. Extruded products contain low levels of protein, which makes it necessary to fortify them with protein-rich diets. One of the possible ways for alleviating this problem is to utilize fish and fish proteins to enrich cereal-based extruded products. Formulation of appropriate types of products using fish meat and fish portions will add value to the low-cost and underutilized fish and shellfish, thus promoting their utilization. Attractive packaging for the products and market studies are needed for the popularization of such products. These products can command very high market potential particularly among the urban elites.

Product diversification is becoming mandatory for effective marketing and currently, speciality products that are more convenient viz., ready to cook/eat are getting more consumer acceptance. The most popular products under the speciality product category include those like stretched shrimp (Nobashi), sushi (Cooked butterfly shrimp), skewered shrimp, shrimp head-on cooked (centre peeled), fish wafers, fish crackers, fish soup powder etc.

Ethnic seafood products are those that are region specific and are being prepared and consumed by different people since ancient times. Some of these EFP are preserved or processed using centuries-old indigenous knowledge of fermentation/drying/smoking etc. Globalization has resulted in high demand for these ethnic food products and hence approaches towards its popularization by adopting various processing techniques can bring a huge market potential for these commodities.

Fermentation, a traditionally been used method to preserve fresh fish, especially in tropical climates, enhances its nutritive value, improve appearance and taste, destroy undesirable factors, and also reduce the energy needed for cooking. However, it takes long duration to develop the characteristic features of fermentation. Similarly, smoking of fish is done primarily for the unique taste and flavour, however the texture of flesh may be affected during the smoking process. Hence, preparation of flavoured products with typical flavour extracts may be advised to reduce the process time and can be projected as a minimal processing protocol with product diversification scope for variety of fish species.

Curing and drying, even though an age-old practice, opens up new dimensions and possibilities towards value addition in domestic as well as overseas markets. In India as per the estimates, about 17-20% of the total catch is converted to dried products and dry fish export contributes to about 7.86% of total fish exports. The major importing countries are Sri Lanka, Malaysia, Indonesia, Singapore and United Arab Emirates. However, there are several factors hindering the addition of dried fishery products to the product profile. The major one being, drying is still considered a traditional method of processing, and hence standard operating procedures are seldom followed. Moreover, there is a general conception that drying is a secondary method for preserving low value varieties and quality compromised materials. Attempts towards improving the handling practices right from the point of raw material harvesting till marketing, popularisation of improved packaging practices, use of hygienic energy efficient mechanical driers, and adequate extension services can facilitate better adoption of drying practice in seafood sector.

Different methods of processing and preservation of seafood guarantees quality and safety to different extent. Of this, one of the most obvious method that can be adopted is keeping them alive till it reaches the table. Marketing of fish in live condition not only attracts customers for its quality but also provide an important avenue for farmers to obtain high profit margins. A number of internal as well as external factors need to be considered critically for improving the survival of fish during their transportation throughout the food chain. Careful handling practices coupled with thorough knowledge on the tolerancy conditions of fishes are mandatory for effectual transportation and storage protocol.

Market Scenario

Seafood processing and marketing has become highly competitive that the exporters are shifting towards value addition for increased margins. Marketing value-added seafood products requires a deep understanding of consumer behavior, effective packaging and presentation, targeted advertising, and the ability to adapt to changing market dynamics. Successful marketing strategies can help seafood producers differentiate their products and compete effectively in the market.

Summary

Seafood product diversification by value addition of main and rest raw material can augment marketing of these commodities in food and nutraceutical sector. Simultaneously, it leads to reduced post-harvest losses contributing to global economic growth and nutritional security. Technology up-gradation remains to be the key element in value addition domain, supported by minimal processing options, innovative smart packaging concepts, intelligent quality monitoring systems.

Suggested Readings

Balachandran.K.K. 2001. Post harvest technology of fish and fish products. Daya Publishing House.

Devadasan.K. 2003. Value added fish and fishery products. *Fishing Chimes.* Vol.**23(1)**, P-131-136.

Gopakumar S. 2000. Fish Processing Technology. 2000. Daya publishing house.

Gonçalves, A. A. and Kaiser, C. (2011). Value-added seafood products: a challenge or a necessity?. *INFOFISH International*, 41.

Parvathy U., Binsi P. K., Ashok Kumar K. and Ravishankar C. N. (2020). Seafood value addition: Current trends and future prospects. Indian Farming, November, 70(11): 38-40

Venugopal.V. 2003. Value addition to Aquacultured Fishery Products. *Fishing Chimes.* Vol.23 (1), P-82-84.

Venogopal. V. and Shahidi. F. 1995. Value added products from under-utilized fish species. *Critical Review in Food Science and Nutrition*. Vol. 35(5), P-431-453.

****

13

CURED FISHERY PRODUCTS

Sreepriya Prakasan, Parvathy U. and Sreelakshmi K. R.
Fish Processing Division
Email ID: ***sreebfsc@gmail.com***

Fish preservation by means of traditional methods is still being used widely to keep the fish edible for longer time. The term “Curing” includes the method of drying, salting, smoking, marination and fermentation. These are the oldest and cheapest methods used alone or in combination to preserve the fish. As fish is highly perishable in nature efficient post harvest handling and preservation is critical to retain its freshness until it reaches the consumer. Proper handling and quicker preservation will help to reduce the physical and quality loss of fish.

Advantages of fish curing

The method of curing is simple, cheap and can be easily adopted

It enhances the shelf life of products

Helps to preserve fish when available in glut for lean period

Source of protein for areas where fresh fish is not available

Less capital intensive

Less energy requirement for storage of end products

Disadvantages of fish curing

Usually, secondary quality fish is used for curing

Poor quality final product due to unhygienic handling and production

Use of low quality inputs such as salt further degrades the quality of the products

Presence of physical contaminants such dirt and sand in the final products

Dried fish products

Drying is one of the oldest and widely used methods of fish preservation in which the moisture content of fish is removed by evaporation to arrest microbial and enzymatic spoilage. In general, the term 'drying' implies the removal of water by evaporation. In fish, water constitutes about 70-80% and since water is essential for the activity of all living organisms its removal will facilitate retardation of microbial and autolytic activity as well as oxidative changes and hence can be used as a method of preservation. Fish drying can be done by natural and artificial means. In any process of drying, the removal of water requires an input of thermal energy. The thermal energy required to drive off the water can be obtained from a variety of sources, e.g.,

the sun or the controlled burning of oil, gas or wood. The thermal energy can also be supplied directly to the fish tissue by microwave electromagnetic radiation or ultrasonic heating.

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:

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.

Air temperature: the evaporation of water produces a cooling effect. At the beginning of drying, the temperature of the fish is reduced below ambient; after a short while it reaches a steady value. At this steady value, 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.

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; the rate of drying will, therefore, be faster.

Falling rate drying phase

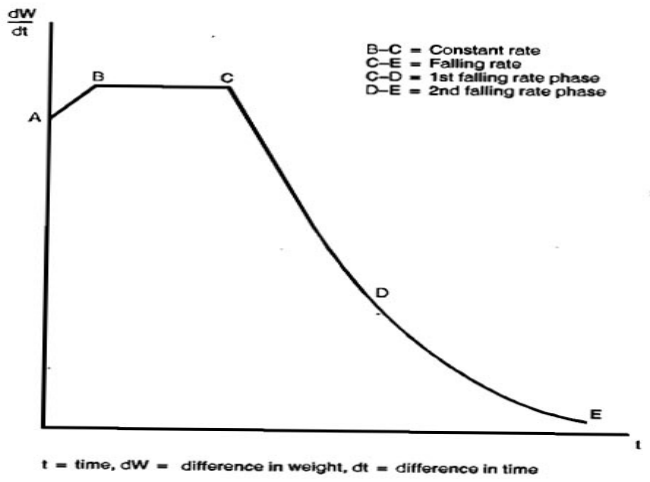
Once the free surface moisture has been removed, the rate of drying depends on the movement of moisture from interior to the surface of the fish. Several factors influence the rate of drying:

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.

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. In recent times, the controlled artificial dehydration of fish has been developed so that fish drying can be carried out under controlled conditions, regardless of weather conditions.

Natural drying or sun drying is the process in which fishes are dried under sunlight. Here solar and wind energies are utilized as the energy sources 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. Different methods include:

Drying on the ground

Rack Drying

Solar tent dryers

Solar cabinet dryer

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. Cleaned, gutted and beheaded fishes are dried in dryers where temperature, humidity and air velocity is controlled.

Artificial / Mechanical Dryers

Hot air dryers

- *Cabinet dryer*
- *Tunnel dryer*
- *Multi deck tunnel*

Contact Dryers

- *Vacuum dryers*
- *Rotary dryers*
- *Drum dryers*
-

Fish can be dried with or without the addition of salt. Dry fish products with and without salt is available in the markets. Small fishes like anchovy, sole, pony fish etc. can be dried directly after thorough washing or they can be dried after giving a drip treatment in brine. Dip such small fishes in 5-10% brine for 5-10 min depending on size of fish and dry to moisture content less than 15%. It is better to use fine salt that dissolves faster. Small shrimps like *Metapenaeus dobsoni* can be dried after giving dip in boiling water containing salt and citric acid to get an attractive pink color to dried shrimp. The shrimp need to be dried to moisture content 10-15%. Layer salting is preferred for medium and bigger-sized sized fishes before drying. The salt is applied in a salt: fish ratio of 1:3 (bigger fish) to 1:7 (smaller fish) depending upon the size of the fish. The salting should be carried out for a period of 16-18 hours, preferably overnight. The salted fishes should be dried to moisture content less than 30% after giving a wash to remove excess salt in the surface layers using potable water (preferably saturated brine solution). Crystal salt alone or fine and large crystal salt in combination can be used for salting. Mechanical drying of fish should be carried out at a temperature of 45-55°C (Ideal). It is better to avoid use of temperature above 60°C to reduce the cooking effect and case hardening. Mechanical drying usually requires 7-10.30 hours depending on the size and kind of the raw material to reduce the moisture content to safe levels.

Salted fish products

Salting is one of the oldest methods of preservation of fish. Salting is usually done as such or in combination with drying or as a pretreatment to smoking. 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 are 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 will prevent the activity of most spoilage bacteria; the removal of some water from the tissue during the salting process will also reduce the activity of the 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 and properties

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 up to 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. One group of halophiles, 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. They do not grow when the fish are fully immersed in brine or when they are fully dried. Halophilic moulds can grow on fully dried fish and cause the formation of dark patches, which is called 'dun'. Halophilic moulds tend to occur more frequently in rock salt.

Physical properties

Fine grain salt dissolves more rapidly in water and is preferred for making brines. If fine grain salt is used directly on a fish, it may cause 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'. For dry salting, a mixture of large and small grain sizes 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 up to 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 a short 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 salt thereby causing less contamination with 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. These are 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 up to 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 cleaned gutted fish which is further stacked in airtight wooden barrels filled with brine. Fishes cured by this method has a shelf life for up to 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 12-15% of salt, 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. This type of spoilage is mainly due to the presence of halophilic bacteria. The source of such bacteria the salt. It is commonly found in tropical countries like India. Spoilage appears on the surface as slimy pink patches. These bacteria are not harmful by nature. They are aerobic and proteolytic in nature, grows best at 36ºC by decomposing protein and giving out an ammoniacal odour. Usage of good quality salt will avoid this. This spoilage is mostly found in heavily salted fish and absent in unsalted fish.

Dun: Halophilic moulds tend to grow on fully dry fish, causing dark patches. These are called "dun". Fungus usually grows well on unsalted and salted dried fish, which has high moisture content. Moulds usually grow at relative humidity above 75%. The optimum temperature for growth is 30-35 degree C. In salted fish, brownish black or yellow brown spots are seen on the fleshy parts. This is mainly caused by growth of halophilic mould called *Sporendonema epizoum*. This gives the fish a very bad appearance. During the initial stages of appearance of moulds on the fish, it is possible to remove them manually. In advanced stages when it has penetrated the flesh nothing can be done. To avoid the mould growth it is necessary that the fish be dried properly to pack the fish in required type of packaging material and keep it in a cool and dry place from 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 and no penetration of salt to the interior of the fish.

Case hardening: Under certain conditions, where the constant rate drying has been very rapid, 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 that is dry on the surface and looks, to all intents and purposes, fully dry but the centre will be wet and spoiled.

Rancidity: This is caused by the oxidation of fat, which is present in the fishes. Rancidity 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 this is known as rust. This change results in an unpleasant flavour and odour to the product, thus leading to consumer rejection. Though a certain degree of rancidity can be accepted, it is seen that the nutritional value of these fishes are much lower than non-oxidized ones. These fatty fishes continue to become rancid during storage. Certain impurities in salt and traces of copper accelerate this.

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. They develop mainly when conditions are favorable with adequate moisture and intermittent rain. This results in both economic and nutritive loss to the fish processor. 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. They are very minute and bring about powdering of the product thereby giving it a white appearance. *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 45ºC.

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. It is necessary that fresh fish be used as raw material to ensure a good finished product.

Smoked fish products

Smoking is a popular method of fish preservation especially in North-eastern states of India. Smoked fishes are known for its unique aroma, texture and its golden yellow colour imparted by wood 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 known as an intermediate step in fish canning.

Marinated fish products

Value of fresh, frozen, salted, and dried fishes 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. Seafood such as squid, mussel, shrimp, sea snail, cuttlefish, and octopus etc. are also used for marination. These products are attracting customers because of their typical flavour, and textural properties. Tenderizing agents like acetic acid is added for texture modification and for better absorption of masala into the fish. The masala used can be theme based, for example mint based, fruit and vegetable based etc. After marinating with the masala, garnishing agents like dry mango, onion etc. can be used to make the product appealing. Selected extracts can be further used for augmenting the taste.

Marinated fish products serve as a good additive in the diet as these products are rich in essential nutrients required. Marinated products are rich source of essential amino acids which are also responsible for flavour and taste. They also contain significant amount of n-3 long chain polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid.

Traditionally, marinades are products with typical flavour prepared from fish by treating it with edible acid and salt and these can be put in a medium such as brine, sauce or oil. The process of marination involves an increase in ionic strength and a decrease in pH which brings desirable change in taste, texture, flavour of marinades. Along with preservative effect acid gives characteristics succulence and tenderness to the marinated fish. Addition of salt aids in extraction of salt out from the fish tissues, helps in coagulation of protein and proteolysis happens in a desired level.

As most of the marinated products contain acid, it should be done in a glass, ceramic or stainless steel container. Aluminium containers should be avoided. Further, the products should be properly covered and refrigerated.

Flavour enhancers used in marination

Other than acid and salt, different flavour enhancers are used in these semi preserves to augment the palatability and shelf life. Different kinds of sauces are among the major flavour enhancers used in the marinated products. Tomato sauce, pomegranate sauce and olive lemon juice sauce are suitable for the marinated products. Sauces with spices, salt, condiments and sugar further increases the quality of the marinated products. As discussed, additives such as spice, sauce, cream, oil, mayonnaise, parsley and dill is found to have essential effects on quality of marinades. Other than this, different vegetables and sauces like garlic, pasteurized hot pepper etc. are also suitable for the purpose. Commercial lemon pepper and eugenol extract are also used as flavour enhancers. Another category of additives are oils. Oils such as sunflower oil, corn oil, essential oil, and vegetable oil are also suitable for the marinated products. Use of plant extracts like myrtle, rosemary, and nettle extracts with brine have preservative effects when added in the marinated products.

Marination of fish has greater scope as these products have huge demand in the market due to its typical taste, texture, and flavour properties. New flavours can be added to attract the modern customers in domestic and overseas markets.

Fermented fish products

Fermentation is an age old process in which complex protein molecules in the fish are broken down by the action of organic catalysts, enzymes or ferments into simpler molecules which are stable at normal temperatures of storage. This is suitable for both freshwater and marine fishes. However in India this technology is confined to north-eastern states where there is high demand for fermented fish products because of its unique aroma. Fermented products have a meaty flavour and they are rich in nutrients. Protein breakdown can be done by both exogenous and endogenous enzymes. Endogenous enzymes present in the guts and intestines of fish. In some processes salt is added to control the extent of fermentation and here a partial breakdown of protein takes place. Microorganisms like lactic acid bacteria are also involved in the process.

Fermented products are of three distinct types:

1. Products in which fish retains its original form eg. cured fish
2. Products in the form of paste- Fish is minced and partially dried
3. Products in the form of liquid that is fish sauce

Fermented products are categorized into three based on the usage of salt

1. High salted products: 20% or more salt eg. fish sauce, cured fish and fish paste
2. Low salted products: 6-18% eg. lactic acid fermentation and acid pickling
3. No-salt: eg. Alkaline fermentation

Endogenous enzymes in fish are active at or near neural pH. In some products various cereals and plants are added to boost the fermentation reaction as the digestive enzymes from these sources also aids in the reaction. In products were salt is used the degree of fermentation depends on the proportion of salt used, fat content of the fish, dressing of the fish like complete or partial removal of gut, nature of additives used and the temperature maintained.

Three types of products obtained when salt is used in a concentration more than 20%. If the fermentation is complete that means all the protein compounds are converted into water soluble compounds, the resultant liquid is called fish sauce. If it is partial then the fish substantially retains its original form, the final product is called cured fish. This cured fish can be minced and partially dried to get fish paste. In the products where salt is less than 20%, there is higher chance for microbial attack. So any other means of preservation other than salt is required in such kind of products. In such instance lactic acid fermentation along with added source of carbohydrate like rice, milk, sugar is used to achieve fermentation. In another method, low salted fermented fish is added with vinegar known as acid pickling at low temperature. Fermentation without salt is not a common practice. Examples are alkali

fermentation and fermentation by propagation of mould on dried fish. Leafy plants ash as a source of alkali is used to ferment the half spoiled fishes.

Another classification of fermented fish is based on the technique employed. One is process involving hydrolysis by enzymes and the other one is product preserved by microbial fermentation. Proteolytic and lipolytic enzymes from the surviving microflora in the fermentation media can boost up the reaction. They also contribute to the characteristic aroma and flavour. Examples for traditional fermented fish products of Northeast India are Sheedal, Hentak, Ngari, Tungtap etc.

Conclusion

Curing is one of the oldest and traditional methods of fish preservation. These are cost effective technologies, which can be opted for a wide range of communities, with minimum investment. However a major drawback in 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 preserving low value varieties and quality compromised materials. 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.

Suggested readings

Balachandran, K. K., 2001. Post-harvest Technology of Fish and Fish Products. *Daya Publishing House,* Delhi*.,* pp. 1-433.

G. M. Hall., 1997. Fish Processing Technology (Second edition), *Published by Blackie Academic and Professional, an imprint of Chapman & Hall, 2-6 Boundary Row, London, UK.*

Behera, S. S., Madathil, D., Verma, S. K. and Pathak, N., 2020. Seafood marination-A review. *International Archive of Applied Sciences and Technology*, 11(3), pp.165-168.

****

14

SMOKED FISHERY PRODUCTS

Sathish Kumar K.
Fish Processing Division
Email ID: ***sathishcife@gmail.com***

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.

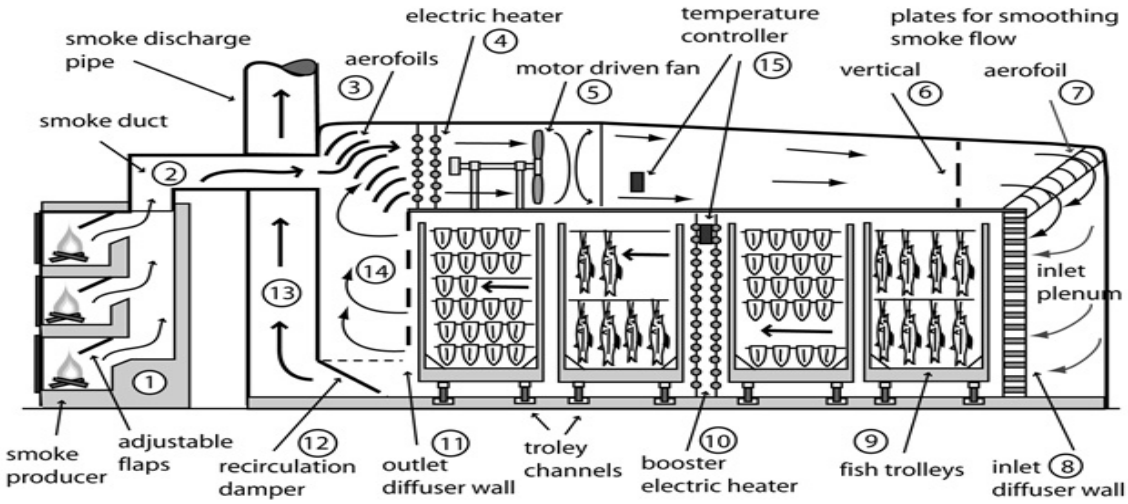


Fig. Illustration of the hot smoke airflow in the Torry smoking kiln

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.

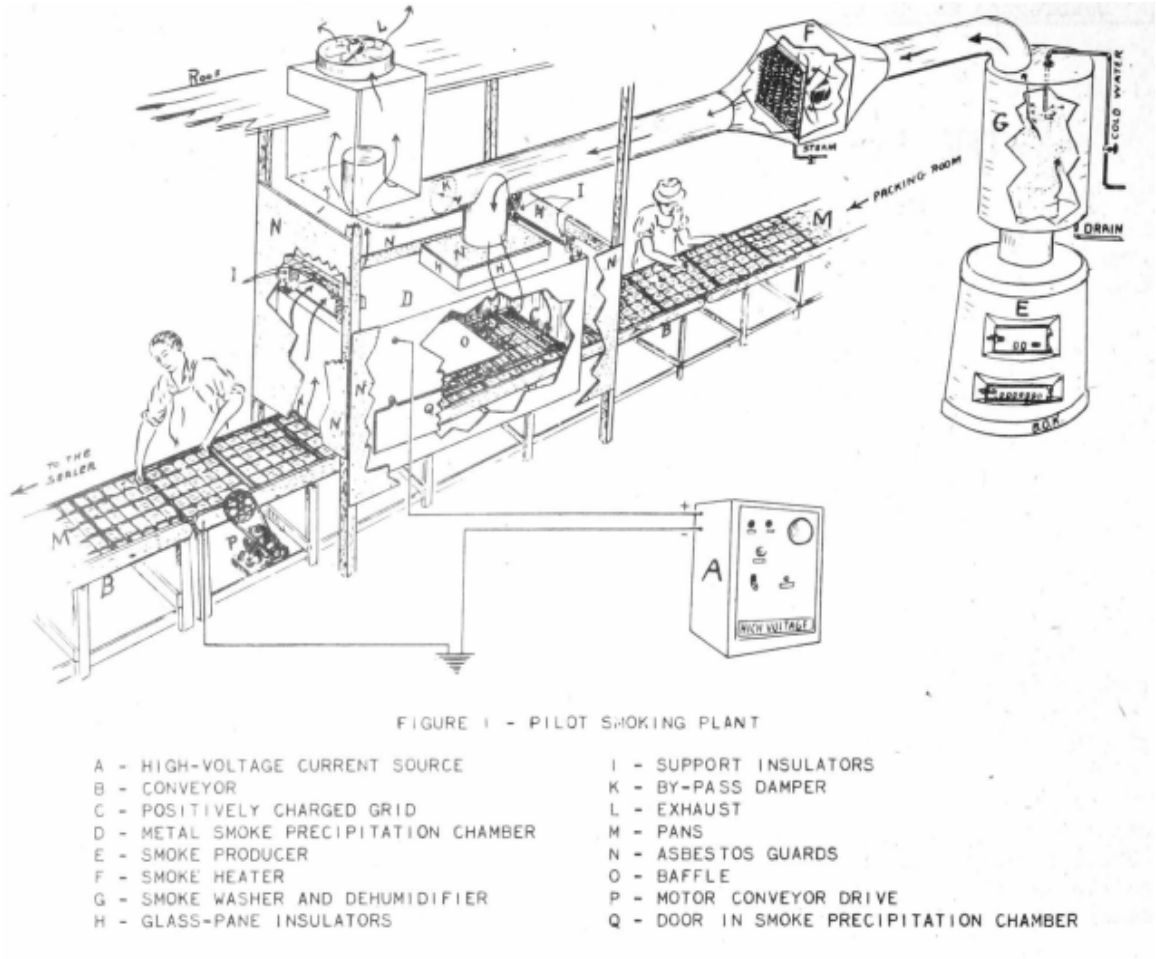


FIGURE 1 - PILOT SMOKING PLANT

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.

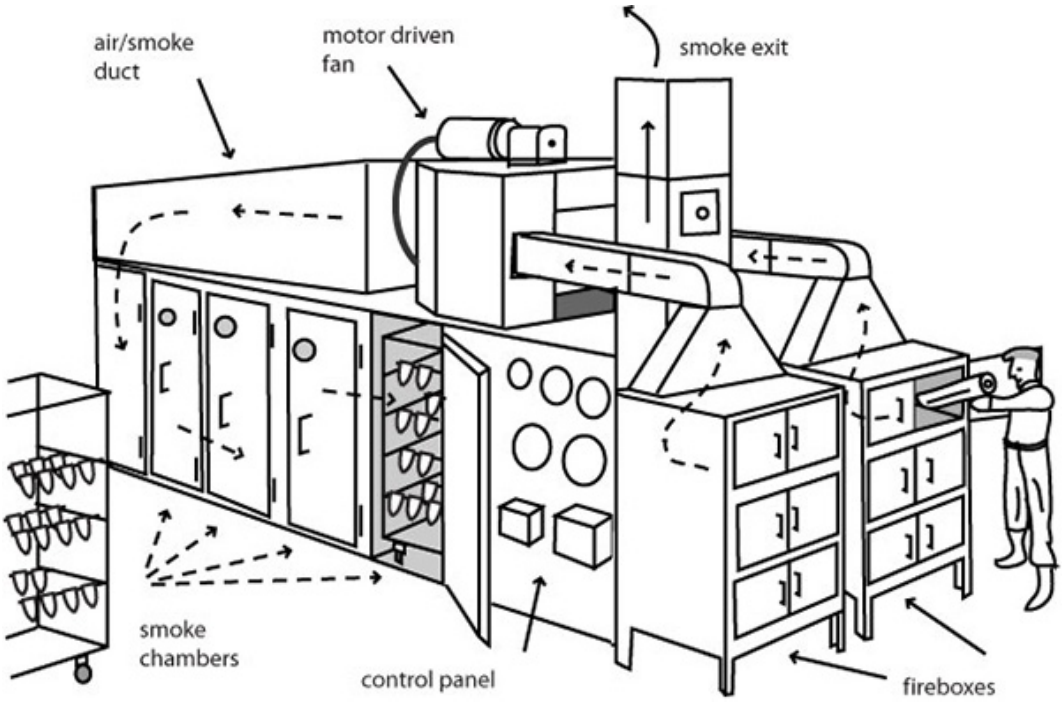


Fig. Smoking kiln

Potential hazards associated with smoking of fish

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.

Chemical hazards

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.

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.

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.

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.

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.

****

15

BATTERED AND BREADED PRODUCTS

Sreelakshmi. K.R.
Fish Processing division
Email ID: ***sreecift@gmail.com***

A coated food product is one that is coated with another foodstuff. Coating acts as a moisture barrier, minimizing moisture losses during frozen storage and microwave re-heating and retains the natural juices of foods, thereby ensuring a final product that is tender and juicy on the inside and at the same time crisp on the outside. The first commercially successful coated product was fish finger.Consumers are looking for better alternative for conventional fresh food that offers time-saving preparation. These food items are called convenient products and the global demand for such products is increasing rapidly. Battering and breading enhances the consumer satisfaction by improving the nutritional value, organoleptic characteristics and appearance of the products. The most important advantage of coating is value addition as it increases the bulk of the product. Also this paves way for better utilisation of low cost or underutilised fishes.

Steps in preparation of coated products

Portioning / forming

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

Predusting

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

Battering

Batter is defined as the liquid mixture composed of water, flour, starch, and seasonings into which the fish products are dipped prior to breading. Two types of batter are there- adhesive batter and tempura batter. The adhesive batter is a fluid, consisting of flour and water.

Tempura batter is the puff-type batter containing raising/leavening agents. This forms a crisp, continuous, uniform layer over the food. The predusted portions are applied with wet batter and excess batter can be blown off by a current of air. The batter mix helps in governing the amount of bread to be picked up and it contributes to flavour of the final product. Specific ingredients are used to aid viscosity, texture and adhesion.

Breading

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

Pre-frying/ flash frying

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

Freezing

The first step in preparing the fried fish portion for freezing is air-cooling. This is usually accomplished with the use of a fan or a series of fans. This allows the coating temperature to drop, while at the same time allowing the batter coating to recover from the frying shock and also to stabilize itself. The coated fish portions are then fed to the freezer through conveyor belts. Since the fried portions are fragile, care should be taken to avoid contact between the portions while loading in the freezer. Freezing is usually carried out in spiral freezers. Other types of IQF freezers can also be used depending on the product and convenience. Freezing is completed when the internal and external temperature of the fish portion drop to about – 40°C.

Packaging and storage

The common deteriorative changes taking place during frozen storage of battered and breaded fish products are desiccation, discolouration, development of rancidity etc. Application of proper packaging prevents/retards these changes to a great extent. Conventional packaging materials like flexible plastic films are not suitable for these products as they provide little mechanical protection to the products and as a result the product gets damaged or broken during handling and transportation. Hence thermoformed containers are commonly used for this purpose. The packed coated products are usually stored at –20°C.

Coated fish products

Coated fish fillets

Fried coated fish fillet is a prominent food item in the European markets. Along with fried potato chips it forms a substitute for lunch for majority of the floating population in Europe. Fish fillet of table size and having minimum fin bones can be used for this purpose. A fish fillet is a skinless, boneless fish loin cut along the central bone frame and trimmed free of loose or hanging meat. Skinless and boneless fish fillets can be prepared manually as well as using filleting machines. While fillet yield is 30 to 40% with machine filleting, manual filleting gives better yield. To fillet, keep the fish on the chopping board and cut from behind the pectoral fin down to the main bone and move the knife along the bone frame with minimum loss of meat. Remove the skin along with scales by passing the knife along the skin layer. Also remove the belly flaps. Trim off any hanging meat from the fillet and make it regular and uniform. Wash the fillets in chilled water and drain. Dip the fillets in 5% brine solution containing 0.1% citric acid for 3-5 minutes depending upon the size grade and then drain off. Fillets are then pre-dusted with a suitable pre-dust or dry batter mix itself. The excess pre-dust adhered to the substrate is then removed either by shaking or using an air blower. The pre-dusted fillets are then coated with batter uniformly. The batter coated fillets are further coated with bread crumbs. Generally medium size porous crumbs having a relatively large granulation are used even though the selection of the crumbs depends upon the requirement of the finished coated product. The bread crumbs are uniformly applied on the product and the excess crumbs are then removed using an air blower. The coating picks up depends on the viscosity of the batter and the type of crumbs and 30-35% is generally obtained. After the application of bread crumbs the fillets are flash fried in hot vegetable oil for 20-30 seconds depending on the size grade of the fillets. The temperature of frying is maintained at 180-200℃. The flash fried fillets are cooled immediately using a fan and then frozen in an IQF freezer preferably a spiral freezer for the required time depending on the size of the fillets. The time is adjusted by regulating the conveyer speed of the freezer belt. The frozen coated fillets are immediately packed in thermoformed containers or pouches made of 12µm plain polyester laminated with 118µm LDPE. A specified number of such consumer packs are then packed in master cartons. The packed cartons of frozen coated fillets are stored in a cold storage maintained at -20°C.

Fish fingers/Fish portions/fish sticks

Fish fingers are regular sized portions cut from rectangular frozen blocks of fish fillet or fish mince. A common size fish block in commercial practice in Europe is 47.9cm long, 25.4 cm wide x 6 cm thick weighing 7.5 kg. On the production line the blocks are subdivided by a series of band saws and subsequently cut into the desired width and shape. Fish fingers are made in to different shapes such as rectangular, square, wedge and french cuts. For small-

scale units, frozen slabs of 1.5 cm thick may be convenient for cutting out fish fingers of uniform size. A typical British fish finger normally weighs about 28 g (1 oz) of which up to 50% of the total weight is contributed by the batter and crumbs. Accordingly, a rectangular piece of 7.5 x 2.0 x 1.5 cm weighing about 15 g may give a final weight of 28 g.

The frozen fish block is prepared by mixing fish fillet/mince with 0.6% sodium tripolyphosphate and 1% sodium chloride, placing in a frame of convenient size, pressing slightly and frozen to form a solid block of fixed dimension. (The removal of pin bones from the fillets of fresh water fish of many species is a difficult task. In such cases it will be better to prepare the fish block from the fish mince after removing the pin bones using a fish meat strainer). The frozen block is cut into suitable uniform sizes. These pieces are given a coating of pre-dust, batter and breading as in the case of coated fish fillets. The battered and breaded fish fingers are flash fried in oil at 180-200°C for 30 seconds. After cooling, the fingers are frozen preferably in an IQF machine and packed in thermoformed trays or pouches and stored at −20°C. The flow chart for production of fish finger is given in Fig. 2.

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

Fish Cutlet

Fish cutlet has become a popular snack at celebrations, household functions, tea times etc. The basic raw material required for preparation of this product is cooked fish meat generally from less costly fishes with white meat or cooked meat from skeletal frame obtained after filleting of fish.

The dressed fish /skeletal frame/mince is cooked in 2% brine for 30 minutes and drain off the water. Remove the skin, scales and bones and separate the meat. Mix the meat well with a little salt and turmeric powder in a homogenizer. Fry chopped onions in oil till brown. Add curry leaves, chilly and ginger in chopped form and mint in blended form and fry. Mix these with the cooked meat. Add mashed potato and spices and mix well with the cooked meat. Adjust the salt content to taste and shape 30 g each in round or oval form manually or using a forming machine. Batter with batter mix dispersed in water in the ratio 1: 2 and roll in breadcrumbs. The cutlets can be preferably frozen in an IQF machine and packed in thermoformed trays/pouches before storage.

Fish Balls

There are several varieties of fish, which do not command a ready market as fresh fish, but are comparable to many table fish in nutritive value and other attributes. One of the ways of ensuring effective utilization of such fish is to process ready-to-serve or ready-to-cook value added ‘convenience' products, for which there already exists great demand. Fish ball is one such product prepared using fish mince and starch that can be processed as a coated

product or as a heat-processed product in a suitable fluid medium. Coated fish ball is a palatable and nutritious product prepared from mince of low cost fishes. The preparation of fish ball is simple and requires only few locally available ingredients. Hence it is an ideal product for small scale units.

Preparation of Specialty Products from Shrimp

Centre-peel shrimp

Raw Material: Prawn 26/30 to 31/40 counts/kg

Process: Wash the whole shrimp in potable water. Peel at the centre retaining the head, the last segment and the tail fans. De-vein by inserting a pointed needle or pointed bamboo stick between the segments dorsally and lifting off the vein. Remove the telson by gently raising upwards. Trim off the head and tail fans to reduce the sharpness to avoid damage of the package. Arrange in PVC/polystyrene trays and vacuum pack in laminated pouches. Blast freezing at –40°C & storage below -18°C in master carton.

Cooked centre peel shrimp

Raw Material: Prawn 26/30 to 31/40 counts/kg

Process: Wash the whole shrimp in potable water. De-vein by inserting a pointed needle or pointed bamboo stick between the segments dorsally and lifting off the vein. Remove the telson by gently raising up wards. Cook the shrimp in 1% boiling brine for 2-3 minutes depending on the size grades. Cool in chilled water. Peel at the centre retaining the head, the last segment and the tail fans. Trim off the head and tail fans to reduce the sharpness to avoid the damage of the package. Arrange in PVC/polystyrene trays and vacuum pack in laminated pouches. Blast freezing at –40°C & storage below -18°C in master carton.

Shrimp skewer

Raw Material: Prawn 26/30 to 31/40 counts/kg

Process: Wash the whole shrimp in potable water and remove the head. Remove the telson by gently raising upwards. Peel the shrimp completely, including the tail fans and de-vein. Arrange 4-5 pieces in a skewer in an inverted "U" shape. Arrange the skewered shrimp in PVC/polystyrene trays and vacuum pack in laminated pouches. Blast freezing at –40°C and storage below -18°C in master carton.

Major Markets: Japan, US and Europe

Fantail round

Raw Material: Prawn 26/30 to 31/40 counts/kg

Process: Wash the whole shrimp in potable water and remove the head. Remove the telson by gently raising up wards. Peel the shrimp leaving the shell intact on the last segment and the tail fans. De-vein the shrimp and trim the tail fans using a pair of scissors. Arrange in PVC/polystyrene trays and vacuum pack in laminated pouches. Blast freezing at –40°C and storage below -18°C in master carton.

Coated fantail round

Raw Material: Fantail round shrimp pre-dust, batter and bread crumbs.

Process: Coat the Fantail round shrimp with a thin layer of pre-dust either manually or using a pre-dusting machine. Coat the pre-dusted shrimp either with a conventional (adhesive) batter or a tempura type batter, depending upon the market. Coat the battered shrimp with breading (Japanese style light coloured coarse crumbs for Japan Markets and darker coloured crumbs (yellow-orange) for European and US Markets. Arrange in PVC/polystyrene trays, preferably in "well" trays and vacuum pack in laminated pouches. Blast freezing at –40°C and storage below -18°C in master carton.

Butterfly shrimp

Raw Material: Prawn 26/30 to 31/40 counts/kg

Process: Wash the whole shrimp in potable water and remove the head. Remove the telson by gently raising up wards. Peel the shrimp leaving the shell intact on the last segment and the tail fans. De-vein the shrimp and trim the tail fans using a pair of scissors. Cut through the dorsal side length-wise using a sharp scalpel or knife (Butterfly cut) to partially separate the lateral muscle block. Gently open up the cut surface to reveal the butterfly shape. Arrange in PVC/polystyrene trays and vacuum pack in laminated pouches. Blast freezing at –40°Cand storage below -18°C in master carton.

Coated butterfly shrimp

Raw Material: Butterfly shrimp pre-dust, batter and bread crumbs.

Process: Coat the butterfly shrimp with a thin layer of pre-dust either manually or using a pre-dusting machine. Coat the pre-dusted shrimp either with a conventional (adhesive) batter or a tempura type batter, depending upon the market. Coat the battered shrimp with breading (Japanese style light coloured coarse crumbs for Japan Markets and darker coloured crumbs (yellow-orange) for European and US Markets. Arrange in PVC/polystyrene trays, preferably in "well" trays and vacuum pack in laminated pouches. Blast freezing at –40°C and storage below -18°C in master carton.

Butterfly "sushi" shrimp

Raw Material: Prawn 26/30 to 31/40 counts/kg

Process: Wash the whole shrimp in potable water and remove the head. Remove the telson by gently raising upwards and de-vein. Insert bamboo skewer along the dorsal side length-wise up to the last segment so as the stretch the shrimp completely. Blanch/lightly cook in 1% boiling brine for 1-2 minutes depending on the size grades. Cool in chilled water. Peel the cooked shrimp completely, including the tail fans. Cut the gently down the ventral side length-wise up to the last segment using a sharp scalpel or knife without damaging the lateral muscle blocks on either side. Gently open up the cut surface to form the butterfly shape. Arrange in

PVC/polystyrene trays and vacuum pack in laminated pouches. Blast freezing at –40°C and storage below -18°C in master carton.

Stretched shrimp (Nobashi)

Raw Material: Prawn 26/30 to 31/40 counts/kg

Process: Wash the whole shrimp in potable water and remove the head. Remove the telson and trim the tail fans. Peel the shrimp, leaving the shell intact on the last segment and the tail fans. Make three or four parallel cuts, across or diagonally on the ventral side using a sharp razor. Stretch the shrimp to the desired length by gently pressing it using a stainless steel mould. Arrange in PVC/polystyrene trays, preferably in "well" trays and vacuum pack in laminated pouches. Blast freezing at –40°C and storage below -18°C in master carton

Breaded "Nobashi"

Raw Material: Stretched shrimp (Nobashi), pre-dust, batter and bread crumbs.

Process: Coat the stretched shrimp with a thin layer of pre-dust either manually or using a pre-dusting machine. Coat the pre-dusted shrimp either with a conventional (adhesive) batter or a tempura type batter, depending upon the market. Coat the battered shrimp with breading (Japanese style light coloured coarse crumbs for Japan Markets and darker coloured crumbs (yellow-orange) for European and US Markets. Arrange in PVC/polystyrene trays, preferably in "well" trays and vacuum pack in laminated pouches. Blast freezing at –40°C and storage below -18°C in master carton.

Shrimp single kebab (barbecue)

Raw Material: Prawn 26/30 to 31/40 counts/kg

Process: Wash the whole shrimp in potable water and remove the head. Peel the shrimp completely and devein. Insert a bamboo skewer along the dorsal side length-wise up to the last segment so as to stretch the shrimp completely. Arrange the skewered shrimp in PVC/polystyrene trays and vacuum pack in laminated pouches. Blast freezing at –40°C below -18°C in master carton

Shrimp vegetable kebab

Raw Material: Shrimp (any species), carrots, onion and capsicum.

Process: Wash the whole shrimp in potable water, remove the head, Peel and de-vein. Blanch in 1% boiling brine for 15-30 seconds and cool in chilled water. Wash the vegetables in potable water and dice to approximately 2 cm cubes or cut into square pieces and blanch in 1% boiling brine for 30-60 seconds and cool in chilled water. Arrange in skewer, shrimp alternating with diced vegetables. Arrange the skewered shrimp vegetables in PVC/polystyrene trays and vacuum pack in laminated pouches. Blast freezing at –40°C and storage below -18°C in master carton.

****

16

THERMALLY PROCESSED FISHERY PRODUCTS

Mohan C. O., Remya, S., Sreelakshmi, K. R.
Fish Processing division
Email ID: ***comohan@gmail.com***

7Thermal processing is an age old well established food preservation technology. The Indian seafood canning industry, once at its boom has collapsed due to non-availability of suitable cheaper containers and competition from other countries. The development of domestic market is very essential to sustain the growth and development of this industry. In recent years there is an increasing demand for convenience products in India due to modernization of the country with supermarkets and hypermarkets across urban places. The demand is also expected to increase due to population growth and increasing disposable income. Market size of canned seafood in India is very small and highly fragmented compared to frozen seafood sector. However, its demand is increasing in most of the countries including in Asia. Europe and North America are the largest market for canned fish products followed by Asic-Pacific and Middle East & African countries. Dongwon, Century Pacific Food INC, Wild Planet, Frinsa, Natural SLA and Bumble Bee are the key players in this sector.

The concept of thermal processing has come a long way since the invention of the process by French confectioner, Nicholas Appert. Later on Bigelow and Ball developed the scientific basis for calculating the sterilization process for producing safe foods. Today, thermal processing forms one of the most widely used method of preserving and extending shelf life of food products including seafood's. Thermal processing involves application of high temperature treatment for sufficient time to destroy all the microorganisms of public health and spoilage concerns. Normally, thermal processing is not designed to destroy all microorganisms in a packaged product, which may result in low quality product which destroys important nutrients. Instead of this, the pathogenic microorganisms in a hermetically sealed container are destroyed by heating and a suitable environment is created inside the container which does not support the growth of spoilage type microorganisms. Several factors must be considered for deciding the extent of heat processing which include,

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

b) pH of the food
c) heating conditions
d) thermo-physical properties of the food and the container shape and size
e) storage conditions

Thermal processing is designed to destroy different microorganisms and enzymes present in the food. Normally in thermal processing, exhausting step is carried out to before sealing the containers. In some cases, food is vacuum packed in hermetically sealed containers. In such cases very low levels of oxygen is intentionally achieved. Hence, the prevailing conditions are not favorable for the growth of microorganisms that require oxygen (obligate aerobes) to create food spoilage or public-health problems. Further, the spores of obligate aerobes are less heat resistant than the microbial spores that grow under anaerobic conditions (facultative or obligate anaerobes). The growth and activity of these anaerobic microorganisms are largely pH dependent. From a thermal-processing standpoint, foods are divided into three distinct pH groups which are given below. Changes in the intrinsic properties of food, mainly salt, water activity and pH are known to affect the ability of microorganisms to survive thermal processes in addition to their genotype. Due to health related concerns on the use of salt, there is increased demand to reduce salt levels in foods. The United States Food and Drug Administration (FDA) have classified foods in the federal register (21 CFR Part 114) as 3 categories:

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).

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.

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 1/10th 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. Nowadays, retort pouch processing is very popular. The retort pouches are flexible in nature and they easily withstand high temperatures used during thermal processing. They also provide good barrier against moisture and gases. The most common retort pouch is 3 layered laminate. The 3 layers are joined with adhesive lamination. These three layers are:

a) Polyester layer which helps in providing strength and abrasion resistance
b) Aluminium foil for providing barrier against moisture, gases and light
c) Polypropylene/ polyethylene for heat sealing properties.

Containers used for thermal processing

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

- 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

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

Spoilage of canned fishery products:

Major spoilage encountered in the canned fish products are listed below.

Identifiable evidences of spoilage:

1. Flipper
2. Springer
3. Swell
4. Flat sour

5. Hydrogen swell
6. Buckles
7. Leakers
8. Panelled cans

Spoilage due to physical or chemical reasons:

1. Black discolouration
2. Blue discolouration
3. Mush
4. Honey combing
5. Stack burning

Spoilage due to microbial growth:

1. Gaseous spoilage
2. Non-gaseous spoilage

****

17

NON-THERMAL FISH PRESERVATION TECHNIQUES

Remya S.
Fish Processing Division
Email ID: ***remya03cof@gmail.com***

The emergence of non-thermal technologies for enhancing shelf life and ensuring food safety has sparked a revolution in the food processing sector. Traditional methods of food processing involving heat can lead to undesirable changes in food, such as the loss of temperature-sensitive nutrients, alterations in food texture due to heat exposure, and modifications in the sensory qualities of the food. Non-thermal food processing, on the other hand, refers to techniques that achieve microbiological inactivation in food without direct heat application. These technologies, relatively recent in their development, employ mechanisms beyond conventional heating to reduce or eliminate microorganisms, offering an alternative to traditional thermal processing.

Irradiation

Irradiation refers to the deliberate exposure of an object to radiation. In the context of food, irradiation involves applying low levels of ionizing radiation to food materials to either sterilize them or extend their shelf life. This process effectively deactivates food spoilage organisms, including bacteria, moulds, and yeasts. Irradiation proves highly effective in prolonging the shelf life of fresh fruits and vegetables by controlling the typical biological changes associated with ripening, maturation, sprouting, and aging. Furthermore, it plays a crucial role in destroying disease-causing organisms like parasitic worms and insect pests, which can damage food during storage. It's important to note that while irradiation involves radiation exposure, it is not harmful or noxious to humans when used for food pre-treatment. The radiation dose applied to food is low and safe for consumption. Food irradiated under approved conditions does not become radioactive.

In the realm of agri-food applications, two key terms are often used i.e., Radicidation and Radurization.

Radurization: This application involves applying an ionization dose sufficient to preserve food quality by significantly reducing the number of spoilage bacteria.

Radicidation: Radicidation entails applying an ionization dose to food that is adequate to reduce the specific number of viable pathogenic bacteria to a level so low that they are undetectable by any known method. This term also applies to the destruction of specific

parasites. Radicidation and Radurization are used for applications involving less than 10 kGy doses.

Radappertization: Additionally, there is a high-dose application known as Radappertization, which involves subjecting food to a high dose of ionization (ranging from 10 to 60 kGy) to reduce the number and/or activity of living microorganisms to a level where none are detectable by any recognized method. Foods treated with Radappertization can then be stored for extended periods, up to 2 years, at room temperature in sealed plastic packaging.

Supercritical Carbon Dioxide Technology ($SC\text{-}CO_2$)

Supercritical carbon dioxide ($SC\text{-}CO_2$) technology has emerged as an innovative non-thermal extraction method with a wide range of applications. It offers the potential to enhance shelf life, reduce microbial contamination, eliminate lipase-producing microorganisms, deactivate endogenous enzymes responsible for food spoilage, recover health-promoting compounds from plant materials, minimize alteration of phytochemicals, and reduce mycotoxin and pesticide residues. One of the key advantages of $SC\text{-}CO_2$ is its dual nature, behaving as both a gas and a liquid. This unique property allows it to easily penetrate complex matrices and extract compounds effectively. $SC\text{-}CO_2$ treatment is known to eliminate various pathogenic and vegetative microorganisms in food products within a fraction of a second, resulting in extended shelf life, improved texture, and enhanced sensory attributes. Despite its many benefits, there are challenges to adopting $SC\text{-}CO_2$ technology on a commercial scale, mainly due to the expense of equipment and operating SC systems. Nevertheless, researchers have reported positive outcomes using high-pressure CO_2 for preserving the colour, enhancing extraction efficiency, and retaining bioactive compounds in various food products such as fresh-cut fruits and vegetables, dairy products, meat, and fruit and vegetable juices. Compared to other high-pressure processing methods, $SC\text{-}CO_2$ operates at lower pressures (typically 10–20 MPa), which still provides higher diffusivity and mass transfer rates. This results in minimal nutrient degradation, improved yield, shorter processing times, and the preservation of crucial substances in food, making $SC\text{-}CO_2$ a promising technology for the food industry.

Ultraviolet Radiation

Ultraviolet (UV) Radiation is an economical non-thermal technology employed for decontaminating and enhancing both the shelf-life and safety of food products. This method is primarily used to reduce the microbial load on the surface of food materials that are indirectly exposed to radiation due to its limited penetration depth. UV radiation falls under the category of non-ionizing radiation and is known for its germicidal properties, particularly within the wavelength range of 200–280 nm, often referred to as UV-C. UV irradiation has proven effective not only in reducing microbial contamination but also in deactivating enzymes present in plant products.

Pulsed Light Preservation

Pulsed Light (PL) Preservation is an alternative method to continuous ultraviolet treatment for both solid and liquid food products. PL involves the sequential emission of high-power light pulses, characterized by short bursts of intense, broad-spectrum white light. In comparison to regular continuous UV light, PL possesses significantly greater strength, exceeding it by a factor of a thousand. Pulsed xenon UV, a component of PL, harnesses the entire ultraviolet light spectrum to disperse germ-killing energy. This spectrum encompasses wavelengths ranging from 180 to 1100 nm, with a notable concentration of light in the short-wave UV spectrum. Like other non-thermal food processing technologies, PL demonstrates the potential for effectively deactivating or eliminating microbes in food. PL finds applications in various food types, including fish, vegetables, fruits, and meat. Additionally, it can be integrated with other innovative technologies to create a multifaceted approach in the fight against surface microbes on food products.

Pulsed Electric Field Processing

Pulsed Electric Field (PEF) processing is a highly efficient non-thermal technique used in food processing, involving the application of short, high-voltage pulses. Its primary purpose is the inactivation of spoilage and pathogenic microorganisms in various food products, achieved by subjecting them to electric pulses that destroy harmful bacteria. Microbial inactivation through PEF is accomplished by causing dielectric breakdown of bacterial membranes. The food material is positioned between electrodes, with the field intensity typically ranging from 20 to 80 kV/cm. Exposure times are incredibly brief, often measured in milliseconds or even nanoseconds. One of the significant advantages of PEF is its ability to extend the shelf life of food without compromising its quality. This process operates on a mechanism known as electroporation, wherein very short, high-voltage electric pulses are applied to the food. These pulses create tiny pores in the cell membrane of the food, allowing for microbial inactivation without damaging essential compounds like vitamins. PEF processing is typically applied to liquid foods or semi-solid foods that can easily flow. A standard PEF device comprises a food treatment chamber, a control system, and a pulse generator. The food is placed within the treatment chamber between two electrodes, which are usually constructed from stainless steel.

High-Pressure Processing

High-Pressure Processing (HPP), also referred to as high hydrostatic pressure (HHP) or ultra-high pressure (UHL) processing, stands out as a non-thermal method for cold pasteurization. In this approach, food is carefully sealed within flexible, water-resistant packaging and exposed to significant hydrostatic pressure, reaching levels as high as 600 MPa (87,000 psi). This pressure is applied for a span ranging from a few seconds to several minutes (1 – 20 min), with water serving as the medium to convey this force onto the food product. The key advantage of HHP lies in its ability to promptly apply isostatic pressure, typically between

100–1000 MPa, while maintaining low temperatures. Through this process, HHP accomplishes preservation effects that can be equated to those achieved through thermal processing, effectively deactivating unwanted microorganisms and enzymes. An HPP unit comprises a dedicated pressure chamber designed to house the food product, facilitating the introduction of water, which is subsequently utilized to exert pressure on the food, thus ensuring its preservation and safety.

Ultrasound Processing

Ultrasound (US) processing involves the use of compressional waves with a frequency exceeding 20 kHz, which is beyond the range of normal human hearing (typically above 20 kHz). In the food industry, the frequencies of ultrasound used for microbial inactivation typically span from 20 kHz to 10 MHz. The bactericidal effects of ultrasound primarily stem from a phenomenon known as cavitation, where microbubbles are generated and then collapse within a liquid medium. During cavitation, temperatures can soar to as high as 5500 °C, and pressures can surge up to 100 MPa, leading to localized microbial sterilization. Ultrasound achieves microbial inactivation through various mechanisms, including the breaking of cell walls, disruption and thinning of cell membranes, and the generation of free radicals resulting from the collapse of cavitation bubbles.

Cold Plasma Technology

Plasma, considered the fourth state of matter, is formed when the energy applied to gases exceeds a certain threshold, leading to the ionization of gas molecules. Cold plasma, one of two main types of plasma (the other being thermal plasma), is a non-thermal treatment method operating within a temperature range of 25–65 °C. Cold plasma exhibits robust antimicrobial activity and an effective capability to inactivate enzymes. The composition of reactive species within the plasma largely depends on the gas used for ionization, with common choices including argon, helium, oxygen, nitrogen, and air. Cold plasma is produced by subjecting gases to various forms of energy, such as thermal, electrical, or magnetic fields. This process generates plasma containing positive and negative ions, as well as reactive species like ozone and singlet oxygen. Several methods are employed for cold plasma generation, including radio frequency plasma, dielectric barrier discharges, gliding arc discharge, microwave irradiation, and corona discharges.

Ozone Treatment

Ozone is a colourless gas with a distinctive odour, composed of three oxygen molecules and chemically represented as O_3. It forms when molecular oxygen (O_2) combines with singlet oxygen (O). Ozone is highly reactive and notably unstable, making it impractical for storage. It must be generated on-site when needed. Ozone is widely utilized as a potent antibacterial agent against various bacteria in food. Its efficacy stems from its strong oxidizing potential and its capacity to target cellular components, giving it a broad-spectrum disinfection capability.

Ozone treatment is a chemical approach to food decontamination. This method involves exposing contaminated food items, including fruits, vegetables, beverages, spices, herbs, meat, fish, and more, to ozone in both aqueous and gaseous phases to eliminate contaminants and enhance food safety.

Conclusion

Consumer demand for safe and nutritious food has driven the development of non-conventional processing technologies. Non-thermal treatments offer high-quality, healthy, and safe food products, but they come with potential disadvantages, including undesirable changes in food if exposed for too long or at high intensities. Nevertheless, these technologies have numerous advantages over thermal processing. With careful planning and mitigation of limitations, non-thermal technologies hold significant potential for broader development and commercialization in the food processing industry.

****

18

MARINE BIOMOLECULES FOR NUTRACEUTICAL AND COSMECEUTICAL INDUSTRIES

Binsi P. K
Fish Processing division
Email ID: ***binsipillai@gmail.com***

Marine biomolecules have the potential to provide significant therapeutic and preventive benefits against a wide range of common lifestyle disorders. As the public becomes more aware of the health benefits associated with fish and seafood in general, the groundwork has been laid for the development of traditional nutraceutical products. There are currently only a few well-known marine-derived nutraceutical products on the market. Some of the most well-known nutraceutical products include fish oil (mainly omega-3 PUA), algal oils, shark liver oils, squalens, chondrotin salts, and collagen, gelatine, collagen peptides, chitins, and related compounds. In recent years, marine-derived cosmetics have become increasingly popular in the cosmetics industry due to the numerous benefits they offer to human skin health and beauty. These cosmetics are known for their nourishing and rejuvenating properties, and their ability to provide a variety of health benefits

Chitins

In India, the shrimp processing industry produces over 2 lakh tones per year of head and shells waste that can be economically transformed into chitins and their derivatives. Chitins are the second most abundant polymer after cellulose. Chitins are linear polymers of N-acetyl-D glucose. Chitins can be hydrolysis converted into glucose hydrochloride from chitins. Currently, glucose hydrochloride is marketed as food supplements and glucosamine sulfate as food supplements. Chitins and its derivatives have various applications such as: Germination of seeds; Enhanced protection against pathogens in plants; Inhibition of chitinases and protenases in soil; Antimicrobial action; Antiviral activity; Interactions with living tissues; Skin and bone replacement; Oral delivery as wound healing etc.

Chitin is mainly used in cosmetic formulations as abrasive and as a bulking agent. However, it could also be used as a cosmetic excipient, as it has good surface-active and film-forming properties. Another promising derivative is oligosaccharide, which is soluble in an aqueous medium at physiological pH, and has many bioactive properties that can be used in cosmetic and Cosmeceutical products. Chitosan, and its derivatives, can also be used as UV filters for various cosmetic and skincare products. UV radiation damages the skin by producing reactive oxygen species and causing inflammation. However, antioxidants and anti-inflammation have

been found in chitosans, which may help to reduce the damage caused by UV radiations to the skin. In addition, it can absorb UV rays below 400nm in range.

Fish collagen/gelatin/collagen peptides

Collagen is the main structural protein in connective tissue. The collagen extracted from fish can be used in cosmetic products, foodstuffs, biomedical applications, etc. CIFT developed the method to prepare absorbable surgical suture from fish gut. The hydrolysis form of collagen is called gelatin. Gelatin is used in the development of bio-degradable packaging, foodstuffs and pharmaceuticals. Since both collagen and gelatin are very high molecular weight proteins (about 300 kDa), a large portion of them is not available to the human body for biological function. In recent years, there has been a lot of focus on small molecular weight peptide development from the native collagen. This can be done by hydrolysis where the native collagen / gelatin molecules are broken down to small fragments (less than 5 kDa). Collagen peptides can be incorporated in a variety of food products such as protein bars, cereal bar, protein drink, smoothie, yogurt, cold desserts, soup, cured meats, etc. Collagen / gelatin peptides are gaining more and more attention as they exhibit various biological activities like anti-oxidants, antioxidants, anti-hypersensitivity, proliferative, anti-diabetes, anti-anti-proliferative, anticoagulant, calcium-binding, anti-obesity, anti-diabetic activities and postponement of age-related diseases.

Collagen is a type of connective tissue protein that is distributed throughout the body. It plays an essential role in the structure of the skin, skeletal system and blood vessels. As we get older, our body's production of collagen diminishes. This can lead to various health problems such as osteoporosis, osteoarthritis, etc. In addition, the appearance of the body can be seriously affected. Some of the most common issues related to the appearance of a person's body are the appearance of wrinkles and loss of elasticity; hyperpigmentation; skin sagging; loss of gloss; etc. In recent years, researchers have clinically demonstrated that oral ingestion and topical application of collagen hydrolysed helps to reduce the signs of aging and reverse the damage to the skin. Types of Collagen Types of collagen used in cosmetic industry are Type I and III. Type I collagen provides tensile strength, structure and elasticity to the skin and bone whereas Type III collagen provides elasticity and resiliency to the skin, muscle and blood vessels. Types of collagen used in cosmetics are derived directly from bovine, chickens, fish and molluscs. Alternatively, collagen synthesis in the body can be increased by including more collagen-boosting nutrients in the diet such as proline, lysine, L-arginine, vitamin C, vitamin A, anthocyanin, manganese, copper, zinc etc.

Fish calcium

Calcium is abundant in marine ecosystems, mainly in calcium carbonate, calcium phosphate, and other skeletal elements of the teleost, exoskeleton, or coral deposits. A large proportion of total fish catches are discarded as processing residues each year, including

trimmings/fins/frames/heads/skin/viscera. The bone fraction (15-20% body weight) of fish contains high levels of calcium and calcium phosphate (2% dry weight). Fatty fish generally have lower ash content compared to lean fish species. Filleting wastes from tuna and other larger fish are very rich sources of calcium when it comes to calcium. Bone structure also differs between species, as a large proportion of the teleosts (teleoskeletal bones) are acellular, meaning they have no enclosed osteocytes. Cellular bones are limited to a few groups of fish, such as Salmonidae. Acellular fish bones have a higher surface-to-volume ratio, so they are likely to have higher calcium availability than cellular fish bones. Ash content is highest for lean fish species that have a cell-like structure. Apart from that exoskeleton of mollusks and coral deposits are excellent source of calcium. However, the calcium form these deposits are mainly in the form of calcium carbonate.

Hydroxyapatite (HAp)

Hap is the main mineral of bone and teeth. It has the chemical formula Ca10 (PO4) 6(OH) 2. The composition of Hap derived from biological sources is different from synthetic Hap. The lattice has several ion substitutions, including CO3 (CO3), F (F2), Mg (Mg2+) and Na (Na+). It is a calcium phosphate group member with a 1.67 (Ca/P ratio) stoichiometric value. Hap is one of very few materials that are bioactive and support bone growth and osteointegration in mammalian bone and mammalian hard tissues when used for orthopedic and dental applications. Fish bones and scales are rich sources of Hap. The content of Hap in fish skeleton can range from 40% to 60%. Hap extraction from bone is usually done at very high heat and the higher the temperature, the stronger the HAp structure is. The high heat also burns away organic molecules like collagen protein. About 65% to 70% of fish bone consists of inorganic Hap. Most of these inorganic Hap is composed of calcium and phosphorous.

Squalene:

Squalene is an unsaturated hydrocarbon. It is found in liver oil of deep sea sharks such as Centrophorus, Squalidae, and other species. The liver oil from these species contains 90% of squalene which can be isolated, purified, and used as dietary supplements. Squalene belongs to a group of antioxidant molecules called Isoprenoids. It has been found to be a good chemo preventive agent in mice bearing lung cancer. Squalene helps in reviving damaged body cells and helps in the revival of cell generation. Its main function is to protect cells from oxidative reactions. It helps in cleaning, purifying, and detoxifying the blood from toxins. It helps in the purification of gastrointestinal tract and kidneys. It also helps in the improvement of bowel movements and urination. Squalene also helps in the regulation of the female menstrual cycle. It can also improve irregular and abnormal cycles.

Squalene is the main component of skin surface Polyunsaturated lipids. It has several advantages for the skin, such as antioxidant, emollient, hydration, and skin condition. Squalene is a strong oxidizing agent. It is stable against attacks from perioxide radicals. It protects

human skin from oxidative damage from UV light and from other sources. It suppresses superoxide anion production, which may reduce skin irritation. Squalenine has a high rate constant for the quenching of singlet oxygen compared to other lipids on human skin surface. It also has a stable rate constant against attacks from peroxide radicals. When adequate levels of Squalenine are present, Lipid Peroxidation Chain Reaction (LPR) is less likely to occur. Supplementation with Squalenine can reduce erythema in the skin. It absorbs a large amount of erythema from the skin. It helps to prevent skin diseases such as acne, comedones, and wrinkles. As an emollient, it is quickly absorbed into the skin, restoring suppleness and flexibility without leaving an oily residue. Squalene has also been found to increase skin hydration by preventing water loss through occlusion. Squalene supplementation was found to be effective in reversing the detergent induced transepidermal water loss and increased riboflavin penetration in both rat and human skin

Taurine

Taurine (2-Amino-Sulfonic Acid) is an amino acid that contains sulfur and is non-protein. Taurine plays a vital role in neurotransmission and cell volume regulation. It also stabilizes cell membranes and facilitates the transport of ions like sodium, potassium, potassium and magnesium ions in and out of cells. Taurine also plays an important role in detoxifying xenobiotics. It is also essential for the efficient absorption of fat and for the solubilisation of fats. Taurine can be synthesised from methionine or cysteine with Vit B6. Taurine has important functions in the visual pathway, brain and nervous system as well as cardiac function and cholesterol metabolism. Taurine deficiency has been linked to cardiomyopathy (heart disease), retinal (retinal) and tapetum (tapetum) degeneration, immune (nervous system) dysfunction, muscle atrophy (muscle weakness), developmental abnormalities, premature aging and impaired reproduction. However, taurine's importance in biological systems has only recently been recognized and is now considered a 'conditionally essential' amino acid.

Taurine protects against the tissue damage caused by oxidative stress caused by mercury induced toxicity. Taurine plays an important role in the development of the fetus and the infant. Studies have shown that increased intake of taurine reduces the risk of hypertension. Deficiencies of taurine do not cause immediate health problems, but long-term lack of taurine can affect a wide range of metabolic pathways. Taurine is a key component of bile and plays a vital role in maintaining normal gastrointestinal development and function. Taurine can be found in higher concentrations in almost all animal products. Meat, breast milk and dairy products, fish, and shell fish contain higher concentrations of taurine than fin fish. According to Zhao et al., (1998) crustaceans and mollusks have the highest taurine content, ranging from 300 to 800 mg per 100 g of meat. Red algae is considered a good edible source for taurine. It is proposed that taurine may play a beneficial role in the prevention of Parkinson's and

Huntington's disease by reducing oxidative stress and promoting apoptosis. Eventhough, 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

Glycosaminoglycan (GAG) is a linear polysaccharide with repeating disaccharide sequences. Each GAG contains an amino sugar (usually N-acetyl-glucosamine) and an uronic acid (usually glucuronic acid, iduronic acid, or galactose). The major members of the GAG family are: Hyaluronic Acid (HA), Keratin Sulfate (KS), Chondroitine (CS), Dermatan Sulfate (DS), Heparin and Heparin Sulfate (HS). Hyaluronic acid has a high molecular weight (typically 2-10 x 107 Da) and a chain length of 2-25 μm. Other GAGs have a short chain length (typically 15-20 kDa). Hyaluronan does not have sulfate groups and does not covalently bind to protein. The other GAGs have sulfates at different positions and are covalently bound to a protein core. Dermatan sulfate differs from chondroitine sulfate in that it contains iduronic acid and keratan sulfates. 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 these pigments against the oxidative alteration of LDL cholesterol can be studied by incorporating them into health drinks. Carpetenoids are one of the most sought after natural food colours in the market. Cephalopode ink is another underutilized reservoir of a spectrum of bioactive substances with curative and curative properties. It is a complex combination of melanin, black pigment, glycocyanidoglycan, protein, lipids and minerals. It has been shown to have anti radiation action, antitumour action, immune modulatory action, anti coagulant action, prothrombotic action, anti-cancer action, anti-hypertensive action, anti IDA etc.

Melanin

Cephalopods (squid, octopus, etc.) are an important resource of the world's oceans and their economic value is increasing day by day. Cephalopods are an important part of the marine products and are considered a major delicacy for export markets in recent years. While a few products are made (fillets, tube, rings etc.) of cuttlefish, octopus, a large number including the ink sac are disposed of as waste. It is interesting to note that cephalopode ink was found to be the most useful source for the commercially relevant pigment melanin. In essence, squid ink is a mixture of melanin and proteins, as well as lipids and carbohydrates, as well as various minerals. Melanin is the predominant component of the ink and is composed of ~1 g melanin and Protein-polysaccharide complex. Melanin accounts for ~15% of total wet weight of the 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-ulcerogeneic properties and anti-inflammatory acivity 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 noice-induced hearing loss. Recently, the anti-ageing property of melanin was demonstrated in mice model, suggesting its use in neutracosmetic formulations. Eventhough melanin is a part of normal human diet, research on dietary intake of melanin is not much explored.

Melanin as such or melanin coated pigments can be utilised in various skincare products, such as lotions, soaps, creams, as well as in make-up products such like eye-shadows, eyeliners, foundations and lipsticks. Melanin is naturally present in hair and offers protection against damage caused by UV radiation and other environmental stressors. Incorporating melanin in haircare products, such as rinses, shampoos and hair dyes, can further improve the overall appearance of hair (Honda et al., 1995). When added to shampoo, melanin can help to strengthen and protect the hair, improve its overall appearance, and reduce damage caused by styling tools and environmental factors. In addition, melanin's antioxidant properties can also protect hair from oxidative damage, promoting healthy hair growth and reducing hair breakage. Since melanin is mostly found in the cortical matrix, its natural protection is mainly limited to matrix layer of hair. However, the cuticle, the outer layer of hair that is exposed to various physical and chemical factors including UV radiation, is responsible for the aesthetic appearance of hair. Exposure to UV radiation has the greatest impact on the endocuticle and the cell membrane complex (Richena & Rezende, 2016). This can cause photodamage, causing breakage, cuticle deformation and exfoliation, ultimately leading to tweaker hair shafts (Bloch et al., 2019). Furthermore, prolonged exposure to sunlight can convert eumelanin to oxymelanin (Draelos, 2006), which fades the natural colour and glossiness of hair. (Binsi et al., 2022)conducted a study to evaluate the photoprotective effects of cuttlefish melanin on human hair. The researchers coated the cuticle surface of hair fibers with melanin and found that UV irradiation caused much greater damage to uncoated hair fibers than to melanin-coated ones. Based on these findings, the study suggests that cuttlefish melanin could be an effective photoprotective agent in hair-care products. There are various synthetic hair-care products available commercially that claim to protect hair from photoaging. The UV filters can be organic or inorganic; the organic filters such as Para-Aminobenzoic acid (PABA) absorb UV radiation, while inorganic filters such as titanium dioxide scatter and reflect UV rays. While these synthetic UV filters are effective, they can have serious adverse effects like photo-allergenicity and gene toxicity (Gilbert et al., 2013).

Astaxanthin

Astaxanthins are carotenoids that are naturally found in shrimp, salmon, and algae. These carotenoids are known for their powerful antioxidant properties and are often used as an ingredient in cosmetics. Astaxanthins have different chemical compositions depending on their source. In marine environments, microalgae is the primary source. The most common types of microalgae used to produce astaxanthins include H. placulatus, Chloraplum ssp, and Chiplococcum ssp. H. placulatus is considered to be the most promising natural source for the production of acanthin. Acanthin produced from H. placulatus is a di-keton composed of 2 hydroxyl group and 2 ketone group, esterified by fatty acids (Palmitic Acid, Oleic Acid, Linoleic Acid, etc. The yield of acanthin from h. placulatus ssp. can range between 0.1%-4% dry biomass depending on the cultivars and extraction methods used.

Astaxanthin is found in the shells and exoskeleton of crustaceans, including shrimp and krill. Shrimp astaxanthin is free carotenoids and does not esterify with fatty acids. The chemical structure of shrimp astaxanthin has two hydroxyl group and two ketone group. However, the yield of shrimp waste astaxanthin can vary according to the species of shrimp. The yield of krill astaxanthin depends on the extraction method. Astaxanthin produced from krill can be as low as 0.1% by weight and as high as 0.3%. Phaffia Rhodozyma is a yeast that produces astaxanthin. It is a di-ketone esterified with hydroxyl groups (2 hydroxyl groups) and ketone groups (2 ketone groups). The yeast can be grown using various carbon sources.

Astaxanthin has become popular in the cosmetics industry because of its many skin-protective properties. It helps protect the skin against oxidative damage. Ascanthin is a compound that has been shown to be effective in accelerating wound healing. In a study in mice with fully-thickened dermal wounds, wounds treated with Ascanthin showed a significant increase in the expression of biological markers related to wound healing, such as collagen type I alpha1 and bFGF. It has also been shown to increase skin hydration and elasticity, improve skin texture, and delay the appearance of wrinkles. In a study of 65 healthy female patients, it was found that a dose of 6 mg (or 12 mg) of Ascanthin dose decreased the secretion of an inflammatory cytokine (IL-1) and an inflammatory protein (MMP-1), both of which are known to reduce the appearance of hyperpigmentation on the skin. Additionally, skin elasticity improvements were observed in the high-dose astaxanthin group compared to the placebo group, especially in participants with high skin moisture content.

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.

Future Dimensions

The key to successful recycling and management of seafood waste lies in the development of appropriate environmentally friendly reprocessing technologies that can convert all the valuable components of the waste into valuable products and reduce the amount of waste requiring disposal. However, there are many challenges that must be overcome to achieve this goal.

1) Consumer awareness and education is one of these challenges. Without consumer acceptance of food waste reduction approaches, no sustainable, environmentally friendly food waste recovery and management strategy can succeed. This requires appropriate efforts by research and extension organisations.
2) The fisheries sector is a poorly organised sector. The widely dispersed seafood processing plants (in the domestic market and processing plants) pose collection and processing problems.
3) Seafood is inherently perishable due to its unique richness in proteins, peptides, enzymes, and microbial flora. This often leads to massive public resistance to starting a business in the area.
4) Strict legal and environmental restrictions from regulators, as seafood waste is not classified as “inactive/inert” waste, is a major barrier for entrepreneurs to invest in this resource
5) Inadequate cold chain management from source of production to processing, as processors are least interested in investing further in residues
6) There is no baseline data on the availability and economics of production collected in recent years, leading to uncertainty about the economics and market demand for secondary products
7) The lack of a clear legal classification of secondary products in the international market is another major challenge for investors
8) The lack of uniform quality assurance protocols (such as HACCP) for secondary products leads to frequent rejections by buyers.

Suggested readings

Anon (2005) Assessment of Harvest and Post-Harvest Losses in Marine Fisheries, Central Institute of Fisheries Technology, Cochin: 122 p.

Binsi P. K., Visnuvinayagam, S. Viji P., Zynudheen A.A. and Chakrabarty. R. 2013. Fish scale: a biomaterial with versatile applications. *Infofish international*. 3. Pp.41-43.

Binsi P.K., Zynudheen AA.,George Ninan, Mohan C.O., Ashok Kumar K.2015. Proceedings of National Seminar on 'Chitin in Agriculture, Medicine AND Allied fields, SOFTI, India. ISBN: 978-81-930245-5-3.

Binsi PK., Viji P., zynudheen AA., Ravishankar CN., Srinivasa Gopal TK. 2014. Fish Collagen Peptide. Central Institute of fisheries technology, ICAR p1-117

Binsi, P. K., Nayak, N., Sarkar, P. C., Joshy, C. G., Ninan, G., & Ravishankar, C. N. (2017). Gelation and thermal characteristics of microwave extracted fish gelatin–natural gum composite gels. *Journal of Food Science and Technology*, *54*(2), 518-530.

Gopakumar, K (2002) Fishery byproducts, In: *Textbook of Fish Processing Technology* (Gopakumar, K., Ed.) Indian Council of Agricultural Research, New Delhi

Hall G.M and Reid .C.L; Scale up of lactic acid fermentation process for the recovery of chitin from prawn waste. In MB Zakaria, W.M Wan Muda and MP Abdulla (eds) *Chitin and Chitosan. The versatile environmentally friendly modern materials* National university of Malaysia, Kualalumpur, 1994 pp48-51

Madhavan, P. and Nair, K.G.R. (1974) Utilisation of prawn waste - Isolation of chitin and conversion to chitosan, *Fish. Technol*. 11: 50-53

Madhavan, P. and Nair, K.G.R. (1978) Metal binding property of chitosan from prawn waste, In: *Proc. First International Conference Chitin and Chitosan* (Muzzarelli, R.A.A. and Parisier, E.R., Ed.), Massachusetts Institute, Cambridge

Mathew, P.T. and Nair, K.G.R. (1978) Hypocholesterolemic effect of chitin and its hydrolysed products on albino rats, *Fish. Technol*. 35: 46-49

Mukundan, M.K., Stephen, J. and Devadasan, K. (1989) Collagen fibres from fish gut, In: Resent Trends in Processing Low Cost Fish (Balachandran, K. K, Pergreen, P.A., Madhavan, P. and Surendran, P.K., Eds.), Society of Fisheries Technologists, Cochin. pp 187-191

Muraleedharan V (2003) Surimi and surimi-based products byproducts In: *Winter School Manual on Product Development and Seafood Safety* (Joseph, J., Mathew, P.T, Joseph, A.C., and Muraleedharan, V., Eds.), p. 133-138, Central Institute of Fisheries Technology, Cochin

Nair, K.G. R, Mathew, P.T, Madhavan, P. and Prabhu, P.V. (1993) Effect of feeding chitin on poultry, Fish Technol. 30: 24-27 .traction. *Fish. Technol.* 11(1): pp.60-63)

Nair, K.G.R., Mathew, P.T., Joseph, A. Xavier, M. and Krishna, A. (2004) Livestock feed from cephalopods processing waste, In: *Report of National Agricultural Technology Project on Development of Value added Products and Byproducts from Low Cost fish and*

Processing Waste from Fish and Shellfish, Central Institute of Fisheries Technology, Cochin pp. (1-11)

Nair, K.G.R., Mathew, P.T., Madhavan, P. and Prabhu, P.V. (1987) Chitin as a feed additive for broiler chicken*, Indian J. Poultry Sci*. 22: pp 40-44.

Ninan, G., Joseph, J., Zynudheen, A.A., Mathew, P.T. and Geethalakshmi, V. (2009) Optimization of gelatin extraction from the skin of freshwater carps by response surface methodology, *Fish. Technol*. 46(2): 123-138

Raghunath, M.R. (2002) Fish silage, In: *Text book of Fish Processing Technology* (Gopakumar, K., Ed.), p. 263-268, Indian Council of Agricultural Research, New Delhi

Rajalakshmi, M. and Mathew, P.T. (2007) Colour and textural parameters of threadfin bream *surimi* during frozen storage as affected by cryoprotectants and chitosan, *Fish.Technol.* 44: 55-64

Santhosh, S. (2006) Studies on the Anti-ulcerogenic and Anti-hepatotoxic Potential of Chitosan and Glucosamine in Experimental Rats, *Ph.D. Thesis,* Cochin University of Science and Technology, Cochin

Santhosh, S. and Mathew, P.T. (2008) Preparation and properties of glucosamine and carboxymethyl chitin from shrimp shell, *J. App. Poly. Sci.* . 107, 280-285)

Santhosh, S., Anandan, R., Simi, T.K., Mathew, P.T. and Thankappan T.K (2005) Biochemical studies on the anti-ulcer effect of glucosamine defense status in experimentally induced peptic ulcer in rats, *J. Clin. Biochem and Nutr.* 37: 61-66

Sini T.K. Application of fermentation techniques in the utilization of prawn shell waste, Phd Thesis approved by Cochin University of Science and Technology, Cochin

Sini T.K., Santhosh S., and Mathew P.T. (2005) Study of the influence of processing parameters on the production of carboxymethyl chitin, *Polymer* 46: 3128-3133.

Thankamma, R. (2003) Specialty products from fish, In: *Winter School Manual on Product Development and Seafood Safety* (Joseph, J., Mathew, P.T, Joseph, A.C., and Muraleedharan, V., Eds.), p. 139-148, Central Institute of Fisheries Technology, Cochin

Thankamma, R., Gopakumar, K., Nair, L., A Shenoy, A.V. and James, M.A. (1979) Preparation of hydrolysate from miscellaneous fish, *Fish. Technol.* 35: 71-75

Venugopal V Alur M.D and Nerker D.P (1989) Solubilisation of fish proteins using immobilized microbial cells *Biotechnol. Bioeng*. 33:1098-1103

****

19

CHITIN AND ITS DERIVATIVES

Elavarasan, K.

Fish Processing division

Email ID: elafishes@gmail.com

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.

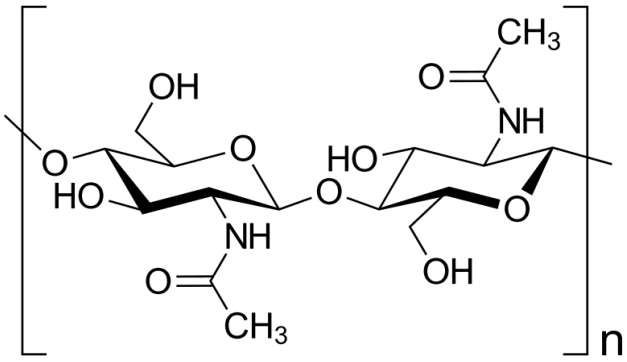


Fig.1 Chemical structure of chitin

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 sysnthesis 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

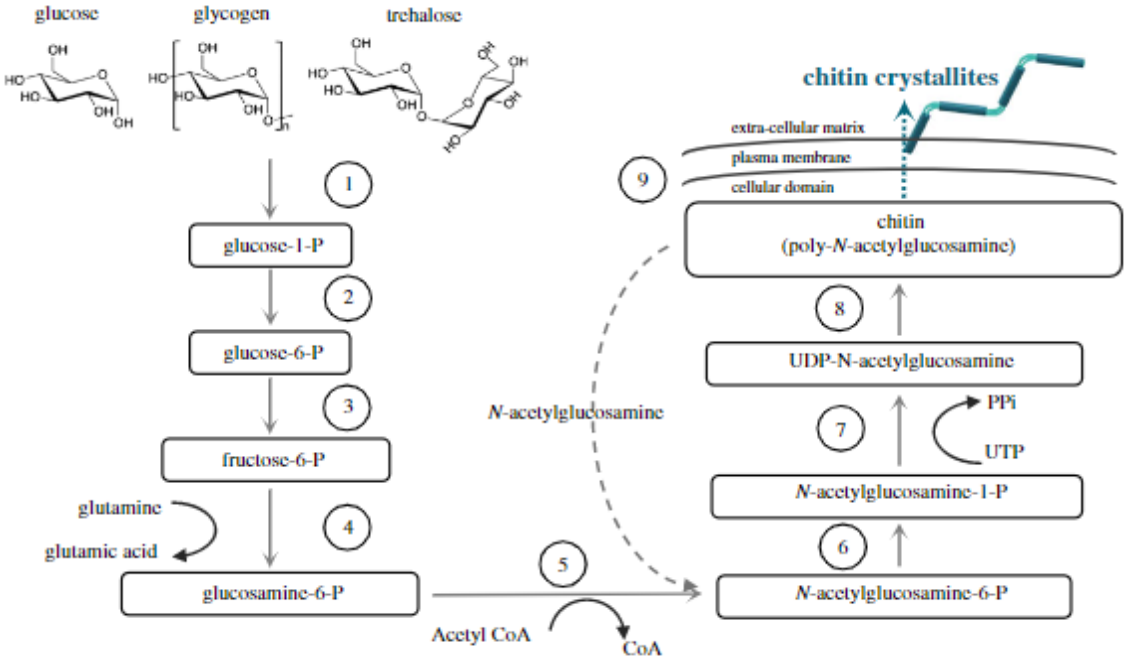


Figure 2. Steps involved in biosynthesis path way of chitin in fungi and insects (Ref: Hou J,Aydemir BE,Dumanli AG. 2021)

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.

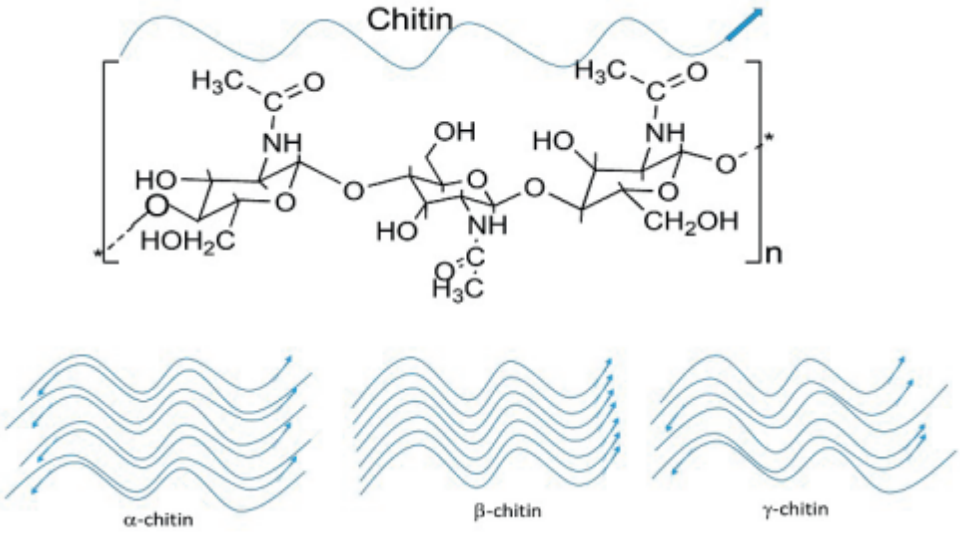


Figure 3. Polymorphic forms of Chitin (Roy et al., 2017)

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:

- Multilayered composite tissue
- Consisting mainly of chitin with various proteins
- Chitin and protein polymers are linked through covalent bond.
- Chitin-protein fibrils are biominerlized with calcium carbonate
- Spacing between the fibers 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.

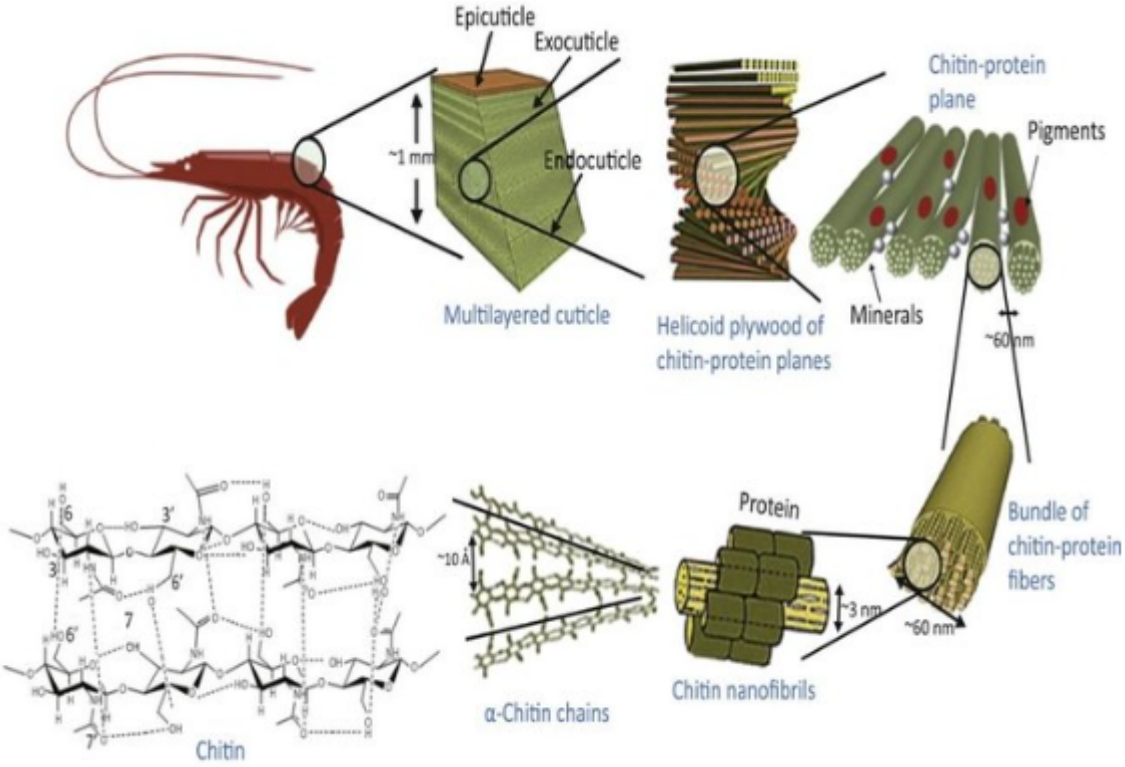
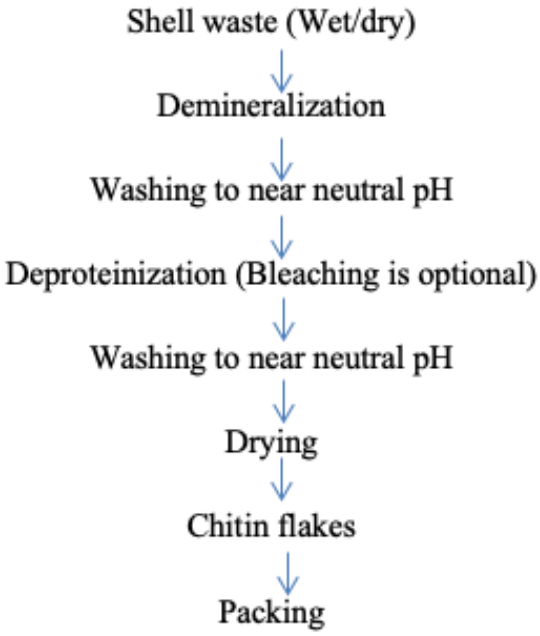


Fig.4 Shell structure and chemical composition (Adapted from Bradic, 2020)

Chitin process

The process for chitin production basically aims to eliminate other chemical constituents like proteins and minerals. For removing these constituents, concventionally chemical process is employed using diluted acid and alkali for demineralization and deproteinisation, 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 deminarlization 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. ***Deproteinisation***

   Deproteinisation 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 deproteinisation influence the extent of deproteinisation. Generally, sodium hydroxide is the

most preferred and cost effective in deproteinisation. 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 deproteinisation 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 deproteinised 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 deproteinised 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 an another important derivative has received attention. As there are functional group in the structure of chitin like hydroxyl, aminoacetyl as well as free amino group in chitosan, many number of derivetives can be manufactured through various chemical reactions.

Other products like chitosan sponges, chitosan hydrogel, electrospun nanofibres are all receiving interest for their medical uses. Recently chitooligosaccharides is an 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

1. Hou J, Aydemir BE, Dumanli AG. 2021) Understanding the structural diversity of chitins as a versatile biomaterial. Phil.Trans.R.Soc.A379: 20200331 (Doi:10.1098/rsta.2020.0331).

2. Roy, J.C., Salaün, F., Giraud, S., Ferri, A., Chen, G. and Guan, J., 2017. Solubility of chitin: solvents, solution behaviors and their related mechanisms. *Solubility of polysaccharides*, *3*, pp.20-60.
3. Raabe, D., Sachs, C. and Romano, P.J.A.M., 2005. The crustacean exoskeleton as an example of a structurally and mechanically graded biological nanocomposite material. *Acta Materialia*, *53*(15), pp.4281-4292.
4. Kumar, M.N.R., 2000. A review of chitin and chitosan applications. *Reactive and functional polymers*, *46*(1), pp.1-27.
5. Rinaudo, M., 2006. Chitin and chitosan: Properties and applications. *Progress in polymer science*, *31*(7), pp.603-632.
6. Kurita, K., 1998. Chemistry and application of chitin and chitosan. *Polymer Degradation and stability*, *59*(1-3), pp.117-120.
7. Bradic, B., 2020. *Isolation of chitin from marine biomass using eutectic solvents and studies of N-deacetylation kinetics to chitosan* (Doctoral dissertation, Univerza v Mariboru).

****

20

FISH MEAL AND FISH OIL: PRODUCTION, PROPERTIES AND STANDARDS

Jeyakumari A, Binsi P K and Elavarasan K
Fish processing division
Email ID: ***jeya131@gmail.com***

Fish meal

Fish meal is a solid product that is obtained by cooking the fish/fish by-products, removal of water and some oil. Fish meal is generally sold as a powder, and is used mostly in compound foods for poultry, pigs and farmed fish; it is far too valuable to be used as a fertilizer. Fish meal is regarded as a highly concentrated nutritious supplement in feeds that contains high-quality proteins, vitamin B, minerals, etc.

Raw material

The raw material used for the production of fish meal varies from region to region depending on the availability. In general, three types of raw materials are used in fish meal manufacture which includes oily pelagic fish (oil sardine), low value whole fish containing more bones, inedible parts of fish and shellfish. Region-specific raw materials are anchovy in Peru, Menhaden in USA, Pilchards in South Africa, Herring and Capelin in Norway, and oil sardine in India. Oil sardine is most commonly used for fish meal and fish oil production in India due to its availability.

Production of fish meal

Methods of production of fish meal include;

• Dry rendering
• Wet rendering

The dry rendering or dry reduction method is suitable for lean fish containing less than 2-3% oil. It is not a continuous process. The wet reduction method is continuous and can be used for the production of fish meal from fatty fish. Wet rendering is a commonly used method of fish meal production throughout the world.

Process of Fish Meal Production: The following steps will be followed for fish meal production;

Cooking: The material is cooked at a temperature of 100°C for 20 minutes in indirect steam. This process stops microbiological and enzymatic activity in the product and helps to separate the oil.

Pressing: In this process, mechanical pressing is done to separate the material into two types of phases. The liquid phase and the solid phase.

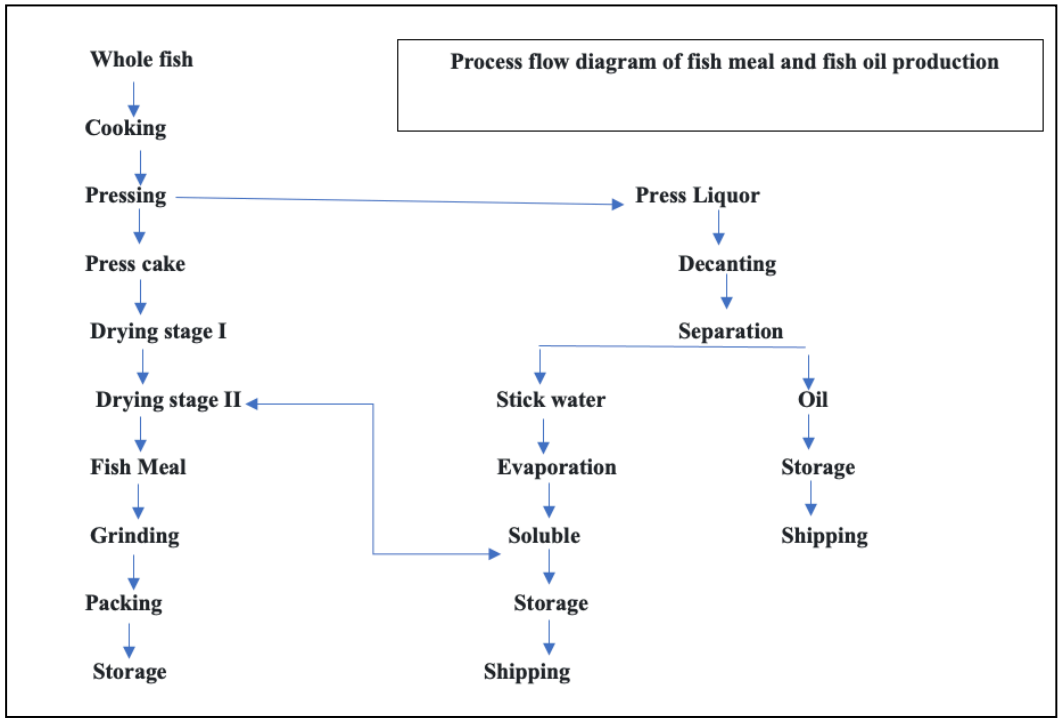
Decanting: In this stage, the liquid phase is decanted to recover more solid products and add them to the solid phase.

Centrifugation: In this procedure, the liquid phase is centrifuged. As a result, oil and water will be obtained.

Evaporation: the evaporation is done in the "tailwater" which is excess liquid, it is intended to reduce the volume of the product to concentrate it better and obtain solids.

Drying: The solids remaining from centrifugation are mixed with the solid cake obtained from pressing until a paste is obtained. Drying extracts more water from this mixture until the moisture content is reduced to 5-10%. This prevents bacteria growth and reduces chemical reactions.

Packaging: Fish meal is stored at ambient temperature either in HDPE bags. The fish meal does not require any refrigerating during storage. Additives such as antioxidants are added to fishmeal.



Physical and nutritional properties of fish meal

Physical Properties

- Color – light to dark brown, should be free from any evidence of scorching or burning
- Odor – shall be characteristic of fresh fish meal and free of rancidity
- Texture – fine granules and powder - generally free-flowing, but less so with higher fat meals

- Appearance – meals should be free of any visible signs of mold, clumps, or contamination Composition of fish meal varies considerably depending on the raw material and processing parameters.

Nutritional properties

- In general, chemical components in fish meals are protein, fat, ash and moisture which are 50-70%, 5-10%, 12-33%, and 6-10% respectively.
- Proteins in fish meal are rich in all essential amino acids which are not synthesized in the body and need to be supplied from the diet. All essential amino acids present in fish meal make it highly nutritive. Fish meal contains lysine in rich quantities which is deficient in cereals and legumes.
- Fish meal supplies vitamins such as riboflavin, niacin, pantothenic acid, choline, and Vitamin B12 in addition to fat-soluble vitamins such as Vitamin A and D. Oil present in fish meal contributes to energy for fish and other animals.
- Average values of vitamins in fish meal are riboflavin – 7.3 mg/100 g of fish meal, niacin 126 mg/100 g of fish meal, pantothenic acid - 30.60 mg/100 g of fish meal, Vitamin B12 – 0.25 mg/100g of fish meal, pyridoxine – 5.7 mg/100 g and choline – 4000 mg/100 g of fish meal. Fish meal also contains a significant quantity of Vitamin D due to residual oil in fish meal (5000 IU/ kg of fish meal).
- Inorganic constituents of fishmeal accounts for 11%. Indian fish meal exhibits higher proportions of phosphorus to calcium 1:1 against 1:2 proportions in other fish meals.
- Fish meal made from whole fish containing bones is rich in calcium, phosphorus and magnesium which are essential for growth. Mineral content in fish meal ranges from 25 to 30%. Mineral composition of fish meal involves zinc – 70 mg/kg, iodine – 70 mg/kg, iron – 250 mg/kg, copper – 7 mg/kg, manganese - 4 mg/kg, cobalt – 0.1 mg/kg
- Fish meal contains lower amounts of crude fibre in their diet which is good for proper digestion and absorption of nutrients in poultry and fish feeds.

General requirements for quality fish meal

Protein: Its value should be between 55 and 65% to ensure a product of good quality and high nutritional value.

Fat: This parameter should not exceed 13% in fishmeal. Higher values favor flour deterioration.

Humidity: This measure should be between 5-10%. Excess moisture affects shelf life and sanitary quality because water favors the replication of bacteria and fungi. On the other hand, values below this favor the heating and combustion of the fishmeal.

Ash: Its value should be less than 20%. Fishmeal is recognized for having a high value of calcium and phosphorus.

pH: should not be less than 5, it indicates the degree of chemical and physical reactions that occur in the flour.

Sodium Chloride: should be a maximum of 3%. Salt is used to preserve the raw material when there is no good storage chain.

Total Volatile Basic Nitrogen (TVBN): should be less than 125mg/100g. Flour should be stored at low temperatures to avoid degradation of nitrogen compounds that increase this parameter.

Fiber: this value must be less than 1% since it indicates the digestibility of the product. This is of utmost importance in poultry farming due to the size of 1-day-old chicks consuming feed.

Rancidity: should be less than 13meq/kg, evidence of packaging failure when oxygen enters the bags. This failure increases peroxides that degrade fishmeal.

Histamine: less than 20mg/10g alterations and hygienic-sanitary quality in conservation, bacteria grow and form toxic amine.

Microbiological analysis: Fishmeal should be free from of *Salmonella* bacteria and ensure the safety of the product.

ISI- Requirements for fish meal as poultry feed ingredient

- Fish meal shall be in the form of powder ground to such fineness that 99 percent of material shall pass through 2.80mm IS Sieve.
- The material shall have the characteristic odor and shall be free from any off-odor indicative of spoilage.
- The material shall be free from adulterants, arthropod infestation, visible fungal growth and any harmful material.
- Fish meals shall be packed in high-density polyethylene bags or jute bags with polyethylene lining inside. The mouth of each bag shall be either machine-stitched or rolled over and hand-stitched.
- Each bag shall be suitably marked or labeled with the following information: a) Name and grade of material, b) Name of the manufacturer, c) Batch or code number indicating the date of manufacture. d) Net mass in kg, and e) Guaranteed composition

ISI- Requirements for fish meal as livestock feed ingredient		
Parameters	**Grade I**	**Grade II**
Moisture (%max)	10	10
Crude protein (% min)	60	50
Ammoniacal nitrogen (% max.)	0.5	0.5
Crude fat (% max.)	10	10
Acid insoluble ash (% max.)	3	3
Chloride (as NaCl) (% max.)	4	5

Fish oil

Fish oil, which was previously a by-product of fishmeal used for animal feed, is now recognized as the primary source of these fatty acids. Fish oil can be extracted from whole fish and liver. Fish oil extracted from both resources has industrial and medicinal uses. Methods to extract fish oil include cooking, use of solvents and, recently, extraction by supercritical fluids, by enzymatic procedures and by chemical (i.e. applying acids) or biological silages. The extraction of fish oil by wet pressing is the most commonly used method for production on an industrial scale. The press liquor is the oil-water emulsion containing dissolved proteins and other substances as well as particulate matter. The press liquor is passed through a series of settling tanks or a series of centrifuges. The amount of particulate matter depends on the degree of cooking, the condition of the fish when processed and also the manner of pressing.

Fish oil Extraction by settling tank system

The settling tanks are heated to assist the breakup of the emulsion and prevent solidification of the stearin portion of the liquids. In a series of five or more heated tanks, the press liquor is admitted to the first at a point below the surface. Oil rises to the top and is passed to the bottom of the second tank containing water and the process is repeated in succeeding tanks. Finally, oil is heated to evaporate the remaining water.

Centrifuge system of extracting fish oil

In this system, a centrifuge is heated and the water phase is spun off and almost clean oil is obtained. Further to get a clean bright oil, oil is heated to about 94 degrees, mixed with clean water of the same temperature and passed to the polishing centrifuge. Oil produced through a centrifuge system is finer, cleaner and brighter and has a lower moisture content than oil from a settling tank system.

Major species used for fish oil extraction	
Species	**Country**
Anchovy	Peru
Jack mackerel, anchovy, sardines	Chile
Various species, anchovy	China
Various species, trimmings	Thailand
Menhaden, Alaska pollock, trimmings	USA
Blue whiting, herring, sprat, trimmings	Iceland
Blue whiting, capelin, herring, sand eel, trimmings	Norway
Sand eel, sprat, blue whiting, herring	Denmark
Sardine, pilchard, various species	Japan
Sardine, various species	India

Purification/Hardening of fish oil

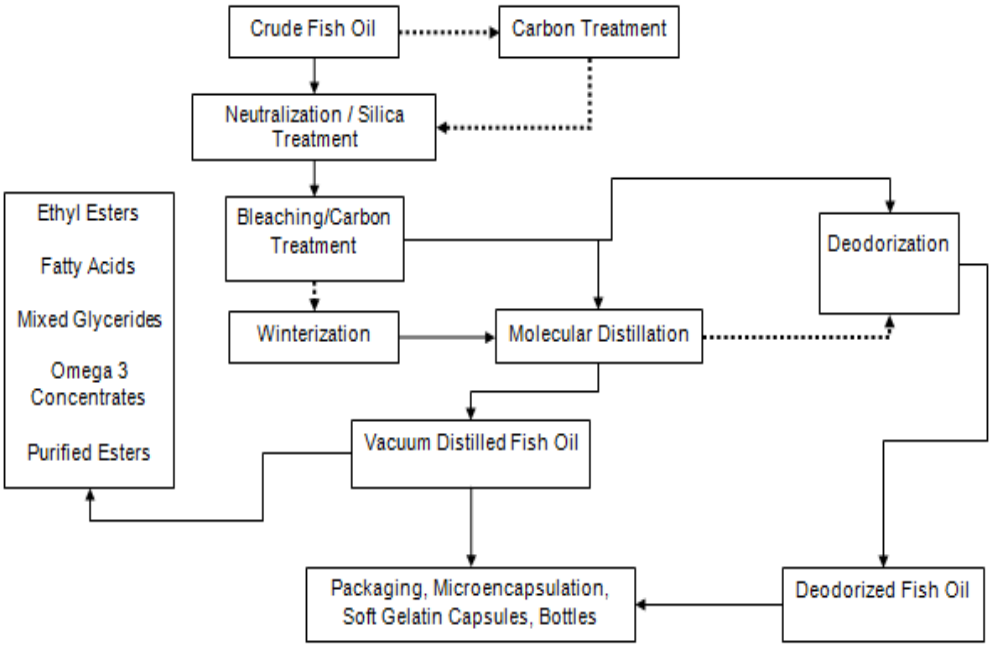
Once fish oils are extracted they require a purification process to achieve the quality characteristics that make them acceptable for human and animal consumption. Crude oil has a number of impurities such as free fatty acids, phospholipids, diglycerides, monoglycerides, pigments, pigment decomposition products, oxidation products, sulfur compounds, proteinaceous compounds, aldehydes, ketones, pesticide residues (preferably organochlorine pesticides and polychlorinated biphenyls. Fish oil may be contaminated with toxic heavy metals (such as Cd, Hg, Pb, Cu, Zn, etc. Cu and Zn are known for their distinct prooxidant effect. The Major steps involved in the purification of fish oil are given below;

Bleaching: Bleaching materials commonly used are natural or activated clays and activated carbon. It removes any colored matter, natural pigments and some of the suspended mucilage. It is also effective in the reduction of oxidation products, phosphorus, to a lesser extent sulfur compounds and heavy metals and non-metals. As an alternative to activated clay and carbon, the treatment of fish oil dissolved in hexane with silica gel gives a nearly colorless oil.

Winterization: It is a cold clearing process, an additional operation for refining fish oil. When the oil is cooled sufficiently, the saturated triglycerides (which have high melting points) commence to solidify and separate out. Cooling must be gradual by circulating cold brine

Refining: Treatment of oil with an aqueous alkaline solution which reacts with the free fatty acids to form soaps and remove any mucilage.

Deodourization: It is done by steam distillation under high vacuum (2 – 5 mm absolute pressure). Dry steam (free from oxygen and temp range of 170 – 230^{0}C) is used, which is passed through the oil under a vacuum for a prolonged period (maybe up to 5 h in a batch process). This step removes free fatty acids, decomposition products of hydroperoxides such as aldehydes and ketones, odoriferous and other volatile impurities. This step is a very critical one. If time and temperature are not strictly controlled as per schedule, the most valued components of the fish oil undergo distinct deterioration. When retention of EPA and DHA are the concern then a temperature not exceeding 170^{0}C is recommended.



Process flow for Production of edible and pharmaceutical-grade fish oils and derivatives

The following additives may be used in fish oil as per the codex guidelines

Additive	**Additive name**	**Maximum level**
Antioxidant		
E300	Ascorbic acid, L	GMP
E 304, 305	Ascorbyl esters	2500 mg/kg, as ascorbyl stearate
E307a, b, c	Tocopherols	6000 mg/kg, singly or in combination
Emulsifier		
E322	Lecithin	GMP
E471	Mono- and diglycerides of fatty acids	GMP

Physical and Chemical properties of fish oil

Physical property	**Value**	**Chemical properties**	**Value**
Specific heat (cal/g)	0.50–0.55	Moisture and impurities (%)	Usual basis 0.5 up to 1 % maximum
Heat of fusion (cal/g)	ca. 54	Free fatty acids (% oleic)	Range 1–7 % but usually 2–5 %
Calorific c value (cal/g)	ca. 9,500	Peroxide value (meq/kg)	3–20
Slip melting point (°C)	10–15	Anisidine number	4–60
Flashpoint (°C)		Totox value	10–60
as glycerides	ca. 360	**Iodine value**	
as fatty acids	ca. 220	Capelin	95–160

Boiling point (°C)	>250	Herring	115–160
Specific gravity at		Menhaden	120–200
15 °C	ca. 0.92	Sardine	160–200
30 °C	ca. 0.91	Anchovy	180–200
45 °C	ca. 0.90	Jack mackerel	160–190
Viscosity (cp) at		Sand eel	150–190
20 °C	60–90	Color (Gardner scale)	Up to 14
50 °C	20–30	Iron (ppm)	0.5–7.0
90 °C	10	Copper (ppm)	Less than 0.3
Refractive index (n D 30)	1.46–1.48	Phosphorus (ppm)	5–100

Nutritional properties of fish oil:

The interest in PUFAs of fish oil in human diets has led to the intense use of fish oil in human and animal diets. It is a good source of energy and also possesses many health benefits. Vitamins A and D occur in the oil of most fish, but many species store large amounts of vitamins A and D in their livers (cod, halibut, and tuna). The body oils of fish generally contain vitamins in minute amounts and are not consumed for that purpose. Fish oils contain varying amounts of vitamin E, which also acts as an antioxidant. The tocopherol levels reported in crude fish oils are 30 μg/g in menhaden oil, over 60 μg/g in anchovy oil, and 25 μg/g in capelin oil.

Application of fish oil

- **Food:** Foods fortified with fish oil are emerging as a novel food category promoted as containing omega-3. These products include margarine, milk, bread, cheese, yogurt, infant formulas etc.
- **Feed:** Pig, poultry, cattle, sheep, fish and pet foods
- **Pharmaceutical:** Fish oil has important roles in the prevention of some types of cancer, including colon, breast, renal, prostate, pancreatic cell, and liver. Several in vitro and animal experiments have clearly shown that the LC omega-3 PUFAs, EPA and DHA, are responsible for the inhibition of promotion and progression of cancer. There has been tremendous growth in the use of fish oil in the pharmaceutical industry including improve the heart health, lower blood fat, ensuring bone, brain, eye and skin health etc.
- **Others**: Leather, paint, fuel, lubricants, printing ink, soaps

Recommended Intake of Omega-3

Several international scientific authorities have published recommendations for the daily intake of omega-3 PUFAs. The UK Government has recommended that people eat fish twice a week, including oily fish, to provide 3 g of LC omega-3 weekly. A similar recommendation has been made by the US Heart Association.

Organization	Recommended level
Health and Welfare Canada	1.0–1.8 g omega-3 PUFAs/day
International Society for the Study of Fatty Acids and Lipids (ISSFAL)	0.22 g DHA and EPA/day
British Nutrition Foundation (BNF)	1.4 g DHA and EPA/day (males) 1.1 g DHA and EPA/day (females)
Institute of Medicine (IOM)	0.5 g omega-3 PUFAs/day (for infants)
United States Food and Drug Administration (US FDA)	3 g DHA and EPA/day

FSSAI standards for Fish oil

Fish oil shall also conform to the requirement given in table below

Sr. No	Characteristics	Requirement
1.	Free fatty acids as % oleic acid, w/w, max	1.0
2.	Moisture, % by weight, max	0.5
3.	Iodine Value	145-180
4.	Saponification value	185-205
5.	Unsaponifiable matter, %, w/w, max	2.0
6.	Refractive Index (40°C)	1.4739-1.4771
7.	The product shall be a bright and clear liquid when heated to a temperature of 40°C.	
8.	The product shall be free from foul and offensive putrefactive odour and should have only characteristic fish- oil odour	

Codex Standards for fish oil (329-2017)

- Acid value <3mg/KOH/g
- Peroxide value < 5 milli equivalent of active oxygen/kg oil
- Anisidine value<20
- Total oxidation value $(ToTox)^2$<26

Fish oils with a high phospholipid concentration of 30% or more such as krill oil shall comply with the following

- Acid value <45mg/KOH/g
- Peroxide value < 5 milli equivalent of active oxygen/kg oil

****

21

PACKAGING OF FISH AND FISHERY PRODUCTS

J. Bindu
Fish processing division
Email ID: ***bindujaganath@gmail.com***

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

Types of Packaging Material

Glass

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

Cans

Most frequently used container for packing food for canning is tin plate can. Tin plate containers made their appearance in 1810. The base steel used for making cans is referred as CMQ or can making quality steel. Corrosion behavior, strength and durability of the tin plate depend upon the chemical composition of the steel base. The active elements are principally

copper and phosphorous. The more of these elements present the greater the corrosiveness of steel. Cans are traditionally used for heat sterilized products and different types are standard tin plates, tin free steel and vacuum deposited aluminium on steel and aluminium cans. For food products packing they are coated inside to get desirable properties like acid resistance and sulphur resistance. But care has to be taken to avoid tainting of the lacquer.

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

Paper

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

Paper board

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

Polymer Packaging

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

Low Density Polyethylene (LDPE)

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

High Density Polyethylene (HDPE)

HDPE resins are produced by low-pressure process. HDPE posses a much more linear structure than LDPE and has up to 90% crystallinity, compared with LDPE which exhibits crystallinities as low as 50%. 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.

Linear Low Density Polythene (LLDPE)

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

Polypropylene (PP)

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

- Cast polypropylene (CPP)

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

- Oriented, Heat set Polypropylene (OPP):

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

Polystyrene

The material is manufactured from ethylene and benzene, which are cheap. The polymer is

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

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

Although many films can be metallized, polyester is the most commonly used one. Metallization results in considerable improvement in barrier properties. A fast growing application for polyester is ovenable trays for frozen food and prepared meals. They are preferable to foil trays for these applications because of their ability to be micro wave processed without an outer board carton.

Polyamides (Nylon)

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

Polyvinyl Chloride (PVC)

The monomer is made by the addition of reaction between acetylene and hydrochloric acid. It must be plasticised to obtain the required flexibility and durability. Films with excellent gloss and transparency can be obtained provided that the correct stabilizer and plasticizer are used. Thin plasticized PVC film is widely used in supermarkets for the stretch wrapping of trays containing fresh red meat and produce. The relatively high water vapour transmission rate of PVC prevents condensation on the inside of the film. Oriented films are used for shrink-

wrapping of produce and fresh meat. Unplasticized PVC as a rigid sheet material is thermoformed to produce a wide range of inserts from chocolate boxes to biscuit trays. Unplasticized PVC bottles have better clarity, oil resistance and barrier properties than those made from polyethylene. They have made extensive penetration into the market for a wide range of foods including fruit juices and edible oils.

Copolymers

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

Aluminium foil

Aluminum foil is defined as a solid sheet section rolled to a thickness less than 0.006 inches. Aluminum has excellent properties like thermal conductivity, light weight, corrosion resistance, grease and oil resistance, tastelessness, odourlessness, heat and flame resistance, opacity and non-toxicity. 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.

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.

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.

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.

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.

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.

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.

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.

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.

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, good bursting and tearing strength , heat sealability and long shelf life.	Prone to easy attack by insects, costly.
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.

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.

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 are 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.

Fish pickles

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

Fish soup powder

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

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

Extruded products

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

Surimi and surimi based products

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

Fish Sausage

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

Glucosamine hydrochloride

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

Chitin and Chitosan

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

Fish Hydrolysate

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

Fish Meal

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

Fish oils

Fish oils are highly unsaturated and easily susceptible to oxidation when exposed to air. Hence they have to be packed in containers which have high barrier properties which are moisture proof, oil resistant and impermeable to oxygen. Larger quantities of fish oil are mainly packed

in LLDE/Nylon films or in glass bottles. Bulk transportation food grade flexitanks made of 4 layered polyethylene and tubular PP. Advantages of using flexitanks are that they can carry 50% more than bottles and therefore will save on storage space, packaging and transportation cost.

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

Fish silage

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

Shark fin rays

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

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.

22

ENTREPRENEURSHIP DEVELOPMENT IN FISHERY PRODUCTS

C. O. Mohan and George Ninan
Fish processing division
Email ID: *comohan@gmail.com*

An entrepreneur is an individual who takes an idea or product or service and creates a business, a process known as entrepreneurship. The entrepreneur is someone who has the ability and desire to establish, administer and succeed in a startup venture along with risk, whereas a startup or start-up is a company or project undertaken by an entrepreneur(s) to seek, develop, and validate a scalable business model. Entrepreneurs focus on finding the most efficient route to profitability whereas startup founders are dedicated to growing their company and making an impact on the world. Entrepreneurship needs innovation as much as it does resources, but perhaps what it needs most of all is access to support functions, mentorship and resources that would provide new enterprises a springboard to stability. It is true that there is no dearth of unique ideas that may solve a problem, target a certain market segment or create a successful and profitable business, but the journey on that path is long and arduous. A helping hand is welcome and often the only means of survival. This is where the role of incubators comes in.

Business incubator is a broad umbrella term referring to any organization that provides physical workspace, management and technical assistance, access to financing and other supporting services to young firms and helps them survive and grow during the startup stage. The Business Incubators acts as catalysts for economic growth by combining the features of technology commercialization, entrepreneurship and business facilitation. The Indian Council of Agricultural Research (ICAR) started the business incubation drive, designed for the Indian agricultural sector to promote agribusiness in 2009, by utilizing the vast research and development facilities and knowledge available with its research institutions. As part of translating the research results arising from the field of fisheries and, ICAR set up a unique Agri-Business Incubation (ABI) Centre at ICAR-Central Institute of Fisheries Technology, Cochin. The ABI Centre helps prospective entrepreneurs, by providing pro-active and value-added support in terms of technical consultancy, infrastructure facility, business support services, expert's guidance and training to develop technology based business enterprises. It provides a platform for the speedy commercialization of the technologies developed by the ICAR Institutes, by creating an interfacing and networking mechanism between R&D institutions, industries and financial institutions. The fisheries technologies available with ICAR includes harvest and post harvest technologies, new and improved aquaculture methods, seed production technologies of finfish and shrimp, cost-effective and nutritious feed formulations for fish farming, test kits / diagnostic methods, ready-to-cook and ready-to-serve

value added products, waste minimization/utilization technologies, byproduct utilization, pharmaceutical and biotechnological products, food packaging techniques etc.

Business incubation at ICAR-CIFT

The ABI Centre at ICAR-CIFT aims at establishment of Agribusiness enterprises through IPR enabled ICAR technologies. It focuses on finding new ways of doing business in agriculture and allied fields by providing access to unexplored markets for achieving the objectives of increased productivity, poverty alleviation, nutritional, livelihood and income security. The objectives of the ABI Centre are:

Commercializing technologies developed by ICAR-ICAR-CIFT

Helping entrepreneurs to commercialize business ideas utilizing the R&D back up of the institutes

Providing pilot level production facilities in fisheries to entrepreneurs

Imparting training for creating prospective entrepreneurs and value added manpower

Process of Incubation

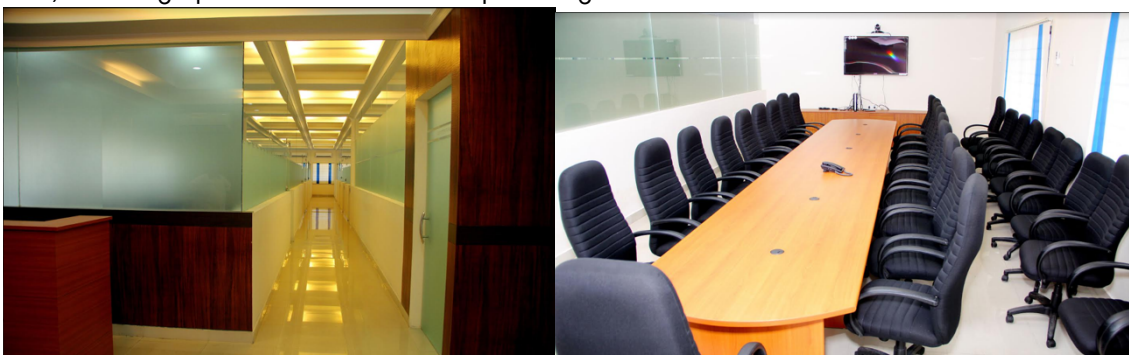
The ABI Centre targets entrepreneurs, from fledgling startups in need of basic small scale processing capacity to sophisticated businesses in need of R&D back up, office infrastructure and pilot/test market processing facility for the development of new products. It possesses good infrastructure facilities suitable for providing direct incubation of about 9 entrepreneurs in a corporate environment within the premises of ICAR-CIFT, at a time. The purpose of direct incubation is to support emerging companies through their infancy. It also provides virtual incubation to budding entrepreneurs who want to start new enterprises as well as to established entrepreneurs who need to increase the efficiency of existing ventures. The Unit regularly conducts awareness and technology promotional programmes for sensitization of entrepreneurs and to identify interested potential candidates for physical and virtual incubation. The residency period for direct incubatees will be normally for one year, extendable by another year in special cases, depending on the progress of incubation. As the business venture becomes mature enough, the concessions and the facilities provided to the incubatee companies will be gradually withdrawn.

Incubation facilities under one roof

The major facilities provided by the business incubator for de-risking are listed below:

1) Provides technology and know-how backed up with scientific results
2) Initial assessment of product and business
    - Assess the commercial viability of the business plan
    - Benchmark against best practices in the industry
    - Identify technology gaps and requirements
3) Regulatory, compliance and standards support
    - Training in quality regulations and related aspects
    - On-site inspections and formulate remedial measures
    - Provides assistance to secure regulatory and standards certifications
4) Infrastructure and production unit
    - State-of-the- art pilot level production facility
    - Well-furnished office space at prime business location

5) Training and skill development
6) Product development and testing
7) Formulation of company policies
8) Setting up of new facilities and up-scaling



Incubation Office facility for Clients

Pilot level production facility in fisheries

A state-of-the-art generic semi-commercial production facility is made available to incubating entrepreneurs for developing value added products from fish. The ABI Centre provides facilities and staff support on a fee for use basis to assist companies and individuals, with production and testing of product formulations provided by the client. The user can experiment with new equipment and processing steps on old products or to link old equipment in new ways. For incubatees, the pilot plant is an ideal testing arena to determine commercial run viability of new products. The pilot plant also serves as a process lab, a place to see how processing equipment impacts food products under varying conditions. The pilot plant facility at ICAR-CIFT includes production lines for

- pre-processing / cooking
- retort pouch processing
- solar drying
- canning / curing / smoking
- sausage production
- extruded products
- chitin & chitosan
- breading & battering
- product packaging

Plant Plant Facility

Categories of entrepreneurs approaching the agri-business incubator

The fishing industry includes any industry or activity concerned with culturing, harvesting, processing, preserving, storing, transporting, marketing or selling fish or fish products. The commercial activity is aimed at the delivery of fish and other seafood products for human consumption or as input factors in other industrial processes. Directly or indirectly, the livelihood of over 500 million people in developing countries depends on fisheries and aquaculture. The commercial sector of the fishing industry comprises the following chain: (i) Commercial fishing and fish farming which produce the fish (ii) Fish processing which produce the fish products and (iii) Marketing of the fish products. The clients approached the centre during the period 2010-19, for various services are classified based on the area of their expertise and represented as Fig: 1.

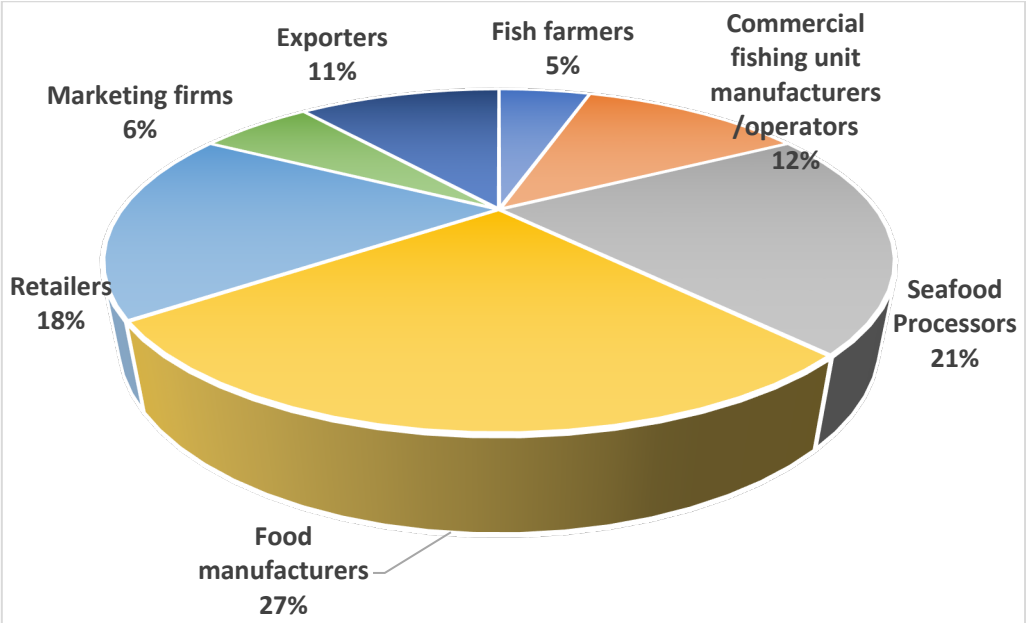


Fig 1: Categories of entrepreneurs incubated at ABI Centre

Conclusion

An outline of the initiative taken by ICAR, in establishing the first fisheries business incubator in India is provided here. It gives an overview of the activities of the ABI Centre at ICAR-CIFT, Cochin and the wide variety of technical, managerial, and administrative supports offered to the incubating entrepreneurs. The incubation unit is expanding its activities at a rapid pace and trying to build a foundation for new technology based industries, establish a knowledge-based economy and create new jobs in the agricultural sector by bridging the gap between private and public sectors, and finding suitable mechanisms for ensuring good entrepreneurial climate.

Contact details

The Director
ICAR – Central Institute of Fisheries Technology
CIFT Junction, Willingdon Island, Matsyapuri P.O., Kochi-682 029, Kerala, India
Ph : 0484-2412300; Email : cift@ciftmail.org; Website : www.cift.res.in

For enquiries on Agi-business Incubation / Technology transfer, mail to ztmcfisheries@gmail.com

****

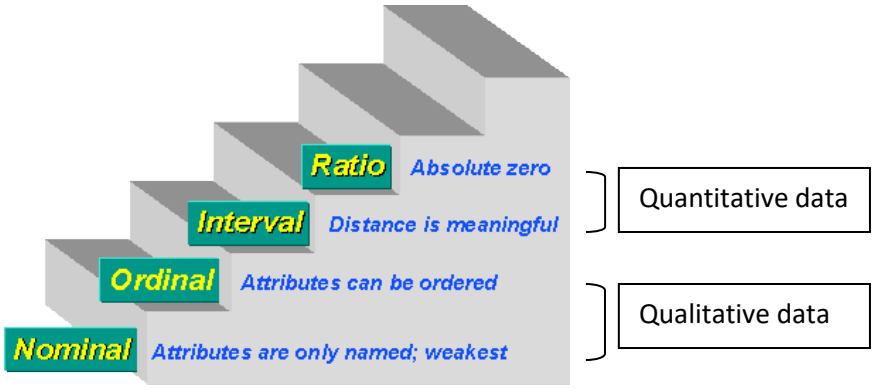
23

DESCRIPTIVE AND BASIC EXPLORATORY STATISTICS

Joshy C. G.
Fish processing division
Email ID:*cgjoshy@gmail.com*

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 summarize 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

1) Range
2) Variance
3) 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 diference 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

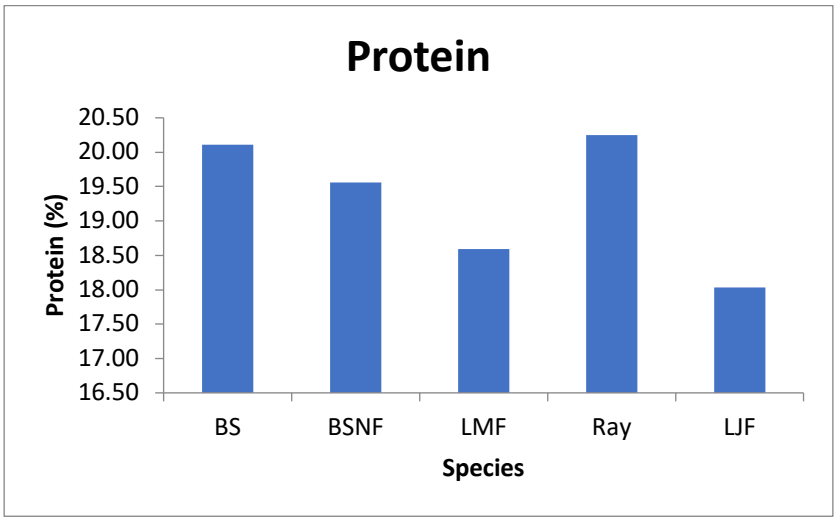
A frequency polygon represents the shape of the data. It can be conceptualized by connecting the midpoints of the classes at the height specified by the frequency.

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.

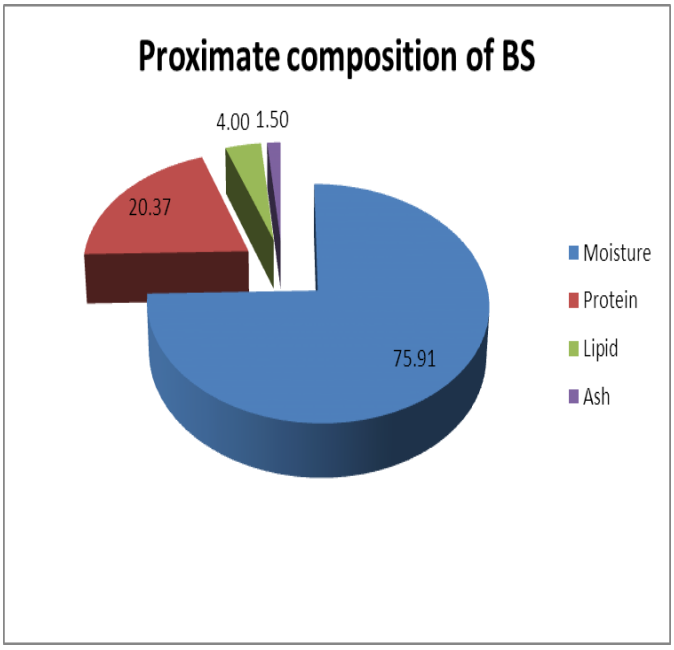
- The qualitative data is summarized in a frequency, relative frequency, or percent frequency distribution
- On the horizontal axis, the labels used for each of the classes are specified
- On the vertical axis, frequency is specified
- The bars are separated to show that each class is a separate category



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.

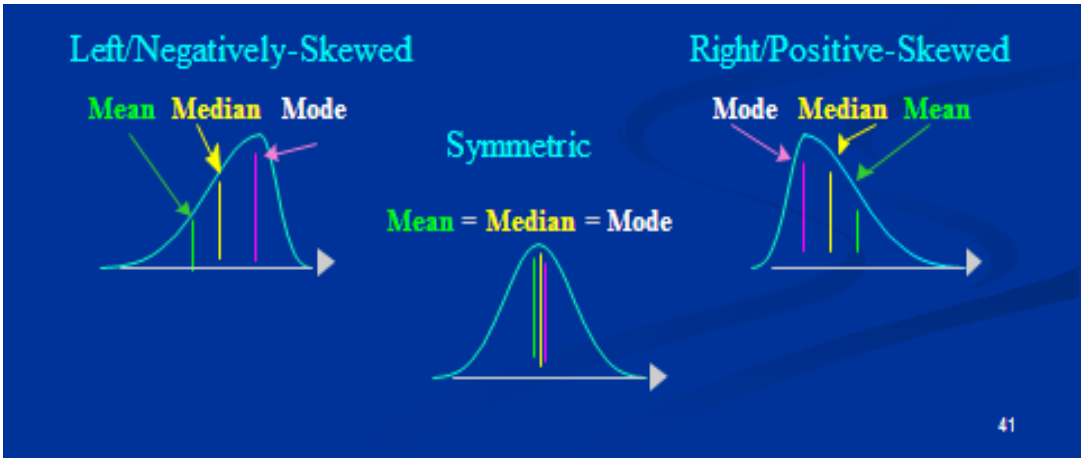
- Commonly used graphical device for presenting relative frequency distributions for qualitative data
- Use the relative frequencies to subdivide a circle (360^0) into sectors that correspond to the relative frequency for each class
- A class with a relative frequency of 0.25 would take $0.25(360) =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

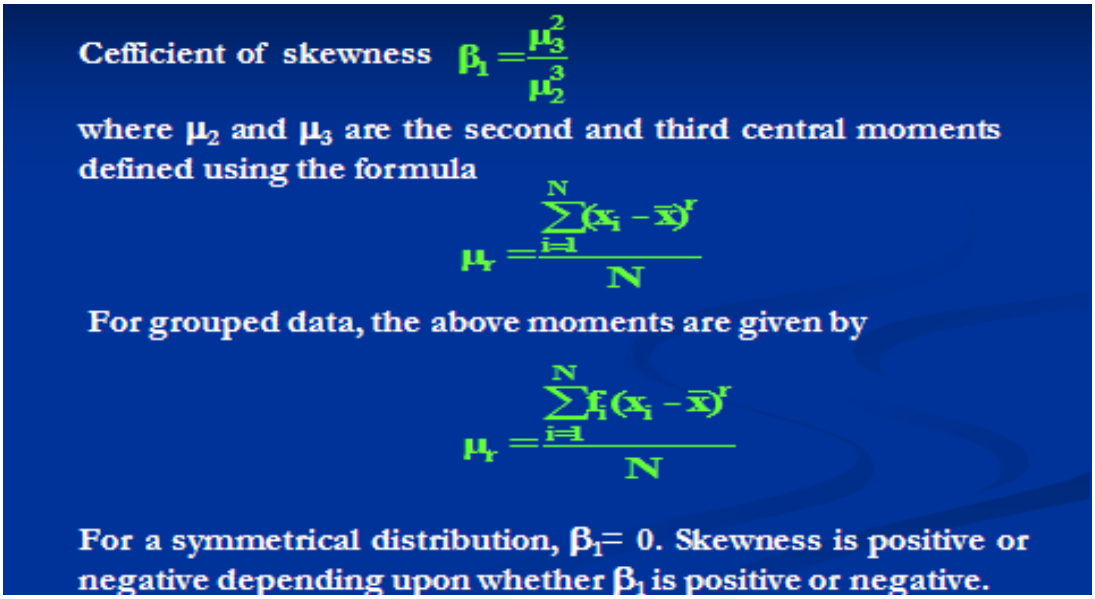


Distribution of a given data

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 = 50th percentile
- Lower quartile Q_1 = 25th percentile
- Upper quartile Q_3 = 75th 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

References

Kothari,C.R. 1990. Research Methodology: Methods and Techniques. Second edition. Wishwa prakashan. New Delhi.P 468

Snecdecor. G.W. and Cochran. W.G. 1989. Statistical Methods. John Wiley & Sons. New York. P 503.

****

24

SEAWEEDS – NUTRITION FACTS AND HEALTH BENEFITS

Suseela Mathew
Biochemistry and Nutrition division
Email ID: s*useela1962@gmail.com*

The well-established correlation between food habits and health status points out to the possibilities of improving the overall health through the consumption of tailor made foods. Furthermore, consumers all over the world are becoming increasingly aware of the food-health relationship and started demanding for foods that can provide health promoting effects in addition to the essential nutrients. Hence, researchers all over the world have started exploring newer food sources for the development of nutraceuticals to cater the ever increasing consumer demand. Marine ecosystems are considered as rich repository of structurally diverse bioactive compounds with immense potential in both biomedical and functional food arenas. Marine organisms such as fish, crustaceans, molluscs, sponges, tunicates, bryozoans, bacteria, cyanobacteria, microalgae and macro algae are often utilized for the extraction of biomolecules. Among the marine organisms, seaweeds represent as one of the richest sources of bioactive molecules. It was mainly employed as a food source in the recent past and currently, the focus has been shifted to the isolation of bioactive molecules owing to the richness of bioactive compounds in them. They are often recognized as good source of healthy food and have even gained the reputation of 'superfood" in the recent past. The bioactive compounds from seaweeds, its bioactivities, application in biomedical and nutraceutical industries are summarized here.

Seaweeds – Source of nutrients

Seaweeds, usually named as macroalgae, are an extensive group of macroscopic marine organisms that comprise of a few thousand species (Kim et al., 2008; Kumari et al., 2010). Based on the pigmentation, seaweeds are categorized into three groups: Phaeophyceae (brown), Rhodophyceae (red) and Chlorophyceae (green). Among these, the highest phytochemical content in terms of terpenes, carotenoids and phenolic compounds have been reported from brown seaweeds (Gupta and Abu-Ghannam, 2011). However, the biochemical composition of different species may vary depending on the sampling site, the season of harvest and the environment (Bourgougnon & Stiger-Pouvreau, 2012; Holdt & Kraan, 2011).

Seaweeds are recognized as treasure house of bioactive compounds as they produce wide variety of biologically active components with different structural features and functional

properties (Choi et al., 2002; Kim & Bae, 2010). The bioactive components of seaweeds include polyphenols, peptides, polysaccharides, dietary fibres, proteins, sterols, carotenoids and numerous other structurally unparalleled secondary metabolites such as monoterpenes, sesquiterpenes, diterpenes, meroterpenoids, C15-acetogenins, phlorotannins etc. (Kladi et al., 2008, Kamenarska et al., 2002). They are also reported to have rich contents of micronutrients such as vitamins (A, B1, B2, B3, B6, B12, C, D, E,B5, B7), sterols, minerals (e.g. calcium, magnesium, potassium, iodine, sodium, phosphorus, nickel, chromium, selenium, iron, zinc, manganese, copper, lead, cadmium, mercury and arsenic) (Patarra et al., 2011; Peña-Rodríguez et al., 2011, Ferraces-Casais et al., 2012, Lopes et al., 2011). These bioactive compounds were reported to possess wide range of bioactivities such as antibacterial, antioxidant, anti-inflammatory, antiviral, , anticoagulant and antitumor properties (Nylund et al., 2010; Vairappan et al., 2010, Li et al., 2007, Matloub and Awad, 2009, Xu et al., 2004a). It has been already reported that the regular consumption of marine seaweeds can prolong life expectancy and reduce the risk of CVDs and one possible reason for this can be due to the presence of the bioactive compounds and its bioactivities.

In general, seaweeds are reported to possess 15–76% of its dry weight as polysaccharides, proteins (1–50% dry weight), lipids (0.3–5% of dry weight) and mineral (11–55% dry weight, in the form of ash). Because of its high nutritional and pharmaceutical values, seaweeds are used as food source and as herbal medicine for treating gall stones, stomach ailments, eczema, cancer, renal disorders, scabies, psoriasis, asthma, arteriosclerosis, heart disease, lung diseases, ulcers, etc. (Ortiz et al., 2006; Besada et al., 2009; Cruz-Suárez et al., 2010; Lee et al., 2011). Later, the focus of utilization of seaweed has been shifted from a mere food source to source of high value nutraceuticals. Different extraction protocols were designed by various researchers for the extraction of these bioactive moieties and these isolated biomolecules were employed for development of nutraceuticals and pharmaceuticals. However, the extraction methods used will vary depending on different factors such as physicochemical properties, molecular size and solubility of the compounds.

Bioactive compounds from seaweeds

Polysaccharides and Sulphated Polysaccharides

Polysaccharides are a class of macromolecules which are garnering attention in the biochemical and medical areas due to their immunomodulatory and anticancer effects. Different polysaccharides are found in seaweeds and their major role is to confer strength and flexibility. The composition may differ according to several intrinsic and extrinsic factors such as seaweed species, geographic area, season, age and parts of the seaweed collected etc. The major polysaccharides from phaeophyceae (brown algae) include alginates, laminarin, sargassan, fucoidans, sulphated galactofucans and ascophyllans. Polysaccharides derived from Rhodophyceae (red algae) include floridean starch, agars, carrageenans, xylans,

galactans, sulphated galactans and sulphated rhamnans. Chlorophyceae contain sulphated galactans, xylans and ulvans as the major polysaccharides. Among the seaweed polysaacharides, the one which have spurred great deal of interest in the last decade is fucoidan.

Fucoidans

Fucoidans are mainly found in many species of brown seaweed, especially in the cell wall matrix of marine brown algae, some terrestrial plants, animals, and microorganisms and fucoidans are sulfated hetero-polysaccharides. Fucoidans is made up of polymeric carbohydrate structures that consist of monosaccharide units linked by glucosidic bonds and enriched in fucose monomers (Holdt and Kraan, 2011). Researches across the globe have well documented various biological activities of fucoidans such as antiviral, anti-inflammatory, anti-coagulant, anti-angiogenic, immunomodulatory, and antiadhesive activity. Research in the biomedical sector has proven the potential use of fucoidans for the development of a novel drug against tumor cancer. Further, fucoidans may play a role as dietary fiber uptake contributing to lower cancer incidence risk (Tiwari et al., 2015).

Proteins and aminoacids

Seaweeds are widely recognized as cheaper protein alternative source due to its high-value proteins containing essential amino acids. The protein content in brown, green and red algae is 1–24%, 4–44% and 5–50% of the dry weight respectively. The major proteins in seaweeds include lectins and phycobiliproteins (Aneiros & Garateix, 2004). Phycobiliproteins are water-soluble and coloured components of the photosynthetic system in red macroalgae.

Lipids

The lipid content in seaweeds is generally low, however, almost half of lipids present are polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and arachidonic acid (AA), which can regulate blood pressure and reduce the risk of cardiovascular diseases, osteoporosis, diabetes etc. (Maeda et al., 2008). Furthermore, green seaweeds like *Ulva pertusa* are reported to have sufficient amounts of hexadecatetraenoic, oleic, and palmitic acids (Norziah and Ching, 2000; Ortiz et al., 2006).

Minerals

Seaweeds are reported to contain significant amounts of essential minerals such as sodium, calcium, potassium, magnesium and trace elements such as iron, zinc, manganese and copper. These minerals which have major role in building human tissues and as cofactors of many metalloenzymes due to their cell surface polysaccharides. Because of the richness of mineral content, seaweeds can be used as food supplements to provide the daily intake of some minerals and trace elements (Teas et al., 2004; Villares et al., 2002).

Vitamins

Seaweeds contain most of the vitamins such as l-ascorbic acid, thiamine, riboflavin, cobalamin, folic acid, and its derivatives (Mišurcová, 2011). It has been reported that vitamin C is present in very high amounts of 2000 mg/kg dry matter in red seaweed *Eucheuma denticulatum* and 3000 mg/kg dry matter in green seaweed *Enteromorpha flexuosa* (McDermi and Stuercke, 2003). Furthermore, B group vitamins, especially thiamine and riboflavin are found in substantial amounts in most red and brown seaweeds (MacArtain et al., 2007) whereas vitamin E content is higher in brown seaweed. Hence, it can be said that seaweeds are good source of vitamins also.

Fucoxanthin

Fucoxanthin is a xanthophyll, found as an additional pigment in the chloroplasts of the brown algae. Fucoxanthin and its de-acetylated metabolite depict anti-inflammatory, anti-nociceptive, and anti-cancer effects (Lee et al., 2013). Fucoxanthin and its metabolites can be used as a novel drug in the field of the bio-medical sector.

Bioactive properties of seaweeds

Antioxidant property

Marine algae are considered as one of the richest sources of antioxidants among the marine organisms. Seaweed phlorotannins by virtue of its eight interconnected rings are considered as very powerful free radical scavengers. These compounds have been isolated and purified from the brown algae *E. bicyclis, E. kurome, H. fusiformis* and *E. cava* and they have shown potent antioxidant activity against hydrogen peroxide induced cell damage (Kang et al., 2006). Some of the phlorotannins like eckol, phlorofucofuroeckol A, dieckol, and 8, 8-bieckol have shown anti-oxidant capacity in phospholipid peroxidation (Shibata et al., 2008). Because of their strong anti-oxidant activities they are even comparable to anti-oxidants such as ascorbic acid and tocopherol. Therefore, phlorotannins from seaweeds can be considered as potent anti-oxidants with wide applications in food and pharmaceutical industries (Kim et al., 2006).

Anti-coagulant property

Anti-coagulants are therapeutics which have ability to prevent blood coagulation or stop the formation of blood clots (Desai, 2004). Heparin, a sulfated polysaccharide is one of the most common anti-coagulant drugs used in the world against thromboembolic disorders (Fan et al., 2011). However, because of the several side effects associated with it, scientists are looking for suitable alternatives for heparin. Sulfated polysaccharides of anti-thrombotic and anti-coagulant properties have been isolated from different marine algae. Fucoidan, a sulphated polysaccharide from seaweeds are reported to display strong anticoagulant properties. The degree of its anticoagulant property is related to its sulphate and polysaccharide content. It was reported that C-2 sulfate and C-2, 3 disulfate in fucoidans is mainly associated with anti-coagulant activity (Chevolot, Mulloy, & Racqueline, 2001). The outcomes of many studies have proposed that fucoidans can be used as suitable alternatives to heparin and even certain

fractions of fucoidan can be qualified as heparinoids (i.e. molecules derived from heparin) (Mourao, 2004).

Anti-inflammatory property

Of late, it has been reported synthetic anti-inflammatory drugs can cause gastrointestinal irritations and hence forth the search for suitable and safer alternatives from natural sources is ongoing (Nguemfo et al., 2007). Sulfated polysaccharides from marine algae are reported to have effects on innate immunity by modulating the ability of immune cells to produce nitric oxide and thereby reducing inflammation (Leiro, Castro, Arranz, & Lamas, 2007). The two important biomolecules from marine algae, fucoidan and arabinogalactan are reported to have immunomodulating effects. As fucoidan can influence the activation and maturation of human monocyte-derived dendritic cells, it can be used for cancer immunotherapy (Yang et al., 2008). Fucoxanthin, another marine bioactive compound, has shown anti-inflammatory activities both in vitro and in vivo assays. Because of its strong anti-inflammatory properties, it can be comparable with predinisolone, a commercially available steroidal anti-inflammatory drug (Shiratori et al. 2005). Apart from this, certain phlorotannins isolated from marine algae such as phlorofucofuroeckol A, eckol, 8, 80-bieckol and dieckol are also reported to have strong anti-inflammatory effects.

Anti-tumor effects

Marine algae are reported to possess an extensive range of bioactive compounds which can be used to cure various types of cancers (Frestedt, Kuskowski, & Zenk, 2009). Many of these compounds have been found to destroy tumor cells by initiating apoptosis or activating signalling enzymes that affect cell metabolism and eventually lead to cell death (Sithrangaboopathy & Kathiresan, 2010), however the clinical trials are limited due to the risk factors. The extracts of Nostocmuscorum and Oscillatoria spp. have shown anti-tumor activity in vitro due to their inhibitory effect on the human hepatocellular cancer cell line (HepG2) and Ehrlich's Ascites Carcinoma Cells (EACC) (Tripathi, Fang, Leong, & Tan, 2012). Microcolin-A, extracted from Lyngbya majuscule, is a linear peptide and can be used as immunosuppressant (Koehn, Longley, & Reed, 1992). Curacin- A, peptide isolated from L. majuscule have shown anti-proliferative properties in various tumor cell lines like renal, colon and breast (Carte, 1996). Borophycin, a boron-containing metabolite purified from marine cyanobacterial strains of *N. spongiaeforme* and *Nostoc linckia* shown cytotoxic effects against human colorectal cells (Banker & Carmeli, 1999). Certain species of algae can inter-convert fatty acids to complex eicosanoids that can play a significant role in curing ailments like cancer, heart disease, asthma, psoriasis, arteriosclerosis and ulcers (Carte, 1996).

Anti-hypertensive effects and cardio-protection properties

Chlorellais is reported to lower the blood pressure by regulating the renin-angiotensin-aldosterone system in hypertensive rat model (Ko et al., 2012). Certain other polysaccharides

from marine algae such as fucoidans could be used for their cardioprotective activity (Mayakrishnan et al., 2013).

Anti-allergic effects

The seaweed extracts are proven to have anti-allergic effects. Sugiura et al. (2007) have reported that dried powder of the brown seaweed exhibits a strong anti-allergic effect on Brown Norway (BN) rats, an allergic model animal. Further, research in this line is required to isolate and characterize the bio-active compounds from seaweeds having anti-allergic effects.

Antimicrobial activities

Bio-active molecules such as phlorotannins, terpenes, and lipophilic compound extracted from seaweeds depict anti-oxidant and antimicrobial activity against gram positive and negative bacteria. Hence, they find their applications in the field of biomedicals as a natural antimicrobial agent.

Angiotensin-converting enzyme (ACE-I) inhibitors

High blood pressure plays a major role in the development of cardiovascular disease and it can be controlled by using pharmaceuticals and derivatives of synthetic drugs which inhibit Angiotensin-converting enzyme (ACE-I)**.** Diet and dietary components including peptides could play a vital role in the control and prevention of High blood pressure, CVD and other diseases without any side effects (Tiwari et al., 2015). Researchers have reported that peptides from seaweed having ACE-I inhibitory activity could possibly reduce high blood pressure.

Tyrosinase Inhibition

Phlorotannins which are the products of secondary metabolism consist of polymers of phloroglucinol. Novel application of seaweed phlorotannis is its use as an antityrosinase in cosmetic products. Tyrosinase is an enzyme with diverse physiological roles related to melanin production in human skin. In the recent past tyrosinase inhibitors has gained much attention in cosmetic sector as whitening agents. Extraction of phlorotannin from the brown seaweed *Ecklonia stolonifera* Okamura (*Laminariaceae*) found to have tyrosinase inhibitors activity (Kang et al., 2004). In addition, fucoxanthin extracted and isolated from *L. japonica* has also been reported to suppress melanogenesis in UVB-irradiated mice (Thomas and Kim, 2013). As per the scientific findings, phlorotannins and other bio-active compounds from seaweed can be used in the cosmetic sector.

Conclusions

The diversity of bioactive compounds in seaweeds which are capable of exerting bioactivities made it as a promising candidate in the development of nutraceuticals and functional foods. Though there are reports available about the bioactive compounds and its bioactivities, lot of efforts and researches still have to be carried out for the complete utilization of seaweeds. Not only the isolation of bioactive compounds will be sufficient, the structural elucidation of the

molecules along with the possible mechanisms of action also has to go hand –in-hand for developing nutraceuticals and health care compounds. Apart from all the scientific aspects, another important lacuna associated with the seaweed research is the considerably low consumer awareness about health benefits of seaweeds. Hence, these all issues need to be properly addressed before this resource can be trapped for its benefits at a large scale.

References

Aneiros, A. and Garateix, A., 2004. Bioactive peptides from marine sources: pharmacological properties and isolation procedures. Journal of Chromatography B, 803(1), pp.41-53.

Banker, R. and Carmeli, S., 1999. Inhibitors of serine proteases from a waterbloom of the cyanobacterium Microcystis sp. Tetrahedron, 55(35), pp.10835-10844.

Besada, V., Andrade, J.M., Schultze, F. and González, J.J., 2009. Heavy metals in edible seaweeds commercialised for human consumption. Journal of Marine Systems, 75(1), pp.305-313.

Bourgougnon, N. and Stiger-Pouvreau, V., 2011. Chemodiversity and bioactivity within red and brown macroalgae along the French coasts, metropole and overseas departements and territories. Handbook of Marine Macroalgae: Biotechnology and Applied Phycology, pp.58-105.

Carte, B.K., 1996. Biomedical potential of marine natural products. Bioscience, 46(4), pp.271-286.

Chevolot, L., Mulloy, B., Ratiskol, J., Foucault, A. and Colliec-Jouault, S., 2001. A disaccharide repeat unit is the major structure in fucoidans from two species of brown algae. Carbohydrate Research, 330(4), pp.529-535.

Cruz-Suárez, L.E., León, A., Peña-Rodríguez, A., Rodríguez-Peña, G., Moll, B. and Ricque-Marie, D., 2010. Shrimp/Ulva co-culture: a sustainable alternative to diminish the need for artificial feed and improve shrimp quality. Aquaculture, 301(1), pp.64-68.

Fan, L., Jiang, L., Xu, Y., Zhou, Y., Shen, Y., Xie, W., Long, Z. and Zhou, J., 2011. Synthesis and anticoagulant activity of sodium alginate sulfates. Carbohydrate polymers, 83(4), pp.1797-1803.

Ferraces-Casais, P., Lage-Yusty, M.A., de Quirós, A.R.B. and López-Hernández, J., 2012. Evaluation of bioactive compounds in fresh edible seaweeds. Food Analytical Methods, 5(4), pp.828-834.

Frestedt, J.L., Kuskowski, M.A. and Zenk, J.L., 2009. A natural seaweed derived mineral supplement (Aquamin F) for knee osteoarthritis: a randomised, placebo controlled pilot study. Nutrition Journal, 8(1), p.7.

Gupta, S. and Abu-Ghannam, N., 2011. Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. Innovative Food Science & Emerging Technologies, 12(4), pp.600-609.

Holdt, S.L. and Kraan, S., 2011. Bioactive compounds in seaweed: functional food applications and legislation. Journal of Applied Phycology, 23(3), pp.543-597.
Holdt, S.L. and Kraan, S., 2011. Bioactive compounds in seaweed: functional food applications and legislation. Journal of Applied Phycology, 23(3), pp.543-597.
Kamenarska, Z., Gasic, M.J., Zlatovic, M., Rasovic, A., Sladic, D., Kljajic, Z., Stefanov, K., Seizova, K., Najdenski, H., Kujumgiev, A. and Tsvetkova, I., 2002. Chemical composition of the brown alga Padina pavonia (L.) Gaill. from the Adriatic Sea. Botanica marina, 45(4), pp.339-345.

Kang, H.S., Kim, H.R., Byun, D.S., Son, B.W., Nam, T.J. and Choi, J.S., 2004. Tyrosinase inhibitors isolated from the edible brown algaEcklonia stolonifera. Archives of pharmacal research, 27(12), p.1226.

Kang, K.A., Lee, K.H., Chae, S., Zhang, R., Jung, M.S., Ham, Y.M., Baik, J.S., Lee, N.H. and Hyun, J.W., 2006. Cytoprotective effect of phloroglucinol on oxidative stress induced cell damage via catalase activation. Journal of cellular biochemistry, 97(3), pp.609-620.

Kim, M.M., Van Ta, Q., Mendis, E., Rajapakse, N., Jung, W.K., Byun, H.G., Jeon, Y.J. and Kim, S.K., 2006. Phlorotannins in Ecklonia cava extract inhibit matrix metalloproteinase activity. Life Sciences, 79(15), pp.1436-1443.

Kim, S.K. ed., 2011. Handbook of marine macroalgae: biotechnology and applied phycology. John Wiley & Sons.

Kladi, M., Vagias, C., Stavri, M., Rahman, M.M., Gibbons, S. and Roussis, V., 2008. C 15 acetogenins with antistaphylococcal activity from the red alga Laurencia glandulifera. Phytochemistry Letters, 1(1), pp.31-36.

Koehn, F.E., Longley, R.E. and Reed, J.K., 1992. Microcolins A and B, new immunosuppressive peptides from the blue-green alga Lyngbya majuscula. Journal of natural products, 55(5), pp.613-619.

Lee, J.B., Takeshita, A., Hayashi, K. and Hayashi, T., 2011. Structures and antiviral activities of polysaccharides from Sargassum trichophyllum. Carbohydrate polymers, 86(2), pp.995-999.

Lee, J.C., Hou, M.F., Huang, H.W., Chang, F.R., Yeh, C.C., Tang, J.Y. and Chang, H.W., 2013. Marine algal natural products with anti-oxidative, anti-inflammatory, and anti-cancer properties. Cancer Cell International, 13(1), p.55.

Leiro, J.M., Castro, R., Arranz, J.A. and Lamas, J., 2007. Immunomodulating activities of acidic sulphated polysaccharides obtained from the seaweed Ulva rigida C. Agardh. International Immunopharmacology, 7(7), pp.879-888.

Li, K., Li, X.M., Ji, N.Y. and Wang, B.G., 2007. Natural bromophenols from the marine red alga Polysiphonia urceolata (Rhodomelaceae): Structural elucidation and DPPH radical-scavenging activity. Bioorganic & medicinal chemistry, 15(21), pp.6627-6631.

Lopes, G., Sousa, C., Bernardo, J., Andrade, P.B., Valentão, P., Ferreres, F. and Mouga, T., 2011. Sterol profiles in 18 macroalgae of the portuguese coast1. Journal of phycology, 47(5), pp.1210-1218.

MacArtain, P., Gill, C.I., Brooks, M., Campbell, R. and Rowland, I.R., 2007. Nutritional value of edible seaweeds. Nutrition reviews, 65(12), pp.535-543.

Maeda, H., Tsukui, T., Sashima, T. and Miyashita, K., 2008. Seaweed carotenoid, fucoxanthin, as a multi-functional nutrient. Asia Pacific Journal of Clinical Nutrition, 17(S1), pp.196-199.

Matloub, A.A. and Awad, N.E., 2009. Chemical composition of some Sargassum species and their cytotoxic and antimicrobial activities. Planta Medica, 75(09), p.PE27.

Mayakrishnan, V., Kannappan, P., Abdullah, N. and Ahmed, A.B.A., 2013. Cardioprotective activity of polysaccharides derived from marine algae: An overview. Trends in food science & technology, 30(2), pp.98-104.

McDermid, K.J. and Stuercke, B., 2003. Nutritional composition of edible Hawaiian seaweeds. Journal of Applied Phycology, 15(6), pp.513-524.

Mourão, P.A., 2004. Use of sulfated fucans as anticoagulant and antithrombotic agents: future perspectives. Current pharmaceutical design, 10(9), pp.967-981.

Nguemfo, E.L., Dimo, T., Azebaze, A.G.B., Asongalem, E.A., Alaoui, K., Dongmo, A.B., Cherrah, Y. and Kamtchouing, P., 2007. Anti-inflammatory and anti-nociceptive activities of the stem bark extracts from Allanblackia monticola STANER LC (Guttiferae). Journal of ethnopharmacology, 114(3), pp.417-424.

Norziah, M.H. and Ching, C.Y., 2000. Nutritional composition of edible seaweed Gracilaria changgi. Food chemistry, 68(1), pp.69-76.

Nylund, G.M., Persson, F., Lindegarth, M., Cervin, G., Hermansson, M. and Pavia, H., 2010. The red alga Bonnemaisonia asparagoides regulates epiphytic bacterial abundance and community composition by chemical defence. FEMS Microbiology Ecology, 71(1), pp.84-93.

Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernández, J., Bozzo, C., Navarrete, E., Osorio, A. and Rios, A., 2006. Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds Ulva lactuca and Durvillaea antarctica. Food chemistry, 99(1), pp.98-104.

Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernández, J., Bozzo, C., Navarrete, E., Osorio, A. and Rios, A., 2006. Dietary fiber, amino acid, fatty acid and tocopherol

contents of the edible seaweeds Ulva lactuca and Durvillaea antarctica. Food chemistry, 99(1), pp.98-104.

Patarra, R.F., Paiva, L., Neto, A.I., Lima, E. and Baptista, J., 2011. Nutritional value of selected macroalgae. Journal of Applied Phycology, 23(2), pp.205-208.

Peña-Rodríguez, A., Mawhinney, T.P., Ricque-Marie, D. and Cruz-Suárez, L.E., 2011. Chemical composition of cultivated seaweed Ulva clathrata (Roth) C. Agardh. Food chemistry, 129(2), pp.491-498.

Shibata, T., Ishimaru, K., Kawaguchi, S., Yoshikawa, H. and Hama, Y., 2008. Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. Journal of Applied Phycology, 20(5), p.705.

Shiratori, K., Ohgami, K., Ilieva, I., Jin, X.H., Koyama, Y., Miyashita, K., Yoshida, K., Kase, S. and Ohno, S., 2005. Effects of fucoxanthin on lipopolysaccharide-induced inflammation in vitro and in vivo. Experimental eye research, 81(4), pp.422-428.

Sithranga Boopathy, N. and Kathiresan, K., 2011. Anticancer drugs from marine flora: an overview. Journal of oncology, 2010.

Sugiura, Y., Matsuda, K., Yamada, Y., Nishikawa, M., Shioya, K., Katsuzaki, H. and AMANO, H., 2007. Anti-allergic phlorotannins from the edible brown alga, Eisenia arborea. Food Science and Technology Research, 13(1), pp.54-60.

Teas, J., Pino, S., Critchley, A. and Braverman, L.E., 2004. Variability of iodine content in common commercially available edible seaweeds. Thyroid, 14(10), pp.836-841.

Vairappan, C.S., Anangdan, S.P., Tan, K.L. and Matsunaga, S., 2010. Role of secondary metabolites as defense chemicals against ice-ice disease bacteria in biofouler at carrageenophyte farms. Journal of applied phycology, 22(3), pp.305-311.

Villares, R., Puente, X. and Carballeira, A., 2002. Seasonal variation and background levels of heavy metals in two green seaweeds. Environmental Pollution, 119(1), pp.79-90.

Xu, N., Fan, X., Yan, X. and Tseng, C.K., 2004. Screening marine algae from China for their antitumor activities. Journal of Applied Phycology, 16(6), pp.451-456.

Yang, M., Ma, C., Sun, J., Shao, Q., Gao, W., Zhang, Y., Li, Z., Xie, Q., Dong, Z. and Qu, X., 2008. Fucoidan stimulation induces a functional maturation of human monocyte-derived dendritic cells. International immunopharmacology, 8(13), pp.1754-1760.

****

25

SUPERCRITICAL EXTRACTION AND ITS APPLICATION IN ISOLATION AND CHARACTERISATION OF MARINE BIOACTIVE MOLECULES FOR HUMAN HEALTH

K.K. Anas., Lekshmi R.G.Kumar., C.S. Tejpal., R. Anandan., Suseela Mathew.

Biochemistry and Nutrition division

Email ID: ***anaskk40@gmail.com***

By definition, extraction is the removal of soluble material from an insoluble residue, either liquid or solid after treating with a solvent. Rate of diffusion of the solute through the liquid boundary layer at the interface is the controlling factor in the process. The extract obtained by conventional procedures is more over impure liquids, powders intended only for external use. So high quality extraction procedures are of considerable interest to obtain improved yields of drug derived from plant as well as animal sources. However currently available conventional extraction methods are time consuming, requiring different solvents which are costly, often needing concentration step to improve yield. Limited selectivity and degradation of thermally labile compounds are also associated disadvantages. The solvents used in the extraction and the waste generated as a result is also creating environmental hazards. An ideal extraction method should be swift, environmentally safe, yield quantitative recovery without degradation, and the extracts should be easily separated from the solvent. Henceforth, it is the need of the hour to replace conventional extraction methods with alternative green technology with improved extraction efficiency and low environmental impact. Supercritical fluid extraction (SFE) technology offers many features that overcome many limitations of conventional extraction methods. Hence laboratories engaged in innovative research are developing SFE methods to replace conventional methodologies for routine analyses utilizing the high solvent power of supercritical fluids (SFs).

Supercritical Fluid

Matter exists in three most common phases which are solid, liquid, and gas. The phase of a pure simple substance depends on the temperature and pressure. Phase diagram shows a substance's phase at a given temperature and pressure as well as show the temperatures and pressures at which any two phases can coexist in equilibrium (Fig.1). The critical point refers to the temperature and pressure at which above which the substance can no longer be condensed into a liquid. Beyond the critical point, there is no longer an equilibrium curve to

divide the liquid and gaseous regions; thus, the liquid and gas phases are no longer distinguishable. This region of the phase diagram is called the supercritical fluid region.

A supercritical fluid can be defined as a form of matter in which the liquid and gaseous phases are indistinguishable. Supercritical fluids are having more densities comparable to liquids. As a result, these fluids have solvating power. Supercritical fluid exhibits physicochemical properties intermediate between those of liquids and gases. Both liquid-like and gas-like characteristics of supercritical fluids make them unique for chemical separation. In particular, supercritical fluid densities, diffusivities, and viscosities fall into ranges between those of liquids and gases. Properties of supercritical fluid are given below:

(i) Supercritical fluids behave like gases and liquids in an interesting manner.

(ii) Supercritical fluids can lead to reactions, which are difficult to achieve in conventional solvents.

(iii) For most of the solutes, supercritical fluids have solvent power similar to light hydrocarbons.

(iv) Solubility of SFs increases with increasing density.

(v) The SFs are commonly miscible with permanent gases (e.g. N_2 or H_2) and this leads too much higher concentrations of dissolved gases than can be achieved in conventional solvents.

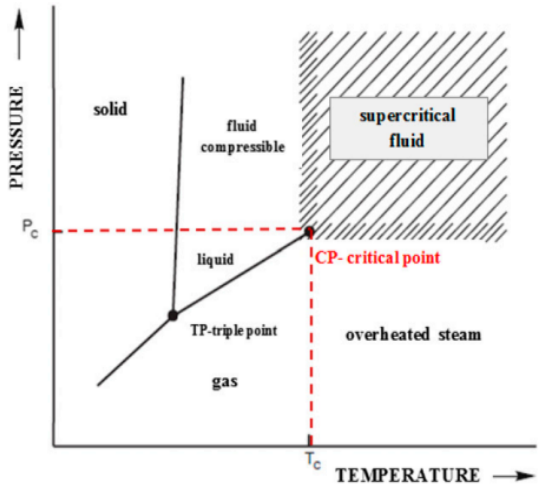


Figure 1: CO_2 Pressure - Temperature Phase diagram

Available Supercritical Fluids

The most popular SFE solvent is carbon dioxide. It is inexpensive, non-flammable, relatively nontoxic, low critical temperature and commercially available even at high purity. The SFE solvent supercritical CO_2 have extraction conditions above the critical temperature of 31°C and critical pressure of 74 bar. Supercritical CO_2 is having density of around 200 bar pressure is close to that of hexane. The solvation characteristics are also similar to hexane since it acts as a non-polar solvent. Around the supercritical region, CO_2 can dissolve triglycerides at concentrations up to 1% mass. Other SFE solvents used are nitrous oxide (laughing gas),

nitrogen, propane, ammonia, fluoroform, freons, and water. But in all the cases the number of disadvantages outweighs the advantages. Carbon dioxide does have a few disadvantages even though it is practically the only solvent for SFE.CO_2 has limited solvating power and expensive instrumentation is required to maintain high critical pressure. Critical properties of various solvents used in SFE are given below:

Critical properties of various solvents (Reid et al., 1987)

Solvent	Molecular weight (g/mol)	Critical temperature (K)	Critical pressure MPa (atm)	Critical density (g/cm^3)
Carbon dioxide (CO_2)	44.01	304.1	7.38 (72.8)	0.469
Water (H_2O) (acc. IAPWS)	18.015	647.096	22.064 (217.755)	0.322
Methane (CH_4)	16.04	190.4	4.60 (45.4)	0.162
Ethane (C_2H_6)	30.07	305.3	4.87 (48.1)	0.203
Propane (C_3H_8)	44.09	369.8	4.25 (41.9)	0.217
Ethylene (C_2H_4)	28.05	282.4	5.04 (49.7)	0.215
Propylene (C_3H_6)	42.08	364.9	4.60 (45.4)	0.232
Methanol (CH_3OH)	32.04	512.6	8.09 (79.8)	0.272
Ethanol (C_2H_5OH)	46.07	513.9	6.14 (60.6)	0.276
Acetone (C_3H_6O)	58.08	508.1	4.70 (46.4)	0.278

Supercritical Fluid Extraction (SFE)

SFE can be defined as the process of segregating one component from the matrix by using supercritical fluids as the solvent. Extraction is usually done from a solid matrix, but also possible from liquids. SFE is useful as a sample preparation step (for analytical purposes) or to strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils). In SFE, the mobile phase is subjected to pressures and temperatures near or above the critical point for the purpose of enhancing the mobile phase solvating power. The process begins with CO_2 in vapour form. It is then compressed into a liquid before becoming supercritical. While supercritical, the extraction takes place.

Supercritical Fluid Extraction (SFE) System extracts chemical compounds using supercritical carbon dioxide instead of an organic solvent. The supercritical fluid state occurs when a fluid is above its critical temperature (Tc) and critical pressure (Pc), when it is between the typical gas and liquid state (Raventós *et al.*, 2002). Manipulating the temperature and pressure of the fluid can solubilize the material of interest and selectively extract it. The sample is placed in an extraction vessel and pressurized with CO_2 to dissolve the sample. Transferred to a fraction collector, the contents are depressurized and the CO_2 loses its

solvating power causing the desired material to precipitate. The condensed CO_2 can be recycled.

In SFE, the applications of supercritical carbon dioxide was having biggest interest, because it has a near ambient critical temperature (31^0C), thus biological materials can be processed at temperatures around 35^0C. The advantage here is that with a slight reduction in temperature or a slightly larger reduction in pressure can lead to precipitation of the entire solute. In addition, supercritical fluids can extract a product with minimal solvent residues. Utilization of SFE technology in decaffeinated coffee, cholesterol-free butter, low-fat meat, evening primrose oil, squalene from shark liver oil and many more. The solvation characteristics of supercritical CO_2 can be modified by the addition of an entrainer like ethanol (Doane-Weideman and Lieschesk., 2004).

Supercritical Fluid Extraction- Instrumentation

The instrumentation required to perform a successful SFE is commercially available. The process begins with a clean source of fluid, which in most cases is a high-pressure cylinder of CO_2. A pump is used to increase the pressure of the fluid above its critical pressure. The working extraction pressure is determined by the density required to dissolve the target analytes from the sample. The sample is contained in the extraction chamber, which is heated to the desired extraction temperature above the critical point. The pressurized fluid is brought to temperature by the chamber and allowed to flow through the sample matrix to extract the analytes. After the sample, the analyte fluid flows to a restrictor, this controls the flow rate of the fluid. The restrictor maintains the high pressure of the fluid in the chamber. At the restrictor, the supercritical fluid loses its solvating strength as its pressure drops to atmosphere. After the restrictor, the analytes can be collected for analysis (Sapkale *et al.*, 2010). Figure 2 shows a block diagram of a complete SFE system.

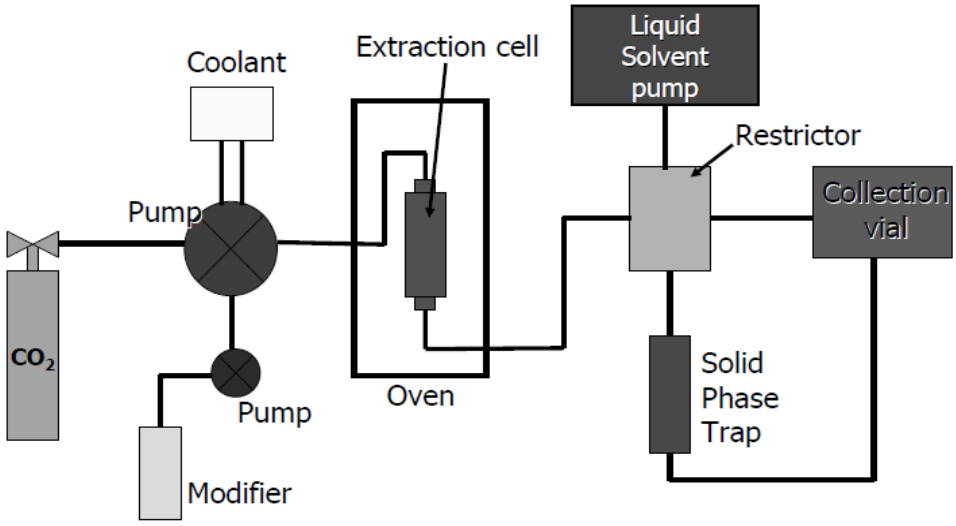


Fig.2: Supercritical fluid extraction apparatus

Advantages of SFE

- Environmental safety: SFE is a substitute to liquid extraction which uses organic solvents such as hexane or dichloromethane. There is always chance of solvent residue in the extract and matrix and there is always some level of environmental contamination from their use. Whereas carbon dioxide is easy to remove simply by reducing the pressure, leaving almost no trace, and it is also environmentally benign. The use of SFE with CO_2 is also approved by the Soil Association for organic products. The CO_2 used is largely a by-product of industrial processes or brewing, and its use in SFE does not cause any extra emissions.
- Selectivity: By changing the pressure and the temperature, the solvent strength of a supercritical fluid can be altered. For example, volatile oils can be extracted from a plant with low pressures (100 bar), whereas liquid extraction would also remove lipids. By SFE, lipids can be removed using pure CO_2 at higher pressures, and then phospholipids can be removed by adding ethanol to the solvent.
- Speed: It is a fast process and completed in 10 to 60 minutes. It is a diffusion-based process, with the solvent required to diffuse into the matrix, and the extracted material to diffuse out of the matrix into the solvent.
- Purity: A supercritical fluid can be separated from an analyte by releasing pressure so that the product will be almost pure.
- Recovery: Recovery of analytes is simpler as compared to conventional techniques.
- Supercritical fluids are cheap, inert and nontoxic. Thus, they are readily disposed off after an extraction is completed by allowing them to evaporate into the atmosphere.

Efficiency in sample preparation

Because SFE has several distinct physical properties, it is regarded as a promising alternative technique to conventional solvent extraction. Some of its major advantages are summarized as follows:

(1) Super critical Fluids have higher diffusion coefficients and lower viscosities than a liquid solvent. So, solubility and diffusivity in such fluids tends to be much higher than in liquids, resulting in comparatively fast reactions (Mira *et al.*, 1999).

(2) In Super critical Fluid extraction, the solvation power of the fluid can be controlled by changing pressure (P) or temperature (T); so, it may achieve a remarkably high selectivity. This solvation power of SFs is useful for the extraction of complex samples.

(3) In Super critical Fluid extraction, fresh fluid is continuously passes through the sample; therefore it can provide complete extraction (Stashenko *et al.*, 196).

In addition to these benefits, another advantage of SFE over conventional methods is that, it involves less duration and minimal usage of organic solvents. It was shown that SFE for 30–60 min provides higher recoveries than several hours of Soxhlet extraction (Reindl *et al.*, 1994).

Applications:

SFE applications in the food, pharmaceutical, and fine chemical industries:

- Decaffeinating of coffee and tea
- Extraction of essential oils (vegetable and fish oils)
- Extraction of flavors from natural resources (nutraceuticals)
- Extraction of ingredients from spices and red peppers
- Extraction of fat from food products
- Fractionation of polymeric materials
- Extraction from natural products
- Photo–resist cleaning
- Precision part cleaning

Supercritical fluid extraction of bioactive compounds

The pursuit for bioactive compounds from natural sources has been driven by scientific research of the targeted molecules against a wide range of diseases and also can use as natural food additives. Many compounds extracted from natural sources have been shown to possess several bioactive applications, such as antimicrobial, antibacterial, antifungal, antiviral, anti-inflammatory, antitumor, anti-obesity, phagocytotic, insecticide, and antioxidant functions. Supercritical fluid extraction (SFE) provides attractive features overcoming most of the limitations of conventional extraction of bioactive compounds.

Since carbon dioxide is a gas at room temperature, when the extraction is completed and the system decompressed, the elimination of CO_2 is achieved, yielding a solvent-free extract. On an industrial scale, when carbon dioxide consumption is high, the operation can be controlled to recycle it. However, because of its low polarity, CO_2 is less effective in extracting more polar compounds from natural matrices, and modifiers (also called cosolvents) are commonly used in order to overcome this problem (Barbosa *et al.*, 2014). These are polar compounds that, added in small amounts, can induce substantial changes of the solvent properties of pure supercritical CO_2 (M. Herrero *et al.*, 2006). Supercritical extraction basically occurs in two steps: the solubilization of the chemical compounds present on the solid matrix and its separation into the supercritical solvent. During the extraction, the solvent flows through the packed bed, solubilizing the existing compounds present in the matrix. Afterwards the solvent exits the extractor carrying the solubilized compounds, and by pressure reduction and/or temperature increase, the extract becomes solvent free.

The bioactivities from natural compounds obtained by SFE were mainly antioxidant (41%), antitumor (18%) and antibacterial activity (10%), followed by antiviral, antimicrobial, anti-inflammatory and anticholinesterase (in a total of 5%). In order to achieve a higher specific yield or higher bioactivity capabilities, 43% of the work applied an extraction temperature range of 40 to 50 °C, followed by 33% at 50 to 60 °C, while the pressure trend was 37% for a

pressure range of 200 to 300 bar followed by 28% at 300 to 400 bar. The proportion and the type of modifier also have an important role on the extraction, allowing the manipulation of the solubility of the target compounds in the supercritical fluid extraction. Consequently, 47% of the reviewed works applied modifiers in order to enhance the extraction of their targeted bioactive compounds, where ethanol was the modifier of choice with a proportion that could vary between 5% and 30% (Da Silva *et al.*, 2016).

Conclusions and future trends

Supercritical fluid extraction technology can offer attractive features for obtaining bioactive compounds and overcome many limitations that exist in other extraction methodologies. SFE allows the control of fluid density by changing its pressure and/or temperature thus providing faster extraction rates. Accordingly, it is expected that the integration of single and combined technologies will lead to higher extraction yields and greater selectivity of such bioactive compounds with significant interest to the pharmaceutical industry. SFE can be regarded as a more sustainable, cleaner and environmental friendly extraction process in the research of bioactive compounds, while providing tools and technology output for future laboratorial and industrial development. Smart, systematic development of SFE can be expected to consolidate it into an advantageous alternative to conventional solid-liquid extraction, so that its real, great potential can be fully realized. Supercritical fluid extraction cannot be considered as a fully mature technology. Knowledge of the chemical properties of both the analyte and the matrix is important for SFE. In addition, one must ensure that mechanics of SFE have been optimized.

References

Da Silva, R.P., Rocha-Santos, T.A. and Duarte, A.C., 2016. Supercritical fluid extraction of bioactive compounds. TrAC Trends in Analytical Chemistry, 76, pp.40-51.

Barbosa, H.M., de Melo, M.M., Coimbra, M.A., Passos, C.P. and Silva, C.M., 2014. Optimization of the supercritical fluid coextraction of oil and diterpenes from spent coffee grounds using experimental design and response surface methodology. The Journal of Supercritical Fluids, 85, pp.165-172.

Herrero, M., Cifuentes, A. and Ibañez, E., 2006. Sub-and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. Food chemistry, 98(1), pp.136-148.

Mira, B., Blasco, M., Berna, A. and Subirats, S., 1999. Supercritical CO 2 extraction of essential oil from orange peel. Effect of operation conditions on the extract composition. The Journal of supercritical fluids, 14(2), pp.95-104.

Reindl, S. and Hoefler, F., 1994. Optimization of the parameters in supercritical fluid extraction of polynuclear aromatic hydrocarbons from soil samples. Analytical Chemistry, 66(11), pp.1808-1816.

Stashenko, E.E., Puertas, M.A. and Combariza, M.Y., 1996. Volatile secondary metabolites from Spilanthes americana obtained by simultaneous steam distillation-solvent extraction and supercritical fluid extraction. Journal of Chromatography A, 752(1-2), pp.223-232.

Sapkale, G.N., Patil, S.M., Surwase, U.S. and Bhatbhage, P.K., 2010. Supercritical Fluid Extraction: A Review. International Journal of Chemical Sciences, 8(2).

Raventós, M., Duarte, S. and Alarcón, R., 2002. Application and possibilities of supercritical CO_2 extraction in food processing industry: an overview. Revista de Agaroquimica y Tecnologia de Alimentos, 8(5), pp.269-284.

Doane-Weideman, T. and Liescheski, P.B., 2004. Analytical supercritical fluid extraction for food applications. Oil Extraction and Analysis: Critical Issues and Competitive Studies, p.69.

****

26

SEAWEEDS CHROMATOGRAPHY- PRINCIPLES AND APPLICATION IN NUTRIENT PROFILING

Renuka. V
Biochemistry and Nutrition division
Email ID: ***renucift@gmail.com***

Chromatography is the technique used for the separation of the components in a sample mixture. It is the process of analyzing the different components present in a mixture qualitatively and quantitatively by separating them from each other. Chromatography was introduced by Mikhail Tswett in 1906. The name chromatography was derived from the terms "Chroma" which means colour; and graphein means written, as this technique was initially used for the separation of coloured compounds. Later, this technique became applicable even to colourless compounds. Chromatography can separate large as well as small amounts of compounds.

Principles

Chromatography is based on the principle where molecules in a mixture are applied onto the surface or into the solid, and the fluid stationary phase (stable phase) separates from each other while moving with the aid of a mobile phase. Chromatography allows the separation of components of a mixture on the basis of their nature, structure, size, and other properties. The essential components of chromatography are stationary phase and mobile phase.

Stationary phase

The majority of materials used as stationary phases include pores, which enable components to adhere during chromatography. Depending on the type of chromatography being used and the characteristics of the components that need to be separated, a stationary phase must be chosen. The stationary phase can be made of gel beads, thin uniform paper, silica, glass, certain gases, or even liquid components, depending on the type of chromatography being utilised.

Mobile phase

Depending on the nature of the components to be separated and the kind of chromatography, materials employed as mobile phases are chosen for a chromatographic procedure. The mobile phase in various chromatographic procedures is frequently made up of alcohol, water, acetic acid, acetone, or certain gases. If the mobile phase is liquid it is termed liquid chromatography (LC), and if it is gas then it is called gas chromatography (GC). Gas

chromatography is applied for gases, mixtures of volatile liquids, and solid materials. Liquid chromatography is used especially for thermal unstable, and non-volatile samples.

Term	Definition
Mobile Phase or carrier	Solvent moving through the column
Stationary Phase or absorbent	Substance that stays fixed inside the column
Eluent	Fluid entering the column
Eluate	Fluid exiting the column (that is collecting in flasks)
Elution	The process of washing out a compound through a column using a suitable solvent
Analyte	A mixture whose individual components have to be separated and analyzed

Separation mechanism

The separation of compounds is achieved by the differential partition between the stationary phase and the mobile phase. During differential partition, the compounds distribute or partition between the two phases, depending on their relative affinity to the phase. Relative affinity depends on their molecular structure and weak intermolecular forces such as hydrogen bonding or van der waals forces. If the affinity of the compounds is greater for the stationary phase, slower will be movement and vice-versa. This migration results in the separation of the compounds.

The time taken for the distribution of the compounds between the phases much be rapid compared to the velocity of mobile phase. The stationary phase, which is an immobile matrix contains sites, in which, the compounds passed along the mobile phase can bind. If the compounds interact or bind to the solid matrix, their movement through the stationary phase is retarded. This is called “impedance”.

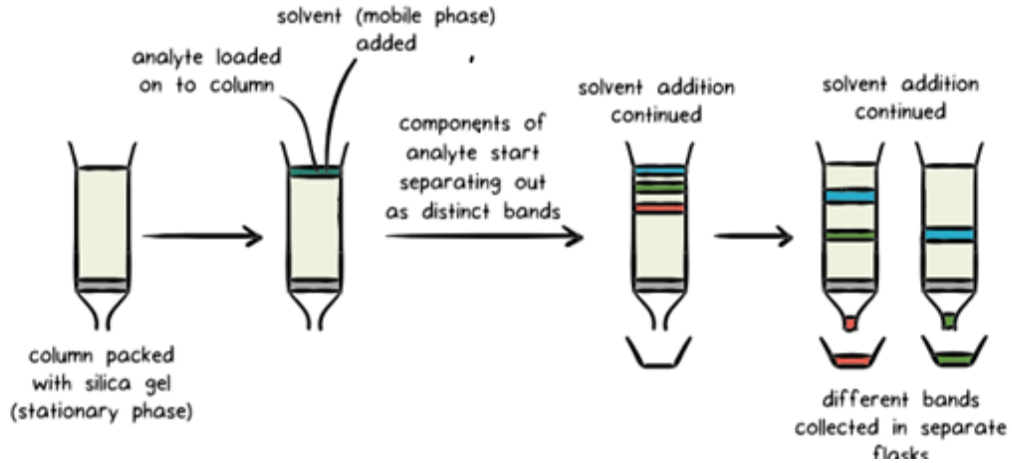
Factors influencing separation

There are two major factors that affect the resolution of the compound ina chromatographic separation. They are

- Distribution coefficient
- Sharpness of compound band

The distribution coefficient or partition coefficient (K_d) is defined as the ratio of the concentration of the compound in the mobile phase (c_m) to the concentration of the compound in the stationary phase (c_s). The K_d of the compound depends on its molecular structure, the nature of two phases, and temperature of the chromatographic column or system.

Separation mechanism of compounds



$K_d = c_m / c_s$

The sharpness of the compound band depends on the number of equilibrations. The number of equilibrations is also termed as “Theoretical plates”. If the number of theoretical plates is more, the compound band will be sharp and the column will be more efficient for the separation.

Types of chromatography

The chromatography techniques are broadly classified into adsorption chromatography and partition chromatography.

Adsorption chromatography

Adsorption chromatography or liquid-solid chromatography, first discovered by Tswett in 1903, is probably the oldest mode of chromatography. The adsorption chromatography is used for solid-gas chromatography and solid-liquid chromatography. Adsorption Chromatography involves the separation of a chemical mixture based on the interaction of the adsorbate with the adsorbent. In this process, the mixture of gas or liquid gets separated on the adsorbent bed which adsorbs different compounds at different rates. Column chromatography is an example of adsorption chromatography. Methods for vitamin K analysis in seafood use a combination of adsorption chromatography for sample clean-up, and reversed-phase LC on C-18 supports for the quantification.

Factors affecting the adsorption chromatography

- Choice of the adsorbent
- Selection of the solvent for the mixture

- The rate of flow of the solvent
- The temperature of the system
- The column height for the procedure.

Types of Adsorption Chromatography

There are four types of adsorption chromatography.

1. Thin Layer Chromatography
2. Solid Liquid Chromatography
3. Gas-Liquid Chromatography
4. Column Chromatography

Partition chromatography

Partition chromatography was introduced by Martin and Synge in 1941 for the separation of acetylated amino acids and was first applied to the separation of alkaloids by Evans and Partridge in 1948. The stationary phase in partition chromatography is a liquid or semi-liquid immobilized on the surface of a solid support with the help of a substance (polymer) to form a thin film. A filter paper or a column can act as a solid support. The mobile phase is usually "liquid". The separation is achieved based on the partition of the compound between two solvents i.e. stationary and mobile phases. E.g. Paper chromatography, high-pressure liquid chromatography, and gas chromatography.

Types of chromatographs used in nutrient profiling

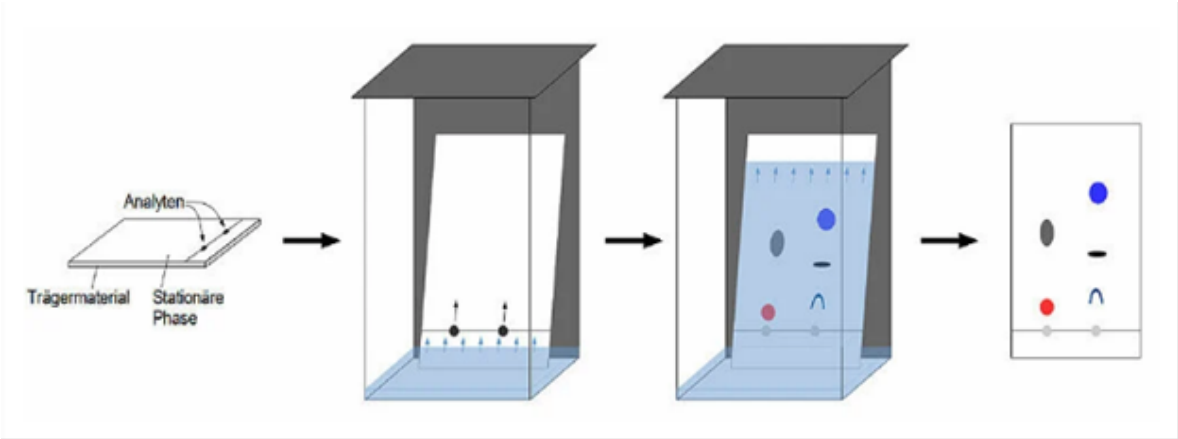
Chromatography can be classified based on mobile phase, stationary phase, forces of separation, or method of separation. There are several types of chromatography techniques, each with its own principles and applications. Chromatography techniques are widely used in seafood analysis to separate, identify, and quantify various compounds such as contaminants, flavor compounds, and nutritional components. These techniques are crucial for ensuring the nutrients, safety, quality, and authenticity of seafood products. Here are some of the most common types of chromatography used in nutrient profiling.

Preliminary screening and qualitative analysis of seafood extracts

Thin-layer chromatography (TLC)

Thin-layer chromatography is a separation technique where the stationary phase is applied as a thin layer on a solid support plate with a liquid mobile phase. This chromatography technique is based on the principle that components of a mixture are separated when the component having an affinity towards the stationary phase binds to the stationary phase. In contrast, other components are eluted with the mobile phase. The substrate/ ligand is bound to the stationary phase so that the reactive sites for the binding of components are exposed. Now, the mixture is passed through the mobile phase where the components with binding sites for the substrate bind to the substrate on the stationary phase while the rest of the components are eluted out with the mobile phase. After separation, the molecules are seen as spots at different location

throughout the stationary phase. The detection of molecules is performed by various techniques. TLC is a simple and cost-effective technique for separating and identifying compounds in seafood extracts. It is often used in preliminary screening and qualitative analysis.



Thin layer chromatography

Amino acid and vitamin analysis

High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography is a modified form of column chromatography where the components of a mixture are separated on the basis of their affinity with the stationary phase. This technique is based on the principle of differential adsorption where different molecules in a mixture have a varying degree of interactions with the absorbent present on the stationary phase. The molecules having higher affinity remain adsorbed for a longer time decreasing their speed of movement through the column. However, the molecules with lower affinity move with a faster movement, thus allowing the molecules to be separated in different fractions. This process is slightly different from the column chromatography as in this case; the solvent is forced under high pressures of up to 400 atmospheres instead of allowing it to drip down under gravity. HPLC is one of the most commonly used chromatography techniques used in the determination of amino acid and fat-soluble vitamins. Using UV detection for amino acids in most cases requires using the absorption of the carboxyl group (-COOH) in the 200 to 210 nm range. Some amino acids with benzene rings can also be detected in the 250 to 280 nm range, but in general, they are difficult to analyze as it is sufficient sensitivity and selectivity. The most commonly used detectors in liquid chromatography are Diode-Array Detector (DAD), Fluorescence Detector (FLD), Mass spectrophotometry (MS), and evaporative light scattering detector (ELSD).

LC-MS combines liquid chromatography with mass spectrometry detection and is used for the analysis of complex mixtures in seafood, including the detection of various contaminants,

pesticides, and toxins. LC-MS is highly sensitive and can provide structural information about the compounds.

Volatile fatty acids and Volatile organic compound analysis

Gas Chromatography (GC)

Gas chromatography is a separation technique in which the molecules are separated on the basis of their retention time depending on the affinity of the molecules to the stationary phase. The sample is either liquid or gas that is vaporized in the injection point. Gas chromatography is based on the principle that components having a higher affinity to the stationary phase have a higher retention time as they take a longer time to come out of the column. However, the components having a higher affinity to the stationary phase have less retention time as they move along with the mobile phase. The mobile phase is a gas, mostly helium that carries the sample through the column. The sample once injected in converted into the vapor stage is then passed through a detector to determine the retention time. The components are collected separately as they come out of the stationary phase at different times.

Gas Chromatography-Mass Spectrometry (GC-MS): GC-MS is employed for the analysis of volatile and semi-volatile compounds in seafood, including the detection of flavor compounds, lipid oxidation products, and contaminants like PCBs, dioxins, and pesticides.

Polysaccharide and protein analysis

Size-Exclusion Chromatography (SEC)

Size-exclusion chromatography (SEC) is the conventional name for a separation method used most frequently for the fractionation and analytical characterization of macromolecules of biological or synthetic origin and less frequently for the separation of colloidal particles. Size exclusion chromatography (SEC) separates molecules based on their size by filtration through a gel. The gel consists of spherical beads containing pores of a specific size distribution. Separation occurs when molecules of different sizes are included or excluded from the pores within the matrix. The invention of small porous particles with a typical diameter between 1 and 10 μm brought about an important technological improvement in SEC. The consequent miniaturization of the columns allowed the reduction of the analysis time to minutes or even to tens of seconds. The development of this separation method is reflected by the numerous names that have been given to this process; for example, gel filtration, gel chromatography, gel filtration chromatography, gel exclusion chromatography, gel permeation chromatography, restricted diffusion chromatography, size separation chromatography, and molecular sieve chromatography. SEC is employed for the separation and quantification of macromolecules like proteins and polysaccharides in seafood products. It is useful for assessing protein quality and freshness.

High-Resolution Mass Spectrometry (HRMS)

High-resolution mass spectrometry (HRMS) is an analytical technique that is used to determine the exact molecular masses of compounds present in a sample. The highly accurate nature of HRMS makes it ideal for the identification of molecular structures, ranging from small organic molecules to large biological macromolecules. HRMS analysis begins by passing a sample into the spectrometer, where it is ionized. The formed ions will travel along the spectrometer's length, separated by their relative charges and masses. Once the ions reach the end of the spectrometer, they are picked up by a detector, and the information is logged on a computer.

Conclusion

Chromatography techniques have diverse applications in various fields, including chemistry, biochemistry, pharmaceuticals, environmental science, and food analysis, among others. These chromatography techniques, when appropriately selected and applied, enable seafood analysts to assess the safety, quality, and nutritional value of seafood products, as well as to detect and quantify contaminants and flavor compounds that are critical for consumer satisfaction and regulatory compliance.

****

27

FISH BIOMOLECULES IN FIGHTING MALNUTRITION

Lekshmi R.G.Kumar, Tejpal C.S., Anas K.K., Renuka V., Anandan R.
Biochemistry & Nutrition Division
Email ID: *lekshmirgcof@gmail.com*

Malnutrition can be a serious condition that develops when the diet doesn't contain adequate amount of vital nutrients such as vitamins, minerals, proteins, carbohydrates, lipids etc. In general, malnutrition can be of two types: (1) overnutrition wherein the body gets extra nutrition leading to symptoms like obesity (2) undernutrition wherein the body is deprived of essential nutrients. Undernutrition is often reported to have a significant impact on people's health including stunted growth, low body weight and muscle wasting than the former one. Several factors contribute to malnutrition such as health status, body function, ageing, education, health state, food security, economic and environmental condition, political situation etc. This all combinedly may contribute to the suboptimal dietary intake causing malnutrition. Reports state that there is an increased risk of malnutrition among children and the elderly. It has been shown that deficiencies of vitamin A and zinc result in deaths. Further deficiencies of microelements such as iodine and iron are reported to contribute to the delayed growth and mental development in children. It also results in deteriorating health, increased morbidity, and mortality in elderly and chronically ill patients. In this regard, several strategies and nutrition related programmes are being undertaken nationally and internationally to curb malnutrition. The important programme among this is specifically focusing on the first 1000 days of life, as stunting often begins in utero and continues for at least the first 2 years of postnatal life.

Functional foods offer a potential opportunity to fully meet food needs. Hence, development of tailor-made functional foods to address various nutritional requirements is one of the promising ways to address malnutrition. In this context, fish/marine-derived molecules can be of significant importance as they are reported to be rich in essential nutrients in adequate proportions. Further, globally there is an increase in awareness among the consumers about seafood and its health/nutritional benefits. Hence, scientists constantly explore these bioactive constituents/therapeutic potentials to improve populace health and quality of life.

Fish is considered as a good source of high quality, easily digestible protein rich in essential aminoacids. Moreover, fats and oils from fish is an excellent dietary sources of long chain highly unsaturated fatty acids of omega-3 type such as as Eicosapentaenoic acid (EPA C20:5) and Docosahexaenoic acid (DHA C22:6). Omega-3 fats are long chain polyunsaturated fats

containing methylene-separated double bonds starting from the third carbon atom counted from the methyl-terminus. These fatty acids are required by humans, but cannot be synthesized endogenously and hence considered as essential fatty acids. Therefore, the requirements for these fatty acids must be obtained from the diet. The International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends an adequate intake of omega-3 LC PUFA to be 0.65 g of DHA plus EPA per person per day (as minimum 0.22 g of each). The American Heart Association recommends adults eat fish (in particular fatty fish) at least two times per week

The two important omega-3 fatty acids -EPA and DHA play a key role in: (1) cell membrane formation, integrity, and functions; (2) functioning of brain, retina, liver, kidney, adrenal glands, and gonads; and (3) local hormone production for the regulation of blood pressure and immune and inflammatory responses. EPA and DHA play a major role in maintaining health of the young children by modulating the lipid metabolism. These ω-3 fatty acids also regulate prostaglandin metabolism, which regulates the vascular functions in growing children. They also have influence on kidney function by modulating the retention of water and removal of excess sodium, which plays a major role in the behavior of kids. DHA is critical to normal eye and vision development in the early and later parts of the human beings. Along with linoleic acid it makes > 1/3rd of FA in human brain and retina. DHA also increases memory power of young children. A person can expect good health if he or she consumes 0.5-1g of PUFA/day.

Further, fish and seafood are also reported to be rich in easily digestible proteins. The amount of protein in fish and seafood muscle is usually between 15 and 22%. The types of proteins found in fish muscle can be categorized into three groups: (1) Myofibrillar proteins (Structural protein such as actin, myosin and regulatory protein such as tropomyosin, troponin, and actin) (2) Water-soluble sarcoplasmic proteins (myoalbumin, globulin, and enzymes) (3) Stroma proteins (collagen and elastin). It is reported that fish-based protein could modulate several regulatory factors including lowering insulin resistance, leptin, and tumor necrosis factor-(TNF-) α, improving hyperglycemia, and decreasing adipose tissue oxidative stress in animal models. Further, in terms of nutrition, the human body readily digests and absorbs fish proteins, so as to deliver important biological effects. Bioactive peptides (called hydrolysates) have been isolated from different species of fish which possess important metabolic activities, from antioxidant, antimicrobial, to antihypertensive aspects.

Apart from this, fish are also an important source of vitamins and minerals, such as vitamin D, selenium, zinc, phosphorus, and calcium. They are reported to be rich source of minerals such as sodium, potassium, calcium, phosphorus and magnesium. Fish bone which is often discarded after the removal of protein is an excellent source of calcium and hydroxy apatite. Being rich in minerals, fish bone powder can be fortified into several food products. However, for fortification, the fish bone should be converted into an edible form by softening its structure

by pre-treatment with hot water or hot acetic acid or superheated steam. Calcium powder processed from the backbone of tuna is a potential nutraceutical. It can be used to combat calcium deficiency in children. Other than fish bone calcium, certain other minerals such as selenium, potassium, iodine, zinc, magnesium are more abundant in seafood than in meats.

Conclusion

Because of their high nutritional value, marine fish consumption is associated with many health benefits from foetal life to adulthood. These benefits include neurodevelopment at the embryonic stage, cognitive and visual development during infancy and childhood, and lowering the risk of cardiovascular diseases during adulthood. In this context, fisheries and aquaculture programs can address and mitigate issues of malnutrition in the world by increasing the access to fish due to its nutritional value. Therefore, increasing fish production could increase the access to fish products and improve the nutritional status in children which has the potential to end malnutrition and food insecurity.

****

28

IR DRYING OF FISH AND FISHERY PRODUCTS

Aniesrani Delfiya, D.S.
Engineering Section
Email: ***delfy.lenin@gmail.com***

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.

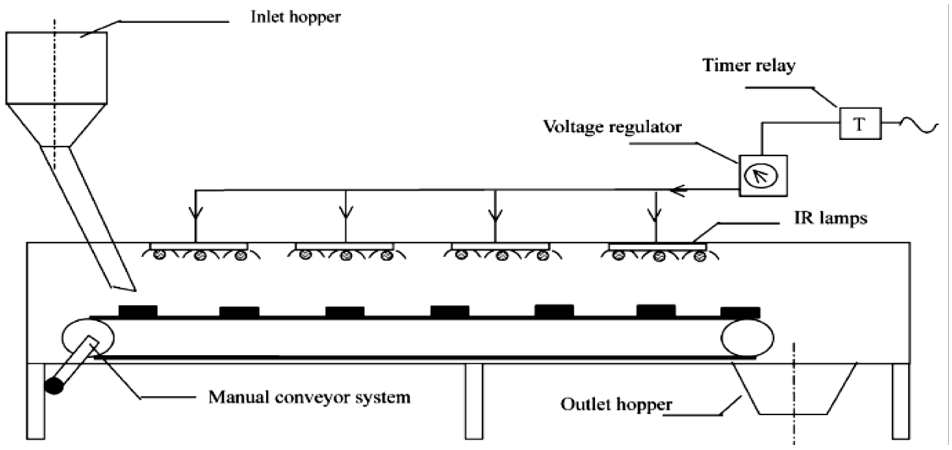


Fig. 1 Conveyor type IR drying system (Ratti and Mujumdar, 1995)

Drying of anchovy fish using IR dryer

IR drying of anchovy fish was carried out using the hot air-assisted continuous IR dryer (Fig. 2) with the IR intensities of 1,000, 2,000, and 3,000 W/m^2, IR source to sample distances of 5, 10, and 15 cm, drying temperature of 65°C, and air velocity of 1.5 m/s. Prior to the drying experiment, dryer was allowed to run for 30 min to achieve the set conditions. One kilogram of cleaned anchovy fish was filled in the feed hopper after reaching the required conditions. The hot air-assisted continuous IR dryer is having the input capacity of 0.2 kg/min for the continuous mode of operation. However, in case of anchovy fish drying the residence time of 1.5-3 h inside the dryer is essential to achieve the final moisture content of less than 15%. Hence, the conveyor belt was switched on for 8 min to spread the fish in the top layer of the dryer and switched off for 45 min to 1.5 h to ensure proper exposure of anchovy fish to IR radiation. This on/off time was obtained through prior experimental trials and the same operation was continued till the fish reaches the required moisture content. The drying chamber temperature was measured using a J-type thermocouple and product temperature was measured using a handheld IR thermometer with a precision of ±2°C. A hot wire anemometer with an accuracy of ±0.1 m/s was used to measure the air velocity. Total energy consumption during each drying experiment was measured using an energy meter in kWh (Make: Schneider, Model- Conzerv EM 1000). Drying was continued till the anchovy attains about less than 15 %wb moisture content. Because the moisture content of 15% or less retards the growth of mold and prolongs the shelf life. After the drying process, the dried anchovy fish were packed in laminated polyethylene polyesters, weighed, and then stored at room temperature (28 ± 2°C).

Fig. 2 Hot air-assisted continuous infrared dryer

The drying process of small and large size anchovy fish in a hot air-assisted continuous infrared dryer revealed the following results:

- The moisture content of small and large size anchovy fish was reduced to the final moisture content of 11.02 to 13.26 (%wb) in 3.5 h.
- Drying occurred in the falling rate period in all combinations of drying.
- The maximum drying efficiency of 46 % and 38.8% for small and large anchovy fish, respectively was observed at 2000 W/m^2 and 5 cm.
- The lowest SEC values in small and large anchovy fish were 1.36 and 1.61 kWh/kg, respectively at 2000 W/m^2 and 5 cm.
- IR intensity and distance between the IR source and the sample were significantly ($p<0.05$) influenced the drying and quality characteristics of anchovy fish.
- The lowest water activity, shrinkage, colour change and maximum rehydration ratio were observed at 2000 W/m^2, 10 cm and 2000 W/m^2, 5 cm for small and large anchovy fish, respectively.

Hence, it was concluded that hot air-assisted continuous IR drying is a promising method for anchovy fish drying and the hot air-assisted continuous IR dryer can be adopted for the production of dried small and large anchovy fish.

Drying of shrimp using IR dryer

Performance evaluation of continuous IR dryer (Fig. 2) was carried out by analyzing the drying characteristics of shrimp under three operating modes: hot air (HA), infrared radiation (IR) and combination of infrared radiation and hot air (IR-HA). Infrared power, drying air velocity, distance from infrared radiation source to sample and air temperature values were selected based on literature review and preliminary trials. Hot air (HA) drying was performed at 45°C and 1.5 m/s with infrared heaters in off condition. Infrared radiation (IR) mode of drying was conducted with the infrared power of 4500 W (Intensity is 3000 W/m^2), air velocity and temperature of 1.5 m/s and 28±2°C and infrared radiation source to sample distance of 10 cm. Combination of IR-HA drying was done at distance from infrared radiation source to sample of

10 cm, air temperature 45°C, infrared power of 4500 W and drying air velocity of 1.5 m/s. Dryer was allowed to run for 30 min before each experiment to achieve the set conditions.

For each drying experiment 4 kg of cleaned samples were taken and filled in feed hopper. The chamber temperature was measured using J-type thermocouple and shrimp temperature was measured using handheld infrared thermometer with an accuracy of ± 2°C. Relative humidity of air was ranged from 70±1% to 35±1% during drying. Hot wire anemometer with accuracy of ±0.1 m/s was used to measure the air velocity. Total energy consumption during each drying experiment was measured using energy meter in kWh (Make: Schneider, Model – Conzerv EM 1000). Drying was continued up to 10-15 %wb moisture content. Dried shrimps were packed in laminated polyethylene polyesters and stored at room temperature (28±2°C). The photograph of shrimp drying in the hot air-assisted continuous infrared dryer is presented in Fig. 3.

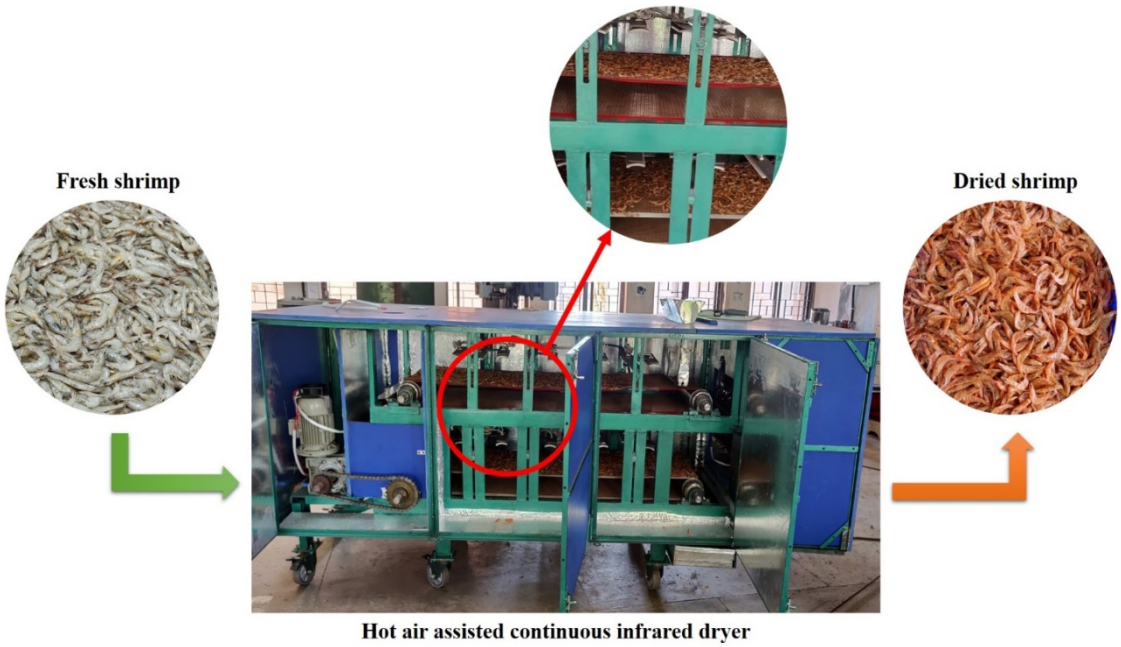


Fig. 3 Shrimp drying process in the hot air-assisted continuous infrared dryer

The hot air assisted continuous infrared dryer was found to be the most suitable dryer for shrimp drying in combined IR-HA mode of operation than HA and IR drying. The shrimp drying process occurred in the falling rate period in all the drying modes. Lowest drying time (2 h), specific energy consumption (1.80 kWh/kg of water evaporated), shrinkage (7.27%), water activity (0.57) and maximum drying efficiency (36.25%), sensory score (8.33) was observed in combined IR-HA drying. Overall, the drying of shrimp in IR-HA drying was quicker, consumed less energy and superior quality product in terms of physical, sensory characteristics and proximate composition. This dryer can be used for the production of high-quality dried shrimp with economical viability and it can be scaled up for commercial-scale continuous production. Similarly, other products like dried clam and squid rings, marinated and dried anchovy, shrimp, squid rings can also be prepared using this IR dryer.

Reference

Ratti, C., & Mujumdar, A. S. (1995). Infrared drying. In A. S. Mujumdar (Ed.), Handbook of industrial drying (pp. 567–588). New York: Marcel Dekker.

Sakai, N. and Hanzawa, T. 1994. Applications and advances in far infrared heating in Japan. Trends in Food Science & Technology. 5: 357–362.

Sandu, C. (1986). Infrared radiative drying in food engineering: a process analysis. Biotechnology Process, 2, 109–119.

****

29

EQUIPMENT AND INSTRUMENTS FOR SMALL-SCALE FISH PROCESSING INDUSTRIES

S. Murali
Engineering Section
Email: ***murali.s@icar.gov.in***

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 in post-harvest operations are the design and development of fish processing equipment and machinery, the design of indigenous electronic gadgets/instruments, and energy and water optimization techniques for fish processing industries. The equipment used for different unit operations in post-harvest handling of fish and fishery products can be broadly classified into the following areas.

- Preprocessing operations– Cleaning, sorting, skinning, scaling, gutting, beheading, cutting, and filleting operations
- Processing operations – Drying, steaming, baking, blanching, frying, retorting
- Preservation – Chilling, freezing, ice manufacturing
- Packaging – Band sealer, hand sealer etc.

1. Equipment and machinery for preprocessing operations

1.1. Size grader and washer

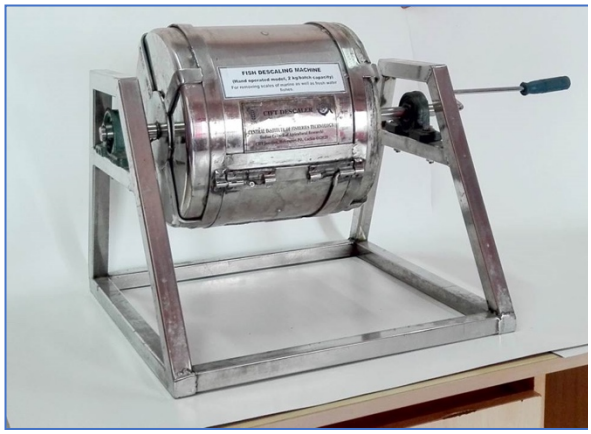
The preprocessing operation of fish starts from cleaning followed by grading of fish by species and/or size. Sorting by species or based on freshness and physical damage are still manual processes, but grading of fish by size is easily done with equipment. Mechanical graders yield better sorting precision for fish and fish products. In the size graders, two smooth rotating rollers are installed above the surface of the conveyor belt and the distance between the rollers and belt can be adjusted according to the maximum thickness of the fish to be sorted. Thinner fishes fall off the belt while the thick ones are retained on it until the end of the line. The device serves a dual purpose simultaneously by being a grading machine and a conveyor system.

Fish washing machine

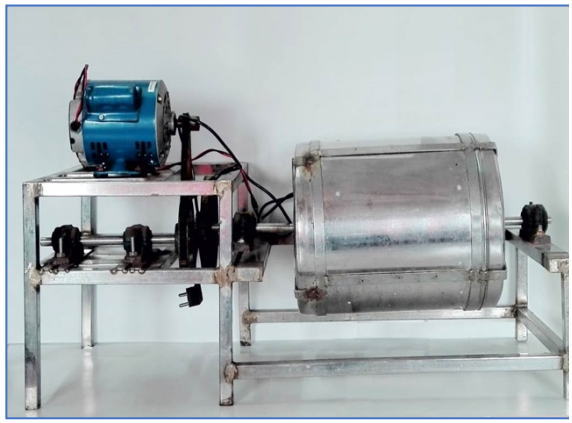
Fish size grader

1.2. Fish descaling machine

The fish descaling machine is designed and fabricated to remove the scales of fish easily. This equipment can remove scales from almost all types/sizes/ species of fishes ranging from marine to freshwater species like Sardines, Tilapia to Rohu. The machines are made of SS 304 and have a 5-10 kg capacity. It comes in two variants. In the hand-operated machine, a pedal is fitted on the side to rotate the drum manually and the same machine is motorized with a 0.5 hp motor with fixed rotation of the drum.



Hand operated machine



Motorized machine

2. Equipment and machinery for processing operations

2.1. 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. 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 specimens 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.

Fish meat bone separator

2.2. Solar hybrid dryers

ICAR-Central Institute of Fisheries Technology (CIFT), Cochin, has already developed low-cost, energy-efficient, and eco-friendly dryers like Solar cabinet dryers, Solar tunnel dryers, Infrared dryers, etc. for uniform and hygienic drying of fish. These dryers are also suitable for drying agricultural products like fruits, vegetables, spices, and condiments. The design of solar dryers varies from simple direct dryers to more complex hybrid designs. Hybrid model solar dryers have LPG, biogas, biomass, or electricity as alternate backup heating sources for the continuous drying of fish even under unfavourable 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-LPG hybrid dryer

Solar-electrical hybrid dryer

2.3. Battering and breading machine

The basic purpose of a coating machine is to achieve a uniform coating. Also, it is necessary to make all the operations in a uniform style till the product is packed. Battering and Breading Machine is a conventional machine where the two applications viz. battering and breading can be carried out continuously. This equipment is a combination of one battering unit and a breading unit coupled together so that after the application of the batter, the fish portions are transferred to the breading unit by the conveyor system.

Battering and breading machine with integrated forming unit

2.4. Fryers

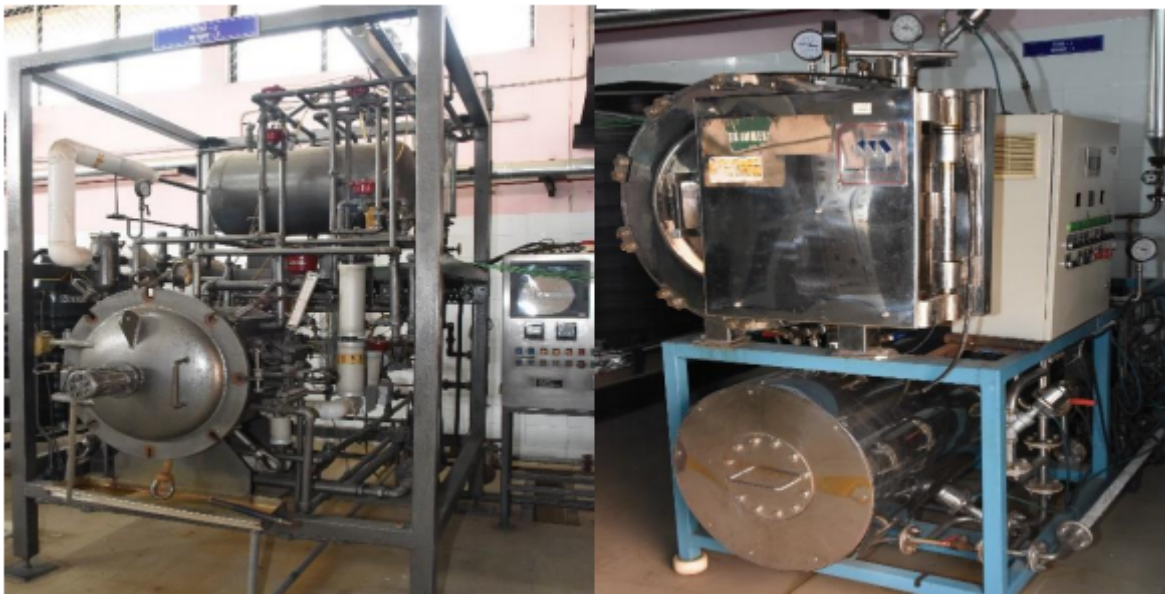
Frying is one of the fastest heat transfer methods available for cooking. It is a simple and commonly used technique for developing flavour, colour and unique product characteristics that cannot be duplicated by any other methods. Frying can be accomplished in a batch or continuous system. A batch system is recommended for small-scale production and a

continuous system for large-scale commercial production. The type of product and its sensory qualities and physical dimensions all have to be considered while selecting a frying system.

Fryer

2.5. Retorts

All canned fish products are sterilized at temperatures above 100 ºC. Thermal process sterilization takes place in retorts, with or without water. Overpressure is between 2-3 kg/cm^2. The simplest and most common retorts today are horizontal, or vertical, batch retorts. The most frequently used style of retort found in commercial fish canneries today is the static batch system for processing cans in saturated steam. The most significant difference between static retorts and continuous systems is that the latter must have container transfer mechanisms to regulate the movement of cans at a predetermined rate through the heating and cooling sections. Batch retorts heated with water under pressure are vertical or horizontal and are most frequently used for sterilization of products packed in aluminium cans with score-line easy open ends.



Steam retort and water immersion retort

Horizontal retort

3. Equipment and machinery for fish preservation

3.1. Plate freezers

In a contact freezer or plate freezer the fish is frozen by direct contact with a refrigerated surface, typically between two hollow metal plates cooled by a refrigerant, such that the distance between the plates can be varied up to 100 mm or more. Horizontal and Vertical types of plate freezers are available. Horizontal freezers are generally used in processing plants in which fish, especially in flat packs such as laminated blocks, is frozen between two or more hollow, horizontal, parallel plates through which refrigerant passes. In a vertical plate freezer, the refrigerated, parallel plates are vertical and it is used mainly at sea or onshore for freezing large 25 or 50-kg blocks of whole, gutted, or headed gutted fish.

Horizontal plate freezers

Vertical plate freezer

3.2. Air blast and tunnel freezer

In an air blast freezer, fish is frozen in a stream of high-velocity cold air either in a batch or continuously, typically in a duct or tunnel in which a stream of cold air is guided over the product on shelves (batch) or a conveyor (continuous air blast freezer); also called blast

freezer, freezing tunnel, tunnel freezer. The advantage of the blast freezer is its versatility. It can cope with a variety of irregularly shaped products and whenever there is a wide range of shapes and sizes can be frozen. Continuous air blast freezers and batch air blast freezers are used. The equipment has a food-grade conveyor belt passing through an insulated chamber. It has an air-cooling system and an air blower to blow the air through the tunnel. Cold air is blown to the tunnel counter to the movement of the belt. The product to be frozen is passed through the belt. Circulating cold air at high speed enables the product to be frozen at a moderately rapid rate. Usually, the air temperature is between 18 and -34°C or lower. The moving of the product counter current to the cold air at a speed of 1 to 20 meters/second enables freezing to take place at a rapid rate. It is the popular method to prepare frozen fish products as IQF (Individually Quick Frozen).

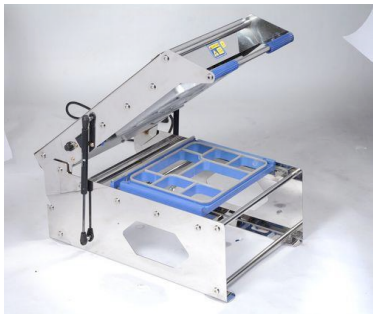
Air blast freezer

Tunnel freezer

4. Machinery for packaging of fish and fishery products

4.1. Sealers

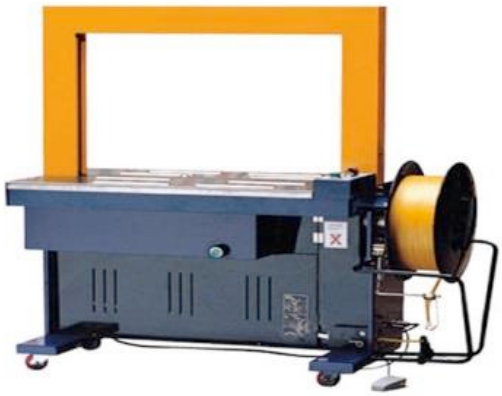
Sealers are used in multiple forms of flexible packaging applications. A heat sealer uses heat to melt plastic or adhesive together to seal off a package. Heat sealers are used for many different products to help protect from product tampering and contamination. They can be used in small operations and fully automatic operations. Heat sealing systems use a combination of heat, time and pressure to create a seal with a set of crimp seal heating bars. When the jaws come together, this melts a layer of plastic and bonds the two layers of film together. There are different types of sealers such as band sealers, hand sealers, blister sealers tray sealers, vacuum sealers and vertical form fill sealing machines.



Hand sealer **Band sealer** **Tray sealer**

4.2. Labelling, coding and bundling machines

Labelling and coding machines are used for industrial and retail packaging applications. Most packaged products use some form of labelling or coding. Labelling machines are used for applying branded labels for advertising and/or bar codes for inventory and batch management. The most popular use for strapping machines is a reinforcement of heavy boxes during shipping and retail sales. Polypropylene strapping is commonly used. Strapping machines use heat to mend ends together for durable reinforcement. Another use for a strapping machine is bundling applications. Strapping can help unitize multiple products together and secure products for transport.



Labelling and coding machine **Strapping and bundling machine**

References

Mohan, C. O., Elavarasan, K., Sreejith, S. and Sreelakshmi, K. R. (eds) (2021) Fish and Marine Products Processing, Central Institute of Fisheries Technology, Cochin, India.

Bykowski, P. and Dutkiewicz, D. Freshwater fish processing and equipment in small plants.FAO Fisheries Circular.No. 905. Rome, FAO. 1996. 59p.

A.Gonçalves and F. Blaha (2010) Cold chain in seafood industry. In: Refrigeration: Theory, Technology and Applications, M. E. Larsen (ed), Nova Science Publishers. pp.287-367

Graham, J., Johnston, W.A., Nicholson, F.J.Ice in Fisheries. FAO Fisheries Technical Paper.No. 331. Rome, FAO. 1992. 75p.

****

30

ENERGY AND EXERGY ANALYSIS OF SOLAR-ELECTRICAL HYBRID DRYER

K. C. Neethu
Engineering Section
neethu.c@icar.gov.in

Drying is one of the ancient methods of food preservation. It is one of the unit operations in the postharvest phase. Drying is the removal of water from a solid, semi-solid or liquid to a predetermined level by virtue of simultaneous heat and mass transfer. Drying is preferably done to increase the storage life of food. Drying reduces the water activity of food which in turn helps to arrest or delay the microbial activity and chemical reactions thus dried food can be stored for longer time. Drying also enhances the product flavours and texture to provide definite sensory properties. Dried foods are more suitable for handling and reduces the cost of packaging, transportation and storage. Dried foods are delicious, nutritious, lightweight and easy-to-store and use (Ahmed et al. 2013).

Drying operations use a lot of energy, accounting for 7–15% of the sector's overall energy consumption in some nations and possibly as much as 33%, and they have only 25–50% thermal efficiency (Dincer and Ezzat 2018). There are numerous methods of drying. The predominant food drying methods are open sun drying and shade drying. Other improved conventional methods are hot air drying and cabinet or oven drying. Non-conventional methods of drying include solar drying, microwave drying, infra-red drying, freeze drying, etc.

The traditional methods of drying such as open sun drying have disadvantages like longer drying period, contamination and adversely affected by weather conditions. Sun drying is being the most widely practiced method throughout the world for drying agricultural commodities. Sun drying is just spreading things in out-doors in the open sun and letting them dry. It is one of the cheapest methods drying and energy is freely available, renewable and abundant. But it takes quite a few days to dry foods in out-doors. As the weather conditions is not controllable, sun drying can be uncertain. And also, direct exposure of the food material to unhygienic open conditions may cause dust, excreta, pests, insects and microbial infestations and yield inferior quality product.

Fish or shrimp is being the most cost-effective animal protein source and because of its high perishability, and sun drying were used to dry fish and to preserve the same (Paul et al. 2018). To overcome the drawbacks of sun drying, mechanical dryers with electric heating system are generally used. But this involve running costs due to electricity consumption and are not

recommended due to exploitation of non-renewable sources of energy and leaving carbon footprint. 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 avoid the disadvantages of open sun drying.

Solar energy is freely available abundantly and can be easily harnessed. Solar energy is radiant energy from sun in the form of light and heat that can be collected using a range of technologies to generate electricity and thermal energy. Solar energy can be effectively utilized for drying purpose in an efficient way using solar thermal collectors.

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. Quicker drying process reduce the menaces of spoilage or microbial attack. Even though farmers were using sun drying for several centuries, but recently, solar drying has been widely accepted over open sun drying, as it is more effective. Solar dryers use solar energy which is renewable and freely available 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 thermal energy storage systems etc. Among all, solar drying is most effective for fish or shrimp drying as it is using renewable energy for drying. And also, many studies have proven that solar drying is a method of food preservation as the food is dried under controlled conditions and fully protected from infestation of rain, dust, insects, pests and animals during drying.

Solar energy is used either as the sole heating source or as a supplementary heating source. Solar dryers use atmospheric air to get heated by the help of solar thermal energy collectors. The hot air flow can be made either by forced or natural convection. The energy of from solar radiations used to heat the air that flows into the dryer through the material in the dryer. As the air is heated up, its humidity decreases and is capable of holding more moisture. The drying will happen by the passage of the hot air through the food kept in the chamber or by directly exposing the food to solar radiation in the thermal collectors, or a combination of both. The heat transfer to the moist food from hot air will occur by convection and conduction which is at temperatures above that of the food, or by radiation from the sun and to some extent from the surrounding hot surfaces, or by conduction from heated surfaces in contact with the food.

Solar dryer can perform drying for food preservation only during sunny days, and hence the drying efficiency depends largely on climatic conditions and the season (Nukulwar and Tungikar 2022). Hybrid solar dryers are more reliable as there is a back-up system to provide heating in it. Solar-electrical hybrid dryer is more trustworthy as auxiliary system is electrical heating coil. Hybrid solar dryer would lessen the drying costs (in comparison with the elecrical dryer's costs) and also there is a possibility to improve the quality of the dried food by controlling the drying condition in solar hybrid dryer (Ferreira et al. 2007).

Solar hybrid dryers use different energy sources for auxiliary or back-up heating. Studies are already done on the solar hybrid dryers with solar water heaters and electric water heating coil (Amer et al. 2010) biomass back-up (Dhanuskodi et al. 2014; Sonthikun et al. 2016), sensible heat storage using water heaters and LPG back-up (Murali et al. 2021), biogas powered air heaters (Rupnar et al. 2020), steam based solar hybrid dryers (Nukulwar and Tungikar, 2020), ohmic heating (Richa et al. 2021) and black pebble-based sensible heat storage (Andharia et al. 2023) based solar dryers.

Design and working of solar-electrical hybrid dryer

The solar-electrical hybrid dryer (SEHD) comprises a solar air collector, a drying cabinet, blowers and fans, an electric heating coil, and a temperature sensor. The solar air collector is connected to the cabinet of the dryer. The cabinet is fabricated with a stainless-steel box with insulated PUF (polyurethane foam) walls and five trays, each capable of holding 2 kg of fish. The drying trays of food-grade quality (ss 304) was used to hold the materials in the cabinet and make it easier to (un)load or shift the trays. The door of the dryer was appropriately sealed to stop any heat loss and for accessing the products inside the chamber. The collector's hot air will enter the bottom dryer cabinet. The fluctuation in the drying air temperature depending on the incident solar radiation at the flat-plate collector, was regulated by a supplementary heat source. The blower supplies the air through the heating coil placed inside the drying chamber's double frame structure, and heated air will enter the drying chamber through the perforations made at the side bottom of the cabinet beneath the trays. Then the heated air will pass through the material over the trays removing the moisture from the material and throwing it out with the help of exhaust fans. If the dryer temperature was not attained, the heating coil will be heated up to attain the desired temperature in the cabinet, which is controlled thermostatically.

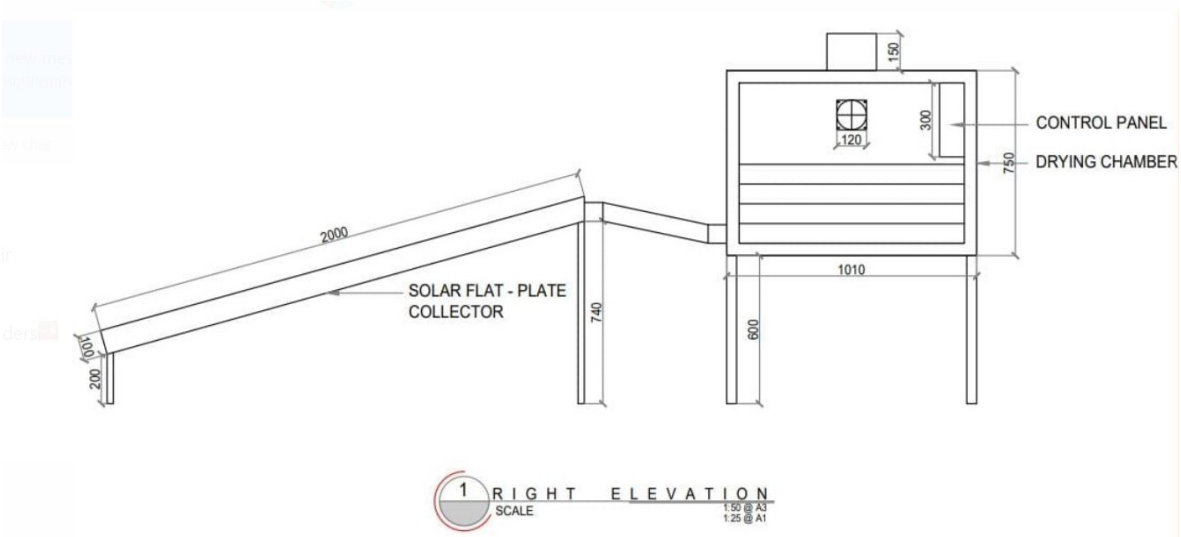
Energy analysis

Initially, the parameters such as the initial moisture content, final moisture content and total mass of the fish to be dried was estimated to calculate the quantity of moisture to be removed during drying (M_w, kg).

$$M_w = M \times \frac{Mi - Mf}{100 - Mf} \quad (1)$$

The heat energy required to dry the material is calculated by taking into account of weight of material to be dried (kg), quantity of moisture to be evaporated (kg), specific heat of water (kJ/kg°C), latent heat of vaporization (kJ/kg) and the difference in temperature between ambient and drying conditions.

$$Q = M C_{pw} \Delta T + M_w \lambda \quad (2)$$



Schematic Diagram of the solar-electrical hybrid dryer

The drying efficiency of SEHD was calculated by the amount of energy required to remove the moisture from the material to the energy supplied (by the solar collector electrical coil). The sensible and latent heat is the total energy required to dry the material. The energy required to raise the temperature of the food to a dryer temperature is called sensible heat. The latent heat of vaporization is the energy required to vaporize at drying temperature (Leon et al. 2002). The energy absorbed by the solar air collector and energy utilized by the heating coil, exhaust fans, blowers, etc., is the energy supplied. The drying effieciency was calculated using the following equation (Vieira et al. 2007).

$$The\ Drying\ Efficiency = \frac{(Energy\ required\ to\ remove\ moisture)}{(Energy\ supplied\ by\ heating\ coil + Energy\ supplied\ by\ solar\ collector)} \quad (3)$$

The solar-electrical hybrid dryer was having a drying efficiency of 28.65%, with an average incident radiation of 710 W/m^2. The major part (65%) of thermal energy required for shrimp drying was supplied by solar radiations, and the remaining part (45%) was provided by the electrical heating coil as an auxiliary system when operated in hybrid mode (Cisni et al. 2023).

Exergy analysis

Exergy is the "available energy" or "usable energy" which represents the portion of energy that can be converted into useful work, such as mechanical work or electrical work, under ideal conditions. The remaining energy that cannot be converted into useful work is considered wasted energy. The concept of exergy analysis, which assesses the thermal efficiency of a system, is based on the fundamental principles of the second law of thermodynamics. The concept of exergy is applicable to analysis, design and optimization of energy conversion processes and systems. The exergy output is inherently lower than the exergy input due to the presence of irreversibilities and this loss is directly proportional to the entropy formation within

the process (Mugi et al. 2021). Overall, exergy provides a valuable tool for assessing the efficiency and sustainability of energy systems by considering not only the quantity of energy but also its quality and the potential for useful work extraction.

This approach considers the transfer of mass, heat, and work within the system to evaluate its overall exergy in the process and calculated by the following formulas (Kumar et al. 2023).

$$Ex = \dot{m}C_p\left[(T - T_a) - T_a\, ln\frac{T}{T_a}\right] \quad (4)$$

$$Ex_{loss} = Ex_i - Ex_o \quad (5)$$

$$\eta_{Ex} = \frac{Ex_o}{Ex_i} \quad (6)$$

The exergy efficiency of the drying chamber of SEHD was found to be 28.57%, indicating the portion of available exergy that is effectively utilized (Cisni et al. 2023). Ndukwu et al. (2022), also reported a range of exergy efficiency values from 19.09% to 52%. The exergy loss associated with the drying chamber of SEHD was measured as 0.70, indicating the magnitude of exergy dissipation during the drying process. On the other hand, the exergy efficiency of the solar air collector was found to be 62.50%, demonstrating the effectiveness of energy conversion from solar radiation to usable exergy. The exergy loss attributed to the solar air collector was calculated to be 0.34, representing the extent of exergy degradation within the collector (Cisni et al. 2023).

Fig. 2. Shrimp before and after drying in SEHD

In the same way in the study conducted by Mugi et al. (2021), exergy analysis was performed separately for both the solar air collector and the drying chamber. The low exergy efficiency of

the drying chamber suggests potential areas for improvement, such as enhancing insulation, optimizing air flow pattern, or implementing more efficient heat transfer mechanisms. Similarly, the exergy efficiency of the solar collector in the hybrid dryer was found to be 62.43%. The high exergy efficiency of the solar collector highlights the effectiveness of harnessing solar energy for drying applications due to its advanced design, optimized orientation, and efficient heat transfer mechanisms. The associated exergy loss with the solar collector was measured as 0.34, representing the energy lost during the conversion process. This loss can be attributed to factors such as thermal losses, optical inefficiencies, and the unavoidable limitations of energy conversion technologies.

Abbreviations

M Weight of shrimp to be dried (kg)

M_w Amount of water to be removed (kg)

M_i Initial moisture content (% w. b.)

M_f Final moisture content (% w. b.)

Q Heat energy requirement (kJ)

C_p Specific heat of air (kJ/kg℃)

C_{pw} Specific heat of water (kJ/kg℃)

ΔT Temperature change between ambient and drying conditions (℃)

λ Latent heat of vaporisation (kJ\kg)

ṁ Mass flow rate of air (kg/s)

Ex Exergy

Ex_i Exergy Input

Ex_o Exergy Output

Ex_{loss} Exergy Loss

η_{Ex} Exergy Efficiency

T_a Atmospheric Temperature

References

Ahmed N, Singh J, Chauhan H, Anjum PGA, Kour H (2013) Different drying methods: their applications and recent advances. Int J Food Nutr Saf 4:34-42

Amer BMA, Hossain MA, Gottschalk K (2010) Design and performance evaluation of a new hybrid solar dryer for banana. Energy Convers.Manag.51:813-820

Andharia JK, Markam B, Dzhonova D, Maiti S (2022) A comparative performance analysis of sensible and latent heat based storage in a small-scale solar thermal dryer, J Energy Storage, Volume 45:103764, https://doi.org/10.1016/j.est.2021.103764.

Cisni F M, Neethu K C, Murali S, Delfiya D S, Ninan G (2023) Design and development of solar-electrical hybrid dryer and its performance evaluation using shrimps. Biomass Conv Bioref https://doi.org/10.1007/s13399-023-04651-6

Dhanuskodi S, Wilson V, Kumarasamy S (2014) Design and thermal performance of the solar biomass hybrid dryer for cashew drying. Facta Univ Ser Mech Eng 12:277-288

Dincer I, Ezzat MF (2018) 3.4 renewable energy production. Comprehensive Energy Systems, Vol. 3–5. Elsevier, pp 126–207

Ferreira AG, Charbel A, Silva JG (2007) Experimental analysis of a hybrid dryer. Therm Eng 6:10.5380/reterm.v6i2.61680

Kumar B, Szepesi G, Szamosi Z (2023) Optimisation techniques for solar drying systems: a review on modelling, simulation, and financial assessment approaches. Int J Sust Energ 42: 182-208, https://doi.org/10.1080/14786451.2023.2185870

Leon MA, Kumar S, Bhattacharya S (2002) A comprehensive procedure for performance evaluation of solar food dryers. Renew Sustainable Energy Rev 6:367–393.

Mugi V R, Chandramohan V P (2021) Energy, exergy and economic analysis of an indirect type solar dryer using green chilli: A comparative assessment of forced and natural convection. Therm Sci Eng Progr 24: 100950.

Murali S, Aniesrani Delfiya DS, Sathish Kumar K, Lekshmi RGK, Ezhil Nilavan S, Amulya PR, Krishnan VS, Alfiya PV, Samuel MP (2021) Mathematical Modeling of Drying Kinetics and Quality Characteristics of Shrimps Dried under a Solar–LPG Hybrid Dryer. J Aquat Food Prod Technol 30:561-578 https://doi.org/10.1080/10498850.2021.1901814

Ndukwu M C, Ibeh M I, Etim P, Augustine C U, Ekop I E, Leonard A, Bennamoun L (2022) Assessment of eco-thermal sustainability potential of a cluster of low-cost solar dryer designs based on exergetic sustainability indicators and earned carbon credit. Cleaner Energy Sys 3: 100027

Nukulwar MR, Tungikar,VB (2022) Recent development of the solar dryer integrated with thermal energy storage and auxiliary units. Therm Sci Eng Prog 29: 101192

Nukulwar MR., Tungikar VB (2020) Design and Development of Steam-Based Solar Dryer for Agricultural Products. Proceedings of International Conference in Mechanical and Energy Technology 174:365–375

Paul PC, Reza MS, Islam MN (2018) A review on dried fish processing and marketing in the coastal region of Bangladesh. Res Agri Livestock Fish 5: 381–390

Richa R, Shahi NC, Lohani UC, Kothakota A, Pandiselvam R, Sagarika N, Singh A, Omre PK, Kumar A (2022) Design and development of resistance heating apparatus-cum-solar drying system for enhancing fish drying rate. J Food Process Eng 45: e13839

Rupnar AK, Jain S, Panwar NL, Jain SK (2020) Design and Development of Solar-biogas Hybrid Dryer for Onion Drying. Int J Environ Clim 10:65-73.

IMPORTANCE OF HYGIENE AND SANITATION IN FISH HANDLING

Femeena Hassan
Quality Assurance and Management Division
drfemeenahassan@gmail.com

Fresh fish has a natural complement of bacteria of aquatic origin. During handling fish gets contaminated with various types of bacteria, some of these bacteria have sanitary significance. These micro-organisms are not originally present in fresh fish caught from offshore waters but contamination occurs during handling of the material in unhygienic conditions. If the time-temperature conditions are favourable, these bacteria can grow and multiply at a fast rate leading to spoilage. Consumption of such fish is dangerous as it can lead to food poisoning and problems of public health. Hygiene and sanitation, therefore play a vital role in fish handling.

Bacterial of public health significance in fish handling

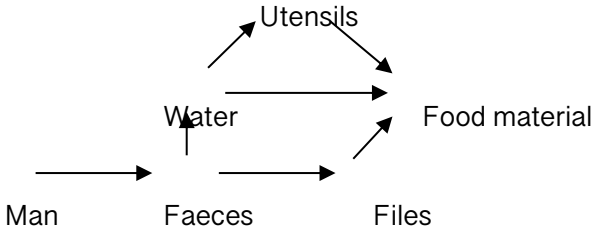
Handling and resultant contamination introduce a variety of bacteria into food. Some of these bacteria which are important from public health point of view, along with their primary habitats are listed in Table 1.

Table 1 Bacteria of public health significance and their habitats

Bacteria	Primary habitat
Escherichia coli (E.Coli)	Gut of man
faecal streptococci	Gut of man & animals
staphylococcus aureus	Skin, sweat, eargum, nasal drips and throat of man
Salmonella	Also present in ulcers, wounds and carbuncles in
Shigella	human beings.
V.cholerae	Gut of man and animals
Clostridium wechii	Gut of man
	Gut of man
	Soil, water, dust and intestinal tract of man and animals

Many of these microorganisms escape from the primary source into nature through excreta of man and animals. through various other vectors such as files, birds, animals and forces of nature like wind and rain they spread in land and water, and finally into food as shown below:

Route of contamination of food materials by bacteria of public health significance



Sanitary and hygiene precautions to be followed in fish handling

If we are careful, we can effectively check these routes and prevent contamination and spreading of diseases. Such preventive measures are called sanitation and hygiene methods, some of which are listed below;

Use only Potable Water or clean Sea Water for washing fish

Harbour waters and shore waters are often contaminated with sewage and harmful enteric bacteria. This water shall not be used for washing fish or fish-contact surfaces. Always use only potable water or clean sea water from outer sea for washing and processing fish.

Never put fish on sand or any unclean surface

In certain areas of the country, these is a practice of sorting on sea beaches where even faecal matter gets dispersed. This is a sure source of contamination of fish with diseases-producing microorganisms. All operations on fish shall be done only on smooth and clean surfaces, which are sufficiently above the ground level and free from wind-blown dust and flies.

Construction and maintenance of buildings for fish processing to achieve sanitation

I. Buildings should be in an area where potable water and electricity are readily available.
II. The building and the sections inside should be oriented in such a way that wind does not blow the dust into the plant or to the food material handled inside.
III. The premises of the building should be kept neat and clean.
IV. The roof of the building shall be of simple construction with smooth and clean surface.
V. It is preferable to gave concrete roofing with smooth and washable surface
VI. The walls shall be of solid construction using brick and cement and shall be smooth and washable but water resistant. Walls shall be preferable lined with white ceramic tiles
VII. The roof-wall joints, wall-floor joints are wall –to-wall joints shall be rounded in order to prevent pests and dirt settling on them.
VIII. The walls shall be well polished and where ever possible fitted with glazed tiles, at least to a height of four feet from floor, to facilitate easy cleaning.
IX. The floor of the hall shall be smooth, cemented and free from crevices.
X. The slope of the floor shall be in such a way that water easily runs into the drain. Preferable the direction of waste water flow shall be opposite to the flow of material.

Where ever possible the water from processing tables and equipment shall be led through pipes into the drain, without spilling on the floor.

Lighting and Ventilation

Lighting shall be adequate for reasons of both safety and efficient working. If natural light is not adequate. It can be supplemented with artificial lights, which are similar to natural light. Light bulbs and fixtures over the product shall be protected to prevent contaminator in case of breakage and harbourage insects and pests. All electrical fittings in the factory building shall be washable.

Ventilation

Adequate ventilation is an important factor in the design and operation of a sanitary fish processing plant. Exhaust fans shall be installed wherever necessary. A relative humidity of 60-70% and a temperature of 20-25°C are the ideal conditions in a fish handling hall, fish stall or market, Ventilation is also essential to prevent condensation of moisture and fungal growth on roof and walls.

Water supply

There shall be plentiful supply of potable water in fish handling and marketing area. The water shall be annually tested and certified for potability /IS 4251/EU Norms. The microbiological quality of the water shall be checked at least once in three months. The level of free chlorine in the process water shall be <2 ppm. Non-potable water supply for fire control and similar purposes shall be through separate lines, which can be identified by different colour code to avoid cross contamination.

Ice

Ice shall be prepared from potable water chlorinated to a residual level of <2 ppm. Dragging of ice through walking floors will lead to bacterial contamination of the ice block and if contaminated ice is used for icing it will in turn contaminate the fish with bacteria of public health significance, Ice shall be stored only on elevated floor where walking shall not be permitted. Preferably ice blocks may be stored in separate insulated rooms, over raised platforms or suitable tubs or boxes. Sawdust, gunny bags, tarpaulins etc. shall not be used for covering or protecting ice.

Ice crushing on floor is not advisable, ice crushing machines may be used for this purpose. Crushed ice shall be collected directly into tubs lined inside with stainless steel or aluminium and ice shall always be stored and handled in such a way that bacterial contamination is avoided.

Utensils for fish handling

It is not advisable to handle fish on the floor. Tables may be used for this purpose. Table tops shall be of stainless steel, or any other noncorrosive, nonreactive, smooth and washable material. All fish contact surfaces shall be smooth, water resistant and free from pits and

r=crevices. They shall withstand normal repeated cleaning. Wooden, enamel and wire mesh utensils are not advisable for handling fish. Bamboo baskets, cane baskets and such other containers that are difficult to be cleaned shall not be used.

Utensils that are used for inedible and contaminated materials shall be separately identifiable by some mark or colour so that they are not used for handling edible products.

Washing of utensils and fish contact surfaces

On constant use, the utensils get a coating of fish slime, which can harbour harmful bacteria. All the utensils and fish contact surfaces shall be washed at frequent intervals using suitable detergents such as teepol, followed by disinfection using sodium hypo chlorite solution having a residual chlorine strength of 100 ppm and giving a minimum contact time of 15 minutes. The washing shall be done before and after each shift as well as whenever there is visible spoilage of any dirt on the contact surfaces.

Workers' hygiene and health

Fish handlers shall be provided with clean uniforms while on work. They may wear clean overalls and head and mouth covers. They shall be trained to be appreciative of the need for a high standard of cleanliness in fish handling. Before starting the work, all those who have to handle fish, shall wash their hands from elbow down using soap followed by disinfection using 20-50 ppm chlorine. The process may be repeated each time they leave their work sport and return for work or when over their hands become contaminated. Talking, spitting, eating and use of tobacco shall be prohibited in the premises where fish is handled.

Workers can be healthy carriers of many dangerous bacteria including *salmonella, shigella, V.cholerae or S.aureus.* These workers will contaminate the material which they handlethereby creating serious public health hazards. The management shall, therefore, take proper care to see that the workers having cut or injury on their palms as well as those suffering from any disease are kept away from work. The workers shall be subjected to a medical examination at an interval of at least one year, particularly to identify the carriers among them. Wherever possible workers shall be provided with prophylaxis against contagious diseases particularly typhoid.

Rodent control measure

Rodents are known to spread many diseases such as plague, endemic typhus fever, infectious jaundice and salmonella food poisoning. Most of these are transmitted from infected rodents to man through their urine and droppings. Therefore, all possible precautions have to be taken to prevent the entry of rodents to the fish handling areas.

Multiplication of rats and mice depends upon the food and harbourage available to them. Therefore, the only permanent and lasting means of control is the elimination of food source and harbourage areas. The processing area shall be made rodent-proof. As a guide to rodent

proofing it may be noted that rodents have exceptional skills to climb jump and squeeze in as indicated below.

- Rodents can squeeze in through a hole of less than 2 cm
- They can climb vertically through wires and pipes
- They can jump both horizontally and vertically from any flat surface.
- They can jump to 15m down without being killed

So, all efforts shall be made to bait, trap and kill rodents in factory premises but without resorting to the use of toxic chemicals.

Fly control measure

Files transmit a number of bacteria from the surroundings to the food. Therefore, the doors and windows of the fish handling areas may be fitted with fly proof net of 4mm mesh size. Further, the doors shall be self –closing. All outside opening doors and chutes shall have automatic air curtain to prevent entry of insects. For rodent and fly control toxic or poisonous substances shall not be used.

Waste disposal

Timely non-disposal of waste from fish handling premises often poses serious problems as it attracts flies and creates an unhealthy surrounding. There should be an efficient and prompt system of waste disposal from such areas.

Storage of toxic substances

Fumigants and other toxic substances meant for use in the fish handling areas shall be stored in locked cabinets and handled only by properly trained personnel having a thorough knowledge of the hazards including the possibility of contamination of food products. Poisonous rodenticides and insecticides are not permitted in fish handling areas. Fumigation shall be resorted to only when there is a need.

Toilet facilities

Proper construction, maintenance and supervision of toilet facilities are also port of sanitation in a food processing plant. Such facilities should be adequate in number and shall be cleaned and disinfected daily at frequent intervals. The roof-wall Joint of the lavatories shall be tight so as to avoid harbouring of flies and rodents. The lavatories shall be fitted with self-closing doors. The recommended number of toilets in relation to the number of workers is given in Table 2. All toilets shall be fly proof.

Table2 toilet facilities required in a food processing factory

Number of workers/shift	Number of toilets required
1 to 9	1
10 to 24	2
25 to 49	3
25 to 100	5

32

PREREQUISITE PROGRAMME REQUIREMNT FOR SEAFOOD PROCESSING PLANT

Priya, E. R
Quality Assurance and Management Division
Email:priyaer@gmail.com

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

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

Employee training and training records

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

The processors must provide adequate facilities, required to keep records that document the training on the principles of food hygiene and food safety for those who supervise or perform

manufacturing, processing, packing, or holding activities for food. Processors must maintain records of this training for at least 2 years.

Good Manufacturing Practices (GMP)

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

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

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

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

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

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

Sanitation Control Procedures (SCPs)

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

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

the processing environment and employee practices that may contribute to food safety hazards.

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

Eight Key Sanitation Areas

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

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

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

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

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

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

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

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

These eight key areas of sanitation should be monitored at a frequency sufficient to ensure conformance. In addition to that the monitoring results and corrections made for any deficiencies must be recorded. The frequency or time for monitoring will vary according to various types of products and the schedule of operations. The SCP monitoring forms or records must include the name and location of the processor, the date and time the monitoring was performed, corrections made and the signature or initials of the person conducting the

monitoring. The sanitation monitoring, corrections, and sanitation control recordkeeping may be performed as part of a firm's HACCP Plan controls, or separately.

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

****

33

INTRODUCTION TO HACCP CONCEPTS IN SEAFOOD INDUSTRY

Devananda Uchoi
Quality Assurance & Management Div
Email: uchoidev514@gmail

Seafood industry is a rapid growing sector where production and consumption is increasing every year. Seafood industry has undergone tremendous expansion in the last two decades. India achieved an all-time high exports of seafood both in terms of volume and value (both US$ and Rupee) by shipping 17,35,286 MT of seafood worth Rs. 63,969.14 crores (US$ 8.09 billion) during FY 2022-23 despite the several challenges in its major export markets like USA (MoCI, 2023). Food safety remains a major concern in the growing seafood industry. The seafood safety is of more concerns in international fish trade due to its vast expansion in recent decades. In 2020, global aquaculture production reached a record 122.6 million tonnes, with a total value of USD 281.5 billion (FAO, 2022). This increase in seafood consumption is a result of being increasingly recognized for its key role in food security and nutrition, not just as a source of protein, but also as a unique and diverse provider of essential omega-3 fatty acids and bioavailable micronutrients.

Therefore, with the rapid growth of the world's fisheries and aquaculture sector, aquatic food consumption has increased significantly in the present decade and is expected to keep increasing in the coming years. Total global fisheries and aquaculture production reached a record 214 million tonnes in 2020, of which 178 million tonnes comprise aquatic animals and 36 million tonnes of algae. Of the overall production of aquatic animals, over 157 million tonnes (89 percent) were used for human consumption. On a per capita basis, consumption of aquatic food grew from an average of 9.9 kg in the 1960s to a record high of 20.5 kg in 2019. In 2020, consumption slightly declined to 20.2 kg per capita. However, with the increase in seafood consumption, the responsibility for maintaining seafood safety has increased many fold. Because seafood has been associated with several foodborne illnesses and disease outbreaks has been reported all around the globe. Hence, issue of seafood safety is a global concern with increasing international fish trade among nations.

The statistics on foodborne illness is alarming as over 200 illnesses, ranging from diarrhea to cancer, are brought on by contaminated food that contains pathogenic bacteria, viruses, parasites, or harmful chemicals. Additionally, it contributes to a vicious cycle of illness and malnutrition that affects young children, the elderly, the sick, and infants in particular. Nearly 1 in 10 people worldwide, or 600 million, are expected to get sick from eating contaminated food, and 420000 people die as a result (WHO, 2021). In low- and middle-income nations, unsafe food costs US$ 110 billion annually in lost productivity and medical bills. With 125000 deaths each year from foodborne illness, children

under the age of five bear 40% of the burden. By straining healthcare systems, destroying national economies, tourism, and trade, food-borne illnesses limit socioeconomic progress to a large extend. These illnesses can be attributed to contaminated food. Hence, a food safety system aimed at ensuring all food is as safe as possible is required.

In this connection, the Hazard Analysis and Critical Control Points (HACCP) system is a single system that has been adopted by national and international bodies for ensuring seafood safety. However, HACCP system is not a standalone programme as it requires prerequisite programmes to work effectively. In present decade, the International Organization for Standardization (ISO) has developed the ISO 22000 family of standards on food safety management systems (FSMS) by taking approach of ISO 9001 as a management system, and incorporates the hygiene measures of prerequisite programmes and the HACCP principles and criteria. The HACCP programme plays a significant role in food safety management system. HACCP can help to achieve the food safety goal when there is an effective cooperation between governments, producers, and consumers. In order to have effective implementation of HACCP programme in any food production system, it is important to understand the potential hazards associated with that particular category of food. HACCP is a scientific and systematic approach to identify, assess and control hazards in the food production process. With the HACCP system, food safety control is integrated into the design of the process rather than relied on end-product testing. Therefore, HACCP system provides a preventive and thus cost-effective approach in food safety.

The HACCP system

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

Pre-requisite programmes (PRPs)

PRPs such as standard operating procedures (SOP), sanitation standard operating procedures (SSOP), good manufacturing practises (GMP), etc. are implemented prior to HACCP plans. PRPs focus on employees, facilities and equipment and deals with illness policy, cleaning and sanitizing procedures, garbage removal, pest control, equipment selection, employee hygiene. It also deals

with control of harvest operation and the overall plant environment which are not directly related to food (e.g. water quality, transportation and storage, plant sanitation, employee training, etc.).

The hazard analysis worksheet

A hazard-analysis worksheet can be used to organize and document the considerations in identifying food-safety hazards. Although there is no specific or required form, the worksheet should document specific information as required by FDA (Food and Drug Administration, USA). The first two principles of HACCP is being taken care by HACCP worksheet. Each worksheet should bear the name and address of the production unit, name of the product, intended use of the product and target consumers and method of storage and distribution. Obviously separate worksheet is required for each class of products. The Seafood HACCP Regulation requires that all seafood processors conduct, or have conducted for them, a hazard analysis to determine whether there are food safety hazards that are reasonably likely to occur in their product and to the preventive measures that a processor can apply to control those hazards (21 CFR 123.6(a)). FDA has found that the use of a standardized Hazard Analysis Worksheet assists with this process.

HACCP plan

It is a document prepared in accordance with the principles of HACCP to ensure control of hazards that are significant for food safety in the segment of the food chain under consideration. It is implemented following pre-requisite programmes. Prior to the application of HACCP to a fish or seafood establishment, that establishment should be operating proper prerequisite programmes according to the Recommended International Code of Practice –General Principles of Food Hygiene (CAC/RCP 1-1969, Revision 2008/2020). Management awareness and commitment are necessary for the implementation of an effective HACCP system. The effectiveness will also rely upon management and employees having the appropriate HACCP knowledge and skills. Therefore, ongoing training is necessary for all levels of employees and managers, as appropriate. If the necessary expertise is not available on-site for the development and implementation of an effective HACCP plan, expert advice should be obtained from other sources, such as trade and industry associations, independent experts and regulatory authorities. Two steps are involved in HACCP plan preparation.

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

Preliminary steps

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

HACCP principles

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

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

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

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

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

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

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

3. Identify the intended use:

The intended use should be based on the expected uses by the end user or consumer. The use and preparation before use greatly influence the safety of the product. Certain products may carry harmful organisms as part of the natural flora. If the processing does not include a killing step, the only possibility to render the product safe is adequate heat treatment (e.g. cooking) during preparation. It is important to identify whether the product is to be used in a way that increases the

risk of harm to the consumer, or whether the product is particularly used by consumers who are especially susceptible to a hazard. In specific cases, e.g. institutional feeding, vulnerable groups of the population, such as elderly and infants, must be considered.

4. Construct a process flow diagram:

A flow diagram should be constructed by the HACCP team to provide a clear and simple description of all steps involved in the operation. When applying HACCP to a given operation, consideration should be given to steps preceding and following the specific operation. Receiving and storage steps for raw materials and ingredients should be included. Time and temperature conditions during processing should be mentioned whenever there is a holding step, e.g. in holding vats, buffer tanks or other areas, where there could be a potential delay or temperature abuse.

5. On site verification of the process flow diagram:

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

Principles of HACCP

1) Conduct a hazard analysis and identify control measures

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

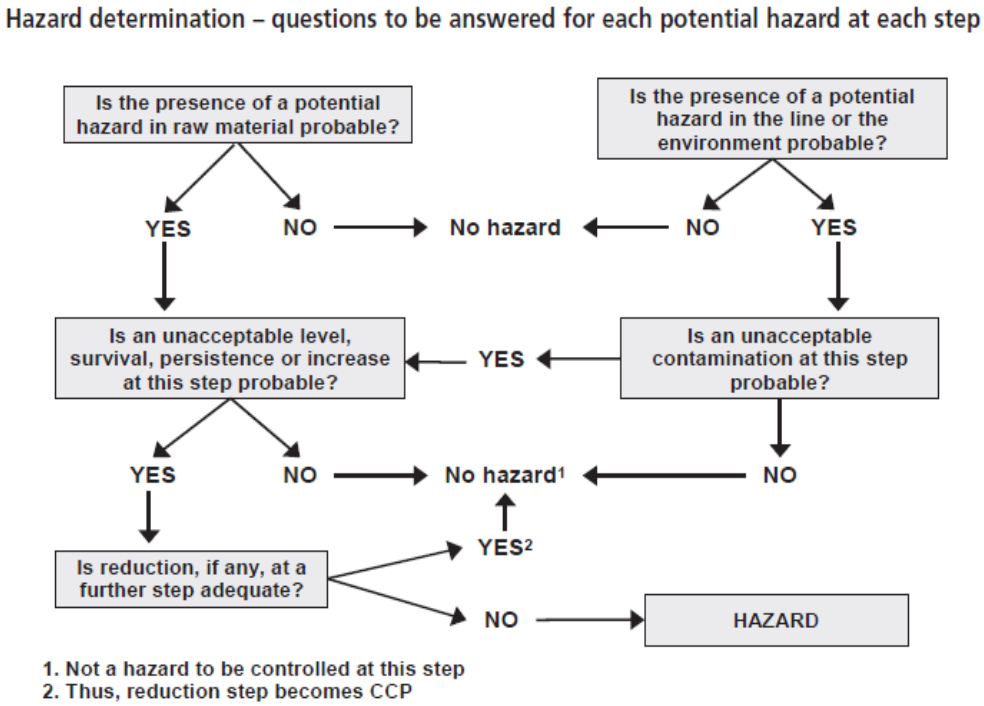


Fig 2. Hazard determination decision tree

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

USFDA suggested following control measure for seafood-borne hazards:

Pathogenic bacteria:

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

Pathogenic viruses:

- Cooking, source control from acceptable region

Parasites:

- Cooking, freezing.

Chemical hazard:

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

Physical hazard:

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

1. Determine CCPs

A CCP is a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level. CCPs are product and process specific. There may be more than one CCP at which control is applied to address the same hazard. Likewise, several hazards can be controlled at a single CCP. Complete and accurate identification of all the CCPs is

fundamental for controlling food safety hazards. The determination of a CCP in the HACCP system can be facilitated by the application of a decision tree.

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

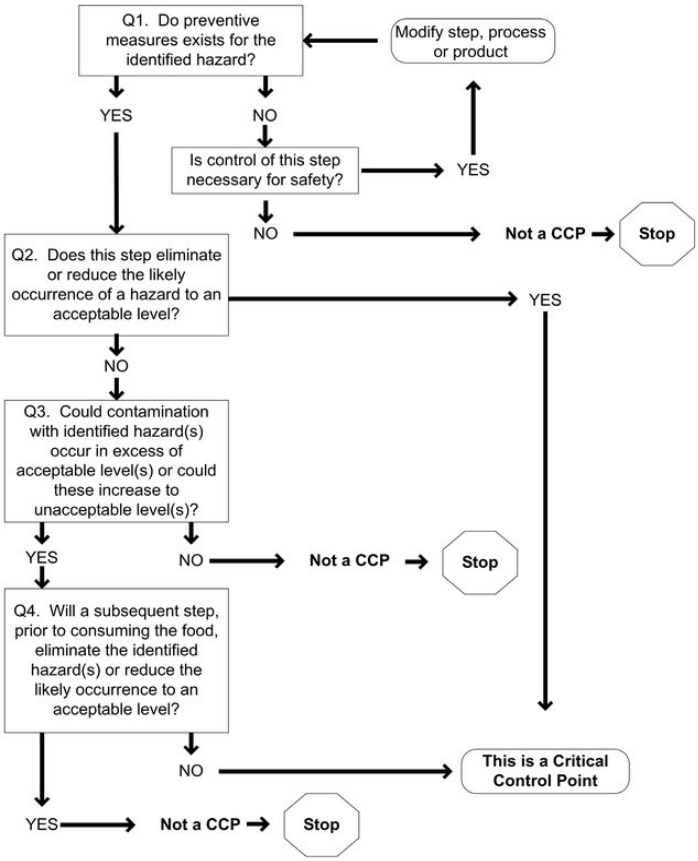


Fig 3. CCP decision tree

2. Establish validated critical limits

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

legal and commercial standards required for the products. Example: There is a cooking (80°C for 2.5 min) step in the process line to control biological hazard. Here predefined time and temperature is the CL.

3. Establish a system to monitor control of CCPs

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

Components:

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

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

As the main reason for implementing HACCP is to prevent problems from occurring, corrective actions should be predefined and taken when the results of monitoring at the CCP indicate a loss of control. Loss of control can cause a deviation from a critical limit for a CCP. All deviations must be controlled by taking predetermined actions to control the non-compliant product and to correct the cause of non-compliance. Product control includes proper identification, control and disposition of the affected product. The establishment should have effective procedures in place to identify, isolate (separate), mark clearly and control all products produced during the deviation period. Corrective action procedures are necessary to determine the cause of the problem, take action to prevent recurrence and follow up with monitoring and reassessment to ensure that the action taken is effective. Reassessment of the hazard analysis or modification of the HACCP plan may be necessary to eliminate further recurrence. The control and disposition of the affected product and the corrective actions taken must be recorded and filed. Records should be available to demonstrate the control of products affected by the deviation and the corrective action taken. Adequate records permit verification that the establishment has deviations under control and has taken corrective action.

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

Verification is the application of methods, procedures and tests, including random sampling and analysis and other evaluations, in addition to monitoring, to determine compliance with the HACCP

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

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

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

- Support documentation, including validation records, for developing the HACCP plan;
- Records generated by the HACCP system: monitoring records of all CCPs;
- Deviation and corrective action records, verification/validation records;
- Documentation on methods and procedures used;
- Records of employee training programmes.

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

Table 1. Hazard analysis worksheet

Product Name

Firm Name:			Product Description:		
Firm Address:			Method of Distribution and Storage:		
			Intended Use and Consumer:		
(1)	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**
Ingredient/ processing step	**Identify potential biological, chemical, and physical hazards associated with this product and process**	**Are any potential food safety hazards significant at this step?**	**Justify your decision for column 3**	**What preventive measure(s) can be applied for the significant hazards?**	**Is this Step a Critical Control Point?**

		(Yes/No)			(Yes/No)

Signature of Company Official: _____Date: _____

Table 2. HACCP plan form

Firm Name:					Product Description:				
Firm Address:					Method of Distribution and Storage:				
					Intended Use and Consumer:				
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Critical Control Point	Significant Hazard(s)	Critical Limits	Monitoring				Corrective Action(s)	Verification	Records
			What	How	Frequency	Who			

Signature of Company Official: _____Date:

_____

Conclusion

The safety of seafood products varies considerably and is influenced by a number of factors such as origin of the fish, microbiological ecology of the product, handling and processing practices and preparations before consumption. However, the food safety hazards and risk in seafood products

cannot be made nil through any approach, it can only be minimized or reduced to an acceptable level. A large number of hazards are related to the pre-harvest situation or raw-material handling and must be under control by implementation of HACCP when the raw material is received at the processing factory.

References:

- Food and Drug Administration. (2022). Fish and fishery products hazards and controls guidance. US Department of Health and Human Services Food and Drug Administration Centre for Food Safety and Applied Nutrition, pp 454.
- https://cdhd.idaho.gov/pdfs/food/HACCP%20worksheets.pdf
- https://southcenters.osu.edu/sites/southc/files/site-library/site-documents/abc/marketprocess_addresources/HACCP%20for%20fin%20fish%20.pdf
- https://www.fda.gov/food/hazard-analysis-critical-control-point-haccp/haccp-principles-application-guidelines

****

34

CHEMICAL CONTAMINANTS IN FISH AND FISHERY PRODUCTS

Laly S.J.
Quality Assurance & Management Division
Email: lalyjawahar@gmail.com

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

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

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

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

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

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

Biotoxins

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

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

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

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

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

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

Heavy metals

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

upon geographic location, species and fish size, feeding pattern, solubility of chemical and their persistence in the environment.

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

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

Histamine in fish

Though all types of biogenic amines can be formed in fish, the most toxic amine detected in fish is histamine. Histamine poisoning is the most common form of toxicity caused by ingestion of fish and is generally due to the ingestion of foods containing unusually high levels of histamine. The commonly implicated incidents of histamine poisoning are associated with the fish families Scombridae and Scomberesocidae. It is also known as Scombroid poisoning. Histamine is a powerful biologically active chemical present in the mast cells and basophils in larger amounts. Histamine poisoning is often manifested by a wide variety of symptoms. Major symptoms affecting the cutaneous system include rashes, urticaria, edema and localized inflammation etc. gastrointestinal effects include nausea, vomiting, diarrhoea and abdominal cramps. Also include symptoms like hypotension, headache, palpitation, tingling and flushing. Severe suffocation and respiratory distress have been reported in severe cases of histamine poisoning. The onset of histamine poisoning can extend from 10 minutes to 1 hour following consumption of contaminated fish and can last from 12 hour to a few days. Histamine concentration required to produce poisoning varies with respect to the susceptibility of each individual. In case of susceptible individuals concentration between 5 and 10 mg/100g can cause symptoms. Many foods contain small amounts of histamine which can be tolerated easily.

As per USFDA guideline the toxicity and defect action level established are 50 mg/100g and 5 mg/100g respectively. According to EU regulation No 2073/2005 mean value all samples (nine) must not exceed 10 mg/100g, two samples may be > 10 mg/100g but < 20

mg/100g and no sample may exceed 20 mg/ 100g. According to USFDA guideline for the control of histamine production a core temperature of 4.4 ºC or less should be achieved and maintained throughout handling, processing and distribution of susceptible species.

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

Antibiotics

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

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

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

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

The monitoring of antimicrobial residues in fish tissues requires sensitive and selective analytical methodologies to verify the accomplishment of the legal framework and reach the desirable high standards of quality and food safety. The methods can be microbiological,

immunochemical or physico chemical. European council directive 96/23/EC, 1996 gives direction on measures of monitoring residues in live and animal products. It specifies spectrometric detection, GC, HPLC, ELISA and LC-MS/MS methods.

Pesticides

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

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

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

Food additives

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

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

Classification of food additives

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

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

****

35

FUNDAMENTALS OF BACTERIOLOGY OF FISH

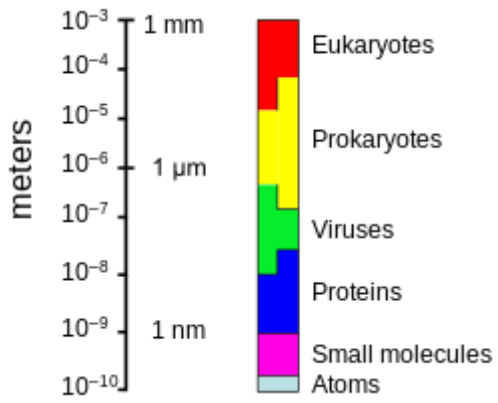
Ranjit Kumar Nadella and Satyen Kumar Panda

Quality Assurance & Management Div

nranjeetnadella @gmail.com

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

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



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

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

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

b) Bacillus Bacteria (Rod-Shaped): Bacillus bacteria have the following traits: Are all rod-shaped, form endospores and are facultative anaerobes. bacillus bacteria are also arranged differently. While some exist as single, unattached cells (e.g. *Salmonella enterica* subsp, *Bacillus cereus*, and *Salmonella choleraesuis*), others are attached. The following are the different types of bacillus arrangements: *Diplobacilli bacteria* - Like Diplococci bacteria, Diplobacilli occur in pairs. Following cell division, the two cells do not separate and continue

existing as a pair. Examples of Diplobacilli bacteria include: *Coxiella burnetii*, *Klebsiella rhinoscleromatis*, *Moraxella bovis*. **Coccibacilli bacteria** - Compared to other bacilli, Coccibacilli bacteria are shorter in length and thus appear stumpy. Examples of Coccibacilli include: *Chlamydia trachomatis*, *Haemophilus influenza*. Unlike cocci and bacilli bacteria, some types of bacteria appear curved when viewed under the microscope. However, they vary in shape making it possible to differentiate them from each other. These include: Vibrio bacteria - Generally, vibrio bacteria are comma-shaped and thus not fully twisted (curved rods). Examples of Vibrio bacteria include: *Vibrio mytili*, *Vibrio anguillarum*, *Vibrio parahaemolyticus*, *Vibrio cholerae*. **Spirochete** - Spirochetes are characterized by a helical shape. Spirochetes are also flexible and have been shown to produce mycelium. The movement involves the use of axial filaments, which is one of the distinguishing features between the bacteria and other types of bacteria. Examples of Spirochetes include: Leptospira, Spirochaeta,
Treponema. **Spirilla** bacteria - Like Spirochetes, Spirilla bacteria possess a helical shape. However, they are more rigid and have the typical flagella found in other types of bacteria. Some examples of Spirilla bacteria include: *Aquaspirillum*, *Campylobacter jejuni*, *Spirillum winogradskyi*.

In microbiology and bacteriology, Gram stain or Gram staining, also called Gram's method, is a method of staining used to classify bacterial species into two large groups: gram-positive bacteria and gram-negative bacteria. The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique in 1884. Gram staining differentiates bacteria by the chemical and physical properties of their cell walls. Gram-positive cells have a thick layer of peptidoglycan in the cell wall that retains the primary stain, crystal violet. Gram-negative cells have a thinner peptidoglycan layer that allows the crystal violet to wash out on addition of ethanol. They are stained pink or red by the counterstain, commonly safranin or fuchsine. Lugol's iodine solution is always added after addition of crystal violet to strengthen the bonds of the stain with the cell membrane. Gram staining is almost always the first step in the preliminary identification of a bacterial organism. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique. Acid-fast staining is the differential staining techniques which was first developed by Ziehl and later on modified by Neelsen. So this method is also called Ziehl-Neelsen staining techniques. Neelsen in 1883 used Ziehl's carbol-fuchsin and heat then decolorized with an acid alcohol, and counter stained with methylene blue. Thus Ziehl-Neelsen staining techniques was developed. The main aim of this staining is to differentiate bacteria into acid fast group and non-acid fast groups. This method is used for those microorganisms which are not staining by simple or Gram staining method, particularly the member of genus Mycobacterium, are resistant and can only be visualized by acid-fast staining.

Growth Curve

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

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

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

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

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

Physical factors that affect microbial growth

a) Temperature: Generally,an increase in temperature will increase enzyme activity. But if temperatures get too high, enzyme activity will diminish and the protein (the enzyme) will denature. On the other hand, lowering temperature will decrease enzyme activity. At freezing temperatures enzyme activity can stop. Repeated cycles of freezing and thawing can denature proteins. In addition, freezing causes water to expand and also forms ice crystals, hence cells begin to rupture. Every bacterial species has specific growth temperature requirements which is largely determined by the temperature requirements of its enzymes. PSYCHROPHILES grow best between -5°C and 20°C, MESOPHILES grow best between 20°C and 45°C and THERMOPHILES grow best at temperatures above 45°C. THERMODURIC organisms can survive high temperatures but don't grow well at such temperatures. Organisms which form

endospores would be considered thermoduric. Some organisms have exotic temperature requirements. *Thermus aquaticus* is a bright orange gram negative rod isolated from hot water and steam vents at Yellowstone Park. This organism grows best at temperatures between 70-75°C (158-167°F). Some of its unique enzymes are in demand for molecular biological and industrial applications.

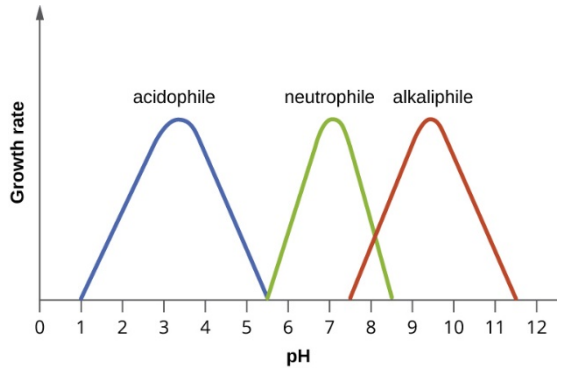
b) **Oxygen**: Microbes display a great diversity in their ability to use and to tolerate oxygen. In part this is because of the paradoxical nature of oxygen which can be both toxic and essential to life. OBLIGATE AEROBES rely on aerobic respiration for ATP and they therefore use oxygen as the terminal electron acceptor in the electron transport chain. Pseudomonas is an example of this group of organisms. MICROAEROPHILES require O_2 for growth but they are damaged by normal atmospheric levels of oxygen and they don't have efficient ways to neutralize the toxic forms of oxygen such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2). The Streptococci are examples of this group. OBLIGATE ANAEROBES will die in the presence of oxygen because they lack enzymes like superoxide dismutase and catalase. Organisms like Clostridium, metabolize through fermentation and / or anaerobic respiration.

AEROTOLERANT organisms like Lactobacillus ferment and therefore do not use oxygen, however they do tolerate it. FACULTATIVE ANAEROBES are the most adaptable. They are capable of both fermentation and aerobic respiration. *Escherichia coli* is an example of this class of organisms. ANAEROBIC PATHOGENS: *Clostridium tetani* - agent of tetanus, puncture wounds, produces a toxin which enters the spinal column and blocks the inhibitory spinal motor neurons. This produces generalized muscle spasms or spastic paralysis. *Clostridium botulinum* - this soil organism is the causative agent of botulism which typically occurs after eating home canned alkaline vegetables which were not heated enough during canning. The neurotoxin blocks transmission across neuromuscular junctions and this results in flaccid paralysis. *Clostridium perfringes* and *Clostridium sporogenes* - these organisms are associated with invasive infections known as GAS GANGRENE. *Clostridium difficile* - the causative agent of pseudomembranous colitis, a side effect of antibiotic treatment which eliminates the normal flora. MICROAEROPHILES: These organisms are all catalase negative, therefore the catalase test is useful in identification. They also have distinctive colonial morphology on blood agar which is differential for them. It is important to note if the colonies are alpha, beta, or gamma hemolytic. Group A Streptococcus - *Streptococcus pyogenes,* This beta hemolytic organism is also bacitracin sensitive. It is the cause of strep throat, rheumatic fever, glomerulonephritis and scarlet fever. Group D Streptococcus - Enterococcus - *Streptococcus faecalis*, This organism is a normal inhabitant of the large intestine. It is also a frequent cause of bladder infections. *Streptococcus pneumonia*, This organism is a normal inhabitant of the respiratory tract. It is a frequent cause of pneumonia in people who have been compromised by other illness.

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

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

Based on pH bacterial requirements are classified as follows:



Most bacteria are neutrophiles, meaning they grow optimally at a pH within one or two pH units of the neutral pH of 7. Most familiar bacteria, like *Escherichia coli*, *Staphylococci*, and *Salmonella* spp. are neutrophiles and do not fare well in the acidic pH of the stomach. However, there are pathogenic strains of *E. coli*, *S. typhi*, and other species of intestinal pathogens that are much more resistant to stomach acid. In comparison, fungi thrive at slightly acidic pH values of 5.0-6.0. Microorganisms that grow optimally at pH less than 5.55 are called acidophiles. Eg. *Lactobacillus* bacteria. Acidophilic microorganisms display a number of adaptations to survive in strong acidic environments. For example, proteins show increased negative surface charge that stabilizes them at low pH. Pumps actively eject H^+ ions out of the cells. At the other end of the spectrum are alkaliphiles, microorganisms that grow best at pH between 8.0 and 10.5. *Vibrio cholerae*, the pathogenic agent of cholera, grows best at the slightly basic pH of 8.0; it can survive pH values of 11.0.

Foodborne bacterial pathogens

Foodborne pathogens are mainly bacteria, viruses, or even parasites that are present in the food and are the cause of major diseases such as food poisoning. Foodborne pathogens are categorized according to the specific foods that are consumed. Foodborne illness occurs when a pathogen is ingested with food and establishes itself (and usually multiplies) in the human host, or when a toxigenic pathogens establishes itself in a food product and produces a

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

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

a) *Clostridium botulinum*

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

b) ***Clostridium perfringens***

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

c) ***Bacillus cereus***

- **Bacteria:** Facultatively aerobic, spore-forming, motile GPR
- **Source:** Soil, dust, raw foods
- **Illness:** 1) diarrheal type (infection, heat-labile toxin); 2) emetic type (intoxication, heat-stable toxin)
- **Symptoms:** 1) profuse watery diarrhea, abdominal pain; 2) vomiting, nausea
- **Foods:** 1) vegetables, salads, meats, casseroles; 2) rice **Transmission:** Spores present in raw foods
- **Control:** time/temperature; reheat cooked foods to >165° F
- **Isolation:** The bacterium is isolated on commonly used microbiological media such as nutrient agar.

C) ***Campylobacter jejuni***

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

- **Identification:** PCR

d) Pathogenic *Escherichia coli* O157:H7

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

e) *Listeria monocytogenes*

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

f) ***Salmonella spp.***

- **Bacteria:** Facultative anaerobic, motile, GNR
- **Source:** Intestine of mammals, birds, amphibians and reptiles
- **Illness:** Infection (gastroenteritis)
- **Symptoms:** Nausea, vomiting, abdominal cramps, fever
- **Foods:** Poultry, poultry salads, meats, dairy products, egg products
- **Transmission:** Cross-contamination, human contamination, sewage pollution of coastal waters
- **Control:** Proper cooking, temperature control, preventing cross-contamination, personal hygiene
- **Isolation:** The bacterium is isolated from the samples by using Buffered peptone water incubated at 37 °C for 24 h followed by enrichment in Rappaport and Vassiliadis broth (incubation at 41.5 °C for 24 h), Muller-Kauffman Tetrathionate Novobiocin broth (incubation at 37 °C for 24 h) and later streaking on XLD agar incubated at 37 °C for 24 h under aerophilic conditions. On XLD agar it produces red colour colonies with black centre.
- **Identification:** Biochemical, serological and PCR

g) ***Shigella spp.***

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

h) ***Pathogenic Staphylococcus aureus***

- **Bacteria:** Facultative anaerobic, motile, GNR
- **Source:** Intestine of mammals, birds, amphibians and reptiles
- **Illness:** Infection (gastroenteritis)

- **Symptoms:** Nausea, vomiting, abdominal cramps, fever
- **Foods:** Poultry, poultry salads, meats, dairy products, egg products
- **Transmission:** Cross-contamination, human contamination, sewage pollution of coastal waters
- **Control:** Proper cooking, temperature control, preventing cross-contamination personal hygiene
- **Isolation:** The bacterium is isolated from the samples by using Baird parker agar supplemented with egg yolk and potassium telurite incubated initially at 35 °C for 24 h under anerobic conditions. *Staphylococcus aureus* is characterized by the formation of black, shiny, convex colonies surrounded by a lightening halo of the egg yolk. Coagulase negative staphylococci are almost completely inhibited and if, however, a culture does appear, areas of thinning would be absent.
- **Identification:** Mannitol fermentation, genotypic characterisation (pvl, spa typing, SCCmec typing) and phenotypic characterization (growth on ORSAB agar)

i) ***Vibrio cholerae***

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

j) ***Vibrio parahaemolyticus***

- **Bacteria:** Facultative aerobic, motile, curved GNR
- **Source:** Naturally occurring in estuaries and other coastal areas throughout the world
- **Illness:** Infection (gastroenteritis)
- **Symptoms:** Diarrhea, abdominal cramps, nausea, vomiting, headache
- **Foods:** Raw, improperly cooked, or cooked and contaminated fish and shellfish

- **Transmission:** Cross-contamination from raw to cooked seafood, consumption of raw seafood
- **Control:** Proper cooking, preventing cross-contamination
- **Isolation:** The bacterium is isolated from the samples by using alkaline salt peptone water incubated initially at 37 °C for 6-18 h under anerobic conditions followed by streaking on TCBS agar incubated under aerophilic conditions (37 °C for 18-20 h). *Vibrio parahaemolyticus* produces colorless colonies with a green center.
- **Identification:** Biochemical tests, pathotyping and PCR

k) ***Yersinia enterocolitica***

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

****

36

NATIONAL AND INTERNATIONAL STANDARDS FOR FISHERY PRODUCTS

Niladri Sekhar Chatterjee and Pankaj Kishore
Quality Assurance & Management Divisi
Email: niladri.sekhar.chatterjee@gmail.co

Healthy foods are need of the todays' consumer and Food Safety is equally important for human health protection. Food safety is negatively affected by a variety of food contamination along food supply chain. Contamination of food products may cause by physical, chemical, biological, and radiological hazards. Controlling such hazards can be possible by a proper control program for food safety, issues such as foodborne illness and food-related injuries can occur. Defining the actual goal of food safety has been an arduous task as there are umpteen interrelated factors that influence the intended goals. Some of the definitions on food safety put forward by international agencies are as follows: a) Concept that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use (ISO 22000:2005), b) A suitable product that when consumed orally either by a human or an animal does not cause health risks to consumer (USDA-FSIS), c) Range of food-related activities from prevention and surveillance to detection and control (ASTHO)

Food Safety also encompasses many aspects of handling, preparation and storage that introduce or control chemical, microphysical and microbiological hazards. Quality of raw material, presence of pathogens, processing methods, climate change and cross-contamination also significantly impact any food safety measure. Seafood is always in the news as it is proclaimed to be the most nutritious and healthy food as well as being linked to an increasing number of foodborne outbreaks across the globe. In the nutritional front, fish accounts for 17 percent of the global population intake of animal protein and 6.7% of all protein consumed (FAO, 2016). The world per capita consumption of fish and fishery products has increased from 9.9 Kg in 1960s to 20 Kg in 2014.

Seafood trade apart from being highly volatile accounts for 10 percent of total agricultural exports and 1 percent of world merchandise trade in value terms. In 2010, the quantum of seafood trade has crossed US$109 billion. Ninety percent of global trade in fish and fishery products consists of processed products, where 39% of the total quantity is traded as frozen. This trend indicates high mobility of the fishery products across the globe, which demands stringent traceability system in place to track the movement of the commodity from harvest to consumers. Nearly 75% of the volume of seafood in international trade is imported by

developed nations and 50% of that is exported by developing nations. Hence, food safety issues concerned with seafood is no more local or restricted to a particular geographical location, but has acquired global dimension. Some of the major food safety concerns linked to seafood are:

- Marine Biotoxins
- Histamine fish poisoning
- Viruses and pathogenic bacteria
- *Clostridium botulinum* in processed products
- High level of environmental pollutants
- Heavy metal contaminations
- Polychlorinated biphenyls and pesticides
- Antimicrobial residues in aquaculture products
- Unapproved additives

The most challenging task for the policy makers has been to link incidences of foodborne illnesses with a particular food commodity. It needs a strong surveillance and monitoring mechanism to unequivocally attribute a particular food commodity. In USA, Centre for Disease Control (CDC) does the massive work of source tracking for major foodborne pathogens through pulse net programmes. The recent report by CDC (Scallan et al., 2011) indicates that 31 major pathogens reported in the United States caused 9.4 million episodes of foodborne illness, 55,961 hospitalizations and 1,351 deaths during 2. Most (58%) illnesses were caused by norovirus, followed bynon-typhoidal *Salmonella* spp. (11%), *Clostridium perfringens* (10%), and *Campylobacter* spp. (9%). Leading causes of hospitalization were nontyphoidal *Salmonella* spp. (35%), norovirus (26%), *Campylobacter* spp. (15%), and *Toxoplasma gondii* (8%). Leading causes of death were non-typhoidal *Salmonella* spp. (28%), *T. gondii* (24%), *Listeria monocytogenes* (19%), and norovirus (11%). In India, the recently established National Centre for Disease Control (formerly, National Institute of Communicable Diseases), Ministry of Health and Family Welfare, Government of India has a similar mandate to undertake activities on outbreak investigation and provide referral diagnostic services.

In absence of etiological data linked to seafood, the export rejection figures provides an indirect account of food safety hazards associated with seafood. Import refusals and rejections from countries like USA, Japan, Russia and EU are on the rise because of presence of biological and chemical hazards in seafood, leading to heavy economic loss by seafood industries. The most common import refusal of seafood by USA is due to presence of *Salmonella*, *Listeria*, filth or illegal veterinary drugs. The RASFF portal of EU indicates alert notifications due to presence of veterinary drug residues, heavy metals, histamine, foreign bodies, biotoxin, defective packaging, incorrect labelling, improper health certificate, unapproved colour and additives and organoleptic aspects. In recent months most of the

rejections from Japan had been due to presence of furazolidone (AOZ) and Ethoxyquin in shrimp. Seafood rejections from Russia are mostly due to presence of high load of mesophilic bacteria, coliforms, pathogens and presence of crystal violet.

Genesis of Food Safety Standards and Regulations

Food safety standards can be classified as regulatory, voluntary, Government/Statutory, private, domestic, international or benchmarked depending upon its scope and range of application. Most of these standards have evolved based upon sanitary and phyto-sanitary (SPS) requirements, economic interest, risk analysis or as precautionary approach. The precautionary approach mostly relies on perception i.e. equivalent level of protection, appropriate level of protection (ALOP) or as low as reasonably achievable (ALARA).

In international trade, sanitary and phytosanitary measures are envisioned to be based on sound scientific principles that ensure food safety and do not anyway compromise the production potential and resources of a particular country. These measures should not be linked to prevent market access based on non-scientific reasons, and are requirements but not sufficient condition of trade. As per the Annex A of WTO Agreement, Sanitary and phytosanitary measures are applied to (i) protect animal or plant life or health within the territory of the Member from risks arising from the entry, establishment or spread of pests, diseases, disease-carrying organisms or disease-causing organisms (ii) to protect human or animal life or health within the territory of the Member from risks arising from additives, contaminants, toxins or disease-causing organisms in foods, beverages or feedstuffs (iii) from risks arising from diseases carried by animals, plants or products thereof, or from the entry, establishment or spread of pests and (iv) to prevent or limit other damage within the territory of the Member from the entry, establishment or spread of pests. WTO encourages members to use accepted international standards by Codex Alimentarius Commission, OIE (World Organization for Animal Health) and IPPC (International Plant Protection Convention). Countries may introduce or maintain SPS measures that provide higher level of protection than the current international or Codex standards.

Salient features of some Export regulations related to Seafood European Union

European Union is the biggest importer of fish and fishery products in the world. The food safety regulations set by EU is harmonised, gets periodically updated, transparent and based on principles of risk assessment. The key elements of EU requirements for import of seafood are (a) certification by a competent authority (b) compliance to hygiene and public health requirements in terms of structure of vessels, landing sites, processing establishments and on operational processes, freezing and storage (c) certified production area for bivalves (d) national control plan on heavy metals, contaminants, residues of pesticides and veterinary drugs (e) approval of establishments.

The legal acts of EU are managed through regulations, directives, decision, recommendations and opinions.

Regulation: A binding legislative act applied in entirety across EU

Directives: A "directive" is a legislative act that sets out a goal that all EU countries must achieve.

Decision: A "decision" is binding on those to whom it is addressed (e.g. an EU country or an individual company) and is directly applicable.

Recommendations: A "recommendation" is not binding act that allows the institutions to make their views known and to suggest a line of action without imposing any legal obligation on those to whom it is addressed.

Opinions: An "opinion" is an instrument that allows the institutions to make a statement in a non-binding fashion, in other words without imposing any legal obligation on those to whom it is addressed.

Some of the important EU legislations related to food safety issues of fish and fishery products are as follows:

Regulation (EC) No 178/2002: General principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety

Regulation (EC) No 852/2004: Hygiene of foodstuffs.

Regulation (EC) No 853/2004: Specific hygiene rules for food of animal origin

Regulation (EC) No 854/2004: Specific rules for the organisation of official controls on products of animal origin intended for human consumption

Regulation (EC) No 2073/2005: Microbiological criteria for foodstuffs

Regulation (EC) No 882/2004: Official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules

Regulation (EC) No 1881/2006: Maximum levels for certain contaminants in foodstuffs

Regulation (EC) No 333/2007: Methods of sampling and analysis for the official controls for the levels of lead, cadmium, mercury, inorganic tin, 3- MCPD and benzo(a)pyrene in foodstuffs

Regulation (EC) No 1883/2006: Methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs

Regulation (EC) No 396/2005: Maximum residue levels of pesticides in or on food and feed of plant and animal origin

Council Directive 96/23/EC: Measures to monitor certain substances and residues thereof in live animals and animal products

Commission Decision (2005/34/EC): Harmonised standards for the testing for certain residues in products of animal origin imported from third countries

Commission Decision (2002/657/EC): Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results

Commission Decision (98/179/EC): Official sampling for the monitoring of certain substances and residues thereof in live animals and animal products

Commission Decision (2004/432/EC): Approval of residue monitoring plans submitted by third countries in accordance with Council Directive 96/23/EC

Council Directive 96/22/EC: Prohibition on the use in stock farming of certain substances having a hormonal or thyrostatic action and of betaagonists

Regulation (EC) No 470/2009: Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin

Commission Regulation (EU) No 37/2010: Pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin

Commission Regulation (EC) No 2023/2006: Good manufacturing practice for materials and articles intended to come into contact with food

Commission Regulation (EC) No 1935/2004: Materials and articles intended to come into contact with food

Commission Regulation (EU) No 1129/2011: Amendment to Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the

Council by establishing a Union list of food additives Commission Regulation (EC) No 1333/2008 : Food Additives

Commission Regulation (EC) No 1334/2008: Flavourings and certain food ingredients with flavouring properties for use in and on foods

Commission Regulation (EC) No 1331/2008: Establishing a common authorisation procedure for food additives, food enzymes and food flavourings

Directive 2000/13/EC: Labelling, presentation and advertising of foodstuffs (until 12 December 2014)

Commission Regulation (EU) No 1169/2011: Provision of food information to consumers, amending Regulations

Commission Regulation (EU) No 1379/2013: Common organisation of the markets in fishery and aquaculture products

USA

In USA both Federal and State Regulatory agencies are involved in ensuring safety and quality of seafood. Multiple federal agencies are involved in regulatory oversight of seafood for both importation and export.

United States Department of Agriculture (USDA) oversees the implementation of country-of-origin labelling (COOL) regulation enacted under the Farm Security and Rural Investment Act of 2002. This law requires that all retailers, such as full-line grocery stores or supermarkets must notify their customers with information regarding the source of certain foods. The COOL regulation for fish and shellfish (7 CFR Part 60) came into force in 2005. Apart from the

country of origin, all fish and shellfish covered commodities must be labelled to indicate whether they are wild caught or farm-raised.

United States Fisheries and Wildlife Service (USFWS) is also involved in regulation of import and export of shellfish and fishery products through Convention on International Trade in Endangered Species (CITES) act (50 CFR Part 23), Endangered Species Act (50 CFR Part 17), General Permit Procedures (50 CFR Part 13), Lacey Act (injurious wildlife) (50 CFR Part 16), Marine Mammal Protection Act (50 CFR Part 18) and Wildlife (import/export/transport) act (50 CFR Part 14). Live farm-raised fish and farm-raised fish eggs are exempted from export declaration and licensing requirements. Imports or exports of any sturgeon or paddlefish product, including meat, caviar, and cosmetics made from sturgeon eggs, dead uneviscerated salmon, trout and char and live fertilized eggs from these salmonid fish require a permit. Aquatic invertebrates and other animals that are imported or exported for human or animal consumption but that do not meet the definition of shellfish such as squid, octopus, cuttlefish, land snails, sea urchins, sea cucumbers and frogs are also covered under these provisions.

National Oceanic and Atmospheric Administration (NOAA) functioning under the United States Department of Commerce (USDC) provides voluntary seafood inspection program for fish, shellfish, and fishery products to the industry as per the 1946 Agricultural Marketing Act. The NOAA Seafood Inspection Programme often referred to as the U.S. Department of Commerce (USDC) Seafood Inspection Programme provides services such as establishment sanitation inspection, system and process audits, product inspection and grading, product lot inspection, laboratory analyses, training, consultation and export certification. NOAA Fisheries is the Competent Authority for export health certification and IUU catch documentation for US seafood products meant for export to EU and non-EU countries.

The U.S. Food and Drug Administration (USFDA) is vested with the primary Federal responsibility for the safety of seafood products in the United States. It operates a mandatory safety program for all fish and fishery products under the provisions of the Federal Food, Drug and Cosmetic (FD&C) Act, the Public Health Service Act, and related regulations. The most important regulation enacted by USFDA was “Procedures for the Safe and Sanitary Processing and Importing of Fish and Fishery Products” published as final rule 21 CFR 123 on 18th December 1995 and came into force on 18th December 1997. It required processors to adopt the preventive system of food safety controls known as HACCP (Hazard Analysis and Critical Control Point). Seafood was the first food commodity in the U.S. to adopt HACCP in USA. For screening imports, USFDA uses a tool “Predictive Risk-based Evaluation for Dynamic Import Compliance Targeting (PREDICT)”, that targets higher risk products for examination and sampling and minimizes the delay in shipments of lower risk products.

Food Safety and Modernization Act (FSMA) is the most important milestone event in the food safety scenario in USA. It was signed in to law on 4th January 2011 which sifted the focus from responding to a contamination to prevention of the actual cause. The salient features of FSMA act are as follows:

Sec. 103.Hazard analysis and risk-based preventive controls

(HARPC): Requires human and animal food facilities to

- evaluate hazards that could affect food safety;
- Identify and implement preventive controls to prevent hazards;
- Monitor controls and maintain monitoring records; and
- Conduct verification activities

Sec. 106.Protection against intentional adulteration

Sec. 111. Sanitary Transportation of Food

Sec. 301. Foreign supplier verification program

• Requires importers to verify their suppliers use risk-based preventive controls that provide same level of protection as U.S. requirements.

Sec. 302. Voluntary qualified importer program

Allows for expedited review and entry; facility certification required

Sec. 303. Certification for high-risk food imports

• FDA has discretionary authority to require assurances of compliance for high-risk foods

Sec. 304. Prior notice of imported food shipments

• Requires information on prior refusals to be added to prior notice submission

• Effective July 3, 2011

Sec. 307. Accreditation of third-party auditors

• FDA can rely on accredited third parties to certify that foreign food facilities meet U.S. requirements

Sec. 308. Foreign Offices of the Food and Drug Administration.

• Establish offices in foreign countries to provide assistance on food safety measures for food exported to the U.S.

Sec. 309. Smuggled Food

• In coordination with DHS, better identify and prevent entry of smuggled food

• Rules on anti-smuggling strategy is already framed

China

In recent years China has strengthened its SPS measures and has taken a number of precautionary steps to ensure safety to its population. Some of the important regulations enacted by Peoples Republic of China are as follows:

- GB 2763—2012: National food safety standard on Maximum residue limits for pesticides in food

- GB 2762—2012: National food safety standard on Contaminants in Food
- GB-2010: National Food Safety Standard for Pathogen Limits in Food (GAIN Report No. 12063)
- GB 2733-2005: Hygienic Standard for Fresh and Frozen Marine Products of Animal Origin
- GB 2760-2011 additives
- GB 10136-1988 Hygienic standard for salt & liquor-saturated aquatic products of animal origin

Russia

Russia has a comprehensive regulatory framework for fish and fishery products. The hygienic requirements are different from other countries as some of the microbiological parameters are expressed as absent in 0.001g or 0.01g. Also some different nomenclature like QMAFAnM is followed instead of APC. The Russian regulation currently in force pertaining to fish and fishery products are as follows:

- Hygienic requirements for safety and nutrition value of food products. Sanitary and epidemiological rules and regulations, sanpin 2.3.2.1078-01

Japan

Compared to other countries, SPS measures followed by Japan is very stringent. Many additives which are in the approved list of Codex are banned or prohibited in Japan. Japan uses a positive list system for MRL of agricultural chemicals in foods. A uniform limit of 0.01 ppm is followed for the compounds for which no risk assessment is done but which are included in the positive list (MHLW Notification No. 497, 2005). MHLW uses a toxicological threshold of 1.5 μg/day as the basis to determine the uniform limit. Substances having no potential to cause damage to human health are specified by MHLW Notification No.498. 2005. The MRL list is mentioned as compositional specification of foods (MHW Notification, No. 370, 1959, amendment No.499 2005, updated as on March 15, 2013). The relevant food safety acts of Japan as enacted by Ministry of Health, Labour and Welfare and other agencies are as follows:

- Food Sanitation Act (Act No.233, 1947): Latest Revision on June 5, 2009, Act No. 49)
- Specifications and Standards for Food and Food Additives, Latest Revision on September 6, 2010, MHLW Notification No. 336
- Japan's Specifications and Standards for Food Additives" (Eighth Edition). Published by the Ministry of Health, Labour and Welfare in 2007
- Food Safety Basic Act (Act No. 48, 2003)
- Agricultural Chemicals Regulation Law (Law No. 82, 1948)

Codex Alimentarius Commission

The Codex Alimentarius Commission (CAC) was established in 1961- 1963 by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) to implement their Joint FAO/WHO Food Standards Programme. CAC has the mandate to formulate food standards, code of practice, guidelines and recommendations to protect health of consumers, ensure fair practices in food trade and to promote coordination of all food standards work undertaken by international governmental and non-governmental organizations. Codex operates through thee standing expert scientific bodies convened under the auspices of FAO and WHO to generate food data and provide risk-assessment type advice:

- Joint Expert Committee on Food Additives (JECFA)
- Joint Meeting on Pesticide Residues (JMPR)
- Joint Meeting on Microbiological Risk Assessment (JEMRA)

Different subject committees and commodity committees, adhoc intergovernmental task forces and regional coordinating committees function and under codex. Codex Committee on Fish and Fisheries Products (CCFFP) is entrusted with the task of formulating standards for different product categories. Although Codex standards on Fish and Fishery Products specifically do not address food safety requirements, but provide a strong framework for production, hygienic requirements and sampling.

Available Codex Standard for Fish and Fishery Products

Code of Practice	
Code of Practice for Fish and Fishery Products	CAC/RCP 52-2003
Guidelines	
Guidelines for the Sensory Evaluation of Fish and Shellfish in Laboratories	CAC/GL 31-1999
Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood	CAC/GL 73-2010
Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food	CAC/GL 79-2012
Model Certificate for Fish and Fishery Products	CAC/GL 48-2004
Guideline Procedures for the Visual Inspection of Lots of Canned Foods for Unacceptable Defects	CAC/GL 17-1993
Guidelines on Good Laboratory Practice in Pesticide Residue Analysis	CAC/GL 40-1993
General guidelines on sampling	CAC/GL 50-2004
Guidelines on the Use of Mass Spectrometry (MS) for Identification, Confirmation and Quantitative Determination of Residues	CAC/GL 56-2005

Codex standard applicable to Fish and Fishery Products

General Standard for Contaminants and Toxins in Food and Feed	CODEX STAN 193-1995
General Standard for the Labelling of Prepackaged Foods	CODEX STAN 1-1985
Standard for Food Grade Salt	CODEX STAN 150-1985
General Standard for Food Additives	CODEX STAN 192-1995
General Methods of Analysis for Contaminants	CODEX STAN 228-2001
Recommended Methods of Analysis and Sampling	CODEX STAN 234-1999
General Methods of Analysis for Food Additives	CODEX STAN 239-2003

Bureau of Indian Standards (BIS)

Bureau of Indian Standards (BIS) functioning under the Ministry of Consumer Affairs, Food and Public Distribution, Government of India. It came into existence on 01 April 1987 through an Act of Parliament on 26 November 1986. It was functioning previously as Indian Standards Institution which was established on 06 January 1947. BIS has so far formulated 64 standards related to fish and fishery products, out of which 33 are active. All these standards are voluntary, which addresses method of production, quality and safety requirements. It also stipulates the method of testing and sampling. There is an attempt by FSSAI to re-draft all BIS standards related to fish and fishery products as most of the food safety requirements are not in sync with the current national standards.

BIS Standards on Fish and Fishery Products

IS 2168	1971	Pomfret Canned in Oil
IS 2236	1968	Prawns/Shrimp Caned in Brine
IS 2237	1997	Prawns (Shrimps) - Frozen
IS 3336	1965	Shark Liver Oil for Veterinary Use
IS 3892	1975	Frozen Lobster Tails
IS 4304	1976	Tuna Canned in Oil
IS 4780	1978	Pomfret, Fresh
IS 4793	1997	Whole Pomfret - Frozen
IS 5734	1970	Sardine Oil
IS 6121	1985	*Lactarius* sp Canned in Oil
IS 6122	1997	Seer Fish (*Scomberomorus* Sp.) - Frozen
IS 6123	1971	Seer Fish (*Scomberomorus* spp.), Fresh
IS 7143	1973	Crab Meat Canned in Brine
IS 7313	1974	Glossary of Important Fish Species of India
IS 7582	1975	Crab Meat, Solid Packed
IS 8076	2000	Frozen Cuttlefish and Squid
IS 9808	1981	Fish Protein Concentrate
IS 10059	1981	Edible Fish Powder
IS 10760	1983	Mussels Canned in Oil

IS 10762	1983	Tuna Canned in Curry
IS 10763	1983	Frozen Minced Fish Meat
IS 11427	2001	Fish and Fisheries Products - Sampling
IS 14513	1998	Beche-de-mer
IS 14514	1998	Clam Meat - Frozen
IS 14515	1998	Fish Pickles
IS 14516	1998	Cured fish and fisheries products - Processing and storage - Code of Practice
IS 14517	1998	Fish Processing Industry - Water and Ice - Technical Requirements
IS 14520	1998	Fish Industry - Operational Cleanliness and layout of market - Guidelines (Amalgamated Revision of IS 5735, 7581 and 8082)
IS 14890	2001	Sardines - Fresh, Frozen and Canned (Amalgamated revision of IS 2421, 6677,8652,8653, 9750 and 10761
4891	2001	Mackerel - Fresh, Frozen and Canned (Amalgamated Revision of IS 2420, 3849,6032, 6033 and 9312)
IS 14892	2000	Threadfin - Fresh and Frozen
IS 14949	2001	Accelerated Freeze Dried Prawns (Shrimps) (Amalgamated revision of IS 4781 and 4796
IS 14950	2001	Fish - Dried and Dry-Salted

Food Safety and Standards Authority of India (FSSAI)

The Food Safety and Standards Authority of India was established under the Food Safety and Standards Act, 2006 as a statutory body for laying down science-based standards for articles of food and regulating manufacturing, processing, distribution, sale and import of food so as to ensure safe and wholesome food for human consumption. Various central acts including the erstwhile Prevention of Food Adulteration Act (1954) were merged under this act The Food Safety and Standards Regulations (FSSR) came into force in 2011, which is divided to following sections:

FSS (Licensing and Registration of Food businesses) regulation, 2011

- FSS (Packaging and Labelling) regulation, 2011
- FSS (Food product standards and Food Additives) regulation, 2011 (part I)
  FSS (Food product standards and food additives) regulation, 2011 (part II)
- FSS (Prohibition and Restriction on sales) regulation, 2011
- FSS (contaminants, toxins and residues) regulation, 2011
- FSS (Laboratory and sampling analysis) regulation, 2011

Recently, standards related to microbiological specifications of fish and fishery products, limit of heavy metals, PAH, PCBs and biotoxins have been incorporated in the FSSR.

****

37

BACTERIA OF PUBLIC HEALTH SIGNIFICANCE

Minimol V.A and Toms C. Joseph
Microbiology, Fermentation & Biotechnology Division,
Email: minimattath*@gmail.com*

Seafood is generally considered microbiologically safe when cooked and offers several health benefits including reduction of cardiovascular diseases, contribution to improving bone strength and congenital developments in infants, reduction of joint pains and inflammations etc. However, when the seafood is consumed in raw form such as fresh, live, partially cooked etc, despite having these advantages, are associated with foodborne illness. Rapid industrialization has resulted in the release of sewage and other industrial effluents into natural water bodies, increasing the chances of seafood borne diseases. The seafood-borne outbreaks are mainly caused by bacteria, viruses, and parasites. The major risk recognised for the contamination of seafood by pathogenic bacteria is by the exposure of food chain to contaminated water. The water runoff from polluted areas such as waste waters from agricultural, industrial and sewage will significantly change the microbial flora of the harvesting water bodies and culture ponds which will result in the contamination of seafood with pathogens like*, pathogenic E. coli,* S*almonella, Campylobacter etc.* or viruses such as Hepatitis A, Norwalk etc. The consumption of raw or partially cooked seafood especially bivalve molluscs can be one of the major contributing factors for the spread of seafood borne pathogens. Another reason for the spread of contaminating pathogens in seafood is the poor personal hygiene of workers and food handlers. Inadequate storage temperature and use of poor-quality raw material in the preparation of seafood etc will increase the risk of illness due to bacteria. Many of the pathogens grow rapidly at room temperature. Fish or fishery product left at ambient temperature is easily spoiled and contaminated with pathogens. This chapter covers the details of major seafood borne bacterial pathogens including emerging pathogens that are causing serious threat to food safety measures.

Salmonella

Infection caused by *Salmonella* continues to be the major cause of seafood borne outbreaks globally. The main sources of contamination are associated with raw oyster, salmon, tuna, value added products of tuna, sole etc. Infection due to *Salmonella* causes gastrointestinal disease and typhoid fever in human. *Salmonella* induced seafood borne outbreaks are reported from several countries worldwide. Non typhoid serovars are generally associated with seafood borne outbreaks. It was reported that USA alone contributes about 1 million cases of

food borne non-typhoid disease globally. In India, the prevalence of *Salmonella* is ranged between 30.5% in fish to 34.1%. The prevalence rates were low in cold temperate regions such US, Spain and Mexico, ranging from 1.5% to 16.4%. The major serovars of *Salmonella* reported from seafood samples of fishing harbours and fish markets in Cochin (India) were *S. Weltevreden, S. Rissen*, *S. Typhimurium* and *S. Derby. Salmonella* infection occurs either through the contact with infected animals, or through the consumption of contaminated seafoods.

Pathogenic *Escherichia coli*

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

Staphylococcus aureus

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

Vibrio parahaemolyticus

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

Vibrio cholerae

The transmission route of *V. cholerae* to human occurs mainly through aquatic environments particularly water. There are reports of this pathogen in fish and fishery products from several parts of the world. Several cases of rejections of consignments of seafood in international trade due to the presence of *V. cholerae* have been reported. Generally environmental strains are non-pathogenic and do not possess any virulence related genes such as *ctx, zot, ace*, and *tcp*A. The survival and evolutionary dynamics of *V. cholerae* in water causes the emergence of diverse Sero and bio variants of *V. cholerae* due to the gene transfer mechanisms. The horizontal and lateral gene transfer mechanism causes the acquisition of virulence genes, antigenic types such as O1 and O139 etc. Toxigenic *V. cholerae* of classical biotype, had been responsible for infections previously and many epidemic outbreaks were reported in the 19th century which was gradually replaced with an emerging strain of the El Tor biotype in 20th century. Re-emergence of classical biotype together with El Tor strains were reported in Bangladesh during 1982 and these strains were frequently reported in gastroenteritis and diarrhoea from this area until 1993. Another epidemic strain of *V. cholerae* carrying O139 antigen was first reported in 1992 in Southern Asia. The incidence of cholera due to O139 and O1 Biotype El Tor strains gradually increased thereafter in India and Bangladesh. Subsequently, the variant of O1 El Tor (hybrid) which carries *tcp*A classical genes or classical *ctx* A or *ctx* B genes have been reported from clinical cases of cholera from Bangladesh. The non-toxigenic strains of O1 are different in terms of its biochemical and serological properties. Clinical and environmental origin of non-toxigenic strains of O1 has been reported from several countries. However, the non-toxigenic strains lacking toxigenic genes also have the

potential of causing diarrhoea in human. The mechanism of virulence and pathogenicity of this strain remains unknown.

Listeria monocytogenes

Listeria monocytogenes is major concern in lightly preserved food products and the prevalence of this bacterium in considerably increased in ready to eat fishery products. Seafood has the highest risk among the minimally processed products. *L. monocytogenes* enters into seafood by cross-contamination and the presence of this pathogen in seafood has been reported from different seafood products. Prevalence rate of this pathogen in seafood products varies from 0 to 17 %. However, the prevalence in seafood is relatively low compared to other food products such as dairy and other animal products. The mortality rate due to *L. monocytogenes* infection is very high ranging from 20% to 30% in immuno-compromised patients and hence an important public health concern. The symptoms of infection include septicaemia, meningitis, gastroenteritis, pneumonia, and spontaneous abortion. Regulatory agencies such as FDA, ISO, WHO, etc. have included this pathogen in zero tolerant categories in processed food products due to its survivability in wide environmental conditions. This pathogen is able to withstand high NaCl concentration of up to 20%, pH range of 4.3 to 9.8, temperature range of 0.5 to 45°C, and low water activity of 0.91. This pathogen is very well adapted to grow in refrigerated condition, and pose serious risk to the chilled and frozen products once it is contaminated.

***Yersinia* spp.**

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

Clostridium botulinum

C. botulinum is grouped under Gram positive bacteria, and are anaerobic spore producing bacilli of important public health concern in seafood industry. This bacterium is autochthonous to the aquatic environment and aquatic sediments and forms major reservoir of this pathogen. The toxigenic types of *C. botulinum* belong to type A, B, E and F. The major risk factors in seafood are due to the presence of these toxigenic types. Botulinum food poisoning is due to the consumption food contaminated with preformed toxins of *C. botulinum* and low oral dose of 70 µg is sufficient to causes illness in human. Its prevalence in seafood depends upon

several factors such as topographical location, culture practices, detection methods etc. The fish poses serious risk due to its direct contact with sediment and the ingestion of spores through contaminated feed/sediment. This bacterium is a major concern in packaged seafood products where cold chain is not maintained during storage, transport and distribution chain. The favourable condition for the growth of *C. botulinum* in preserved products such MAP or vacuum-packed products include, pH of about 4.6, water activity of 0.93%, low salt up to 3%, temperature range of 3°C to 50°C.

Emerging pathogens in seafoods

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

Vibrio vulnificus

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

***Campylobacter* spp.**

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

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

***Cronobacter* spp.**

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

***Arcobacter spp*.**

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

Vibrio mimicus

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

Food safety with respect to seafood pathogens is an important in terms of public health perspectives as over 200 types of diseases are due to the consumption of contaminated foods. (To ensure food safety, routine microbiological screening tests should be validated in real time so that the contaminated food products get detected. National regulations shall be enforced for ensuring food safety that includes the strict implementation of food hygiene and sanitation programme through Hazard analysis and critical control point (HACCP), together with Good

management practices (GMP), standard operating Procedures (SOPs), Sanitation standard operating procedures (SSOPs) practices from production to consumption stages, there by the product becomes safe at all stages of production, processing and distribution levels. The harmonization of these practices in international trade ensures the safety of seafood products, globally.

****

38

ANTIMICROBIAL RESISTANCE IN FOODBORNE PATHOGENS

G. K. Sivaraman
Microbiology, Fermentation & Biotechnology Division,
E-mail:gk.sivaraman@icar.gov.in/gkshivraman@gmail.com

Antimicrobial resistance (AMR) is the ability of the microorganisms (bacteria, fungi, viruses and parasites) resist the action of treatments, it's very difficult to treat the common infections and could cause severe illnesses, chance of spreading of infections and finally lead to death. If we are failure to tackle AMR as a pandemic issue, it could lead to 10 million deaths per year by 2050 and costing $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 highest burdens of AMR and maximal resistance trends in Asia [Murray et al., 2022]. Moreover, AMR threat has long been signalled 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, discovery of new antibiotics are extremely in slow progress. Moreover, the recent SARS-COVID-19 pandemic could have been worsened emergence 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. Now 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 culture farming system is becoming blooming on every years as becomes an intensive and super intensive aquaculture farming system. So the intensive aquaculture often demands the use of formulated feeds, antibiotics, disinfectant's, water, soil treatment compounds, algaecides, pesticides, fertilizers, probiotics and 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] and with high mortalities. So the fish farmers are often bound use antibiotic to

control the diseases. Generally the antibiotics are administered through feed or applied directly into the aquaculture ponds [Heuer et al., 2009, Pham et al., 2015, Okocha et al., 2018]. Moreover, the administered antibiotics are not metabolize completely by the fishes and almost 75% of the consumed antibiotics are excreted in to the pond through faeces and directly applied antibiotics in the ponds will remains for a certain period (varied days of withdrawal period for different antibiotic). As on now, there is no defined antibiotics are produced for the control of fish diseases, 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–Pacific 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 from 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 from 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), shifting to 80% higher than human (91.7 mg kg−1) consumption and remaining 18% higher than terrestrial food producing animal consumption projected 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 influence of antimicrobial resistance (AMR)

AMR is poorly understood in this aquaculture sector in the emergence, re-emergence and spread of AMR. Often the water bodies/ aquaculture system may act as the source of AMR pathogen by collecting from all possible settings and potential source for dissemination of AMR across the settings since its well interconnected system in India. The aquaculture system either use the natural water bodies (rivers, lakes, streams, marine backwater and sea

cage) and human made aquaculture farming (fin fishes and shell fish farming) are frequently getting a chance of contracting with the AMR pathogen, antibiotic residues and AMR contributing factors such as biocides, chemical residues (cu, 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. Whereby the existing potential normal microflora of the aquatic system would acquire these ARGs through HGT or vertical and development resistance against these pollutants and influence the transfer of ARGs between them which lead to the accumulation of AMR pathogens and risk to the clinical settings [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 bacteria species are also harbouring from a few to more than 10 numbers of antimicrobial resistance genes (ARGs) and also harbour virulence and toxigenic genes. So these non-pathogenic antibiotic resistant bacteria species in this aquatic system are either ignored or not monitored properly since these species could act as potential reservoir for the dissemination of AMR. However, a clear cut understanding of the origin and environmental factors that account 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 the environmental selection pressure/ factors and to evaluate of its risks (pathogenicity) to human, animal and aquatic animal health. So thereby prevent the spread of AMR infection 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, promote development of new rapid AMR diagnostics, promote the development of vaccines, immune-modulators, antimicrobial peptides, digestible enzymes in feed, endolysins, hydrolases, and new drugs, enhance 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 have aligned their antibiotics regulations and Maximum Residual Limits (MRLs) with the European Council (EC) and the FDA requirements, to meet export requirements. India, government authorities have listed antibiotic compounds authorized and

banned for use in aquaculture (CAA) have adopted EC MRLs to meet export requirements of the importing consuming countries.

Conclusion

It is imperative to identify and mitigate the source and spread of AMR as they contributing serious antimicrobial resistance, alterations of microbial community, causes of 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 that includes connections between humans, animals, and the environment as a cause and a solution. 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.

References

Aoki, T., 1988.Drug-resistant plasmids from fish pathogens. Microbiological sciences, 5(7), pp.219-223.

Argudín MA, Deplano A, Meghraoui A, Dodémont M, Heinrichs A, Denis O, et al. Bacteria from animals as a pool of antimicrobial resistance genes. Antibiotics.2017;6(12). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC54 85445/

Bondad-Reantaso MG, Subasinghe RP, Arthur JR, Ogawa K, Chinabut S, Adlard R et al. (2005) Disease and health management in Asian aquaculture. Veterinary Parasitology 132: 249–272.

Chi, T.T.K., Clausen, J.H., Van, P.T., Tersbøl, B. and Dalsgaard, A., 2017. Use practices of antimicrobials and other compounds by shrimp and fish farmers in Northern Vietnam. Aquaculture Reports, 7, pp.40-47.

FAO. 2018. The state of world fisheries and aquaculture 2018 - Meeting the sustainable development goals. FAO, Rome. 210 pp.

Hsu, J. How COVID-19 is accelerating the threat of antimicrobial resistance, BMJ 369 (2020) m1983. URL http://dx.doi.org/10.1136/bmj.m1983

Lulijwa Ronald, Emmanuel Joseph Rupia and Andrea C. Alfaro. (2020). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers, Reviews in Aquaculture: 12: 640-663.

Michael I, Rizzo L, McArdell CS et al. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: a review. Water Res 2013;47:957–95.

Muloi D, Ward MJ, Pedersen AB, Fèvre EM, Woolhouse MEJ, Van Bunnik BAD. Are food animals responsible for transfer of antimicrobial-resistant Escherichia coli or their resistance determinants to human populations? A Systematic Review. Foodborne Pathog Dis. 2018;15(8):467–74.

Murrary et al., 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis, The Lancet (Jan. 2022). doi:10.1016/s0140-6736(21)02724-0. URL https://doi.org/10.1016/s0140-6736(21)02724-0.

Okocha, R.C., Olatoye, I.O. and Adedeji, O.B., 2018. Food safety impacts of antimicrobial use and their residues in aquaculture. Public health reviews, 39(1), pp.1-22.

O'Neill, J, Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations, Review on Antimicrobial Resistance, 2014. URL https://books.google.co.uk/books?id=b1EOkAEACAAJ

Pham DK, Chu J, Do NT, Brose F, Degand G, Delahaut P et al. (2015) Monitoring antibiotic use and residue in freshwater aquaculture for domestic use in Vietnam. EcoHealth 12: 480–489.

Rico A, Satapornvanit K, Haque MM, Min J, Nguyen PT, Telfer TC et al. (2012) Use of chemicals and biological products in Asian aquaculture and their potential environmental risks: a critical review. Reviews in Aquaculture 4: 75–93

Romero, J., Feijoo, C.G. and Navarrete, P. Antibiotics in Aquaculture – Use, Abuse and Alternatives, In Health and Environment in Aquaculture, Carvalho, E.D., David, G. S. and Silva, R. J. ISBN 978-953-51-0497-1, 2012.

Rottmann, R.W., Francis-Floyd, R. and Durborow, R., 1992. The role of stress in fish disease (p. 474). Stoneville, MS: Southern Regional Aquaculture Center.

Schar, D., Klein, E.Y., Laxminarayan, R. et al. Global trends in antimicrobial use in aquaculture. Sci Rep 10, 21878 (2020).https://doi.org/10.1038/s41598-020-78849-3.

Sharma C, Rokana N, Chandra M, Singh B, Gulhane R, Gill J, et al. Antimicrobial Resistance: Its surveillance, impact, and alternative management strategies in dairy animals. Front Vet Sci. 2018;4 (237). https://pubmed.ncbi.nlm.nih.gov/29359135/.

Song, Y.L. and Lee, S.P., 1993. Characterization of ecological implication of luminous Vibrio harveyi isolated from tiger shrimp (Penaeus monodon). Bull InstZoolAcad Sin, 32, pp.217-220.

Subasinghe RP, Barg U, Tacon A (2000) Chemicals in Asian aquaculture: need, usage, issues and challenges. In: Use of Chemicals in Aquaculture in Asia: Proceedings of the Meeting on the Use of Chemicals in Aquaculture in Asia 20-22 May 1996 SEAFDEC Aquaculture Department, Tigbauan, Iloilo, Philippines, pp. 1-5.

Van Boeckel T, Brower C, Gilbert M, Grenfell B, Levin S, Robinson T, et al. Global trends in antimicrobial use in food animals.PNAS. 2015;112(18):5649–54.

****

39

PATHOGENIC VIBRIOS OF PUBLIC HEALTH AND AQUATIC ANIMAL HEALTH

T. Muthulakshmi and T. Rajaswaminathan
Microbiology, Fermentation & Biotechnology Division,
E-mail: muthuocean @gmail.com

Classification of Vibrios

Domain - Bacteria
Phylum - Proteo bacteria
Class - Gamma Proteobacteria
Order - Vibrionales
Family - Vibrionaceae
Genus - Vibrio

Vibrios are the diverged group of organism and mostly had the history of pandemics. They are inhabitants of natural aquatic ecosystem like ocean, River, wells and ponds. They are gram negative facultative anaerobes motile by using single polar flagella. Vibrios do not form spores and capsules. Most of the Vibrios are not fastidious and they tolerate high alkaline pH. Vibrios can do both Oxidative and fermentative utilization. They are distributed throughout the world with more occurrences in the tropical region. Vibrio occurrence in the temperate regions is more in hotter months. They have the peculiar ability to go to viable but non-culturable 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. Aquaculture is the food production sectors from water. So any inhabitant in water can affect the production adversely.

The sudden onset of diseases, especially by *Vibrio spp*. is becoming a great concern in larval and juvenile penaeids, and fishes. Hence, the monitoring of aquaculture environments for pathogenic Vibrios is essential to control the spread of Vibrio infections. The members of the genus Vibrio are the most important food-borne and aquatic pathogens which are responsible for illness in humans and cause large-scale mortality in the aquaculture sector. Nowadays in the international trade of marine fishes, testing of *Vibrio species* has become a criterion of

microbiological testing. Even though *Vibrio species* are a common inhabitant of the aquatic environment, some species are emerging as pathogens which can cause up to more than 50% of deaths of all clinical cases. Major Vibrio sp. viz. *V. harveyi, V. parahaemolyticus, V. alginolyticus, V. anguillarium, 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 chemo tactic activity, followed by deployment of the iron sequestering system and eventually damages the fish through extracellular products i.e., hemolysin and protease. Gram negative, curved, comma shaped bacilli

Traditional method of detection of pathogenic Vibrio species

There are well-established isolation and biochemical confirmation procedures for pathogenic *Vibrio* spp. Which were described in ISO and BAM protocol for Vibrios. First stage in traditional detection methods exploits the ability of *Vibrio* species to grow rapidly at relatively high pH values. Media containing sodium chloride and with a pH of about 8.6, such as alkaline saline peptone water (ASPW), are used for enrichment. Typically, a 6-hour preliminary enrichment (at 41.5°C for fresh products, or 37°C for frozen or salted products) is followed by a second enrichment in ASPW at 41.5°C (for *V. cholerae* and *V. parahaemolyticus*) or 37°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°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**

Cholera is a severe diarrheal disease that can lead to rapid dehydration and death if left untreated. It is primarily transmitted through the consumption of contaminated water or food, especially seafood from contaminated waters. Cholera outbreaks often occur in areas with poor sanitation and inadequate access to clean water.

The bacteria produce a toxin known as cholera toxin, which is responsible for the severe watery diarrhea characteristic of the disease. The toxin triggers the loss of fluids and electrolytes from the body, leading to dehydration. *Vibrio cholerae* is the organism responsible for the disease cholera, an acute illness. The diarrhea cause by cholera is specific with rice water stool. The body will become dehydrate and mortality can occur in hours. This can be cultured with alkaline peptone water enrichment and Thiosulphate citrate bile salt sucrose

agar streaking. After 24 h the TCBS will have yellow round flat colonies of 2-3 mm size. *Vibrio cholerae* has more than 200 serotypes with O antigens. Only serogroup O1 and O139 are found to cause cholera epidemics.The 01 serogroup is divided into two biotypes, Classical and El tor, both of which can cause epidemics. The classical bio-types susceptible to Polymyxin, VP negative and do not produce hemolysin to lysed hepatocytes. Whereas, El-tor biotype insusceptible to Polymyxin, VP positive and produce hemolysin to lysed hematocytes. 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 outbreaks 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

This species can cause gastroenteritis, often associated with consuming raw or under cooked seafood, particularly shellfish. Symptoms include watery diarrhea, abdominal cramps, nausea, vomiting, and fever. Infections are typically self-limiting and resolve within a few days in healthy individuals, but they can be more severe in people with compromised immune systems.

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 factors are hemolysin (TDH, TRH) and cytolysin. The TDH is the major toxin present in 95% of the *Vibrio parahaemolyticus* and it can be seen as hemolysin in wagatsuma agar. Thermolabile hemolysin also reported from *Vibrio parahaemolyticus*. This also causes 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 septicemia. The toxin production is correlated with Urease production in the *Vibrio parahaemolyticus*. The disease propagation in cells needs ammonia which can be produced by the Urease positive *Vibrio parahaemolyticus*. More than 800 food-borne disease outbreaks were reported in china, 40 % are from *Vibrio parahaemolyticus* alone.

The *Vibrio parahaemolyticus* is a deadly pathogen for shrimp which cause early mortality syndrome. It causes 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 this bacteria and it mostly follow the fecal oral route. It causes fever, chills. Nausea and water like stools. The shock from the toxin sometime gives death.

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

This bacterium can cause serious wound infections and bloodstream infections, especially in individuals with pre-existing health conditions or weakened immune systems. Wound infections can occur when open wounds are exposed to seawater or raw seafood from contaminated waters. Bloodstream infections can occur when the bacteria enter the bloodstream through a wound or by consuming contaminated seafood.

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

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

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

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

An aquatic Vibrio Disease - Early mortality syndrome

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

Some examples of Vibrio species that can act as exclusive aquatic pathogens include:

Vibrio anguillarum:

This bacterium is a well-known pathogen in fish, particularly in marine and brackish water species like salmon, trout, and eels. It causes a condition known as vibriosis, characterized by symptoms such as skin lesions, hemorrhaging, and systemic infections. Vibriosis outbreaks can lead to high mortality rates among affected fish populations, causing economic losses in aquaculture operations.

Vibrio harveyi

Vibrio harveyi is associated with diseases in marine and freshwater animals, including fish, shrimp, and crustaceans. It can cause vibriosis like luminescent vibriosis in larval stages of shrimps. In hatcheries this is a big threat for the larval stages. This diseases leads to symptoms like lethargy, loss of appetite, and eventual death *V. harveyi* infections can spread rapidly within aquaculture facilities and have a negative impact on production.

Control method for zoonotic Vibrio diseases in aquatic food production sectors

- Water quality parameters should be maintained optimum
- Fish source should be disease free.
- Farm should have bio-security measures
- The disease farm water should be treated with bleaching powder before release
- The handlers should wear gloves while handling diseased fishes
- To reduce the risk of Vibrio infections, it's important to practice proper food safety and hygiene measures, especially when handling and consuming seafood.

- ❖ Thoroughly cooking seafood can help kill Vibrio bacteria. Avoid consuming raw or undercooked seafood, especially if you have underlying health conditions.
- ❖ If you have open wounds or cuts, avoid exposing them to seawater or brackish water, particularly in areas where Vibrio infections are more common.
- ❖ Access to clean and safe drinking water, proper sanitation, and good hygiene practices are crucial for preventing cholera and other waterborne diseases.

****

40

PRINCIPLES OF PCR AND DIFFERENT TYPES

Minimol V A, Sivaraman G K and Toms C Joseph
Microbiology, Fermentation & Biotechnology Division,
E-mail: minimattath@gmail.com

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 enables the synthesis of more than 10 million copies of a target DNA sequence from a few initial copies of the sequence. A PCR technique is used widely in various diagnostics and forensic investigations, and becomes essential for many common procedures such as cloning, sequencing, microarrays etc.

History

PCR was discovered by Dr. Kerry Mullis in 1983 at the Cetus Corporation in Emeryville, CA and he was awarded the Nobel Prize in Chemistry for his work on PCR in 1993. Moreover, the in vitro DNA synthesis using two primers was initiated by Gobind Khorana in 1971, but the main constraints is that primer synthesis and polymerase purification issues. Though the previous techniques were used for isolating a specific gene by gene cloning method, but it's a laborious and time consuming processes. Whereas in the direct sequencing strategy for the gene of interest is quite difficult because of the complexity of the human genome (3 X 109 base pairs) and the primers were added to block the progression of the synthesis of the first primer. Prior to 1988, a PCR reaction was carried out by a series of water baths by adding a fresh aliquot of Klenow fragment of *E. Coli* DNA polymerase I after each denaturation step. These Klenow fragment was not highly specific, upper size limit is only about 400bp and could produce an incompletely pure target product and the target sequence should be confirmed by specific hybridization probe. Obviously, it is a tedious step and was eliminated by the introduction of a thermostable DNA polymerase, the Taq DNA polymerase and is highly specific to the target sequence as well as can withstand in the repeated heating PCR cycling conditions. This increased specificity also could increase the yield of the target sequence and can amplify the PCR products up to 10 kb. The introduction of DNA polymerase enzymatically assembles a new DNA strand from target DNA and nucleotides, for 135 initiation of DNA synthesis. Recently, *Pyrococcus furiosus* (Pfu) DNA polymerases and *Thermococcus Litoralis* (VENT) are becoming more widely used because of the proof reading 3'to 5'exonuclease activity which is lacking in *Taq* polymerase.

Components and Reagents used in PCR Mixture

Typical PCR mixtures consists of a 10× reaction buffer for the Taq polymerase, forward and reverse primers, deoxyribonucleotide triphosphates (dNTPs), Taq polymerase and the template DNA. Each of these components is described below.

Template DNA

DNA template is nothing but the target DNA to be amplified. The quantity, quality and integrity of the template DNA are an important factor to determine the success of PCR. About 1 ng of plasmid or phage DNA and 10-100 ng of pure genomic DNA generally yield expected amplifications. Higher amounts of template DNA usually result in nonspecific PCR amplifications. Still higher quantities might result in the inhibition of amplification. The contaminants such as heparin, heme, formalin, Mg^{2+}-chelating agents, as well as detergents etc if any will also result in the inhibition of amplification process and need to be eliminated.

10× buffer for *Taq* polymerase

10X buffer is a buffer solution, which provide suitable condition for optimum activity and stability of the DNA polymerase. The reaction buffer generally consists of Tris-HCl, EDTA, KCl, Nonidet P40, Tween 20 and glycerol. However, this can vary depending on the type of polymerase and the source. Buffers are supplied in 10× concentration along with the polymerase. It is not advisable to interchange buffers and polymerases from different suppliers, as this may not yield expected results.

$MgCl_2$ concentration

The range of $MgCl_2$ concentration in a reaction mixture varies between 1 to 4 mM, with a standard concentration of 1.5 mM. The 10× buffers are available either with or without $MgCl_2$. If the buffer does not contain concentration of $MgCl_2$, it has to be added separately in to the reaction mixtures. The Mg^{2+} ions form a soluble complex with dNTPs which is essential for dNTP incorporation. It stimulates polymerase activity, and increase the T_m of primer/template interaction (and therefore they stabilise the duplex interaction). The optimal concentration of $MgCl_2$ prevents Mg^{2+} ions to form complexes with dNTPs, primers and DNA templates. Too few Mg^{2+} ions result in a low yield of PCR product, and too many increase the yield of non-specific products.

Primers

Primers are complementary to the 3' ends of each of the sense and anti-sense strand of the DNA target. PCR primers are usually 18-25 nucleotides in length with an average GC content of 40-60%. To avoid non-specific priming, primers should not have more than three G or C nucleotides at the 3'-end. The primer should not be self-complementary or complementary to any other primer in the reaction mixture, in order to avoid primer-dimer and hairpin loop formation. The standard concentration of primer/oligonucleotide is usually 1μM. which will be sufficient for at least 30 cycles of amplification. The presence of higher concentration of

primer can cause amplification of undesirable non-target sequences. Conversely, the PCR is inefficient with limiting primer concentration.

Deoxynucleoside triphosphates (dNTPs)

Deoxynucleoside triphosphates (**dNTPs)** are the building blocks from which the DNA polymerases synthesize a new DNA strand. The equimolar concentration of each dNTP (dATP, dCTP, dGTP, dTTP) is required in a PCR mixture and usually at 20- 200 µM each. The unequal concentrations lead to mis-incorporations of nucleotides resulting in altered sequences which might affect post-PCR experiments such as sequencing, cloning and expression of protein. DNTPs stock solutions (usually 100 mM) should be adjusted to pH 7.0-7.5 with 1 M NaOH to ensure that the pH of the final reaction does not fall below 7.1; however, many dNTPs stock solutions are now supplied with already adjusted pH.

Taq DNA polymerase

Taq polymerase specific to the target sequence and can withstand in the repeated heating PCR cycling conditions. The original method of PCR used the Klenow fragment of *E. coli* DNA polymerase I. This enzyme, however, denatures at temperatures lower than that required to denature most template duplexes. Thus, in earlier experiments, fresh enzyme had to be added to the reaction after each cycle. In addition, samples had to be moved from one temperature bath to another to allow the individual steps of denaturation, annealing and polymerisation. The use of heat-resistant DNA polymerase has obviously facilitated the process because the addition of enzymes after every denaturation step is no longer necessary. Typically, DNA polymerases can only incorporate nucleotides from the 3' end of a polynucleotide. The first thermostable DNA polymerase used was the *Taq* DNA polymerase isolated from the bacterium *Thermus aquaticus*. Even though this enzyme is probably the most widely used in PCR applications, several other DNA polymerases are commercially available. The concentration of *Taq* polymerase is very crucial in a PCR. The recommended concentration is 1-1.5 units in a 50 µl reaction mixture involving amplifications of up to 1 kb. When larger than 1 kb is to be synthesized, the concentration of *Taq* may be increased. It is important to follow manufacturer's recommendations regarding the concentrations to be used since this can vary considerably depending on the source of the enzyme.

PCR Cycle and Principles

The PCR process consists of a repetitive series of three fundamental steps called PCR cycle. The cycling is often preceded by a single temperature step called Initialization at a high temperature (>90°C) and one final elongation for any remaining single-stranded DNA is fully extended. The Final hold step is at 4°C for short term storage of the amplified PCR products.

In PCR, the double stranded DNA is denatured by heat and then the temperature is lowered to allow annealing of two specific primers by complementary base pairing on the opposite strands of the DNA. *Taq* polymerase directs the synthesis of the new strand from the primed

sites in both directions that results in double stranded DNA and the procedure is repeated for 25-40 times in a thermo cycler. 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 subsequent manipulations.

Initial Denaturation

Initial Denaturation ensures complete denaturation of the double stranded DNA at the start of the PCR cycling. A 5 min denaturation at 95°C should be sufficient for templates with a GC content of 50% or less. The denaturation time should be extended up to 10 min for GC-rich templates.

After initial denaturation, the following 3 steps are repeated 30-40 times.

1. ***Denaturation*** The double-stranded DNA template denaturation: The double-stranded DNA template denatured into two complementary single strands of DNA by disrupting the hydrogen bonds between complementary bases. Usually DNA undergoes rapid denaturation at 94–98 °C with 30 seconds to 2 minutes.

2***. Annealing*** Annealing of two oligonucleotide primers to the single-stranded template: After denaturation, temperature is lowered to 50–65°C for 20–60 seconds allowing annealing of the primers to the single-stranded DNA template and preventing immediate re-annealing of long DNA strands. The primers rapidly anneal to the single strands of DNA because of their small size and Taq polymerase will binds to them. Generally the annealing temperature is 136 about 3-5 degrees Celsius below the Tm of the primers used. Once the stable DNA-DNA hydrogen bonds are formed, the polymerase binds to the primer-template hybrid and begins DNA synthesis.

Annealing temperature of the primers is calculated using the following formula

Tm = 4 (G + C) + 2 (A + T)

Annealing temperature (°C) = Tm – 5°C

Where,

Tm = Melting temperature; G, C, A, T =number of respective nucleotides in the primer.

3. ***Extension*** Enzymatic extension of the primers to produce copies that can serve as templates in subsequent cycles: The DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5' to 3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the extending DNA strand. The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified. The DNA polymerase will polymerize a thousand bases per minute. At each extension step, the amount of DNA target is doubled, leading to exponential (geometric) amplification of the specific DNA fragment.

Final extension

After the completion of cycling, a final extension for 5-15 min at 72°C is done to complete incompletely synthesized strands.

Preparation of PCR Reaction Mixture

A master mix can be prepared by adding the required concentration reagents of PCR reaction in a given volume and then be aliquotted appropriately to individual tubes depending upon the testing requirement. This procedure can minimize the possibility of pipetting errors in case of handling low volume and also saves time. Transfer the required water, buffer, $MgCl_2$ dNTPs, primers and *Taq* DNA polymerase in a single tube is aliquotted into individual tubes. Various templates DNA to be amplified are then added to the individual tubes and to be labelled for further recording of the results. A positive control (known DNA sample) & a negative PCR control (water) should also be included to ensure the reliability of the procedure. PCR reaction mix preparation is follows

Thaw all the reagents such as water, buffer, $MgCl_2$ dNTPs, primers and template DNA except *Taq* DNA polymerase (it should keep always under ice) vortex gently and spin all solutions. Prepare master mix by adding the following reagents except template and transfer aliquot to individual tubes (PCR tubes 0.2ml) placed on ice. Then add DNA templates to the respective tubes. The final concentration for 30 ml reaction mixture is given in the table

Component	**Final concentration**	**Volume required (µl)**
Sterile deionised water	-	15.4
10X *Taq* buffer	1X	3
10 mM dNTP mix	0.2 mM of each	0.5
Primer Forward	1 µM	3
Primer Reverse	1 µM	3
Taq DNA Polymerase	1.5 u	0.3
25 mM $MgCl_2$	1.5 mM	1.8
Template DNA	100 ng	3
Total volume		**30**

Vortex each PCR tubes and spin to collect all drops from the walls of tube. Place the PCR tubes in thermo cycler and start PCR. Once the PCR cycle is over, the PCR product is visualized by electrophoresis in agarose gel stained with 0.5 µg/ml of ethidium bromide.

Agarose gel electrophoresis

Agarose gels are cast by melting the appropriate percentage of agarose (1- 2%) in convenient buffer (1X TAE) until a clear, transparent solution is achieved. The melted gel is then cooled (50°C), poured on a tray containing combs and allowed to solidify. Upon solidification, the comb is removed and gel is placed in the electrophoresis chamber and then pours the buffer to cover the gel. DNA samples are loaded into the sample wells by mixing with loading dye (2

μl for 10 μl of the PCR product) and gel is run at a voltage for a time period that will perform optimal separation. When electric field is applied across the gel, DNA migrates toward the anode. When running of the gel is completed, the separated bands on agarose gel are labelled or stained for interpreting. One method of staining DNA is to expose it to the dye ethidium bromide (EtBr) (0.5 μg/ml). EtBr intercalates between the stacked bases of nucleic acids and fluoresces red–orange when illuminated with ultraviolet (UV) light. EtBr is a carcinogen and should be handled with care.

Specialised PCR

In addition to the amplification of a target DNA sequence by the typical PCR procedures already described, several specialized types of PCR have been developed for specific applications.

RT-PCR (or Reverse Transcription 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 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.

RT-PCR can also be very useful in the insertion of eukaryotic genes into prokaryotes.

Because most eukaryotic genes contain introns, which are present in the genome but not in the mature mRNA, the cDNA generated from a RT-PCR reaction is the exact (without regard to the error-prone nature of reverse transcriptase) DNA sequence that would be directly translated into protein after transcription. When these genes are expressed in prokaryotic cells for the sake of protein production or purification, the RNA produced directly from transcription need not undergo splicing as the transcript contains only exons. (Prokaryotes, such as *E. coli*, lack the mRNA splicing mechanism of eukaryotes). RT-PCR is commonly used in studying the genomes of viruses whose genomes are composed of RNA, such as Influenza virus A and retroviruses like HIV.

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.

Quantitative PCR

Quantitative PCR is used to measure the amount or quantity of target nucleic acid (DNA or RNA) in a sample. The amount of fluorescence generated during the the phase of true exponential stage is directly measures the amount of nucleic acid or target. Special thermal cyclers fitted with light source and suitable filters for wavelength selection are used for the real time detection or monitoring of PCR product. Fluorescent dyes used are Sybr Green, or flurorophore-anchored DNA probes, such as TaqMan, to measure the amount of amplified product as the amplification progresses. Quantitative PCR is also used by microbiologists working in the fields of food safety, food spoilage and fermentation and for the microbial risk assessment of water quality (drinking and recreational waters) and in public health protection. The antibacterial assay Virtual Colony Count utilizes a data quantification technique called Quantitative Growth Kinetics (QGK) that is mathematically identical to QPCR, except

bacterial cells, rather than copies of a PCR product, increase exponentially. The QGK equivalent of the threshold cycle is referred to as the "threshold time".

Colony PCR

In Colony PCR, bacterial colonies are screened directly by PCR, for example, the screen for correct DNA vector constructs. Colonies are sampled with a sterile pipette tip and a small quantity of cells transferred into a PCR mix. To release the DNA from the cells, the PCR is either started with an extended time at 95 °C (when standard polymerase is used), or with a shortened denaturation step at 100 °C and special chimeric DNA polymerase.

Applications of PCR

Selective DNA amplification

- PCR allows selective amplification of a specific region of DNA/ gene from genomic DNA which can be utilized for direct sequencing, genomic cloning, DNA typing, detection of infectious microorganisms, site directed mutagenesis, prenatal genetic disease diagnosis and analysis of sequence variations.
- PCR 'fingerprints' methods can be used to identify genetic relationships between individuals (parent child, between siblings and paternity testing) and microbial identification, evolutionary relationships among individuals/ organisms and forensic analysis.
- PCR may also be used in the analysis of ancient DNA.
- Quantitative PCR methods allow the estimation of the amount of a given sequence present in a sample and quantitative determine the levels of gene expression. 2. PCR in diagnosis of diseases
- A diagnostic application in microbiology for the detection of infectious agents and the differentiation of non-pathogenic from pathogenic strains.
- Identification of non-cultivatable or slow-growing microorganism‘s like viruses mycobacterium, anaerobic bacteria.
- Viruses can be detected before the onset of disease and/ or immediately after the infection.
- Utilize for early diagnosis of cancer research
- Amount of virus in an infected patient can be quantified.

****

41

MOLECULAR FINGERPRINTING OF SEAFOOD BORNE PATHOGENS AND BIOINFORMATIC ANALYSIS

Murugadas Vaiyapuri and Radhakrishnan V N
Microbiology, Fermentation & Biotechnology Division,
E-mail: Murugadas.V@icar.gov.in

Antimicrobial resistance (AMR) is becoming a major concern for human health. The World Health Organization (WHO) has prioritised the diseases for which research and development programmes are urgently needed. Pathogens are prioritised depending on the determination to create new antibiotics or preserve current medications for treatment or control techniques. Pathogens are classified as critical, high, or medium priority. Priority 1 bacteria include carbapenems-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and ESBL-producing Enterobacteriaceae; priority 2 bacteria include Vancomycin-resistant Methicillin-resistant *Enterococcus faecium* Vancomycin-intermediate-resistant *Staphylococcus aureus* Cephalosporin-resistant *Staphylococcus aureus*, fluoroquinolone-resistant Fluoroquinolone-resistant *Neisseria gonorrhoeae* Fluoroquinolone-resistant *Salmonellae* Clarithromycin-resistant *Campylobacter* spp. Priority 3 includes fluoroquinolone-resistant Helicobacter pylori. Ampicillin-resistant *Shigella sp*. Penicillin-resistant *Streptococcus pneumoniae*, *Haemophilus influenzae* (WHO, 2017). Gram-negative bacteria, such as *A. baumannii, P. aeruginosa*, ESBL producing Enterobacteriaceae (Klebsiella, E. coli, Serratia, and Proteus), Campylobacter sp, Helicobacter pylori, *Salmonella sp, Shigella sp, N. gonorrhoeae*, and *H. influenzae*, are the key targets for management. *S. aureus, Enterococcus faecium*, and *S. pneumoniae* are three more major Gram-positive bacteria that contribute to the urgency of AMR.

The challenge of regulating AMR begins with giving proof of its prevalence as well as recording the genotype of the prevalent bacteria. Molecular methods remain crucial for understanding regional and global epidemiology, as well as the point of genesis and transmission of infections based on clone relatedness and genetic diversity down to the strain level (Barrett et al., 2006; Vaiyapuri et al., 2019). The valuable evidence gathered will be an important factor in developing methods for their control (Ranjbar et al., 2014). AMR resistance molecular approaches determine the existence of AMR genes or particular mutations linked with antibiotic resistance (WHO, 2019). Molecular approaches may supplement phenotypic methods by giving additional information, such as the particular gene or mutation behind a resistance phenotype, boosting our knowledge of the amount of resistance in a given situation

as well as the underlying processes responsible for resistance (Bissonnette et al., 2017; Burnham et al., 2017; WHO, 2019).

Molecular fingerprinting tools are categorized into 4 based on the approach

1. Sequencing based method
2. Amplification based method
3. Hybridization based method
4. Restriction digestion-based method

1. Sequence based molecular fingerprinting tools

Multi-locus sequence typing

The molecular typing procedure which involves the sequencing analysis of more than a single locus is called multi-locus sequence typing (MLST). The first MLST procedure was demonstrated during 1998 for *Neisseria meningitides* and later on the MLST analysis was extended to more than 100 species or genera of bacteria (Enright & Spratt, 1999; Maiden *et al.*, 1998). The MLST analysis can be used for epidemiological investigations in public health, animal health and food borne disease investigations. This is one of the best molecular tools for fingerprinting of bacterial pathogens. Mostly implemented in global and local epidemiology. The MLST analysis is targeted on housekeeping genes of clinically important bacterial pathogens. The MLST analysis is divided into three steps, amplification of housekeeping genes, sequencing of the amplicons and analysis of sequences for assigning the sequence types. In general, the amplicons range between 450 to 500bp for analysis purposes. In exceptional cases, the amplicon size may vary. This tool has high reproducibility, more discriminating power, portability of data as it is sequence based, speed of the completion, easy interpretation and inter-laboratory comparison. Example of *S. aureus* / MRSA- MLST- analysis. The MLST scheme for the S. aureus (MRSA and MSSA) was developed by Enright et al. (2000). The MLST can be performed by amplification of fragments, elution and sequencing, bioinformatic analysis.

Staphylococcal Protein A typing

Staphylococcal Protein A typing (SPA) is the single locus amplification and sequencing method. The SPA gene's variable region XR domain is the target area for SPA typing in S. aureus or MRSA. It is often used for Staphylococcal protein A (spa) typing (Frenay et al., 1999). This approach involves amplification, sequencing, and SPA type designation, and it is often employed in local epidemiology since it accumulates genetic alterations very slowly (Koreen et al., 2004). Open source or software from StaphType (Ridom GmbH, Wurzburg, Germany) and Based upon Repeat Pattern (BURP) are used to perform minimum spanning tree-based clustering of spa kinds (Harmsen et al., 2003; Sammeth et al., 2006; Aires de Sousa et al., 2006).

Whole genome sequencing

Whole-genome sequencing (WGS) is a thorough approach for studying bacterial isolates' whole genomes. There are many ways for sequencing whole genomes. In the late 1970s, Maxam and Gilbert's chemical cleavage approach and Sanger sequencing by chain-termination method were used to sequence viral and bacterial genomes (Maxam and Gilbert., 1977; Sanger et al., 1977). In 2008, a transition to a faster, automated sequencing approach was made, allowing for the sequencing of bigger bacterial and eukaryotic genomes. Following-Sanger sequencing technologies are referred to as 'next-generation sequencing.' It can generate massive volumes of sequencing data at a very cheap cost and time. 454-sequencing, pyrosequencing, Illumina sequencing, and Sequencing by Oligonucleotide Ligation and Detection are all examples of second-generation sequencing (SOLiD). While Sanger's sequencing operates on the basis of chain termination by the inclusion of di-deoxynucleotides (ddNTPs), A, T, G, and C, the output data is slightly less than one kilo base (kb). NGS is the massively parallel sequencing of millions of DNA fragments at the same time, resulting in the generation of millions of nucleotide short reads. The most prevalent NGS technology is undoubtedly Illumina dye sequencing, which employs a "sequence by synthesis" strategy. The genomic DNA is split at random into small pieces and affixed to the inner surface of a flow cell, where sequencing will occur. A solid-phase PCR is then used to generate clusters of clonal populations from each of the individual DNA strands bound to the flow cell. At the end of each cycle, the incorporating nucleotide's identity is recorded using a photo detector by activating the flurorophore with suitable lasers, followed by enzymatic removal of the blocking fluorescent moieties and progression to the next location (Fedurco et al, 2006; Turcatti et al, 2008; Heather and Chain, 2016)

Amplification based methods

Repetitive extragenic palindromic-PCR (REP-PCR)

Repeating extragenic palindromic -PCR (Versalovic et al., 1991, 1994) is a fingerprinting technology established on the PCR foundation in a bacterial genome that is based on the amplification of these repetitive elements that are particularly distinct to strains within the species of bacteria. These repeating REP elements were found in a variety of Enterobacteriaceae and non-Enterobacteriaceae bacteria, and the REP sequences are palindromic, forming a stem-loop structure (Higgins et al., 1982; Stern et al., 1984). Two sets of primers targeting these REP elements, based on 38-bp sequences having degenerate sequences in six places with a 5-bp variable loop among both sides of a conserved palindromic stem, were used for typing in different bacteria. REP element-based typing of AMR bacteria such as E. coli, Salmonella sp., and others has been described (Qian and Adhya, 2017).

Enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR)

The ERIC sequences, which include a 126-bp sequence that is highly conserved core inverted repeat and are situated in extragenic areas of the bacterial genome, are another type of repetitive DNA sequences utilised for bacterial typing. Initially detected in E. coli and Salmonella Typhimurium, the typing approach based on the ERIC pattern is currently being extended to additional bacteria in the Enterobacteriaceae (Sharples et al., 1990).

Amplified fragment length polymorphisms (AFLP)

The amplified fragment length polymorphism (AFLP) approach is excellent for fingerprinting DNAs of any origin and complexity. AFLP offers various benefits over other DNA fingerprinting techniques. The ability to check a full genome for polymorphism and its repeatability are the most crucial. AFLP has the potential to become a universal DNA fingerprinting technique since it can be used to any DNA sample, including microbial DNA, human, animal, and plant DNA. The polymerase chain reaction is used to selectively amplify restriction fragments from a digest of total genomic DNA in the AFLP approach. Zabeau and Vos were the first to create this approach (1993). The AFLP process consists of four basic steps: DNA digestion, ligation, amplification, and gel analysis. Two restriction enzymes initially degrade genomic DNA. The DNA fragments are ligated using double-stranded oligonucleotide adapters that are identical to one of the 5' or 3' ends formed during restriction digestion. The ligated DNA fragments are amplified by PCR using primers that are complementary to the adaptor and restriction site sequences, as well as extra selected nucleotides at the 3' end. The employment of selective primers minimises the mixture's complexity. Under precise annealing conditions, only fragments with complimentary nucleotides extending beyond the restriction site will be amplified by the selective primers. AFLP is a RAPD variant that may discover restriction site polymorphisms without previous sequence information by employing PCR amplification for restriction fragment detection. Janssen et al. (1996) found considerable support for the use of AFLP in bacterial taxonomy by comparing newly acquired data to findings produced by well-established genotypic and chemotaxonomic approaches like as DNA hybridization and cellular fatty acid analyses. Screening of DNA markers connected to genetic characteristics and microbiological typing, diagnostics of genetically inherited disorders, pedigree analysis, forensic typing, and parentage analysis are some of its possible uses.

Randomly amplified polymorphic DNA (RAPD)

The RAPD approach is a PCR-based discriminating method in which short arbitrary primers anneal to several random target sequences, resulting in the formation of the test organism's fingerprint profile. The target sequence to be amplified is unknown in RAPD analysis, and a 10base random sequence primer is utilised in the experiment to construct the RAPD profile. The low-stringency annealing conditions required for the RAPD-PCR reaction result in the amplification of randomly sized DNA fragments. The RAPD-PCR multiple band patterns are followed by dendrogram analysis to provide fingerprint profiles for the test organism. This technique may be used to identify clonal variants in bacterial strains. Because RAPD patterns are not always reproducible, this approach must be used under carefully controlled settings. The RAPD approach was utilised to identify enteropathogenic E. coli, Salmonella, Shigella, Vibrio, Aeromonas, and Listeria in food and water samples. RAPD has been used by multiple organisations to identify and characterise LAB strains from diverse sources, including human, food, and milk samples.

Hybridization based method

Microarray

A microarray is made up of regularly organised target DNA sequences that are connected to a solid substrate such as glass, silicon wafers, nylon membranes, or other functionalized substrates. The sample's DNA is fluorescently labelled and put to the array (hybridization). A fluorescent microarray detector and a computer application can then identify and evaluate several distinct AMR genes (Holzman, 2003). Fink et al. (2019) created a microarray-based AMR chip that identifies massive ARGs for -lactams and vancomycin. Using 14 probes, an array chip was built for six key classes of antibiotics, including -lactams, macrolides, aminoglycosides, tetracyclines, sulphonamides, and trimethoprim (Card et al., 2014) and found a total of 14 distinct resistance genes conferring resistance to six antibiotic classes. A microarray chip was created for 166 ARGs found in major Gram positive and negative bacteria (Garneau et al., 2010).

Fluorescent in situ hybridization (FISH)

FISH is a method that uses fluorescently labelled oligonucleotide probes to hybridise to the complementary DNA sequences of resistance genes. After the hybridization procedure is completed, any leftover probes are rinsed away. The signal from the bounded probes for ARGs is captured using epifluorescence or confocal laser scanning microscopy (Levsky and Singer, 2003). FISH probe designed to detect ampicillin, macrolide, and chloramphenicol resistance in Escherichia coli, Helicobacter pylori, and Bacillus cereus (Demiray and Yilmas, 2005; Juttner et al, 2004; Laflamme et al, 2009; Lee et al., 2019).

Restriction digestion based fingerprinting methods

Pulsed-field Gel electrophoresis (PFGE)

PFGE is a "Gold standard" approach for bacterial pathogen molecular sub typing. The approach involves in-situ macro-restriction of isolated genomic DNA in an agarose plug and digestion with restriction endonucleases (Barrett et al., 1994). Later, as part of the PulseNet approach, the gold standard technique for disease outbreak analysis from food was applied (Swaminathan et al., 2001). Initially, the Tenover (1995) guideline was employed, and subsequently, software for character-based analysis was created to evaluate genomic DNA based on the band number and positions that emerged in the gel electrophoresis (Barrett et al., 2006). These approaches were also employed for source tracing, as well as local and worldwide epidemiology (Vernile et al., 2009).

Restriction Fragment Length Polymorphism (RFLP)

The restriction enzyme restricts the microbe's chromosomal DNA in RFLP. The approach was originally created and utilised to build linkages in the human genome (Botstein et al., 1980). The same approach may be used to produce bands from any DNA, including PCR products and tagged probes with restriction sites. The fingerprint or banding pattern created by agarose gel electrophoresis shows the availability and distribution of restriction sites throughout the chromosome. The RFLP technique may be used to compare strains within species, and using rare cutting restriction enzymes minimises the number of bands generated when compared to using frequent cutting restriction enzymes. The banded pattern was then utilised in probe hybridization. As a result of restrictions such as time and labour demanding work for pure DNA extraction, restriction digestion and probe-based hybridization, and recording and analysis of bands, this approach has lost its relevance (Ben-Ari and Lavi, 2012; Ranjbar et al., 2014).

Multi Locus Enzyme Electrophoresis

MLEE is a system established using restriction enzymes for multiple loci of housekeeping genes prior to the introduction of MLST. The banding pattern, including the number and location of the bands, is also examined in this manner (Zahner et al., 1994). The use of MLEE approaches has decreased significantly since the development of the MLST scheme (Kotetishvili et al., 2003).

Bioinformatic analysis of fingerprint data

Bioinformatic analysis of fingerprint data is performed as character-based analysis or sequence-based analysis. In the character-based analysis, the number of bands and position of bands are taken into consideration for the normalization and analysis purpose. In this type of character-based analysis, the image quality is very crucial. There is much software used for character-based analysis, bio numeric is the software paid version and GelJ is the open-source software used in the character-based analysis. In the sequence-based analysis, the sequence data is either compared to the public domain or assigned value for the fingerprint data.

Example for character-based analysis ERIC PCR fingerprint analysis. Using GelJ software normalise the banding pattern visually. Construct the phylogenetic tree the selected isolates with GelJ software. Keep the DNA ladder (100bp) for the normalization of banding position. Construct the phylogenetic tree based on the similarity calculated by Pearson correlation between the fingerprints with the tolerance of 1%, and grouping of the fingerprints with the help of the unweighted pair group method using arithmetic averages algorithm (UPGMA) (Rasschaert et al. 2005). Example for sequence-based data is multi-locus sequencing typing data. The assignment of allele's number and allelic profile has to be carried out after analysing the quality of the sequences obtained and after assembling. After assigning the allelic profiles, the sequence type's identification will be carried out in the publmlst public domain. Assigning the clonal complexes and relationship to the already existing MRSA/MSSA strains in the public domain can be carried using the Minimum spanning tree development based on algorithm called BURST (based upon related sequence types) developed by Mellman *et al.* 2008. This will bring you the information on how many evolutionary events happened for your strain and what is the diversity of clones prevalent in your local region. As per the information on https://pubmlst.org/organisms/staphylococcus-aureus, there are now 902, 1091, 954, 607, 937, 855, 1024 alleles were identified and available in public domain for *arc, aroE, glpF, gmk, pta, tpi and yqiL* loci respectively. Detailed information on MLST and analysis for S. aureus can be sought from https://pubmlst.org/organisms/staphylococcus-aureus. And for MRSA from seafood (Murugadas et al., 2017; Vaiyapuri et al., 2019).

****

42

BASICS IN ANIMAL CELL CULTURE – PRINCIPLES AND APPLICATIONS

T. Raja Swaminathan
Microbiology, Fermentation & Biotechnology Division,
E-mail: t.swaminathan@icar.gov.in

Vertebrate cell cultures are *in vitro* models. The term in vitro refers to keeping entities of an organism outside the living body in an artificial environment, in contrast to in vivo, i.e. in the organisms. Primary cultures start from cells, tissues or organs taken directly from organisms. If a primary culture can be divided into new culture vessels and successfully propagated, it becomes a cell line. A cell line may be propagated a limited number of times, in which case it is finite, or indefinitely, in which case it becomes an immortal or continuous cell line.

Although animal cell culture was first successfully undertaken by Ross Harrison in 1907, it was not until the late 1940's to early 1950's that several developments occurred that made cell culture widely available as a tool for scientists. First, there was the development of antibiotics that made it easier to avoid many of the contamination problems that plagued earlier cell culture attempts. Second was the development of the techniques, such as the use of trypsin to remove cells from culture vessels, necessary to obtain continuously growing cell lines (such as HeLa cells). Third, scientists were able to develop standardized, chemically defined culture media that made it far easier to grow cells. These three areas combined to allow many more scientists to use cell, tissue and organ culture in their research. During the 1960's and 1970's, commercialization of this technology had further impact on cell culture that continues to this day. The overall result of these and other continuing technological developments has been a widespread increase in the number of laboratories and industries using cell culture today.

Any interaction of a toxic substance with an organism is initiated at the cellular level. From cells, alterations can translate to changes in tissue, organ function and finally impact on whole organism. Based on the central role of cells in the expression of toxicity, several cell lines or in vitro models have received regulatory acceptance by the organization as alternative to whole animal tests in health sciences. Besides their potential to replace or reduce animals in toxicity tests, cell lines have several advantages compared to whole animal tests. Large numbers of potentially toxic substances can be screened rather quickly in multiple-well plates, which can be analysed rapidly. As well, cells can help identify the mechanisms underlying a toxic response. If, for a particular purpose, a suitable continuous cell line can be used, a donor

animal is never again needed. Based on these reasons, the role of cell lines is expected to significantly increase.

Fish cell line

Fish cell lines have been useful in many areas of research. Originally developed to support the growth of fish viruses for studies in aquatic animal viral diseases, fish cell lines have grown tremendously in number covering a wide variety of species and tissues of origin and an array of applications. Fish immunology, physiology, genetics and development, toxicology, ecotoxicology, endocrinology, biomedical research, disease control, biotechnology and aquaculture are some of the areas in which fish cell lines have made significant contributions.

Fish cell lines being of poikilothermic origin, grow well at room temperatures without the need of thermo regulated incubators, furthermore, an amino acid-rich nutrient medium such as Leibovitz-15 that does not require CO_2 buffering has been successfully used with fish cell lines, thus CO_2 incubators are not necessary and cells can be grown conveniently in any undisturbed areas. Additionally, because of lower metabolic rates than eurythermic cells, fish cells can be maintained with little care for long periods of time.

In 1962, the first teleost cell line was reported in the literature. The first continuous fish cell culture (RTG-2) was originated over 20 year ago from rainbow trout gonad tissue. Subsequently, many other cell lines of poikilothermic origin have been developed. Most fish cell lines have been established and utilized for isolating, identifying, and studying viruses that cause economically important diseases. Consequently, the majority of these cell lines originate from species that are artificially propagated to some extent. Moreover, most such fish cell lines have been developed in North America or Europe. Nowadays, more fish cell lines are available from fishes indigenous to Asia, since aquaculture and fish farming are pursued on a large scale in this part of the world.

Wolf & Mann (1962) enumerated 61 cell lines originating from 36 fish species, representing 17 families. These cell lines were chiefly used for viral diagnostic purposes, and many had not been well characterized or previously reported. Fryer and Lannan (1993) have compiled a new listing of the fish cell lines reported in the literature that have been at least partially characterized. Recently Lakra et al (2010) made a comprehensive review on the characterized fish cell lines of both freshwater and salt water that have been developed after 1993.

Most fish cell lines originate from normal tissues, and embryos or normal fins are most frequently listed as the source of the tissue used in the primary culture. However, few cell lines were initiated from fish tumours, and in some cases, these cells remained tumorigenic *in vivo* following repeated in vitro passage. Traditionally, the chief uses of these cell lines were for detection and study of fish viruses and for diagnosis of the diseases caused by these agents. Today, fish cell cultures are increasingly utilized in research unrelated to disease, and with the recent identification of rickettsial fish pathogens, the diagnostic role of cultured fish cells has

also expanded. Along with the multiplying uses of fish cell culture is a concomitant increase in the need for guidelines for the health and maintenance of fish cell lines.

Fish Cell Culture characteristics

The physiology and the blood plasma constituents of teleost fishes are very much like those of terrestrial vertebrates; therefore the methodology for culture of cells and tissues is also similar. Most fish cell lines are readily propagated in vitro using unmodified media and techniques developed for mammalian cells, with appropriate adjustment of incubation temperatures to reflect the temperature range normal for the donor fish species. Also, osmolarity of the media must be adjusted upward for fishes of marine origin. Most important, fish tissue culture often requires less time for preparation and maintenance. Mammalian cell culture techniques need only be modified to reflect the lower incubation temperature requirements and the slower replication rates of the poikilothermic cells.

Appropriate incubation temperatures for cultured fish cells correspond to the normal temperature range of the fish species from which the cell line is derived. For lines from coldwater fishes, incubation temperatures range from 4-24°Cwith an optimal range of 15-21°C. For lines from warm water fishes, incubation temperatures range from 15-37°C, and the range of optimal incubation temperatures is 25-35°C.

Cell Culture Systems

Once in culture, cells exhibit a wide range of behaviors, characteristics and shapes. Some of the more common ones are described below. Two basic culture systems are used for growing cells. These are based primarily upon the ability of the cells to either grow attached to a glass or treated plastic substrate (Monolayer Culture Systems) or floating free in the culture medium (Suspension Culture Systems).

Types of Cells

Cultured cells are usually described based on their morphology (shape and appearance) or their functional characteristics.

There are three basic morphologies:

Epithelial-like: cells that are attached to a substrate and appear flattened and polygonal in shape.

Lymphoblast-like: cells that do not attach normally to a substrate but remain in suspension with a spherical shape.

Fibroblast-like: cells that are attached to a substrate and appear elongated and bipolar, frequently forming swirls in heavy cultures.

Endothelial cells: Endothelial cells are very flat, have a central nucleus, and are about 1-2 µm thick and some 10-20 µm in diameter.

Other types: Macrophages, neuronal cells, melanocytes, etc.

It is important to remember that the culture conditions play an important role in determining shape and that many cell cultures are capable of exhibiting multiple morphologies.

Development of cell line

Primary Culture

There are several ways with which monolayer cultures of fish cells may be initiated. This is a quick method that employs multiple explants of tissues of either fresh water or marine fish as the simplest way to produce monolayer cultures. When cells are surgically removed from an organism and placed into a suitable culture environment, they will attach, divide and grow. This is called a Primary culture. There are two basic methods for doing this. First, for Explants Cultures, small pieces of tissue are attached to a glass or treated plastic culture vessel and bathed in culture medium. After a few days, individual cells will move from the tissue explant out onto the culture vessel surface or substrate where they will begin to divide and grow.

The second, more widely used method speeds up this process by adding digesting (proteolytic) enzymes, such as trypsin or collagenase, to the tissue fragments to dissolve the cement holding the cells together. This creates a suspension of single cells that are then placed into culture vessels containing culture medium and allowed to grow and divide. This trypsinization method describes warm (1-2 hrs at 37^0 C) and cold (4 ~ to 6~ overnight (16 hr) trypsinization of fish tissues which yields cultivable cells and small aggregates of cells for monolayer cultures. The disaggregated cells obtained by this procedure generally yield more uniform monolayer more quickly than do cultures initiated with minced tissues alone. This method is called enzymatic dissociation.

Before starting the preparation of primary culture, food should be withheld from donor fish for a day or more before use. Healthy specimens free of external lesions are preferred; otherwise there is risk of encountering systemically disseminated bacteria. Cells and tissues should be cultured at a temperature similar to the environmental temperature preferred by the donor species. Extended exposure of tissues from cold-water fishes such as salmon and trout to 30°C can be lethal. In contrast, many and perhaps most fish tissues remain viable even if held for a day or two on ice or at 4°C to 6°C. Internal tissues may be safely removed after thorough topical disinfection; this is conveniently done by total immersion of the fish for several minutes. A solution of liquid household bleach having 500 ppm available chlorine, or a 1:1000 dilution of a quaternary ammonium compound may be used. Excess disinfectant should be rinsed off with chlorinated tap water or sterile water and the surgical site sponged with 70% iso-propanol or ethanol. External tissues such as those of fins, gills, corneas and barbells are severely damaged by such disinfection. Consequently, such tissues should be excised first and decontaminated separately. Immersion for 1 hr in a solution containing 500 IU polymyxin B, 500 µg neomycin and 40 IU bacitracinis suggested, for these are bactericidal antibiotics.

Subculturing

When the cells in the primary culture vessel have grown and filled up all of the available culture substrate (called monolayer) they must be sub cultured to give them room for continued growth. This is usually done by removing them as gently as possible from the substrate with enzymes. These are similar to the enzymes used in obtaining the primary culture and are used to break the protein bonds attaching the cells to the substrate. Some cell lines can be harvested by gently scraping the cells off the bottom of the culture vessel. Once released, the cell suspension can then be subdivided and placed into new culture vessels. Once a surplus of cells is available, they can be treated with suitable cryoprotective agents, such as dimethylsulfoxide (DMSO) or glycerol, carefully frozen and then stored at cryogenic temperatures (below -130°C) until they are needed.

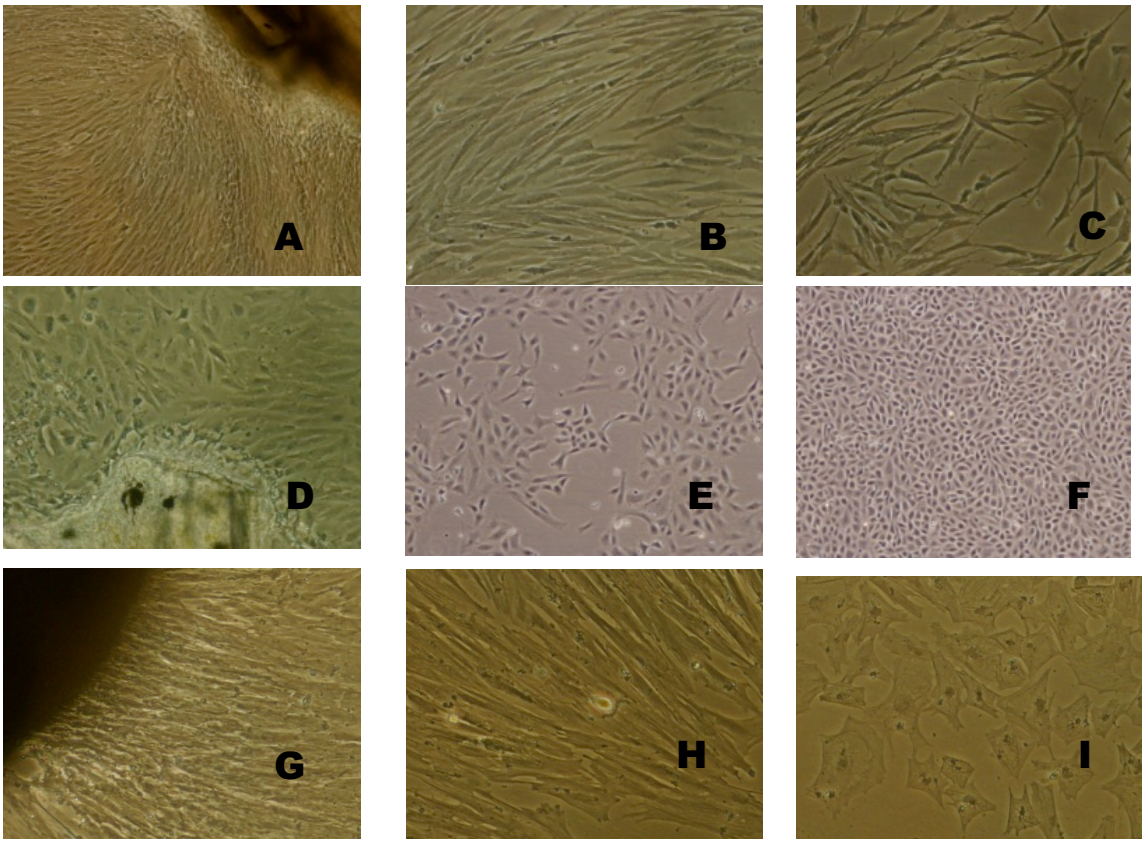


Fig.1. Phase contrast photomicrographs of rohu cells derived from Heart - (A, B, C), Fin - (D,E, F) and Swim bladder - (G, H, I). (A, D, G)-Primary culture on Day 5 following seeding tissue explant; (B, E, H) - subcultured at Passage 10; (C,F,I) - subcultured at Passage 30.

Development of continuous cell lines

Some cell lines may give rise to continuous cell lines. The ability of a cell line to grow continuously probably reflects its capacity for genetic variation, allowing subsequent selection. Genetic variation often involves the deletion or mutation of the p53 gene, which would normally arrest cell cycle progression, if DNA were to become mutated, and over expression of the telomerase gene. Possibly the condition that predisposes most to the development of a

continuous cell line is inherent genetic variation, so it is not surprising to find genetic instability perpetuated in continuous cell lines. The alteration in a culture that give rise to a continuous cell line is communally called *in vitro* transformation and may occur spontaneously or be chemically or virally induced. Immortalization means the acquisition of an infinite life span and transformation implies an alteration in growth characteristics (anchorage independence, loss of contact inhibition and density limitation of growth) that will often, but not necessarily correlate with tumorigenicity

Many (if not most) normal cells do not give rise to continuous cell lines. Normal human fibroblasts remain euploid throughout their life span and at crisis will stop dividing (around 50 generations), although may viable for 18 months. Human glia cells and chick fibroblasts behave similarly. Epidermal cells, on the other hand, have shown gradually increasing life span with improvements in culture techniques and may yet be shown capable of giving rise to continuous growth. Continuous cell line of lymphoblastoid cells is also possible by transformation with Epstein-Barr virus.

Properties of finite and continuous cell line

Properties	**Finite cell line**	**Continuous cell line**
Ploidy	Euploid, eiploid	Aneuploid, heteroploidy
Transformation	Normal	Immortal, growth control altered and tumorigeneic
Anchorage dependence	Yes	No
Contact inhibition	Yes	No
Density limitation of cell proliferation	Yes	No
Mode 0of growth	Monolayer	Monolayer or suspension
Maintenance	Cyclic	Steady phase possible
Serum requirement	High	Low
Cloning efficiency	Low	High
Markers	Tissue specific	Chromosomal, enzymic, antigenic
Special function	May be retained (virus susceptibility, differentiation)	Often lost
Growth rate	Slow	Rapid
Yield	Low	High
Control parameters	Generation number, tissues specific markers	Stain characteristics

Characterization of Cell Lines

In contrast to mammalian cells, are easier to maintain and manipulate, and unlike primary cultures, produce highly reproducible results. This ease of handling and simpler growth requirements makes cross-contamination of cell lines a more likely possibility, Proper characterization and identification of the cell lines are hence critical for scientific usage.

Authentication of a cell line is the sum of the process by which a line's identity is verified and shown to be free of contamination from other cell lines and microbes. Tests used to authenticate cell cultures include iso-enzyme analysis, antigenic markers, karyotyping/ cytogenetic analysis and more recently molecular techniques of DNA profiling. Whilst most of the techniques above are generalized tests and are applicable to all cell lines additional specific tests may also be required to confirm the presence of a product or antigen of interest.

Cell line contamination

Cell line contamination is a major drawback of main cell banks of the world and it has cost of losing important biological products or valuable research. The causative agents are different chemicals, invertebrates, bacteria, fungi, parasites, viral species and even other cell lines. Bacteria, fungi, parasite, viruses, invertebrates and mycoplasmas are main causative agents of cell line contamination.

The bacterial and fungal (including moulds and yeast) contamination of cell lines (except mycoplasmas) can be readily detected, as these organisms cause increased turbidity, shift in media pH (change in medium color) and cell destruction. Some reports have indicated that putative pathogens such as nanobacteria also will not be detected by this method.

In the case of mycoplasmas their cell line contamination is always undetected for many passages. They can proliferate within the cell, tolerate antibiotics and their growth always does not have any obvious microbial evidence like turbidity and pH changes or cytopathic effect. Their contamination also spreads quickly to the other cell lines

Sources of contamination

Another approach to cell culture contamination is sources of contamination. The sources of microbial culture contamination are different and may be grouped under four subjects.

- Contaminated cells, which are used as the primary starting material for cell culture.
- Glassware or apparatus, including storage bottles and pipettes
- Culture media (serum, basal cultural media containing heat-sensitive essential amino acids and vitamins, enzymes like trypsin, pronase and collagenase, and basic salt solutions).
- Airborne modes which can occur anytime the culture vessel is opened or contact is made with culture fluid through a defective culture vessel, stopper, or poor technique

Cross-Contamination and Misidentification

The problem of intra species and interspecies cross-contamination among cell lines has been recognized for half a century. For those scientists working on cell lines derived themselves or

received from a colleague, basic authentication tests such as STR profiling, iso-enzyme analysis, and contamination tests are readily available and should be routinely used. Transferring cell lines to colleagues should be avoided, or when it does occur, accompanied with comprehensive documentation verifying the integrity of the material or tests need to be repeated. Although cross-contamination of fish cells with other cell types has not been widely reported, conveyed the identification of a cell line dubbed Clone 1A believed to be derived from rainbow trout as being CHSE-214, a cell line derived from Chinook salmon embryos. Accordingly, awareness of good laboratory practices and careful vigilance with fish cell cultures as detailed by Lannan should be followed to avoid confusion of cell lines.

Applications of cell culture

Fish cell lines have been useful in many areas of research. Originally developed to support the growth of fish viruses for studies in aquatic animal viral diseases, fish cell lines have grown tremendously in number covering a wide variety of species and tissues of origin and an array of applications. Fish immunologies, physiology, genetics and development, toxicology, ecotoxicology, endocrinology, biomedical research, disease control, biotechnology and aquaculture are some of the areas in which fish cell lines have made significant contributions

Toxicity Testing

Cultured cells are widely used alone or in conjunction with animal tests to study the effects of new drugs, cosmetics and chemicals on survival and growth in a wide variety of cell types. Especially important are liver- and kidney-derived cell cultures.

Cancer Research

Since both normal cells and cancer cells can be grown in culture, the basic differences between them can be closely studied. Since, the normal cultured cells could be induced into cancer cells, the mechanisms that cause the change can be studied. Cultured cancer cells also serve as a test system to determine suitable drugs and methods for selectively destroying types of cancer.

Virology

One of the earliest and major uses of cell culture is the replication of viruses in cell cultures (in place of animals) for use in vaccine production. Cell cultures are also widely used in the clinical detection and isolation of viruses, as well as basic research into how they grow and infect organisms**.**

Cell-Based Manufacturing

Cultured cells can be used to produce many important products, like viral vaccines, genetically engineered protein of medicinal and commercial value and replacement of tissues and organs.

Genetic Counseling

Amniocentesis, a diagnostic technique that enables doctors to remove and culture fetal cells from pregnant women, has given doctors an important tool for the early diagnosis of fetal

disorders. These cells can then be examined for abnormalities in their chromosomes and genes using karyotyping, chromosome painting and other molecular techniques.

Genetic Engineering

The ability to transfect or reprogram cultured cells with new genetic material (DNA and genes) has provided a major tool to molecular biologists wishing to study the cellular effects of the expression of these genes (new proteins).

Gene Therapy

The ability to genetically engineer cells has also led to their use for gene therapy. Cells can be removed from a patient lacking a functional gene and the missing or damaged gene can then be replaced. The cells can be grown for a while in culture and then replaced into the patient. An alternative approach is to place the missing gene into a viral vector and then "infect" the patient with the virus in the hope that the missing gene will then be expressed in the patient's cells.

Further reading

Fryer J L, Lannan C N. 1994. Three decades of fish cell culture: a current listing of cell lines derived from fish. Journal of Tissue Culture Methods 16:87–94.

Freshney R I. 2005. Culture of animal cells: a manual of basic technique. Wiley, New Jersey.

Lakra W S, Swaminathan T R and Joy K P. 2010. Development, characterization, conservation and storage of fish cell lines: a review. Fish Physiology and Biochemistry. DOI 10.1007/s10695-010-9411-x.

****

43

PROBIOTICS IN AQUATIC ANIMAL HEALTH MANAGEMENT

S. Ezhil Nilavan and Toms C Joseph
Microbiology, Fermentation & Biotechnology Division,
E-mail: theezhilnilavan@gmail.com

Aquaculture is a major food-producing activity that helps to cater for 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 worry about finding alternatives that are safe, non-antibiotic-based, and environmentally acceptable prophylactic approaches. 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 the fishes fight diseases and promote well being since they have antibacterial, antifungal, and antiviral capabilities. Probiotics help the fishes fight diseases and promote well being since they have antibacterial, antifungal, and antiviral capabilities. Probiotics are a unique concept in fish farming, and their effectiveness in an aquatic setting is still to be well investigated. This review 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. During 1907, a Russian analyst named Elie Metchnikoff noted that Bulgarian labourers 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 ciliated protozoan. In 1974, Parker defined probiotics as organism and substances 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 which, 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.

Probiotics has 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 labelled as "probiotics" have attempted to capitalize on this theory. The research and potential application of probiotics in aquaculture has continued to grow during last couple of decades. Representatives of roughly 20 bacterial genera have recently been recognized as prospective probiotic candidates, with *Bacillus* spp and the *Lactobacillus* spp (LAB) group representing the bulk of promising species (Knipe et al., 2020).

Characteristics of an ideal probiotic

The vital role of probiotics is to establish or to maintain a health 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 intake all nutrients from the water and the harmful bacteria is 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 which are produced through fermentation
Viable probiotics	These are live microorganisms , have a protocol to be counting, and to be 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 favourable environmental condition (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 gastrointestinal tract despite not being acclimated to the aquatic environment (Merrifield et al., 2010). 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 by 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 probiotics administration. Melo et al., 2021 stated that92.8 % 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 has improved the growth and nutrient utilization in rainbow trout fry.

There are several reports suggesting that probiotics can also be administered through 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* sp through intramuscular route enhanced the immunity in rainbow trout (Laptra et al., 2014).

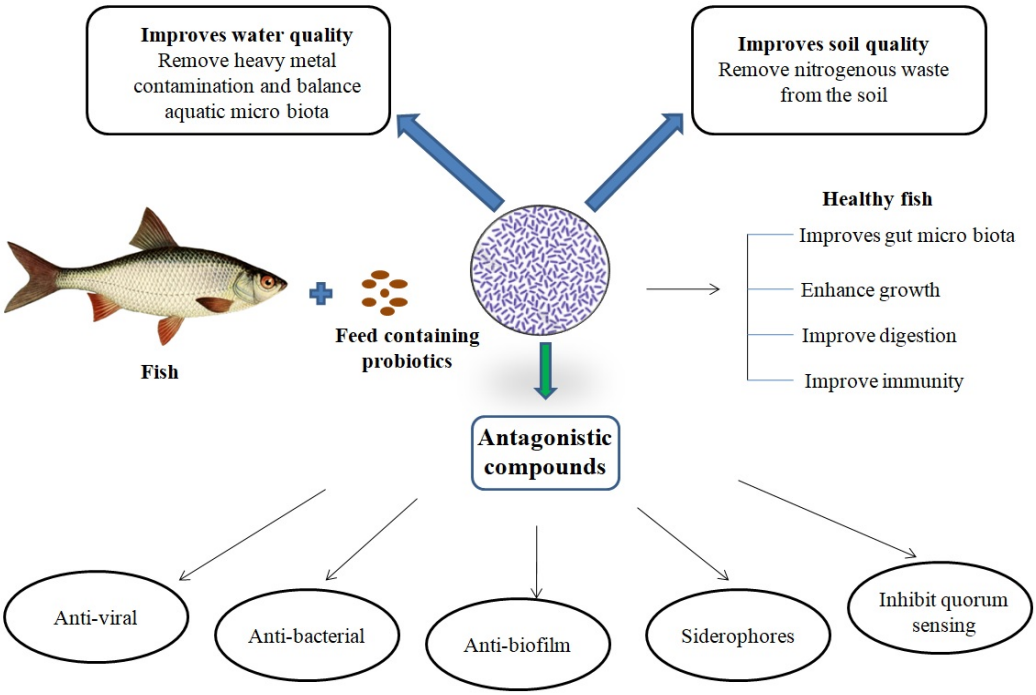
Single and combinations of probiotics

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 over the use of antibiotics, that's not surprise that the probiotics for aquaculture is growing at a quick pace.

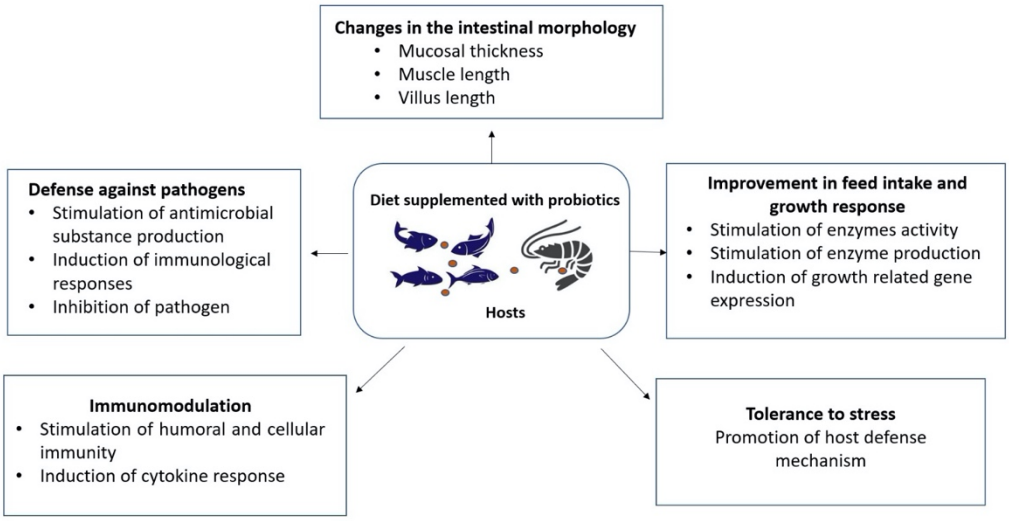
Probiotics have now been recommended by the Food and Agricultural Organization (FAO) for improving aquatic environmental quality by reducing mortality (Subasinghe, 2005).*Bacillus*, *Lactobacillus*, and *Bifidobacterium* are by far the most often used probiotic bacteria. Different species of *Lactobacillus*, *Bifidobacterium* and *Streptococcus* are used as probiotic 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 Indian Aquaculture: Table 1

In the Indian aquaculture industry, intensive and semi-intensive farming practices have emerged 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) have been used as probiotics to improve the water quality. Gram positive bacteria, particularly *Bacillus* species, were shown to be highly efficient at converting organic materials to CO2, slime, or microbial biomass. Gram - positive appear to do superior than 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 favourable 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, haematological parameters, improved feed utilization	Jahan et al.,2021
Lactobacillus fermentum	*Cirrhinus mrigala*	Better growth, haematological 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	*Catlacatla*	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	*Catlacatla*	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

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 around 90% of worldwide production. Aquaculture offers a vital supply of nutritious food for human consumption; however diseases in the fish farming business 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, immunostimulatory abilities of boosting health and wellbeing to enhance growth and yield, strengthen the immune function, hinder QS as a new anti-infective approach, mitigate the adverse affects of reactive oxygen species (ros stressors, and greater resistance. 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.

Reference:

Amoah, K., Huang, Q. C., Tan, B. P., Zhang, S., Chi, S. Y., Yang, Q. H., & Dong, X. H. (2019). Dietary supplementation of probiotic Bacillus coagulans ATCC 7050, improves the growth performance, intestinal morphology, microflora, immune response, and disease confrontation of Pacific white shrimp, Litopenaeus vannamei. *Fish & shellfish immunology*, *87*, 796-808.

Araujo, C., Munoz-Atienza, E., Poeta, P., Igrejas, G., Hernandez, P. E., Herranz, C., & Cintas, L. M. (2016). Characterization of Pediococcusacidilactici strains isolated from rainbow trout (Oncorhynchus mykiss) feed and larvae: safety, DNA fingerprinting, and bacteriocinogenicity. *Diseases of aquatic organisms*, *119*(2), 129-143.

Azad, M. A. K., Islam, S. S., Sithi, I. N., Ghosh, A. K., Banu, G. R., Bir, J., & Huq, K. A. (2019). Effect of probiotics on immune competence of giant freshwater prawn Macrobrachium rosenbergii. *Aquaculture Research*, *50*(2), 644-657.

Balcázar, J. L., Vendrell, D., de Blas, I., Ruiz-Zarzuela, I., Muzquiz, J. L., &Girones, O. (2008). Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. *Aquaculture*, *278*(1-4), 188-191.

Bandyopadhyay, P., Mishra, S., Sarkar, B., Swain, S. K., Pal, A., Tripathy, P. P., & Ojha, S. K. (2015). Dietary Saccharomyces cerevisiae boosts growth and immunity of IMC Labeo rohita (Ham.) juveniles. *Indian Journal of Microbiology*, *55*(1), 81-87.

Banerjee, S., Khatoon, H., Shariff, M., & Yusoff, F. M. (2010). Enhancement of Penaeus monodon shrimp postlarvae growth and survival without water exchange using marine Bacillus pumilus and periphytic microalgae. *Fisheries Science*, *76*(3), 481-487.

Chantharasophon, K., Warong, T., Mapatsa, P., &Leelavatcharamas, V. (2011). High potential probiotic Bacillus species from gastro-intestinal tract of Nile Tilapia (Oreochromis niloticus).

Das, A., Nakhro, K., Chowdhury, S., & Kamilya, D. (2013). Effects of potential probiotic *Bacillus amyloliquifaciens* [corrected] FPTB16 on systemic and cutaneous mucosal immune responses and disease resistance of catla (*Catlacatla*). *Fish and Shellfish Immunology*, *35*(5), 1547–1553.

FAO/WHO, 2001. Report on Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. 1–4 October, Cordoba, Argentina

Giri, S. S., Sukumaran, V., & Oviya, M. (2013). Potential probiotic *Lactobacillus plantarum* VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish, *Labeo rohita*. *Fish and Shellfish Immunology*, *34*(2), 660–666.

Gobi, N., Malaikozhundan, B., Sekar, V., Shanthi, S., Vaseeharan, B., Jayakumar, R., & Nazar, A. K. (2016). GFP tagged Vibrio parahaemolyticus Dahv2 infection and the protective effects of the probiotic Bacillus licheniformis Dahb1 on the growth, immune and antioxidant responses in Pangasius hypophthalmus. *Fish & Shellfish Immunology*, *52*, 230-238.

Gupta, A., Gupta, P., & Dhawan, A. (2016). Paenibacilluspolymyxa as a water additive improved immune response of Cyprinus carpio and disease resistance against Aeromonas hydrophila. *Aquaculture Reports*, *4*, 86-92.

Hai, N. V. (2015). The use of probiotics in aquaculture. *Journal of applied microbiology*, *119*(4), 917-935.

Harikrishnan, R., Balasundaram, C., &Heo, M. S. (2010). Effect of probiotics enriched diet on Paralichthysolivaceus infected with lymphocystis disease virus (LCDV). *Fish & Shellfish Immunology*, *29*(5), 868-874.

Jahan, N., Islam, S. M., Rohani, M. F., Hossain, M. T., & Shahjahan, M. (2021). Probiotic yeast enhances growth performance of rohu (Labeo rohita) through upgrading hematology, and intestinal microbiota and morphology. *Aquaculture*, *545*, 737243.

Jha, D. K., Bhujel, R. C., & Anal, A. K. (2015). Dietary supplementation of probiotics improves survival and growth of Rohu (Labeo rohita Ham.) hatchlings and fry in outdoor tanks. *Aquaculture*, *435*, 475-479.

Kalarani, V., Sumathi, V., Roshan, J. K., Sowjanya, D., & Reddy, D. C. (2016). Effect of dietary supplementation of Bacillus subtilis and Terribacillussaccharophillus on innate immune responses of a tropical freshwater fish, Labeo rohita. *Journal of Clinical and Cellular Immunology*, *7*, 395.

Kesarcodi-Watson A, Kaspar H, Lategan MJ and Gibson L, 2008. Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. Aquaculture, 274(1): 1-14, doi: 10.1016/ j.aquaculture.2007.11.019

Khan, M. I. R., Kamilya, D., Choudhury, T. G., & Rathore, G. (2022). Dietary administration of a host-gut derived probiotic Bacillus amyloliquefaciens COFCAU_P1 modulates immune-biochemical response, immune-related gene expression, and resistance of Labeo rohita to Aeromonas hydrophila infection. *Aquaculture*, *546*, 737390.

Kim, D. H., Brunt, J., & Austin, B. (2007). Microbial diversity of intestinal contents and mucus in rainbow trout (Oncorhynchus mykiss). *Journal of applied microbiology*, *102*(6), 1654-1664.

Knipe H, Temperton B, Lange A, Bass D and Tyler CR, 2020. Probiotics and competitive exclusion of pathogens in shrimp aquaculture. Rev Aquac, 1-29, doi: 10.1111/raq.12477

Krishnaveni, G., Vignesh, S., Vidhyalakshmi, N., Vijay, V., & Ramesh, U. (2021). Effects of dietary supplementation of Lactobacillus fermentum URLP18 on growth, innate immunity and survival against Aeromonas hydrophila ATCC 7966 challenge in freshwater fish Cyprinus carpio (common carp). *Aquaculture Research*, *52*(3), 1160-1176..

Krishnaveni, G., Vignesh, S., Vidhyalakshmi, N., Vijay, V., & Ramesh, U. (2021). Effects of dietary supplementation of Lactobacillus fermentum URLP18 on growth, innate immunity and survival against Aeromonas hydrophila ATCC 7966 challenge in freshwater fish Cyprinus carpio (common carp). *Aquaculture Research*, *52*(3), 1160-1176.

LaPatra, S. E., Fehringer, T. R., & Cain, K. D. (2014). A probiotic Enterobacter sp. provides significant protection against Flavobacterium psychrophilum in rainbow trout (Oncorhynchus mykiss) after injection by two different routes. *Aquaculture*, *433*, 361-366.

Liu, K. F., Chiu, C. H., Shiu, Y. L., Cheng, W., & Liu, C. H. (2010). Effects of the probiotic, Bacillus subtilis E20, on the survival, development, stress tolerance, and immune status of white shrimp, Litopenaeus vannamei larvae. *Fish & shellfish immunology*, *28*(5-6), 837-844.

Melo-Bolívar, J. F., Ruiz Pardo, R. Y., Hume, M. E., & Villamil Diaz, L. M. (2021). Multistrain probiotics use in main commercially cultured freshwater fish: A systematic review of evidence. *Reviews in Aquaculture*, *13*(4), 1758-1780.

Merrifield, D. L., Dimitroglou, A., Foey, A., Davies, S. J., Baker, R. T., Bøgwald, J.,& Ringø, E. (2010). The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture*, *302*(1-2), 1-18.

Mohapatra, S., Chakraborty, T., Prusty, A. K., Prasad, K. P., & Mohanta, K. N. (2014). Dietary multispecies probiotic supplementation enhances the immunohematological responses and reduces mortality by Aeromonas hydrophila in Labeo rohita fingerlings. *Journal of the World Aquaculture Society*, *45*(5), 532-544.

Moriarty DJW, 1998. Control of luminous Vibrio species in penaeid aquaculture ponds. Aquaculture, 164: 351-358, doi: 10.1016/S0044-8486(98)00199-9

Moriarty, D. J. W. (1998). Control of luminous Vibrio species in penaeid aquaculture ponds. *Aquaculture*, *164*(1-4), 351-358.

Mujeeb Rahiman, K. M., Jesmi, Y., Thomas, A. P., & Mohamed Hatha, A. A. (2010). Probiotic effect of Bacillus NL110 and Vibrio NE17 on the survival, growth performance and immune response of Macrobrachium rosenbergii (de Man). *Aquaculture research*, *41*(9), e120-e134.

Munirasu, S., Ramasubramanian, V., & Arunkumar, P. (2017). Effect of probiotics diet on growth and biochemical performance of freshwater fish *Labeo rohita* fingerlings. *Journal of Entomology and Zoology Studies*, *5*(3), 1374–1379.

Nandi, A., Banerjee, G., Dan, S. K., Ghosh, K., & Ray, A. K. (2018). Evaluation of *in vivo* probiotic efficiency of *Bacillus amyloliquefaciens* in *Labeo rohita* challenged by pathogenic strain of *Aeromonas hydrophila* MTCC 1739. *Probiotics and Antimicrobial Proteins*, *10*(2), 391–398.

NavinChandran, M., Iyapparaj, P., Moovendhan, S., Ramasubburayan, R., Prakash, S., Immanuel, G., &Palavesam, A. (2014). Influence of probiotic bacterium *Bacillus cereus* isolated from the gut of wild shrimp *Penaeus monodon* in turn as a potent growth promoter and immune enhancer in *P. monodon*. *Fish and Shellfish Immunology*, *36*(1), 38–45.

Panigrahi, A., Kiron, V., Satoh, S., & Watanabe, T. (2010). Probiotic bacteria *Lactobacillus rhamnosus* influences the blood profile in rainbow trout *Oncorhynchus mykiss* (Walbaum). *Fish Physiology and Biochemistry*, *36*(4), 969–977.

Pannu, R., Dahiya, S., Sabhlok, V. P., Kumar, D., Sarsar, V., &Gahlawat, S. K. (2014). Effect of probiotics, antibiotics and herbal extracts against fish bacterial pathogens. *Ecotoxicology and Environmental Contamination*, *9*(1), 13-20.

Reda, R. M., Selim, K. M., El-Sayed, H. M., & El-Hady, M. A. (2018). In vitro selection and identification of potential probiotics isolated from the gastrointestinal tract of Nile tilapia, Oreochromis niloticus. *Probiotics and antimicrobial proteins*, *10*(4), 692-703.

Ringø, E. Z. Z. V., Zhou, Z., Vecino, J. G., Wadsworth, S., Romero, J., Krogdahl, Å., & Merrifield, D. L. (2016). Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story. *Aquaculture nutrition*, *22*(2), 219-282.

Sahandi, J., Jafaryan, H., Soltani, M., & Ebrahimi, P. (2019). The use of two Bifidobacterium strains enhanced growth performance and nutrient utilization of Rainbow Trout (Oncorhynchus mykiss) fry. *Probiotics and antimicrobial proteins*, *11*(3), 966-972.

Sangma, T., & Kamilya, D. (2015). Dietary Bacillus subtilis FPTB13 and chitin, single or combined, modulate systemic and cutaneous mucosal immunity and resistance of catla, Catlacatla (Hamilton) against edwardsiellosis. *Comparative Immunology, Microbiology and Infectious Diseases*, *43*, 8-15.

Saravanan, K., Sivaramakrishnan, T., Praveenraj, J., Kiruba-Sankar, R., Haridas, H., Kumar, S., & Varghese, B. (2021). Effects of single and multi-strain probiotics on the growth, hemato-immunological, enzymatic activity, gut morphology and disease resistance in Rohu, Labeo rohita. *Aquaculture*, *540*, 736749.

Subasinghe, R. P. (2005). Epidemiological approach to aquatic animal health management: opportunities and challenges for developing countries to increase aquatic production through aquaculture. *Preventive veterinary medicine*, *67*(2-3), 117-124.

Thirumurugan R, Vignesh V (2015) Probiotics: live boon to aquaculture. In: Advances in marine and brackishwater aquaculture. Springer India, pp 51–61

Van Doan, H., Hoseinifar, S. H., Ringø, E., Ángeles Esteban, M., Dadar, M., Dawood, M. A., &Faggio, C. (2020). Host-associated probiotics: a key factor in sustainable aquaculture. *Reviews in Fisheries Science & Aquaculture*, *28*(1), 16-42.

Venkat, H. K., Sahu, N. P., & Jain, K. K. (2004). Effect of feeding Lactobacillus-based probiotics on the gut microflora, growth and survival of postlarvae of Macrobrachiumrosenbergii (de Man). *Aquaculture Research*, *35*(5), 501-507.

Verschuere, L., Rombaut, G., Sorgeloos, P., & Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. *Microbiology and molecular biology reviews*, *64*(4), 655-671.

Weber, B., Chen, C., & Milton, D. L. (2010). Colonization of fish skin is vital for Vibrio anguillarum to cause disease. *Environmental microbiology reports*, *2*(1), 133-139.

Zorriehzahra, M. J., Delshad, S. T., Adel, M., Tiwari, R., Karthik, K., Dhama, K., &Lazado, C. C. (2016). Probiotics as beneficial microbes in aquaculture: an update on their multiple modes of action: a review. *Veterinary quarterly*, *36*(4), 228-241.

****

44

BACTERIAL TOXINS OF SEAFOOD IMPORTANCE, PATHOGENESIS AND DETECTION

T.K. Anupama
Microbiology, Fermentation & Biotechnology Division,
E-mail: anupamatk.tk@gmail.com

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 per capita 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, bio toxins, 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. Centres 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 are 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 processes 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 super antigens 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 vacuum 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 inhalational. 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 catecholamine. 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 pathogen responsible for food poisoning outbreaks worldwide. They are enterotoxin producing pathogenic bacterium and occurring as commensal flora of humans (Alves *et al.,* 2014). They have a great significance in food industry due to the ability of certain strains to produce heat stable enterotoxin and other virulence factors which are responsible for staphylococcal food poisoning (SFP). (Argudin *et al.,* 2012; Tango *et al.,* 2015). Symptoms of SFP include nausea, violent vomiting, and abdominal cramping, with or without diarrhea within 2-4hr of consumption (Chen *et al.,* 2018). The minimum amount of toxins required to have symptoms is about 1ng/g of food. SFP is widely reported on protein rich foods such as meat, dairy and fish products which have extensive manual handling, inadequate heating and inappropriate storage (Adam and Moss 2007). The bacteria can be killed by heat treatment, but toxin produced is very heat resistant and remain in food even after cooking, which can cause food poisoning

SEs (Staphylococcus enterotoxins) belongs to a great family of staphylococcal and streptococcal pyrogenic exotoxins, characterized by common phylogenetic relationships, structure, function, and sequence homology. SEs functions 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 (SEIJ), K (SEIK), L (SEIL), M (SEIM), N (SEIN), O (SEIO), P (SEIP), Q (SEIQ), R (SER), S (SES), T (SET), U (SEIU),W(SEIW)), V (SEIV), X (SEIX), and Y (SEIY).Enterotoxins are encoded in prophages, plasmids, or chromosomal pathogenicity islands.

The location of the SE genes on mobile genetic elements presents an additional risk factor in *S. aureus* food intoxication, due to possible horizontal gene transfer (Cafini *et al.,* 2017; Lindsay, 2014).The transfer of genetic elements in *S. aureus* has contributed to strain variability and enhanced virulence. It is well known that *S. aureus* strains usually carry more

than one SE encoding gene. The stability of toxin is SEA: 3 min at 80 ◦C, 1 min at 100 ◦C; SEB 87 min at 99 ◦C. Stable at wide range of pH and resistant to gastric pH.

Control measures: Adequate control over the health and hygiene of fish handlers. The fish has to maintain at low temperature (below 5°C) during handling and processing. Minimize time/temperature abuse of seafood, especially after cooking

Pathogenic Escherichia coli

E.coli is Gram-negative, rod-shaped, non-spore forming facultative anaerobic bacteria. It is commonly found in the gut of humans and warm-blooded animals. Pathogenic strains of *E. coli* are transferred to seafood through sewage pollution of the coastal environment or by contamination after harvest. Similar concerns occur if contaminated ice used for preservation or the utensils contaminated with *E. coli.* Improperly cleaned boat deck, and containers used in onboard trawlers can also act be source of contamination. There are six categories of pathogenic *E.coli* which include Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E.coli* (EPEC), Enteroinvasive *E.coli* (EIEC), Enterohemorrhagic E. coli (EHEC, Shiga toxin-producing *E. coli* or STEC), Enteroaggregative *E. coli* (EAEC or EAggEc) and Diffusely adherent *E. coli* (DAEC). Among these Shiga toxin-producing *E. coli* (STEC) has been associated with severe foodborne outbreaks of major public health importance in the last years. STEC produces toxins, known as Shiga-toxins because of their similarity to the toxins produced by *Shigella dysenteriae.* Shiga toxins (Stx) can be divided into two categories: Stx1, which is identical to the toxins produced by *Shigella dysenteriae* 1, and Stx2, which is around 60% similar to Stx1. Production of one or more Shiga toxins is essential to cause disease, but the production of Stx2 is more closely linked to the severity of the disease such as hemolytic uremic syndrome (HUS) and HC (Farrokh, *et al.,* 2013). STEC strains can be classified as O157 and non-O157. Serotype O157:H7 is the most common serotype involved in severe infections resulting to HUS and HC, and it has been linked to the majority of large-scale outbreaks of STEC infections. Symptoms of STEC are severe diarrhea, stomach cramps, and vomiting. Diarrhea is often bloody without fever. Symptoms typically appear 3-4 days after eating contaminated product, but can range from 1-10 days. STEC can grow in temperatures ranging from 7°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 extra intestinal 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 faeces 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 susceptible 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

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.

Reference

Adams, M, R., Moss, M. O., Food Microbiology, third ed., RSC, Cambridge, 2007.

Alves, D, R., Gaudion, A., Bean J, E., Perez Esteban, P., Arnot, T, C., Harper, D, R., Kot, W., Hansen, L. H., Enright, M, C., Tobias, A., Jenkinsa, A. 2014. Combined use of bacteriophage K and a novel bacteriophage to reduce *Staphylococcus aureus* biofilm formation. Appl Environ Microb 80:6694–703.

Argudın MA, Mendoza MC, Gonzalez-Hevia MA, Bances M, Guerra B, Rodicioa MR. 2012. ´ Genotypes, exotoxin gene content, and antimicrobial resistance of *Staphylococcus aureus* strains recovered from foods and food handlers. Appl Environ Microb 78:2930–5.

Cafini, F., Thi Le Thuy, N., Román, F., Prieto, J., Dubrac, S., Msadek, T., & Morikawa, K. (2017). Methodology for the study of horizontal gene transfer in Staphylococcus aureus. Journal of Visualized Experiments 121, 1–7.

Centers for Disease Control and Prevention (CDC). (2018). National Outbreak Reporting System (NORS). Retrieved from https://wwwn. cdc.gov/norsdashboard/

Chen, D., Song, Q., Xu, Z., Zhang, D., 2018. Characterization of enterotoxin A-producing Staphylococcus aureus., Infection and Drug Resistance, Page 531–538.

FAO. 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome. Page: 2-8. https://doi.org/10.4060/ca9229en

Farrokh, C., Jordan, K., Auvray, F., Glass, K., Oppegaard, H., Raynaud, S., Thevenot, D., Condron, R., De Reu, K., Govaris,A., Heggum, K., Heyndrickx, M., Hummerjohann,J., Lindsay, D., Miszczycha, S., Moussiegt, S., Verstraete, K.and Cerf, O. (2013) Review of Shiga-toxin- producing *Escherichia coli* (STEC) and their significance in dairy production. Int. J. Food Microbiol., 162: 190-212

Galaviz-Silva, L., Goméz-Anduro, G., Molina-Garza, Z. J., & Ascencio-Valle, F. (2009). Food safety Issues and the microbiology of fish and shellfish. Hoboken, NJ: John Wiley & Sons

Hernández-Cortez, C., Palma-Martínez,I., Uriel Gonzalez-Avila, L., Guerrero-Mandujano, A., Colmenero Solís, R. and Castro-Escarpulli., G. 2017 Food Poisoning Caused by Bacteria (Food Toxins). Poisoning-From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis (eds. Ntambwe malangu).Pp 33-72 Intech open UK http://dx.doi.org/10.5772/intechopen.69953

Iriarte MJ, Ugarte JC, Salazar L. Utility of the airbone endotoxins assay in different occupational environments as a risk indicator by biological agents. Mapfre Medicina. 2001;**12**(4):234-240

Lindsay, J. A. (2014). Staphylococcus aureus genomics and the impact of horizontal gene transfer. International Journal of Medical Microbiology, 304(2), 103–109.

Mi, E., Li, J., & McClane, B. A. (2018). NanR regulates sporulation and enterotoxin production by Clostridium perfringens type F strain F4969. Infection and Immunity, 86, e00416–18.

Peck, M. V. (2005). Clostridium botulinum. In M. Griffiths (Ed.), Understanding pathogen behaviour: Virulence, stress response and resistance (pp. 531–548). Amsterdam, The Netherlands: Elsevier. https://doi.org/10.1533/9781845690229.4.531

Platt-Samoraj, A. ToxigenicProperties of Yersinia enterocolitica Biotype 1A. 2022. Toxins, 14, 118.https://doi.org/10.3390/ toxins14020118

Rajkovic, A., Jovanovic,J., Monteiro, S., Decleer, M., Andjelkovic, M., Foubert, A., Beloglazova, N., Tsilla, V., Sas, B., Madder, A., Saeger, S ., Uyttendaele, M., (2020) Detection of toxins involved in foodborne diseases caused by Gram-positive bacteria *Compr Rev Food Sci Food Saf.* 2020;19:1605–1657.

Rood, J. I., Adams, V., Lacey, J., Lyras, D., Mcclane, B. A., Stephen, B.,Immerseel, F. Van (2018). Expansion of the Clostridium perfringens toxin-based typing scheme. Anaerobe, 53, 5–10.

S. Vidovic, A.K. Mangalappalli-Illathu, D.R. Korber Prolonged cold stress response of Escherichia coli O157 and the role of rpoS International Journal of Food Microbiology, 146 (2011), pp. 163-169

Sánchez-Chica, J., Correa, M. M., Aceves-Diez, A. E., & Castañeda- Sandoval, L. M. (2020). A novel method for direct detection of Bacillus cereus toxin genes in powdered dairy products. International Dairy Journal, 103, 104625

Tango, C. N., Hong S., , Jun Wang, and Deog-Hwan Oh., (2015). Assessment of Enterotoxin Production and Cross-Contamination of Staphylococcus aureus between Food Processing Materials and Ready-To-Eat Cooked Fish Paste. Journal of Food Science. Vol. 80, (12). 2015 page no. 2911-2916.

****

45

WATER QUALITY PARAMETERS IN AQUATIC ANIMAL HEALTH

Ezhil Nilavan. S,
Microbiology, Fermentation & Biotechnology Division,
E-mail: theezhilnilavan@gmail.com

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 between fish variety, age, weight and animal's use. The system's effectiveness and efficacy depends on its capacity to adapt 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 particle, 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 use 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 increase 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 leads 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 an ambient 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

Behavioural 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.

Husbandry

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.

Waste 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 behaviour
- Feeding the fish with artificial or live feed
- Recording each fish length, weight and feeding behaviour
- 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.

****

46

ALTERNATIVE TO ANTIBIOTICS IN AQUACULTURE PRACTICES

S. Visnuvinayagam
Microbiology, Fermentation & Biotechnology Division,
E-mail: visnuvinayagam@yahoo.co.in

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.,*

fructo oligosaccharides, galacto oligosaccharides, and mannan-oligosaccharides, dextrins, Inulin, lignin, waxes, and 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 have been proven to protect channel catfish from enteric septicaemia when injected but not when fed. Furthermore, a prebiotic of brewer's yeast, dairy components, and dried fermentation products was discovered to improve feed efficiency and minimize mortality in hybrid striped bass challenged with bacterial infections.

Synbiotics: Synbiotics a type of chemical that combines prebiotics with probiotics. Synbiotic may function by encouraging the growth of helpful bacteria in the host's gastrointestinal system. No studies in aquatic animals have investigated these products' efficacy, although symbiotic can manipulate gut micro biota 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 bacteriocins are that it is 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 anaesthetic effects.

Organic Acids: The application of the organic acid would lower the pH (around 3.5) of the environment, which provides a favourable environment for the proliferation of beneficial bacteria. E.g. *Lactobacillus* can able to proliferate during acidic conditions. Most of the pathogenic bacteria, such as *V. parahaemolyticus* and *V. cholera,* would die during the acidic condition. Various kinds of research were carried out in the laboratory and found that the organic acids are highly efficient in controlling all pathogenic vibrio. The mechanism action is mostly based on the lower pH. Since the Vibrio prefer to grow in alkaline conditions, all the vibrio species 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 a short chain of amino acids *i.e.*, between 12 to 15 amino acids. Since these molecules are short-chain, they generally thermostable; recently, these antimicrobial peptides are considered 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 zebra fish, piscidins from hybrid striped bass, dicentracin from sea bass, hepcidin from channel catfish and epinician from the groupers.

Plant compounds with antimicrobial activity: Numerous researchhas 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 have 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. However the materials can be used regularly, has significant improvement for disease control, and should not have any negative on the fish/ shrimps as well as consumers.

Nanoparticles: Nanoparticles possess potential antibacterial activity; 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 has 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 benefits 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 to have 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, areresponsible 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. Autogenously 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 shellfish 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. So, good aquaculture practices are a highly efficient way to reduce the

antibiotic-free environment. Office International des Epizooties (OIE) has given clear guidelines for good aquaculture practices.

****

47

ISOLATION OF FISH VIRUSES AND ITS CONFIRMATION IN CELL CULTURE

T. Raja Swaminathan
Microbiology, Fermentation and Biotechnology Division
Email: t.swaminathan@icar.gov.in

Viruses are obligate intracellular parasites that require living cells in order to replicate. Cultured cells, eggs and laboratory animals may be used for virus isolation. Although embroyonated eggs and laboratory animals are very useful for the isolation of certain viruses, cell cultures are the sole system for virus isolation in most laboratories. The development of methods for cultivating animal cells has been essential to the progress of animal virology. Cell lines vary greatly in their susceptibility to different viruses. It is of utmost importance that the most sensitive cell lines are used for a particular suspected virus.

Specimens for isolation on cell culture should be transported to the laboratory as soon as possible upon being taken. Swabs should be put in a vial containing virus transport medium. Bodily fluids and tissues should be placed in a sterile container. Upon receipt, the specimen is inoculated into several different types of cell lines depending on the nature of the specimen and the clinical presentation. The maintenance media should be changed after 1 hour or if that is not practicable, the next morning. The inoculated tubes should be read at least every other day for the presence of cytopathic effect.

Certain specimens, such as urine and faeces, may be toxic to cell lines that may produce a CPE-like effect. If toxic effects are extensive, it may be necessary to passage the inoculated cells. Cell lines that are contaminated by bacteria should either be put up again or passed through a bacterial filter. Cell lines should be kept for at least one to two weeks (longer in the case of CMV). Cell lines should be added with fresh maintenance medium at regular intervals or if required should the culture medium become too acidic or alkaline. When CPE is observed, it may be advisable to passage infected culture fluid into a fresh culture of the same cell type.

Transportation and collection of samples

Pools of organs or of ovarian fluids are placed in sterile vials and stored at 4°C or on ice until virus extraction is performed in the laboratory. However, freezing of samples for testing for subclinical carriers should be avoided. Organ samples may also be transported to the laboratory by placing them in vials containing cell culture medium or Hanks' balanced salt

solution (HBSS) with added antibiotics to suppress the growth of bacterial contaminants (one volume of organ in at least five volumes of transportation fluid). Suitable antibiotic concentrations are: gentamicin (1000 μg ml^{-1}) or penicillin (800 IU ml^{-1}) and streptomycin (800 μg ml^{-1}). Antifungal compounds, such as Mycostatin® or Fungizone®, may also be incorporated into the transport medium at a final concentration of 400 IU ml^{-1}. Serum or albumin (5–10%) may be added to stabilise the virus if the transport time will exceed 12 hours.

Selection of tissue samples

The selection of tissues for virus assays varies according to the size and life stage of fish. The following tissues are the minimum that should be taken for virus assays.

Size/maturity of fish tissue assayed

- Under 4 cm- Entire fish (remove yolk sac if present)
- 4-6 cm- Entire viscera (includes kidney)
- Over 6 cm- Kidney and spleen
- Sexually mature- Ovarian fluid, kidney, and spleen

For fish 4 to 6 cm or over 6 cm, it is recommended (OIE 2013) that brain tissue also be included. The addition of gill filaments to the sample pool may also increase the sensitivity of detection for some viruses.

After tissues and fluid are removed from the fish, they can be pooled; however, no more than five fish should be in one pooled sample of tissue or fluid. Approximately equal volume or weight proportions should be maintained for each specimen in a pool.

Storage of samples

- The samples should be maintained between 4 and 10°C according to the virus (es) suspected. Samples should not be frozen.
- The samples should not be stored longer than 48 hours.
- Tissues may be stored in a buffered solution that contains antibiotics, antifungal, or both. The pH should be maintained within 7.4 to 7.8 or within the range that the suspected viruses (es) are stable.

Preparing samples for virus assays

- The preparation of samples involves the homogenization of tissues and bacterial and fungal decontamination of tissues and fluids.
- Homogenization can be accomplished in several ways; however, sonication is not acceptable for tissues. After homogenization, cellular material should be removed by centrifugation.
- Decontamination can be accomplished either with the use of antibiotics and antifungal or by filtration of the supernatant of centrifuged tissues samples.

- The antibiotics and antifungal that are used should be wide spectrum in their activity, and their concentrations should be effective in decontamination but not adversely affect cell cultures.

The following compounds and concentrations should not be exceeded:

- Gentamicin 1000 µg/mL
- Penicillin 800 IU/mL
- Streptomycin 800 µg/mL
- Fungizone® 40 IU/mL
- Mycostatin® 400 IU/mL

The supernatant from centrifuged tissue samples can also be decontaminated by filtration through a 0.45 µm filter. Passing tissue culture medium supplemented with serum through the filter before filtration of the sample is passed is recommended to minimize virus adherence to the filter.

Inoculating the Samples

Selection of Cell Cultures

Each virus section should be consulted to determine the most sensitive cell line(s) for a given virus. The cells should be normal appearing, rapidly dividing, and mycoplasma-free. Stock cell cultures should be routinely tested for susceptibility to specific viruses and for the presence of *Mycoplasma* Penicillin (100IU/mL) streptomycin (100µg/mL) and antifungal agents such as Mycostatin$^{(R)}$ (50 IU/mL) can be used in media for cell culture and virus assay work.

Inoculation

Direct inoculation; transfer an appropriate volume of the antibiotic-treated or filtered homogenate on to 24 to 48 hour old cell monolayer in tissue culture flasks or multi-well plates. Inoculate at least 5 cm^2 of cell monolayer with 100 µL of the filtered supernatant. Alternatively, make a further tenfold dilution of the filtered supernatant in cell culture medium, buffered at pH 7.6 and supplemented with 2% foetal calf serum (FCS), and allow adsorption for 0.5–1 hour at 18–22°C. Then, without withdrawing the inoculate, add the appropriate volume of cell culture medium (1–1.5ml/5 cm^2 for cell culture flasks), and incubate at 20-25°C. The cell cultures used for sample inoculation should be 80-90% confluent and not older than 48 hours. A minimum of 50 µL of sample should be inoculated per l.0 $cm^2$of cell sheet. Un-inoculated controls must be used. Dilution of original samples should not exceed 1:10 for fluids and 1:100 for tissue samples.

Duration of Assay

The cell cultures should be incubated at 28°C and observed for cytopathological changes for a minimum of 14 days but 21 days incubation is recommended. Cell culture medium should be buffered or cells incubated so that a pH between 7.4 and 7.8 is maintained. A blind pass of 14 days is also recommended. The duration of the assay may need to be longer depending on

which viruses are suspected. When cytopathological changes occur, the cultures should be sub cultured or analyzed by serum neutralization or other confirmatory tests.

If effects have been observed after inoculation of antibiotic-treated homogenate, filter at least 1 ml of the organ homogenate supernatant through a 0.45 µm disposable cellulose acetate filter unit (or unit fitted with a similar low protein binding filter membrane).

Monitoring incubation

Follow the course of infection in positive controls and other inoculated cell cultures by daily microscopic examination at 40×–100× magnification for 14 days. The use of a phase-contrast microscope is recommended.

- Maintain the pH of the cell culture medium at between 7.3 and 7.6 during incubation. This can be achieved by the addition of sterile bicarbonate buffer or HEPES-buffered medium (HEPES = N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid) to the inoculated cell culture medium for tightly closed cell culture flasks.
- If a cytopathic effect (CPE) appears in those cell cultures inoculated with the dilutions of the tested homogenate supernatants, identification procedures must be undertaken immediately.
- If no CPE develops in the inoculated cultures (despite normal progression of CPE in the virus controls), the inoculated cultures should be sub-cultured for a further 14 days. If the virus control fails to develop CPE, the process should be repeated with fresh susceptible cells and new batches of samples.

Viral Plate Observation

Following inoculation of plates, all wells will be monitored on the following day and every other day for the next two weeks for signs of cytopathic effects (CPE), toxicity, and contamination. Plates will be monitored twice during the following week. Total observation period will be 21 d. If no CPE, toxicity or abnormalities are observed within 21 d, the samples are discarded and recorded as negative.

Re-Inoculation

If toxicity, abnormal pH or CPE is observed, one of the replicate wells of that sample will be aseptically aspirated, diluted 1: 10 with medium, filtered through a *0.45µm* filter and re-inoculated onto another 24-well test plate in duplicate and monitored for CPE an additional 14 d. All observations will be documented and recorded by the observer and kept on file with the laboratory records. If no CPE is observed in 14 days after re- inoculation, the sample is discarded and recorded as negative. If CPE is observed, proceed to corroborative methods to confirm identity of suspect virus.

Cytopathic Effects (CPE) of Virus Infection in Tissue Culture Cells

IHNV-induced CPE

- Rounded and granular cells in grape-like clusters.

- Rounded, infected cells also accumulate at plaque margins and can be present within the plaque.

IPNV -induced CPE

- Spindle-shaped or "balloon-on-a-stick"-shaped cells.
- Pyknosis of nuclei (nuclei shrink in size and chromatin condenses).
- Plaques are stellate in a confluent cell monolayer and contain not only live cells but also normal looking cells.

Herpesvirus-induced CPE

- Pyknosis of nuclei and cellular fusion (syncytia).
- Plaques tend to elongate and follow whorl lines of growth if on RTG-2 cells.
- They have relatively clear interiors, but living cells extend into the open area.

VHSV-induced CPE

- The VHSV isolates plaque very similarly to IHNV in EPC cells forming rounded and granular cells in grape-like clusters.
- Number of days following infection with virus that CPE is usually observed in freshly mono layered fish cell cultures.

LMBV-induced CPE

- CPE within 48 h after inoculation.
- Initial CPE - few pyknotic cells, which develop to form circular, cell free areas, with rounded cells at the margins.
- Advanced CPE - Pyknosis, rounding and detached cell sheet. Entire cell sheet affected.

Confirmatory identification

The confirmation of the etiological agent should be done using following methods,

- Confirmation of virus identity by neutralisation
- Confirmation of virus identity by the indirect fluorescent antibody test (IFAT)
- Confirmation of virus identity by enzyme-linked immunosorbent assay (ELISA)
- Confirmation of virus identity by polymerase chain reaction (PCR)

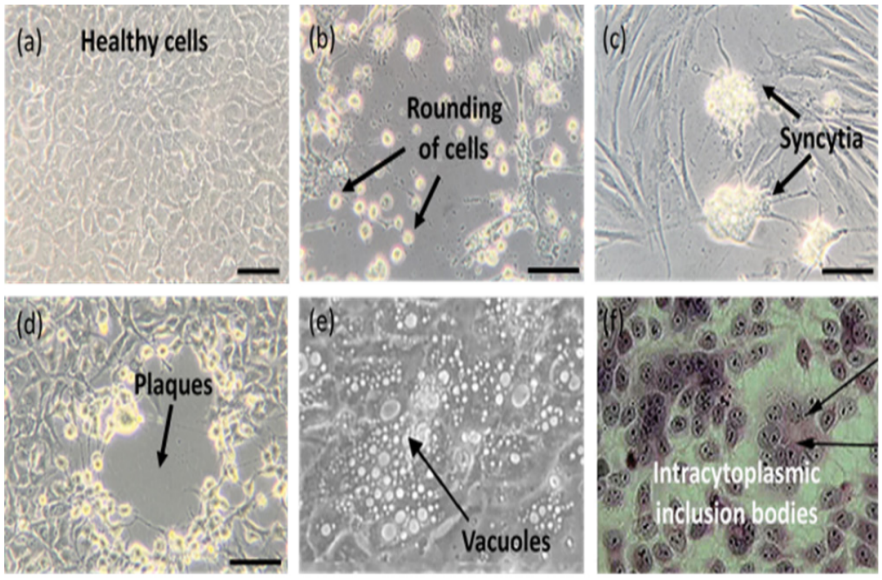
The most reliable method for confirmatory identification of a CPE is by PCR, followed by sequence analysis of the PCR product. The PCR methods recommended for identification of virus are the same methods recommended for direct detection in fish tissues. PCR products for final confirmation of the correct size should be identified as viral in origin by sequence analysis.

Virus preservation and storage

- Centrifuge infected cell cultures at 2–5°C and 2000–4000 *g* for 15 minutes, then dilute the virus containing supernatants in order to obtain virus titres averaging 1–2 × 10^6 PFU ml^{-1}.

- Dispense the resulting viral suspensions into sterile vials at volumes of 0.3–0.5 ml each.
- Freeze and store each series of standard virus stocks at –80°C or liquid nitrogen, and check the titre of each virus stock at regular intervals if it has not been used during that time period.
- *Lyophilisation:* long-term storage (decades) of the seeds of standard virus strains is achievable by lyophilisation. In this purpose, viral suspensions in cell culture medium supplemented with 10% foetal calf serum are mixed (v/v) with an equal volume of cryopreservative medium (20% lactalbumin hydrolysate in distilled water) before processing. Seal or plug under vacuum and store at 4°C, in the dark.

Cytopathic Effects (CPEs) displayed by viruses in susceptible host



Source: Aarattuthodi S, Dharan V, Koshy M (2021) Fish Cell Cultures -

48

INTRODUCTION TO BIOINFORMATICS, SEQUENCING AND BIOINFORMATICS ANALYSIS

Shri. V. Radhakrishnan Nair
Microbiology, Fermentation and Biotechnology Division
Email: vrnair08@gmail.com

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

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

MICROBIOLOGY

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

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

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

BIOTECHNOLOGY

Biotechnology is an applied science, which utilizes biological systems, living organisms to develop or make useful products. In other words, it is the technological application that uses living beings, largely microbial living organisms, or derivatives to make or modify products or processes for specific use. Exploitation of biological processes for industrial and other purposes through genetic manipulation of microorganisms is also scientifically established in the discipline of Biotechnology. For thousands of years now, mankind has been using biotechnology in the field such as agriculture, fisheries, food science and medicine.

The term Biotechnology is believed to have been coined in 1919 by Hungarian agricultural engineer **Karoly Ereky**. In the late 20th and early 21st centuries, Biotechnology has been expanded to include new and diverse scientific disciplines like genomics, recombinant gene techniques, applied immunology, and pharmaceutical therapy.

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

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

Microorganisms are beneficial for microbial biodegradation or bioremediation of domestic, agricultural and industrial wastes and subsurface pollution in soils, sediments and marine environments. The ability of each microorganism to degrade toxic waste depends on the nature of each contaminant. Since sites typically have multiple pollutant types, the most effective approach to microbial biodegradation is to use a mixture of bacterial and fungal species and strains, each specific to the biodegradation of one or more types of contaminants.
The monstrous genera *Thiomargarita* and *Epulopiscium* in which some species of bacteria that measure over 600 to 700 μm in length or diameter and are visible to the naked eye. However, large bacteria are rare in nature, Most of the bacteria size around 0.4 and 2 μm in diameter and 0.5 and 5 μm in length. It is all the more important to see that bacteria are boring, at least in morphological sense. Table1 gives the perception size of some of the bacteria.

SIZE AND SHAPE AND ARRANGEMENT OF BACTERIA

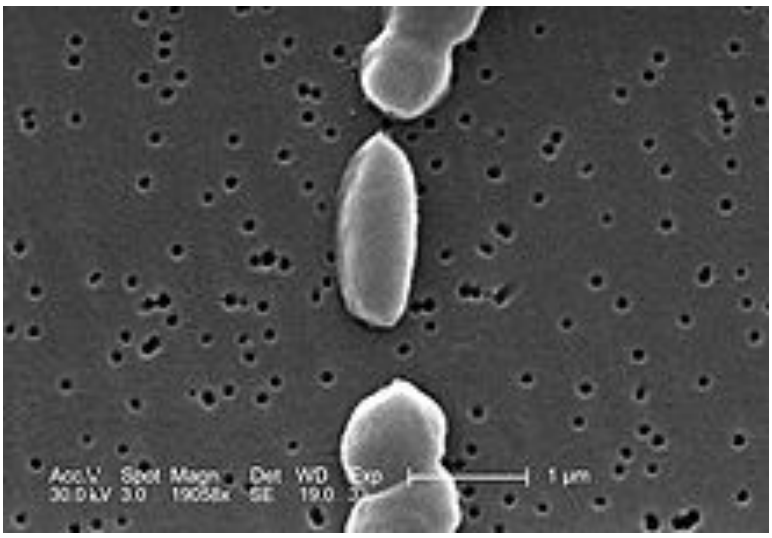
Three basic shapes of bacteria are coccus (spherical shaped), bacillus (rod shaped) and spiral
An average coccus is about 0.5-1.0 micrometer (μm) in diameter. An average bacillus is 0.5-1.0 μm wide by 1.0-4.0 μm long. Spirals come in one of three **forms**, a vibrio, a spirillum, or a spirochete
Some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

Table 1: Size and dimension and of some important bacteria

SL.No.	name	Dimension of size (μm=micron)
1.	*E.coli*	1-3 x 0.4-0.7 μm
2.	*Salmonella*	0.7-1.5 x 2-5 μm
3.	*Vibrio spp.*	0.5 x 1.5-3.0 μm
4.	*Clostridium spp*	0.3-2 x 1.5-20 microns
5.	*Aeromonas hydrophila*	0.3-1.0 x1.0-3.5 μm
6	*Pseudomonas fluorescens*	0.5 μm x 2.0-2.5 μm

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

Figure1: SE Micogram of *Vibrio parahaemolyticus*



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

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

IMPORTANT PATHOGENS AND PROBLEMS WITH FISH/FISHERY PRODUCTS

Bacteria in food may cause illness in human beings. Examples of some of the types of bacteria that may be found in seafood that cause foodborne illness by infection are *Vibrio*, *Salmonella*, *Shigella, and Listeria*. From 1973 to 2006, *Vibrio* species accounted for 38% of the outbreaks associated with seafood and 54% of the illnesses. *Salmonella* and *Shigella* each was associated with about 10% of the reported illnesses, and *Listeria monocytogenes* approximately 1% too. Foodborne intoxications occur when patients consume pre-formed toxins that are produced by certain types of bacteria when they grow and multiply in the food. *Clostridium botulinum* can produce a potent neurotoxin during growth under anaerobic conditions (absence of oxygen) usually associated with vacuum packed, improperly canned, or fermented products. *C. botulinum* toxin was associated with almost one fourth of the seafood related outbreaks from 1973 to 2006 and caused 152 illnesses and 38% of all hospitalizations. Bacteria such as *Staphylococcus aureus* can produce enterotoxins that cause foodborne illness, but less than 5% of the seafood associated

outbreaks and illnesses were associated with this pathogen over the past three decades. Preventing the growth of these bacterial pathogens is important to prevent infection or intoxication when seafood is eaten and is all the more relevant in this Antimicrobial resistance regime.

FISH DISEASES

Many diseases are caused in fishes due to pathogens which cause havocs to fisherman community, fishery industry in general and lay people in particular. Some of the fish pathogens, causing diseases *are Aeromonas hydrophila, A. salmonicida, Pseudomonas fluorescens, P. putrefaciens, Flexibacter columnaris, Edwardsiella tarda, Vibrio alginolyticus* and *V. parahaemolyticus.*

It is highly imperative to identify the pathogens either by biochemical method or by molecular method through the study of genomic sequences to tackle the causative reasons for the diseases prevailing in fish and fishery environment. Though biochemical methods are proven methods for identifying the micro-organisms, molecular methods through the study of genomic sequences of the pathogenic cells is all the more paramount in the present data analytic environment of high-end computing facility. At times it could be used as a mode of corroboration for conventional microbial detection techniques as well.

GENOMICS

Genomics is an interdisciplinary field of science focusing on the structure, function, evolution, of genomes. A genome is the complete set of DNA of an organism, including all of its genes. Unlike in genetics, where the study of individual genes and its roles in inheritance is emphasized, genomics aims at the collective characterization and quantification of genes, which drive and direct the production of proteins with the assistance of enzymes and messenger molecules. In turn, proteins make up body structures such as organs and tissues as well as control chemical reactions carry signals between cells. This has a bearing on our response to different stimuli either, which account for the unique emotional characteristics of all living being. Genomics also involves the sequencing and analysis of genome through uses of high throughput DNA sequencing and sequence analysis tools to study the evolutionary relationships through phylogenetic tree. Identification of organism is also possible by incorporating the logics of the phylogenetic tree in the algorithms of the software module. It also assembles and analyzes the function and structure of all genes of the entire genome to advance the genomic and proteomic study. Advances in genomics have triggered a revolution in microbiological research and systems biology to facilitate the understanding all the organ systems including muscular system and nervous system. This sort of generation of genomic data, its warehousing, its analysis through different analytic tools for generative process for information on biological systems, have emerged as a new scientific discipline viz., **Bioinformatics.**

BIOINFORMATICS

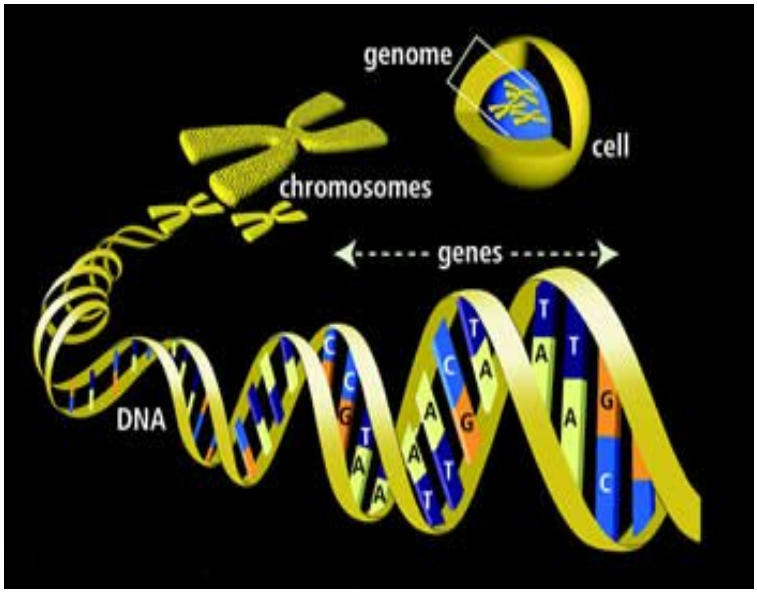
Bioinformatics is an interdisciplinary field of science that develops methods and software tools for understanding biological data. As an interdisciplinary science, bioinformatics combines computer science, statistics, mathematics to analyze and interpret biological data. Bioinformatic tools have been used for *in-silico* analysis (Computer aided analysis) of biological data. These tools are developed based on principles and concepts of mathematical and statistical theory applied in *Data Analytics*.

Bioinformatics is used as an umbrella term for the biological studies based on Database Management System and data analytic tools developed on mathematical and statistical techniques befitting to the software algorithm tailored for the module. Common uses of Bioinformatics include the identification of candidate genes whereby identify a species under microbiological wet lab study. Often, such identification is made with the aim of better understanding the genetic basis of diseases, unique adaptations, desirable properties (esp. in microorganism species), or differences between populations. In a less formal way, Bioinformatics also try to understand the organizational principles within nucleic acid and protein sequences. This will help supplement and corroborate biochemical and other conventional techniques to strengthen microbiology as a discipline of science.

The genomic study, analysis and interpretation of genomic data are based on the concept of **Central Dogma of Molecular Biology**. This dogma encapsulates that "The gene region of DNA in the nucleus of the cell is copied (transcribed) in to the RNA and RNA travels to protein production sites and is translated in to protein. This underscores the fact that DNA and the embedded genes are responsible for morphological characteristics and also the manifestation of response to every stimulus of living organisms. Genomic approaches have opened up new vistas for increasing the quality and their by productivity of biological systems. During the last decade, omics (field of study in biology ending in -omics, such as genomics, proteomics or metabolomics) has witnessed an information explosion. Omics databases contain huge amount of information that are not amenable to traditional analytical approaches. In a multi-disciplinary area with a blend of biology, mathematics and computing science that can be used to derive biological insights from various omics data. It is an application of computing technology along with informatics for the management of biological information.

A diagrammatic representation of genome is shown in fig.2.

Figure 2: Genomics diagram



(Image source.http://www.differencebetween.info/difference-between-gene-and-genome dt.01-07-2022)

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

DEOXYRIBONUCLEIC ACID (DNA)

Deoxyribonucleic acid (**DNA**) is a thread-like chain of nucleotides carrying the genetic instructions used in the growth, development, functioning and reproduction of all known living organisms . DNA and ribonucleic acid (RNA) are nucleic acids; alongside proteins, lipids and complex carbohydrates (polysaccharides), they are one of the four major types of macromolecules that are essential for all known forms of life. Most DNA molecules consist of two biopolymer strands coiled around each other to form a double helix.

The two DNA strands are called polynucleotides since they are composed of simpler monomer units called nucleotides. Each nucleotide is composed of one of four nitogen-

containing nucleobases (cytosine [C], guanine [G], adenine [A]or thymine [T]),a sugar called de oxyribose, and a phosphate group. The nucleotides are joined to one another in a chain by covalent bonds between the sugar of one nucleotide and the phosphate of the next, resulting in an alternating sugar-phosphate backbone. The nitrogenous bases of the two separate polynucleotide strands are bound together, according to base pairing rules (A with T and C with G), with hydrogen bonds to make double-stranded DNA.

The complementary nitrogenous bases are divided into two groups, pyrimidine and purine. In a DNA molecule, the pyrimidines are thymine and cytosine; the purines are adenine and guanine.

DNA stores biological information. The DNA backbone is resistant to cleavage, and both strands of the double-stranded structure store the same biological information. This information is replicated as and when the two strands separate. A large part of DNA (more than 98% for humans) is non-coding, meaning that these sections do not serve as patterns for protein sequences.

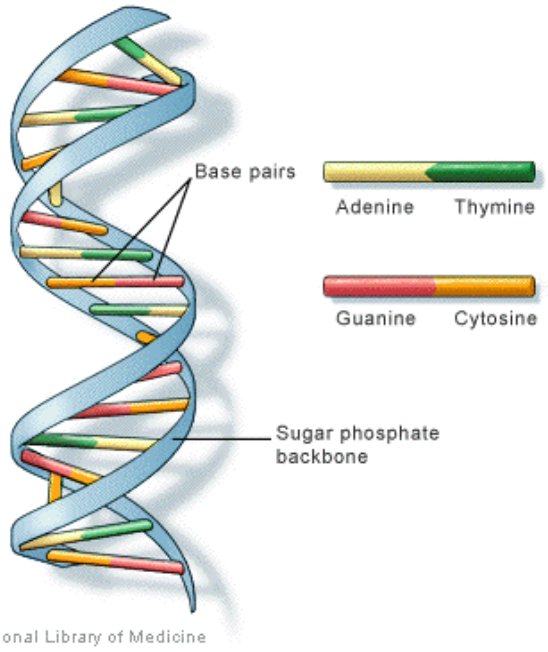
The two strands of DNA run in opposite directions to each other and are thus ant parallel. Attached to each sugar is one of four types of nucleobase. It is the sequence of these four nucleobase along the backbone that encodes biological information. RNA strands are created using DNA strands as a template in a process called transcription. Under the genetic code, these RNA strands are translated to specify the sequence of amino acids within proteins in a process called translation.

Within eukaryotic cells, DNA is organized into long structures called chromosomes. During cell division these chromosomes are duplicated in the process of DNA replication, providing each cell its own complete set of chromosomes. Eukaryotic organisms store most of their DNA inside the cell nucleus and some of their DNA in organelles, such as mitochondria or chloroplasts. In contrast prokaryotes (bacteria and Achaea) store their DNA only in the cytoplasm. Within the eukaryotic chromosomes, chromatin proteins such as histones compact and organize DNA. These compact structures guide the interactions between DNA and other proteins, helping control which parts of the DNA are transcribed.

DNA was first isolated by Friedrich Miescher in 1869. Its molecular structure was first identified by James Watson and Francis Crick at the Cavendish Laboratory within the University of Cambridge in 1953, whose model-building efforts were guided by X-ray diffraction data acquired by Raymond Gosling, who was a post-graduate student of Rosalind Franklin. Anything a cell could possibly want is stored in its DNA. When a cell wants to build a protein, it finds the appropriate piece of DNA, makes a copy of it (called RNA), and uses the instructions in the copy to make the protein. In living organisms, DNA does not usually exist as a single molecule, but instead as a pair of molecules that are held tightly together. These two long strands entwine like vines, in the shape of a double helix. The nucleotide contains both a segment of the backbone of the molecule (which holds the chain together) and a nucleobase (which interacts with the other DNA strand in the helix). A nucleobase linked to a sugar is called a nucleoside and a base linked to a sugar and one or more phosphate groups is called a nucleotide. A polymer comprising multiple linked nucleotides (as in DNA) is called a polynucleotide.

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

Figure 3: DNA double helix structure



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

Proteins are the 'machinery' of a cell. They can perform many functions like transportation, structural support, movement and metabolism. Proteins are made from amino acids. There are twenty different amino acids that are used to build millions of different protein molecules. The principle of bioinformatics is that these molecules can be studied by using computers to analyze the DNA, RNA, and amino acid sequences from which

they are created. Because there are so many different molecules, the best way we have of understanding how the entire system works is to use Bioinformatics.

Base pairing

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

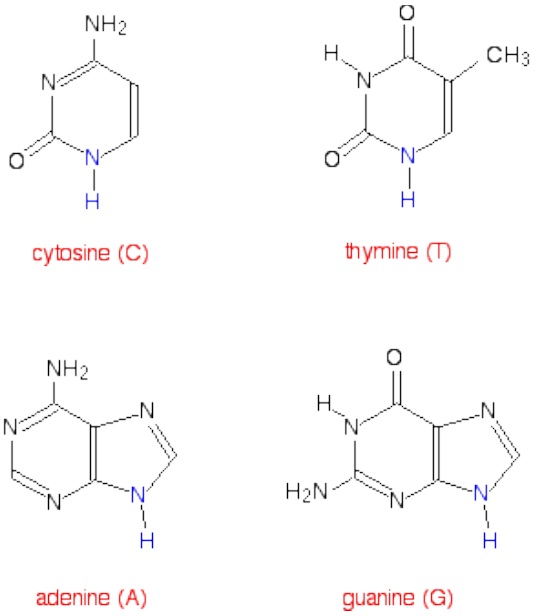


Figure 4: Chemical Structure of nucleobase

Traditionally molecular biology research was carried out entirely at the experimental lab but the huge increase in the scale of data being produced in this genomic era has necessitated incorporating computer and computing science in to research process. Sequence generation and its subsequent storage, interpretation and analysis are solely computer dependent. However, the molecular biology of an organism is a very complex issue with research being carried out at molecular level. The first challenge facing the bioinformatics community today is the intelligent and efficient storage of this massive data by providing reliable access to this data.

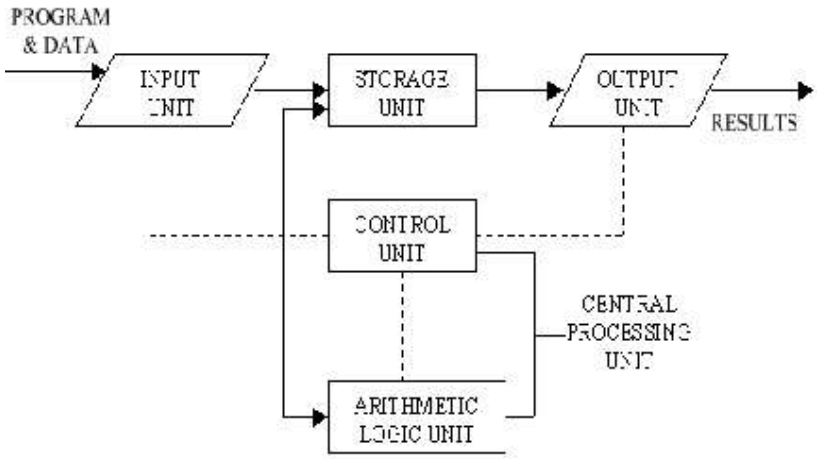
COMPUTER SYSTEMS

A computer system allows users to input, manipulate and store data. It includes hardware like processor, monitor, keyboard, mouse and other peripheral components along with software like operating system and other system and application programmes. All of these components also can be integrated into all-in-one units, such as desktop or laptop computers.

Very high speed and repetition of processing of data, high precision accuracy of result derived after data processing and its capacity storage area are the forte of computer systems which could be harnessed for advancement of Science and research. Though the numerical data

collected are however large or small it be, the processing with very high speed and generation of result with desired accuracy can be achieved through suitable instruction given to the computer system called computer programming. Logical flow of computer system is given in Figure 5.

Figure 5: Logical flow of computer system



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

BIOINFORMATICS AND AVAILABLE WEB BASED GENOMIC DATABASES

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

BIOLOGICAL DATABASES

Biological databases are huge databases mostly sequence data generating from major genome sequencing projects all over the world. The information about DNA, protein and functions of protein must be stored in an intelligent fashion so as to solve problems quickly by

the available information stored in the data bank in the clouds, many of which are accessible by concerned user on internet. Some of the web based databases are available in **table2**

Table2: STANDARD WEB BASED GENOMIC DATABASES

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

Using data banks one can perform all kinds of comparisons and search queries. With this known information we can perform all kinds of comparisons with sequences generated from our wet lab studies for species identification. If you know a protein which causes a disease in human, you might look in to a databank to see if a similar protein has previously been described and what this protein does in human body. This known information has wide pharmaceutical application in Health Science.

The NCBI site is one of the world's premier web site for biomedical and bioinformatics research (http://www.ncbi.nlm.nih.gov/). Based within the National Library of Medicine at National Institute of Health , USA, the NCBI hosts many databases used by medical and research professionals. The service includes PubMed (the bibliographic database), GenBank (the nucleotide sequence database) and the BLAST algorithm for sequence comparison. It is established in 1988 as a national resource for molecular biology information. NCBI creates public databases, conduct research in computational biology, develop software tools for analyzing genome data and disseminate biomedical information all for a better understanding of molecular processes affecting human health and diseases.

On-line software (Bioinformatics tools) for genomic data analysis for species identification and management has revolutionized genomic data analysis of nucleotide and amino acid sequences.

In bioinformatics, BLAST for Basic Local Alignment Search Tool is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences

of proteins or the nucleotides of DNA sequences. A BLAST search enables a researcher to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold. Thanks to the genomic databases available in the databank for comparison with the data under study and the automation of the searching based on the bioinformatics software tools to get the insight of the study.

DIGITAL AND COMPUTER AIDED EQUIPMENTS AVAILABLE FOR MICRO BIOLOGICAL LAB

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

Some of the important and must-have equipments are listed in the **table3** fit microbial study.

Table 3: LIST OF HIGH PRECISION COMPUTER AIDED EQUIPMENTS OF MICROBIOLOGY LAB

Sl. No	NAME OF THE EQUIPMENT	**USE**
1.	Shaking Water Bath	Heating at precision temperature
2.	Automated Colony Counter	Estimate No. of bacteria in a sample
3.	Electronic Colony Counter	Counting the colonies of bacteria in a Petri-dish
4.	Magnetic Stirrer	Dissolving chemical substance effectively
5.	Sonicator	Rupture cells using high frequency waves
6.	Vortex Mixer	Used for thorough mixing of liquids in test tubes
7.	Laminar Flow Chamber	Used for aseptic transfer of sterilized materials, as well as for inoculation of microbes
8.	Electronic Cell Counter	Used to directly count the number of bacteria in a given liquid sample
9.	Microscopes	Used for visual observation of morphology,

		motility, staining and fluorescent reactions of bacteria
10.	Spectrophotometer:	Measuring the differences in color intensities of solutions.
11.	Automatic Bacteria Identification System	Used for automatic computer-assisted identification of bacteria
12.	PCR Thermo cycler	Used to amplify segments of DNA via the polymerase chain reaction (PCR)
13.	Ultra-centrifuge	Precipitating large biological molecules from solution or separate them by their different rates of sedimentation.
14.	Gas Chromatography (GC)	Used for separating and analyzing compounds that can be vaporized without decomposition
15.	High Performance Liquid Chromatography (HPLC)	Technique used to separate, identify, and quantify each component in a mixture.
16.	Thin Layer Chromatography (TLC)	Technique used to separate non-volatile mixtures.
17	Paper Chromatography	A simple technique of separating constituents in a sample solution using a chromatography paper

GENOMIC SOFTWARES

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

CONCLUSION

Most critical task of bioinformatics involves the finding of genes in the DNA sequences of various organisms, developing methods to predict the structure and functions of the newly discovered proteins and structural RNA sequences, clustering protein sequences in to families of related sequences, development of protein models, aligning similar proteins and generating phylogenetic trees to examine the evolutionary relationships. The sequencing of the genomes

of microbes should have enormous benefits for the biological systems including human health in general and fishery eco-system in particular. Computational analysis of this sequence data generated by genome sequencing is critically important. Bioinformatics tools can be used to search for the gene within this genome to understand their functions with the help if high-end computing facilities.

Microorganism enjoys key position in the sustenance of fish farming eco system. Though most of the microorganisms are environment- friendly there is some pathogenic microbe in aquatic system which affects fish health and in turn human health. Microbes play an important role in the degradation of fish products, thus better knowledge of the microbiological conditions throughout the supply chain of fisheries and fish processing industry; thereby optimize fish product quality and fishery resource utilization. Under these circumstances, a regular monitoring of fish health and the quality of fish products in perspective of microbiology with bioinformatics analytic tools is of paramount for better management of the prevailing food safety and security regime.

References:

1. Devey, M.E., M.M. Sewell, T.L. Uren, and D.B. Neale (1999) Comparative mapping in loblolly pine and radiata pine using RFLP and microsatellite markers. Theoretical and Applied Genetics 93:656-662.
2. Kramer JM, Gilbert RJ: *Bacillus cereus* and other *Bacillus* species. P.21. in Doyle MP (ed): Foodborne Bacterial Pathogens. Marcel Dekker, New York, 1989.
3. Norris JR, Berkeley RCW, Logan NA et al: The genera *Bacillus* and *Sporolactobacillus*. p. 1711. In Starr MP, Stolp H, Truper HG (eds): The Prokaryotes A Handbook on Habitats, Isolation and Identification of Bacteria. Vol. 2. Springer-Verlag, New York, 1981.
4. Parry JM, Turnbull PCB, Gibson JR: A Colour Atlas of *Bacillus* Species. Wolfe Medical Atlas no. 19. Wolfe Publishing, London 1983.
5. Crick F. (1970), CENTRAL Dogma of Molecular Biology, *Nature, 227, 561-563*
6. Cervera, M.T., Plomion, C. and C. Malpica (2000) Molecular markers and genome mapping in woody plants. In: Molecular biology of woody plants. Forestry Sciences, Volume 64 (S. M. Jain and S. C. Minocha, eds), pp. 375-394. Kluwer Academic Publishers, the Netherlands.
7. Gion, J. M., Rech, Ph., Grima-Pettenati, J., Verhaegen, D., and C. Plomion (2000) Mapping candidate genes in Eucalyptus with emphasis on lignification genes. Molecular Breeding 6: 441-449.

****

49

INTRODUCTION TO 16S RDNA SEQUENCING AND ANALYSIS, AND GEL DOCUMENTATION

Murugadas Vaiyapuri*, Sreejith V.N., and Vineetha Das
Microbiology, Fermentation and Biotechnology Division
Email: Murugadas.V@icar.gov.in

16s rDNA

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

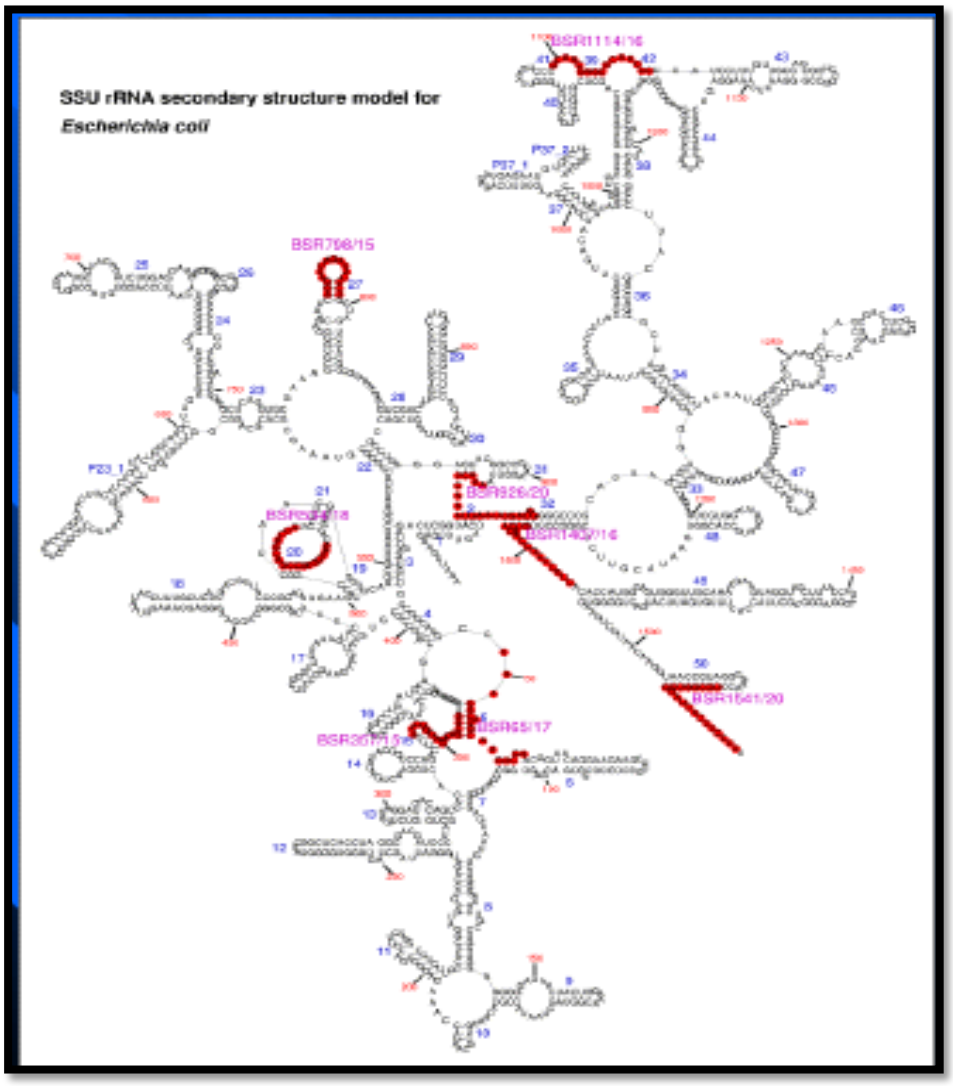
Microbial systematics

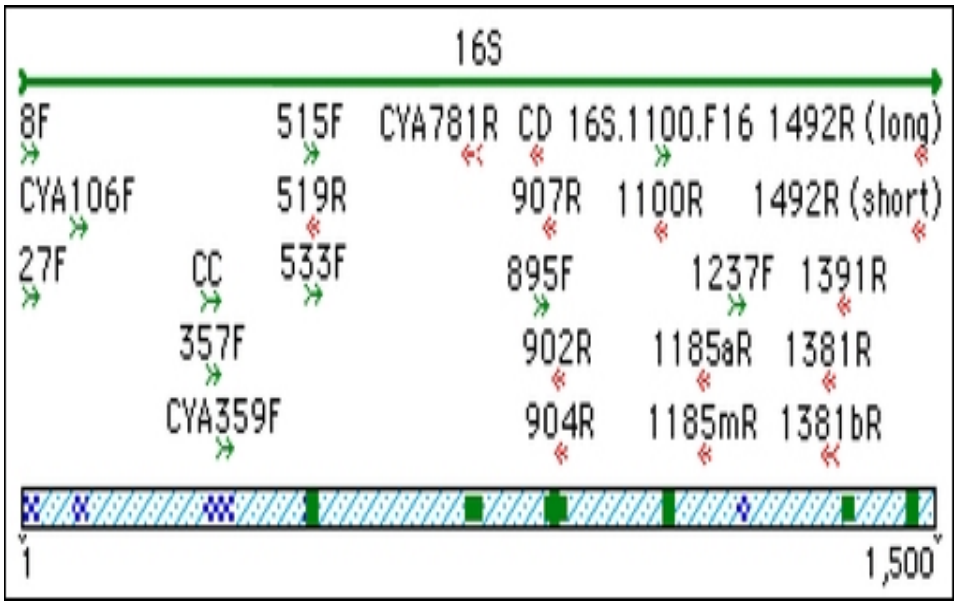
Novel species is recognized using the polyphasic approach - multidimensional aspects of organisms (phenotypic, genotypic and chemotaxonomic traits). Biochemical methods of identification of bacteria are called as phenotypic methods, 16s rRNA sequencing and DNA-DNA hybridization are called as genotypic 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'

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 hyper variable regions, where sequences have diverged over evolutionary time. Strongly conserved regions often flank these hyper variable 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.





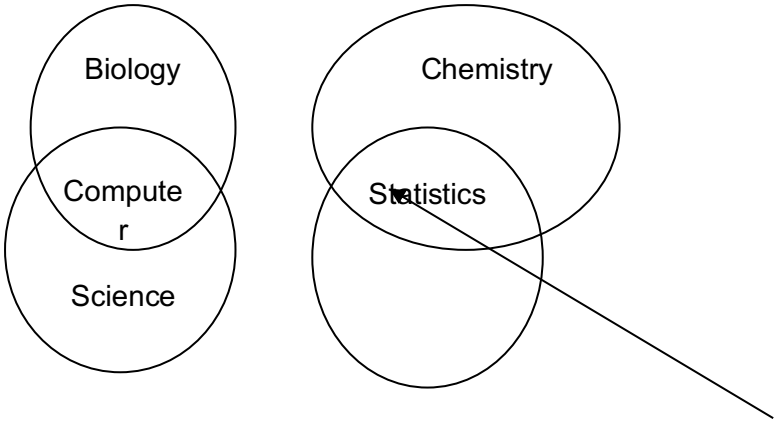
Primer		
Name	Sequence[a]	Amplified hypervariable region
V3F	5′ CCAgACTCCTACGGGAGGCAG 3′ (334–354)	V3 (334–537)[b]
V3R	5′ CGTATTACCGCGGCTGCTG 3′ (519–537)	
V6F	5′ TCGAtGCAACGCGAAGAA 3′ (961–78)	V6 (986–1043)
V6R	5′ ACATtTCACaACACGAGCTGACGA 3′ (1062–85)	
Molecular beacon probe[c]		
Name	Sequence	Target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

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 alignments, 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.

GCAGCGCACGACAGCTGTGCTATCCCGGCGAGCCCGTGGCAGAGGACCTCGCTTG
CGAAAGCATCGAGTACCGCTACAGAGCCAACCCGGTGGACAAACTCGAAGTCATT
GTGGACCGAATGAGGCTCAATAACGAGATTAGCG

Similarly, the protein record infasta as follows

>P31373

MTLQESDKFATKAIHAGEHVDVHGSVIEPISLSTTFKQSSPANPIGTYEYSRSQNPNRENL
ERAVAALENAQYGLAFSSGSATTATILQSLPQGSHAVSIGDVYGGTHRYFTKVANAHGVE
TSFTNDLLNDLPQLIKENTKLVW

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.

Agarose gel electrophoresis and documentation

Recommended agarose concentrations for resolution of linear dsDNA Agarose (%) are as per Size of fragments separated (kb) 0.5% is recommended to separate 1-40 kb sized fragments, and 0.7-0.8% for 0.8-12kb, 1.0% for 0.5-10kb, 1.2% for 0.4-7kb, 1.5% for 0.2-3kb, and 2.0% for 0.05-2kb. In general, for the PCR product electrophoresis purpose 1.5 to 2.0% gel is used. For genomic DNA electrophoresis 0.8% is used. Higher the molecular weight, slower the migration. Greater the concentration of agarose, smaller will be the pore size and consequently, slower the migration (usually concentration ranges from 0.7- 2%). Strength of electric field applied: Usually it ranges from 70-100 V. An increase above this range results in faster movement of the DNA but the process generates greater amount of heat. Composition and ionic strength of electrophoresis buffer: In the absence of ions, electrical conductivity is minimal and DNA migrates very slowly.

Materials required: 1. Agarose 2. Ethidium bromide (10 mg/ml) 3. Molecular weight marker 4. 50X TAE buffer 5. 6X Gel loading dye

50XTAE (1litre) 242 g of Tris base 57.1 ml of Glacial acetic acid 100 ml of 0.5 M EDTA (pH 8.0) D. H2O

6X Gel loading dye 0.25 % Bromophenol Blue 0.25 % Xylene cyanol 30 % Glycerol in water

Procedure (2.0% gel): 1. Prepare gel tray and place comb in gel tray about 1 inch from one end of the tray. 2. To prepare 100 ml of a 2.0% agarose solution, measure 2.0 g agarose into a glass flask and add 100 ml 1X TAE buffer. Microwave until agarose is dissolved and solution is clear. 3. Allow solution to cool to about 42-45^{0}C before pouring. (Ethidium bromide can be added at this point to a concentration of 0.5 µg/ml). Add 5µl Ethidium bromide and mix well without foaming any bubbles. 4. Pour this warm gel solution into the tray to a depth of about 5 mm. Allow gel to solidify about 30-45 minutes at room temperature. (PS: UV will not penetrate efficiently in thicker gels, so make sure to make thin gels.) 5. To run, gently remove the comb, place tray in electrophoresis chamber, and cover (just until wells are submerged) with 1X TAE buffer. 6. To prepare samples for electrophoresis, add 1 µl of 6x gel loading dye for every 5 µl of DNA solution. Mix well and load 6µl of DNA per well. Load a suitable molecular weight marker in one lane. 7. Electrophorese at suitable volts until dye has migrated two third the length of the gel – ***(Electrophoresis Power Pack*** Connect the power, connect ‗BLACK (-)'& ‗RED (+)' pins with respective electrode. Set the ‗VOLT' using ‗▲,▼' keys. Press ‗START'). 8. Document the gel after electrophoresis by using Gel Documentation System. 9. Intact DNA appears as orange fluorescent bands. **Notes**: Ethidium bromide (EtBr) migrates in

the opposite direction of the DNA during electrophoresis. Hence if electrophoresis takes longer time, the visibility of the DNA reduces. Hence the gel will have to be stained again. EtBr is a powerful mutagen and is toxic and so wear gloves while handling the gel and should be decontaminated after the end of experiment. Ethidium bromide should be handled and disposed of as it is considered hazardous waste. This applies to gloves, pipette tips, test tubes, paper towels, etc., that are grossly contaminated with ethidium bromide as well.

Reference

1. Andreas D. Baxevanis and B. F. Francis Ouellette. BIOINFORMATICS-A Practical Guide to theAnalysis of Genes and Proteins. SECOND EDITION. WileysInerscience, A JOHN WILEY & SONS, INC., PUBLICATION. 2001.
2. Des Higgins and Willie Taylor. Bioinformatics-Sequences, structure, and databanks- A practical approach. Oxford University Press. 2000.
3. Andrzej Polanski and Marek Kimmel. Bioinformatics. Springer-Verlag Berlin Heidelberg 2007

****

50

DETERMINATION OF AMR BY PHENOTYPIC METHOD – DISK DIFFUSION ASSAY

G. K. Sivaraman and K. Resmi
Microbiology, Fermentation and Biotechnology Division
Email: gkshivraman@gmail.com

Over 70 years have passed since the disc diffusion testing principle was first applied in microbiology labs. Drs. Bauer, Kirby, Sherris, and Turck meticulously developed by considering all parametersincluding the media, temperature, and depth of agar and were published in the year 1966. The essential operational stages from the Bauer paper's disc diffusion reference approach were adopted by CLSI. The CLSI is approved by FDA-USA and recommended by WHO.

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 = ~1.5×10^8 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 hrs) 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 calliper

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

Adjust pH to ~7.0 after sterilisation,

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°C for 20 min

PROCEDURE

1st Step: Select colonies

Check the bacteria and the quality control strains are pure and well isolated colonies on the grown agar plates and free of any visible contamination. Colonies cannot be more than 18 to 24 hours old when using the direct colony suspension method. Except for staphylococci, most quickly growing organisms are studied using the log phase approach.

2nd Step: Prepare inoculum suspension:

Pick up at least 4 to 5 well isolated colonies with a sterile loop or swab and transfer to the tube of saline and emulsify the inoculum on the inside of the tube to avoid clumping of the cells. Make sure that the microorganism suspension is thoroughly mixed, and vortex it.

3rd Step: Standardize inoculum suspension:

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 accordingly.

4th Step: Inoculate plate:

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. The adjusted suspensions should be used within 15 minutes. 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.

5th Step: Add antimicrobial disks

Apply the disks containing the antimicrobial agents within 15 minutes of inoculating the MHA plate. Dispense the antibiotic disks on the agar surface with a dispenser or sterile forceps (5 disks on a 100 mm plate). To ensure complete, level contact with the agar, firmly press each disc into the surface. Once a disc was placed on the agar, it should not be repositioned. Select the FDA approved products and antibiotic disks with the specified contents as listed in the CLSI standards (Table 1 and 2).

6th Step: Incubate plate

Incubate the plate at 35$\pm$2°C for 18-24hrs within 15 minutes of standardizing the inoculum suspension. For non-fastidious bacteria, incubate in ambient air at 35°C for 16–18 hours (refer as per CLSI recommendations conditions).

7th Step Measure inhibition zones

Check for the growth is even and confluent and the zone of inhibition is of very clear. Measure the diameter of the clear inhibition zones margin. Measure zone of inhibition with respect to each antibiotic using reflected light from the back of the plate for the Enterobacteriaceae, staphylococci, and enterococci (except for oxacillin and Vancomycin). Use transmitted light for Staphylococci with oxacillin and Enterococci with Vancomycin. Measure the zone of inhibition where there is a clear distinction of growth and no growth. Even if the swarm type (*Proteus mirabilis)* is tested, only measure the obvious zone. It may be challenging to interpret zones with trimethoprim-sulfamethoxazole and sulfonamides and Trimethoprim alone since these antibiotics may not prevent bacterial growth until the bacteria have undergone several generations of growth. Therefore, measure the zone at the point when there is an 80%

reduction in growth within the zone.If there is no zone of inhibition; the disk's diameter needs to be recorded as6mm.

8th Step: Interpret and report of the results:

Refer the CLSI Guideline M100-S12: Performance Standards for Antimicrobial Susceptibility Testing, Table 2A- 2I (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints)and report as sensitive (S), intermediate (I) or resistant.

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.

NOTE: The situation with respect to susceptibility testing of bacteria isolated from aquatic animal: The situation with respect to susceptibility testing of this group of bacteria is essentially different. In general there are not a wide number of testing protocols that have been developed by different national agencies. Only one international agency, CLSI, has started to develop standardised testing protocols suitable for bacteria isolated from aquatic animals that require. Incubation at temperatures <35°C or longer than 16-20hrs. Although the CLSI protocols do not yet cover all the diverse species encountered in aquatic animals, the progress they have made is substantial.

Table 1: List of antibiotics for susceptibility testing of *Staphylococci*

Antibiotic	**Disc content**	**Zone diameter interpretive criteria nearest whole mm**		
		S	**I**	**R**
Penicillin	10 units	≥ 29	-	≤ 28
Cefoxitin	30 µg	≥ 22 ≥ 25 (for CONS)	- -	≤ 21 ≤ 24 (for CONS)
Gentamicin	10 µg	≥ 15	13-14	≤ 12
Tetracycline	30 µg	≥ 19	15-18	≤ 14
Ciprofloxacin	5 µg	≥ 21	16-20	≤ 15
Trimethoprim-Sulfamethoxazole	1.25/23.75 µg	≥ 16	11-15	≤ 10
Chloramphenicol	30 µg	≥ 18	13-17	≤ 12
Erythromycin	15 µg	≥ 23	-	≤ 13
Linezolid	30 µg	≥ 21	-	-

Table 2: List of antibiotics for susceptibility testing of *E. coli*

Antibiotic	Disc content	Zone diameter interpretive criteria nearest whole mm		
		S	I	R
Ampicillin	10 µg	≥ 17	14-16	≤ 13
Amoxicillin-clavulanic acid	20µg	≥ 18	14-17	≤ 13
Ceftriaxone	30 µg	≥ 26	23-25	≤ 22
Cefpodoxime	10 µg	≥ 21	18-20	≤ 17
Ceftazidime	30 µg	≥ 21	18-20	≤ 17
Aztreonam	30 µg	≥ 21	18-20	≤ 17
Cefotaxime	30 µg	≥ 26	23-25	≤ 22
Cefoxitin	30 µg	≥ 18	15-17	≤ 14
Imipenam	10 µg	≥ 23	20-22	≤ 19
Amikacin	30 µg	≥ 17	15-16	≤ 14
Tetracycline	30 µg	≥ 15	12-14	≤ 11
Ciprofloxacin	5 µg	≥ 21	16-20	≤ 15
Nalidixic acid	30 µg	≥ 19	14-18	≤ 13
Chloramphenicol	30 µg	≥ 18	13-17	≤ 12
Erythromycin	15 µg	≥ 23	14-22	≤ 13
Colistin	10 µg	≥ 11	-	≤ 10

****

51

APPLICATION OF ICT TOOLS IN FISHERIES

V. Chandrasekar,
Extension, Information and Statistics division
Email: vcsecon@gmail.com

Throughout history, information has played a pivotal role in enhancing the value of every facet of human society. To disseminate information effectively, various digital technologies and tools have become indispensable, reaching diverse segments of society. Initially, the adoption of Information and Communication Technology (ICT) was confined to academic and research institutions due to its high costs. However, over time, ICT has permeated all levels of society, emerging as the most accessible and cost-efficient means of sharing knowledge and information. At first glance, the connection between fishery and the realms of computers, the internet, and communication technology may seem tenuous. However, the fishing industry has witnessed a remarkable transformation driven by information technology, responding to economic, environmental, and regulatory pressures. These pressures have prompted substantial investments in ICT within the fishing sector, fostering sustainability, operational efficiency, and adaptability. In today's world, the Information Communication Technology (ICT) revolution has far-reaching socioeconomic consequences for both developed and developing nations. ICTs play a pivotal role in elevating the Indian fisheries industry at every stage of the supply chain, from catch to consumer. The application of the latest ICT tools promises to revolutionize the lives and livelihoods of fishermen, offering improved profitability, reduced labor, and timely access to critical information, thereby promoting social equity and mainstream integration. The fisheries sector is experiencing rapid expansion and evolution through ICTs. Technologies such as GPS, navigation devices, sonar, fish finders, and high-frequency wireless communication (VHF) have made significant contributions to marine fisheries. Numerous ICT initiatives have been launched to further expand and enhance fisheries technologies for fishing communities. ICT is widely recognized as a fundamental resource for development. Various ICT tools, including mobile phones, television, radio, GPS, and fish finders, have the potential to substantially improve the livelihoods of fishing communities and reduce poverty levels (Kularatne, 1997). ICT also plays a pivotal role in bridging knowledge gaps among stakeholders, fostering better collaboration between researchers, fisheries officials, and other relevant parties. This not only saves time and energy but also helps fishermen secure the best prices for their catches before reaching the landing center. With the aid of ICT, fishermen can venture farther into the deep sea to target high-

value fish, addressing challenges such as rising operational costs, increased investments, declining catch rates, limited infrastructure, and reduced profitability. By integrating ICT applications into fisheries, fishermen can cut operational expenses while boosting their catch quantities. However, it is important to note that rural communities in developing countries, like India, still face challenges related to basic communication infrastructure. In summary, this overview underscores the transformative potential of ICT in the fisheries industry. It highlights how ICT tools can enhance the lives of fishermen, improve profitability, and contribute to the sustainable development of fisheries, while acknowledging the existing communication infrastructure gaps in rural areas.

Definitions:

Information technology (IT) encompasses the utilization of various computing resources, storage systems, networking infrastructure, and physical devices to generate, process, store, safeguard, and exchange electronic data in multiple formats, including letters, photographs, digital sensors, GPS data, and satellite imagery.

Communication, a core function of IT, serves as a conduit for transmitting information from one entity to another, facilitated by technologies such as the internet, mobile networks, and both local and wide area networks.

Information communication technologies (ICT), as defined by UNESCO, encompass a diverse set of technological tools and resources employed to transmit, store, generate, share, or exchange information. ICT comprises a suite of tools that aid in capturing, storing, processing, transmitting, and displaying information through electronic technology means. In the context of the fisheries sector, ICT plays a pivotal role in supporting sustainability by enabling the timely collection, processing, and distribution of crucial information among various organizations.

ICT Technologies Applied in the Fisheries Sector:

In the marine fishing industry, various ICT tools have been adopted to enhance communication and increase fish catch. These tools include messaging applications like WhatsApp, television, radio, mobile phones, Global Positioning System (GPS), General Packet Radio Service (GPRS), echo sounders, Sound Navigation and Ranging (SONAR), Search and Rescue Transponders (SART), Automatic Identification Systems (AIS), Distress Alert Transponders (DAT), internet-enabled personal computers, radar systems, community radio, online portals, and Very High-Frequency (VHF) wireless communication sets.

Identity Technologies Used in the Fisheries Value Chain:

Barcoding: Barcodes, initially represented by varying line widths and spacings, have evolved into 2D barcodes, which can be scanned by mobile devices equipped with cameras. These

barcodes are employed in seafood products to verify authenticity, origin, and additional information like pricing and packing dates.

Vessel Tracking Devices: Devices such as the Pelagic Data Systems (PDS) tracker are employed to determine fishing locations, contributing to a digital record of seafood provenance.

Supply Chain Tracking Software: Various software systems now exist for tracking fish through the supply chain, reducing fraud and ensuring reliable transmission of seafood information to buyers. This may involve labeling fish with unique identifiers, including QR codes, barcodes, or Near-Field Communication (NFC) labels.

Sensors: Sensors are extensively used along the fisheries value chain, particularly in aquaculture farms and fish processing. They monitor water quality parameters and weather conditions in real-time, aiding in the maintenance of aquaculture environments.

Image Processing: Image processing techniques are employed to assess the freshness of fish by analyzing gill tissue segmentation in fish images. Applications like FishAPP use this technology to determine fish freshness through smartphone photos.

Data Management: Web-based seafood export management software simplifies data storage and access, optimizing business productivity and profitability. This system addresses inventory management, yield calculation, product accounting, and various logistical challenges along the supply chain.

Server-Side and Client-Side Components: Web servers, search engines, browsers, and mobile applications comprise the server-side and client-side elements of the IT ecosystem.

Cloud Storage: Cloud services such as Google Drive, iCloud, Dropbox, and SkyDrive provide secure data storage and accessibility from various devices, including desktops, laptops, tablets, and smartphones.

Rephrased:

Fisheries Data Management:

a. ***Fish Base***

Fish Base serves as a global biodiversity information system specializing in finfish. Initially designed to furnish critical population dynamics data for 200 major commercial fish species, Fish Base has since expanded its scope to encompass comprehensive information on all known fish species worldwide. This encompassing dataset includes taxonomy, biology, trophic ecology, life history, and utilization aspects, along with historical records spanning 250 years. At present, Fish Base compiles data on over 33,000 fish species from more than 52,000 references, cultivated through collaboration with over 2,000 contributors. The repository also features a wealth of over 300,000 common names and boasts a collection of over 55,000 images. [Link to Fish Base](https://www.fishbase.de/home.htm)

Identity Management:

a. *AIS (Automatic Identification System)*

The Automatic Identification System (AIS), deployed aboard vessels, operates as a tracking system that autonomously exchanges navigational information among AIS-equipped vessels and coastal authorities. Functioning as a collision-avoidance system, AIS provides details on all nearby ships, including their speed, courses, and contact information (name, callsign, MMSI). This information is publicly broadcast over VHF radio frequencies, accessible to other vessels and shore-based receivers. AIS primarily aims to enhance navigation safety by facilitating efficient ship navigation, environmental protection, and the operation of Vessel Traffic Services (VTS). It accomplishes this by satisfying several functional requirements, including ship-to-ship collision avoidance, providing information to littoral states about a ship and its cargo, and serving as a VTS tool for ship-to-shore traffic management.

Location Recognition:

a. *GPS (Global Positioning System)*

GPS is a constellation of satellites continuously transmitting encoded data, enabling precise Earth location determination by measuring distances from these satellites. The low-power radio signals transmitted by these satellites allow anyone with a GPS receiver to ascertain their Earth-based location. GPS is particularly advantageous for fishermen, enabling them to chart courses to potential fishing areas from any location, even without mobile network coverage.

Fish Finder:

Fish Finders offer valuable information to help locate abundant fishing areas, with features including bottom structure analysis, configurable alarms for depth and fish echoes, and post-processing gain control for all displayed echoes on the screen. Additionally, they allow information sharing and display on chart plotters.

Very High-Frequency Wireless Sets (VHF):

VHF remains a vital communication tool for short-distance marine communications, with a typical range of less than 20 nautical miles. Essential VHF channels include distress, safety, and calling channels, such as Channels 16 (156.8 MHz) and 70 (156.525 MHz).

Application of ICT Solutions in the Fisheries Sector:

Advisories:

Indian Marine Fishery Advisory System (IMFAS) disseminates Potential Fishing Zone (PFZ) advisories using various mediums, including SMS, Interactive Voice Response Systems (IVRS), helplines, voice messages, information kiosks, and electronic display boards. These advisories are made available through location-based electronic display boards, Doordarshan broadcasts, newspapers, emails, websites equipped with Web GIS features, phones, and faxes.

Web-Based Dissemination:

A dedicated website offers multilingual advisories, providing information in eight local languages (Gujarati, Marathi, Kannada, Malayalam, Tamil, Telugu, Oriya, Bengali), as well as Hindi and English. This web platform includes Web GIS functionality for users to retrieve PFZ information for their areas of interest within the Indian Exclusive Economic Zone (EEZ) through simple GIS operations.

Mobile Phones:

Mobile phones empower fishermen with real-time access to market prices and fish quality information, ultimately boosting their income. Additionally, mobile phones facilitate price comparison across different markets. They are particularly transformative in rural India, providing fishermen with weather updates and enhancing safety by helping avoid potential losses to boats and nets. Fishermen also receive weather condition information via SMS before venturing into the sea.

Mobile Applications:

Mobile applications play a crucial role in fisheries, with fishermen using them to receive alerts when crossing borders. Fisheries inspectors utilize mobile apps for reporting cases of illegal, unregulated, or unreported (IUU) fishing. While freely available, customizable fisheries apps were initially scarce, collaborations with service providers enabled the dissemination of PFZ, OSF, and Tsunami warnings through their mobile networks.

PFZ Advisory Mobile Application:

Potential Fishing Zone (PFZ) advisories are essential for coastal fishermen, providing daily insights into chlorophyll presence, sea temperature, and water clarity. These advisories help fishermen locate areas teeming with fish, leading to fuel and time savings.

mKRISHI Mobile Application:

Developed by Tata Consultancy Services (TCS) Innovation Lab, mKRISHI Fisheries is a mobile app created in collaboration with ICAR- Central Marine Fisheries Research Institute and Indian National Centre for Ocean Information Services (INCOIS). This app consolidates Potential Fishing Zone (PFZ) advisories based on remote sensing data from NOAA satellites, sea surface temperature, and phytoplankton presence. It presents advisories in local languages, aiding fishermen in optimizing their fishing activities.

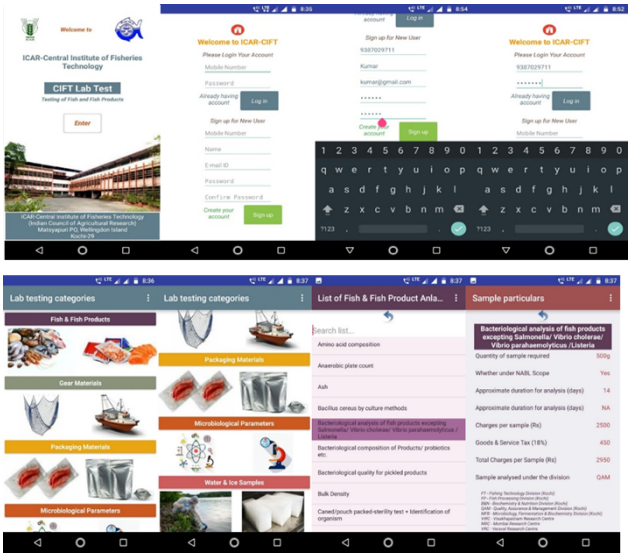
CIFT Lab Test mobile application

The ICAR-Central Institute of Fisheries Technology in Cochin, which holds ISO 9001:2008 certification, has earned recognition as a National Referral Laboratory for Fish and Fishery Products from the Food Safety and Standards Authority of India (FSSAI), operating under the Ministry of Health and Family Welfare within the Government of India.

To enhance accessibility to information pertaining to diverse sample testing and analysis services encompassing fish and fish-derived products, fishing equipment materials, packaging materials, microbiological metrics, as well as quality benchmarks for ice and water samples,

ICAR-CIFT has introduced an innovative mobile application known as "CIFT Lab Test." This mobile app is designed to benefit aquaculture farmers, processing industries, and other stakeholders within the sector. It grants users online access to a comprehensive repository of various lab tests, offering details such as the requisite number of samples, the estimated time for test report generation, associated costs, and more. This information is available round the clock, ensuring convenience and accessibility.

CIFT Lab Test

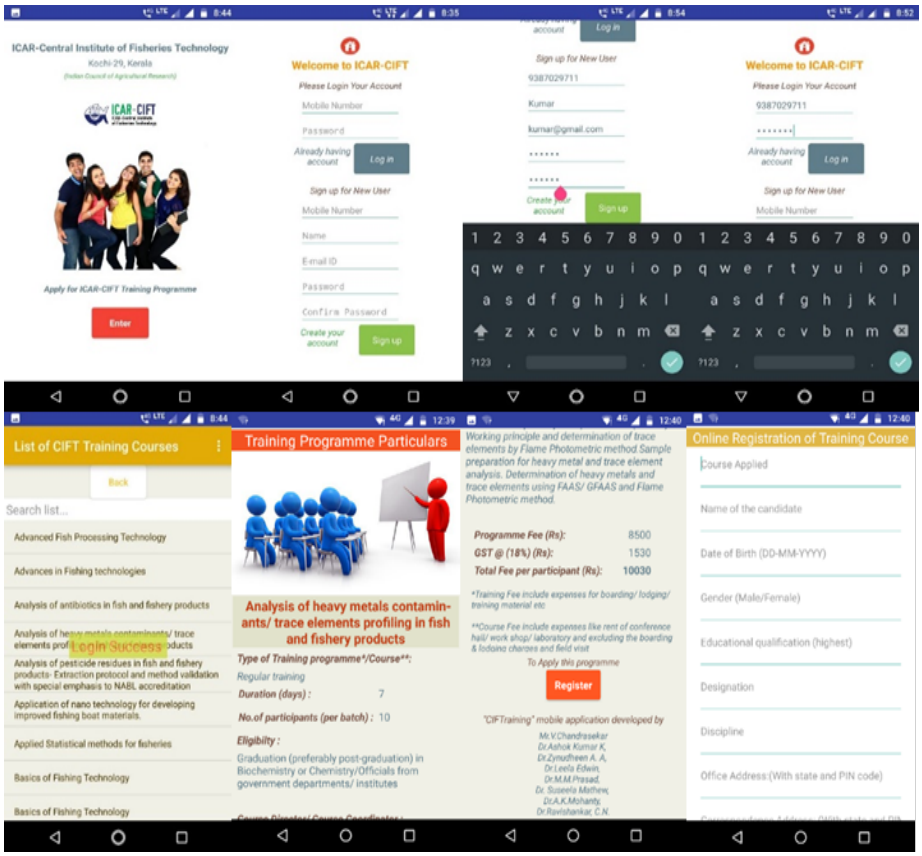


CIFTraining mobile application

The ICAR-Central Institute of Fisheries Technology (ICAR-CIFT) in Cochin has developed an innovative mobile application called "CIFTraining," which serves as a comprehensive information resource for ICAR-CIFT's training programs. This app is a valuable tool for fisheries students, researchers, industry professionals, state extension personnel, fisheries-based entrepreneurs, fishermen, and other stakeholders in the sector. It offers 24/7 access to online information about a wide range of training programs in fields such as Fishing Technology, Fish Processing, Biochemistry & Nutrition, Microbiology, Quality Control, Engineering, Extension & Economics.

The "CIFTraining" mobile app includes a complete list of 68 training programs available at ICAR-CIFT. These programs comprise 60 regular training courses, two comprehensive courses, three specialized courses, and three certified courses, covering various themes across seven divisions. With the "CIFTraining" app, stakeholders can easily search for training programs that align with their interests. They can view detailed information about each

program, including course content, fees, duration, eligibility criteria, and available facilities. This empowers stakeholders to select the most suitable training program to enhance their technical knowledge and skills in their respective fields. Furthermore, the app facilitates online registration for these training programs, streamlining the application process.



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 the Fisheries Industry:

www.marinefishsales.com is an innovative multi-vendor e-commerce platform developed under the NICRA project of ICAR-CMFRI. This platform is available as an Android application for mobile phones, facilitating direct sales between fisherfolk and customers. The aim of the app is to ensure fair pricing through direct sales between fishermen/farmers and consumers.

Daily Fish:

Your journey from 'catch' to 'kitchen' has never been more top-notch than with 'Daily Fish.' This online seafood store offers ready-to-cook seafood that is 'As good as Live,' preserving all the essential nutrients. This aligns with the vision of Baby Marine, the promoters of Daily Fish and a leading marine product exporter from India to various global regions, including Europe, the US, South America, Japan, South East Asia, the Gulf, South Africa, and Australia, spanning over four decades.

Decision Support System (DSS):

A Decision Support System (DSS) is a computer-based application that gathers, organizes, and analyzes business data to facilitate informed decision-making across the fisheries value chain. A well-designed DSS assists decision-makers in consolidating various data sources, including raw data, documents, employee knowledge, and business models. DSS analysis helps companies identify and resolve issues and make decisions, particularly at the farm level.

Types of Decision Support Systems (DSS):

These systems can be categorized into five types: Communication-driven DSS, data-driven DSS, document-driven DSS, knowledge-driven DSS, and model-driven DSS. For instance, Aqua Manager is a comprehensive software solution designed to enhance efficiency in aquaculture industries, supporting all stages of fish production from hatchery to harvest.

Supply Chain Integration:

Integrating technology into the supply chain can be complex, even in the seafood industry, with the introduction of traceability technology that monitors the entire journey of seafood products from water to plate. As consumers increasingly seek information about the origin of their fish, companies are developing advanced solutions to capture, transmit, and receive data across all components of the seafood supply chain, including fishermen, processors, transporters, distributors, and retailers.

Traceability:

Traceability is closely linked to the accuracy of seafood labels that highlight a product's sustainability, origin, authenticity, and other factors significant to consumers. Offering socially responsible products can lead to higher profits, enhanced customer loyalty, and an improved brand reputation. Suppliers are under growing pressure from consumers and retailers to provide traceability for their products. Traceability technology can mitigate risks and minimize the impact of public health incidents. The use of unique ID codes for fisheries and their incorporation into traceability and data-sharing systems, such as the Global Record for Stocks and Fisheries (GRSF), can streamline operations, save time and reduce costs for the seafood supply chain, traceability technology companies, governments, and non-governmental organizations (NGOs).

The Global Record for Stocks and Fisheries (GRSF):

GRSF 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 specifically designed to address intricate problems within a particular domain, leveraging an extraordinary level of intelligence and expertise. The development of the Expert System for Shrimp Aquaculture (ESSHA) followed a structured process encompassing five key steps: problem selection, knowledge acquisition, knowledge representation, system design, and development, culminating in system validation (Zetian et al., 2005).

Expert Systems in the Fisheries Sector:

Expert systems have rapidly gained prominence as integral components of applications across various domains, spanning from conventional manufacturing processes to applications in outer space. In multiple fields, including fisheries and aquaculture, expert systems have demonstrated the potential to significantly enhance traditional approaches, often yielding improvements on the order of magnitude. The return on investment in expert systems can be remarkably high in such domains.

***Social Networking*:**

The internet's widespread penetration and the increasing adoption of social media, particularly among the younger generation, are notable trends. In this context, a study was conducted to assess students' internet and social media usage patterns, as well as their means of accessing professional (fisheries) information through social media platforms. Social media has been categorized into two main types: social networking sites and instant messaging applications, considering both their form and content.

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)
- MPEDA, Fisheries College, Research institute, CMFRI,
- 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

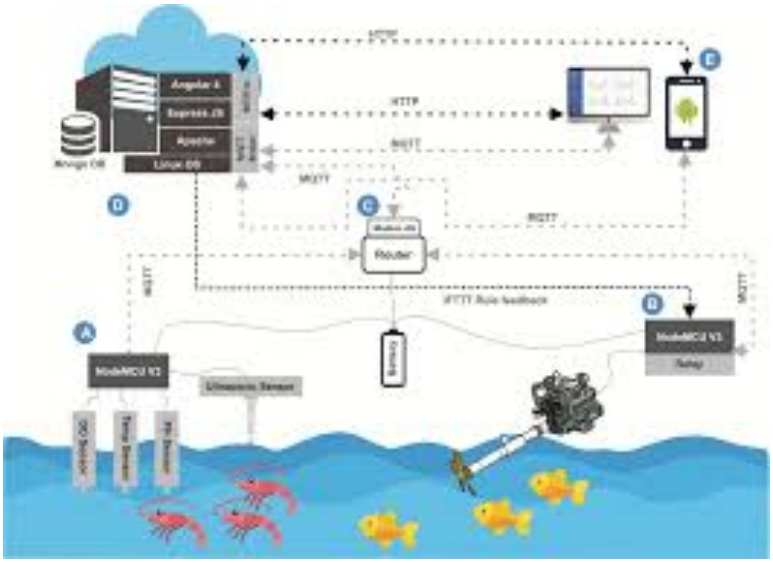
Blockchain technology in fisheries

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,

Aquaculture Automation: Automation in aquaculture involves the use of sensors to monitor water quality parameters like temperature, pH, and oxygen levels. These systems can also automate feeding processes and control environmental conditions in fish farms, ensuring optimal growth and health for the fish.

Remote Sensing: Remote sensing technologies, such as satellites and aerial drones, provide real-time data on ocean conditions and fish populations. This data helps fisheries management make informed decisions about sustainable fishing practices.

Drones (Unmanned Aerial Vehicles - UAVs)**:** Drones equipped with cameras and sensors are used for aerial surveillance of fishing areas. They can monitor fishing activities, detect illegal practices, and collect data on fish stock distribution.

Fish Health Monitoring: Underwater robots equipped with cameras and sensors can inspect fish health and behavior. They help fish farmers detect diseases early, reducing the need for antibiotics and improving overall fish welfare.

Genetic Technologies: Advanced genetic techniques, including selective breeding and genetic modification, are used to develop fish breeds with desirable traits, such as disease resistance, rapid growth, and better feed conversion rates.

Artificial Intelligence (AI) and Machine Learning: AI and machine learning algorithms analyze vast datasets to predict fish stock levels, optimize fishing routes, and improve aquaculture management. They can also identify patterns related to fish health and environmental conditions.

Smart Fishing Gear: Innovations in fishing gear include devices like smart buoys that use GPS and sonar technology to reduce bycatch and minimize environmental impact. These technologies promote sustainable fishing practices.

Aquaponics and Recirculating Aquaculture Systems (RAS): Aquaponics combines fish farming with plant cultivation in a closed-loop system. RAS systems recycle water and nutrients, reducing waste and conserving resources.

Biotechnology: Biotechnology is used for fish health management, including the development of vaccines and treatments for fish diseases. It also plays a role in producing pharmaceuticals and other value-added products from fish.

3D Printing: 3D printing allows for the customization of aquaculture equipment, making it more efficient and cost-effective. It is also used for rapid prototyping of new aquaculture technologies.

Virtual Reality (VR) and Augmented Reality (AR): VR and AR technologies provide immersive training experiences for fisheries personnel and aquaculture workers. They simulate real-life scenarios and enhance learning.

Sustainable Feed Formulation: Software tools use algorithms to formulate fish feeds that meet nutritional requirements while minimizing the environmental impact. This helps reduce overfeeding and waste in aquaculture.

Underwater Robotics: Remotely operated vehicles (ROVs) and autonomous underwater vehicles (AUVs) are used to explore underwater environments, collect data on fish habitats, and conduct maintenance tasks in fish farms and fisheries.

These technologies collectively contribute to the sustainability, efficiency, and innovation of the fisheries and aquaculture sectors, addressing challenges related to environmental conservation, disease management, and responsible fishing practices.

References

De, H.K., Saha, G.S., Srichandan, R. and Vipinkumar, V.P. 2008. New Initiatives in fisheries extension. Aquaculture Asia Magazine. July- Sept. pp 16-19

FAO.2007. Information and communication technologies benefit fishing communities. New Directions in Fisheries- A Series of Policy Briefs on Development Issues, No.07.Rome.12pp

Farell, C.O.2003. Theme paper presented for a seminar on ICT for improving agricultural productivity and competitiveness. 8-12 Sept. Indonesia.

Jensen, R. 2007. The Digital Provide IT, Market Performance and Welfare in the South Indian Fisheries Sector. Quarterly Journal of Economics, 122(3), 879-924.

Jensen, R. and E. Oster.2007. The Power of TV: Cable Television and Women's Status in India, NBER Working Paper 13305.

Rasheed, S.V. 2003. Innovations in agricultural extensions in India. S.D Dimensions, FAO June 2003

Kularatne, E. (1997). Information needs and information provision in developing countries. Information

Development, 13(3), 117-121.

Evoh, C. J. (2009). The role of social entrepreneurs in deploying ICTs for youth and community
development in South Africa. The Journal of Community Informatics, 1 (5), 1-16.
Jensen, R. (2007). The digital provide: information technology market performance and welfare in the
South Indian fisheries sector. Quarterly Journal of Economic Cambridge Massachusetts, 122 (3), 879-924
Mittal, S., & Tripathi, G. (2009). Impact on small farmers and fishermen through use of mobiles in India. In
proceeding of EAAE-IAAE seminar on small farms 26th -27th June University of Kent, UK.
Thangavel V, Shanmugam A. P, Inbaraj L. C. C. E-Coverage of Fisheries Ecosystem and Usage Pattern of Social Media. Curr World Environ 2019; 14(2). DOI:http://dx.doi.org/10.12944/CWE.14.2.10
Zetian, F., Feng, X., Yun, Z. and XiaoShuan, Z. 2005. Pig-vet: a web-based expert system for pig disease diagnosis. Exp. Sys. Appl., 29: 93–103.

****

52

FISH AND SEAFOOD CONSUMPTION AND ITS DETERMINANTS IN INDIA: AN OVERVIEW

Sajeev M.V.
Extension, Information and Statistics division
Email: sajeev.mv@icar.gov.in

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

According to National Sample Survey Organization (NSSO) report, the monthly per capita fish consumption of urban and rural India is 0.27 kg and 0.25 kg. The ICMR recommendation of fish consumption is 12 kg/year, which is yet to be achieved in India with a predicted per capita fish consumption of 6.6 kg in 2030 by World Bank (Msangi et al., 2013). Government of India had also set a target of 20 MT fish production by the year 2022-23 by laying renewed focus on the sector through a flagship scheme "Blue Revolution" (Shasani et al., 2020) which was not met. Tripura (25.53kg), Chhattisgarh (19.7kg), Manipur (18.25kg), Kerala (17.93kg) and Odisha (16.34kg) are the bigger states reporting highest average annual per capita fish consumption while the Union Territory of Andaman and Nicobar Islands reports the highest percapita fish consumption of 77.84kg/year in 2020-21 (DoF, 2022). Identifying the factors influencing consumption of fish and studying consumption behaviour aids government in alleviating hunger and malnutrition among deprived sections (Sajeev et al; 2021).

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

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

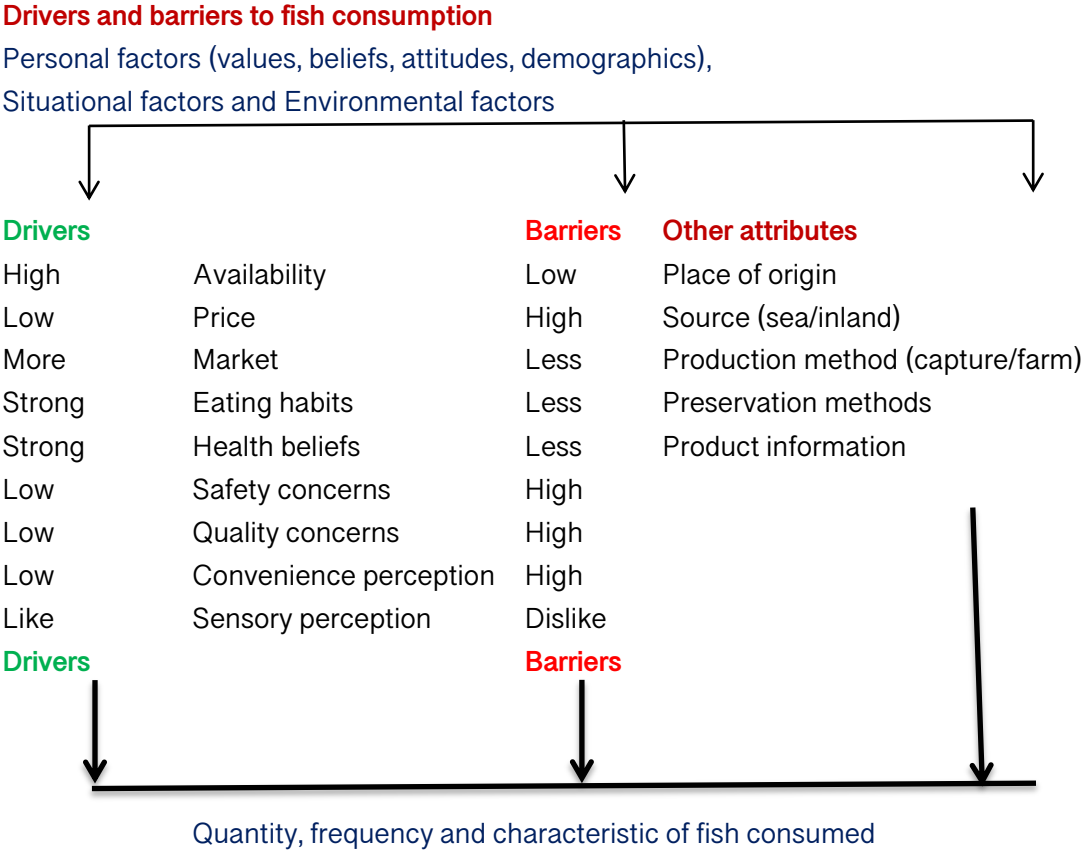
A study on knowledge and perception of fish consumers with respect to health benefits of fish consumption, safety and quality of fish and major drivers and barriers to consumption was done among consumers in Kerala State, India. The state was identified for the study due to its predominantly high fish 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.*, 2019). 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 stakeholders in fishery sector.

References:

Ajzen, Icek (1991). The Theory of Planned Behavior. Organizational Behavior and Human Decision Processes, 50, 179-211.

Bogard, J. R., Thilsted, S. H., Marks, G. C., Wahab, M. A., Hossain, M. A., Jakobsen, J., & Stangoulis, J. (2015). Nutrient composition of important fish species in Bangladesh and potential contribution to recommended nutrient intakes. *Journal of Food Composition and Analysis*, *42*, 120-133.

Bonne, Karijn; Vermeir, Iris; Bergeaud-Blackler, Florence; & Verbeke, Wim (2007). Determinants of halal meat consumption in France. British Food Journal, 109(5), 367-386

Burger, J., Stephens, W. L., Boring, C. S., Kuklinski, M., Gibbons, J. W., & Gochfeld, M. (1999). Factors in exposure assessment: ethnic and socioeconomic differences in fishing and consumption of fish caught along the Savannah River. *Risk Analysis*, *19*(3), 427-438.

FAO. (2020). *The State of World Fisheries and Aquaculture 2020: Sustainability in action*. http://www.fao.org/documents/card/en/c/ca9229en

Gutteling, J.M., Wiegman, O., (1996). The source of risk messages. In: Gutteling, J.M., Wiegman, O. (Eds.), Exploring Risk Communication. Kluwer Academic Publishers, Dordrecht, pp. 151–169.

Jungermann, H., Pfister, H.R., Fischer, K., (1996). Credibility, information preferences, and information interests. Risk Anal. 16, 251–261.

Lowe TE, Brill RW, Cousins KL. Responses of the red blood cells from two high-energy demand

teleosts, yellowfin tuna (Thunnus albacares) and skipjack tuna (Katsuwonus pelamis), to catecholamines. J Comp Physiol [B]. 1998 Aug;168(6):405-418.

Msangi, S., Kobayashi, M., Batka, M., Vannuccini, S., Dey, M. M., & Anderson, J. L. (2013). Fish to 2030: prospects for fisheries and aquaculture. *World Bank Report*, *83177*(1), 102.

Department of Fisheries, Government of India (2022) Handbook on Fisheries Statistics 2022. p. 218.

Olsen, Svein Ottar (1998). Fresh versus frozen seafood as distinct product categories: A qualitative study of Norwegian consumers. Paper presented on the 9th International Conference of the International Institute of Fisheries Economics & Trade. Tromsø, Norway

Olsen, SveinOttar (2001). Consumer involvement in fish as family meals in Norway: An application of the expectance–value approach. Appetite, 36, 173–186.

Olsen, SveinOttar (2003). Understanding the relationship between age and seafood consumption: the mediating role of attitude, health involvement and convenience. Food Qual. Prefer. 14, 199–209.

Olsen, SveinOttar (2004). Antecedents of Seafood Consumption Behaviour: An Overview. Journal of Aquatic Food Product Technology, 13(3), 79-91.

Petrovici, Dan A., Ritson, Christopher & Ness, Mitchell (2004). The Theory of Reasoned Action and Food Choice: Insights from a Transitional Economy. Journal of International Food & Agribusiness Marketing, 16(1), 59-87.

Pieniak, Z., Verbeke, W., Scholderer, J., Brunsø, K., Olsen, S.O., (2007). European consumers' use of and trust in information sources about fish. Food Qual. Prefer. 18, 1050–1063.

Pieniak, Z., Verbeke, W., Scholderer, J., (2010a). Health-related beliefs and consumer knowledge as determinants of fish consumption. J. Hum. Nutr. Diet. 23, 480– 488.

Pieniak, Z., Verbeke, W., Olsen, S.O., Birch Hansen, K., Brunsø, K., (2010b). Health related attitudes as a basis for segmenting European food consumers. Food Policy 35, 448–455.

Saba, Anna, & Vassallo, Marco (2002). Consumer attitudes toward the use of gene technology in tomato production. Food Quality and Preference, 13, 13–21.

Sajeev, M. V., Mohanty, A. K., Sajesh, V. K. and Rejula, K. (2019) A review of drivers and barriers to fish consumption based on theory of planned behaviour. FishTech Report. 5(2): 18

Sajeev, M. V. (2021) E-marketing of Fish and Fish Products. In: Fishpreneurship: Present Status, Challenges and Opportunities (Ravishankar, C.N., A.K. Mohanty and Sajeev, M.V. (Eds.), pp 323-336, Biotech Books (India), New Delhi

Sajeev, M. V., Radhakrishnan, A., Mohanty, A. K., Joshy, C. G., Ali, V. A., Gopika, R., … and Ravishankar, C.N. (2021) Factors Influencing the Fish Consumption Preferences: Understandings from the tribes of Wayanad, Kerala. Indian J. Ext. Educ. 57(4): 23-27

Sajeev, M. V., Joshy, C. G., Rejula, K., Sajesh, V. K., Suresh, A., Mohanty, A. K., & Ravishankar, C. N. (2021). Drivers and Barriers to Online Fish Purchase in Kerala, India.

Sajeev, M. V., Aparna Radhakrishnan, Mohanty, A. K., Joshy, C. G., Akber Ali, V. P., Gopika, R. Suseela Mathew & Ravishankar, C. N. (2021) Factors influencing fish consumption preferences: Understandings from the tribes of Wayanad, Kerala. *Indian Journal of Extension Education, 57*(4), 23-27.

Sajeev, M. V., Mohanty, A. K., Joshy, C. G., Gopika, R., Jabbar, P. A., Mathew, S., & Ravishankar, C. N. (2022). Fresh and Dried Fish Consumption and its Contributory Factors: A Study of Malappuram, Kerala. *Indian Journal of Extension Education, 58*(4), 86-90.

Steptoe, Andrew, Pollard, Tessa M., & Wardle, Jane (1995). Development of a Measure of the Motives Underlying the Selection of Food: the Food Choice Questionnaire. Appetite, 25, 267–284.

Turan, D., Kottelat, M., Kirankaya, S. G., & Engin, S. (2006). Capoeta ekmekciae, a new species of cyprinid fish from northeastern Anatolia (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters*, *17*(2), 147.

Verbeke, W., Vackier, I., (2005). Individual determinants of fish consumption: application of the theory of planned behaviour. Appetite 44, 67–82

****

53

ADVANCED EXTENSION TECHNIQUES FOR HARNESSING POTENTIAL OF FISHERIES SECTOR

Sajesh.V.K
Extension, Information and Statistics division
Email: sajeshvk@gmail.com

Fisheries extension envelops the fisheries development in action (Ananth, 2010). Cole (1977) has opined that that fisheries extension service is mainly intended to achieve all-round development of the fishing sector. In India, though both the central and state governments formulate policy guidelines, the states have the major role in executing the extension programs at field levels through their respective Departments of Fisheries (DoFs). The Union government also provides financial support through its schemes to provide technical, financial and extension support to aqua farmers (Kumaran et al., 2003).

Apart from providing the information and services needed and demanded by fishers and other actors like processors in rural settings, the extension has the onus to carry out different activities to assist them in developing their own technical, organisational, and management skills and practices so as to improve their livelihoods and well-being (GFRAS, 2012).

Table 1: Functions of Fisheries Extension Services

Area of Work	Objectives
Technology transfer	Improved techniques of mariculture and aqua culture Introduction of modern craft and gear material for fishing Scientific post- harvest practices Diversified technology application in fisheries Introduction of innovative technology application methods
Information And support services	Information support to fishermen about prices, types and availability of known and new fishing inputs
Food safety and quality.	Awareness creation on importance and methods of hygienic handling of fish. Promotion of food safety and quality standards among various stakeholders.

Marketing and distribution	Provision of real time marketing information to fishermen about wholesale and retail prices, ultimate market places etc. Strengthening the position of the fishermen against middlemen by organizational and financial support of marketing through fisher women and co-operatives
Sustainable fisheries	Advising and educating fishermen in resource conservation methods and responsible fishing practices
Credit and finance	Facilitating direct contact between banks and fishermen Facilitating indirect institutional finance through self help groups, co-operatives, credit societies etc. Implementing welfare schemes for the development of poorer fishermen Promotion of institutional savings
Organizational and capacity development	Facilitating the development of fishermen organization to promote collective action. Capacity development of various actors in the value chain.
Entrepreneurship development	Identification and promotion of entrepreneurial possibities in fisheries sector Development of entrepreneurial capacity of students, rural youth, fishermen and women Incubation support to potential entrepreneurs Facilitating technology commercialisation
9. Safety measures	Awareness generation about life saving equipments, risk communication devises and survival strategies. Skill development on use of communication devises and survival techniques
10. New extensionist approaches	Networking, promotion of interagency collaboration, facilitation, creating many-to-many relationships among the wide range of actors.

(Sajesh et al,2018)

The scenario mentioned above points to the need for an '*extension- plus*' approach synergising both technology and non-technology services demanded by the fishermen.

Table 2. Extension- plus: Key shifts

From	To	Strategies
Technology dissemination	Supporting rural livelihood	Enabling fishers to develop livelihood assets through skill

		development, facilitating access to capital, community mobilization, hazrd mitigation and infrastructural development
Improving productivity	Improving income	Price information, market intervention, avoid exploitation by middlemen
Forming fishers group	Building independent fisher operated organisations	Reorienting existing fishers organizations and apex agencies for upscaling and outscaling their efforts
Providing services	Enabling fishermen to access services from other agencies	Liaison with agencies in public, private and civil society segments for inputs, credit, research, technology extension, marketing and capacity building
Market information	market development	Forge networking with supply chain actors, processors

(Adapted from Sulaiman & Hall, 2004)

Shifting focus from technology dissemination to supporting rural livelihood; improving productivity to augment the income of the producers; providing service to enable fishermen to access service from various agencies and building independent fishermen-operated groups are some of the key changes required in this context (Sulaiman & Hall, 2004). Operationalisation of such changes requires strategies like skill development, community mobilization, infrastructure development, market intervention, reorienting existing fishermen organizations for upscaling and outscoring their efforts, liasoning with various agencies in public, private and civil society segments, forging linkage with processors and other supply chain actors etc.

Technology dissemination should be the core, but the focus has to be broadened. There needs to be a range of objectives like mobilisation and strengthening of producer collectives, promotion of linkage with various agencies in the public, private and civil society segments, and entrepreneurship development while being sensitive to the ecosystem and environmental protection. In addition to technology transfer, it is important to strengthen locally relevant innovation processes and knowledge systems (Sulaiman & Hall, 2004). Innovations can be in the realms of technology (eg: technologies for responsible fisheries), organization (eg: group mobilization or restructuring), institutions or decision-making (eg: decision to adopt) and need not be promoted by research or extension systems. The innovation capacity of the fishers and

other actors in rural settings depends on the skills to develop and assimilate internal and external resources for problem-solving and to leverage opportunities (World Bank, 2012), which in turn requires harnessing the synergy of pluralistic stakeholders in a complementary manner. It starts with the identification of multiple actors and their roles as well as the ways by which they can be effectively converged for the larger goal of making fishermen better managers of the sector and organizations.

Role of collectives in fisheries extension

Extension- plus approach, to be effective, requires the convergence of various agencies and schemes to optimise their contribution towards the welfare of the fisherfolk. This, in turn, requires a suitable platform for harnessing the strength of diverse actors across the value chain. As discussed earlier, collectives like fishermen/fisherwomen groups have the potential to act as such platforms. The efficacy of such collectives depends on the extent of self-mobilization. These collectives should be linked to larger innovation networks composed of fishermen, fishermen organizations, private and public firms, researchers, extension agents, government agencies, funding agencies and financial agencies. Major activities to foster the emergence of innovation networks include creating trust among potential partners, identifying common goals, establishing the bases of collaboration and developing innovation capabilities (Ekboir, 2012). The extension has an important role to carry out these activities as well as to enhance the ability of other actors to support fishermen in an integrated way. Reorienting the fisheries extension system to address the varied concerns of the sector requires policy-level interventions in terms of human and financial resources (Sajesh et al., 2018) and organisational innovations.

Cooperatives and producers' organizations open a new avenue for the smallscale producers by facilitating various multiple linkages with institution/organization to spread awareness and strengthen the policies and procedures to boost productivity and help farmers to adapt changing organizational conditions. Offering of extension services by cooperatives have positive impact on performance. Beyond that they often offer social services and building of physical infrastructure in rural areas

Research institute-cooperatives linkage for technology dissemination

Research institute-cooperatives linkage for technology dissemination refers to the association between institutes and cooperatives to transfer information and technology for enhancing the production practices and hence to improve the return from farming/ cultivation(Sajesh,2023). Technologies and practices generated at research institutes often fail to reach smallscale producers owing to multiple reasons. Cooperatives, being owned and by the rural producers and farmers, can help in disseminating research outcomes to wide range of end users making use of their networks and membership base.

In addition to technology dissemination, institutes can join hands with cooperatives in the field level evaluation of the technologies and customizing them as per the feedback of its members. In this way the collaboration can facilitate better adoption of technologies by the members of cooperatives as they are involved in the technology assessment and refinement process. Further, the linkage can also promote capacity building for farmers and rural producers. Research institutions can provide technical assistance and skill support to cooperative members on innovative practices. Also, cooperatives can serve as a platform for farmers and rural producers for cross learning and knowledge acquisition.

While fomenting the collaboration, the roles of research institutes and cooperatives can overlap and be complementary. For example, research institutes can work with cooperatives to identify research priorities and to co-design research projects that address the needs and interests of fishers and processors. Cooperatives can provide access to fishing communities and facilitate communication and knowledge exchange between research institutes and fishers. Together, research institutes and cooperatives can collaborate to disseminate new knowledge and technologies, and to promote the adoption of sustainable and socially responsible fishing practices.

Table 3: Roles of Research Institutes and Cooperatives in developing the linkage

Attributes	**Institute**	**Cooperatives**
Research, technology validation and advocacy		
Research	*Conducting research*: based on the research needs of stakeholders, Research institutes can initiate research for solving various problems constraining effective value chain functioning in fisheries	*Identifying research needs:* Identify areas where further research is needed for effective value chain functioning in the agriculture or allied sector *Supporting research projects*: Cooperatives can support research institutes on executing studies by facilitating access to resources and various facilities of the members farmers or producers
Information dissemination	Through publishing research papers, reports, or other	Cooperatives can facilitate transfer of information and

	publications, as well as providing training and educational programs	technologies to their members through trainings, awareness programmes and other outreach activities.
Feedback	To redesign research as per the needs of stakeholders and suiting to the particular environment.	Cooperatives can provide feedback to research institutes and extension agencies on the effectiveness of the innovations being disseminated
Advocacy:	Research institutes can advocate for policies and regulations that support sustainable fisheries management practices	By collaborating with research institutes and extension agencies cooperatives can help in formulating advocacies for policies,practices and regulations for the benefit of fisheries sector.
Extension and Programme implementation		
Beneficiary Identification	Guidelines for beneficiary identification and selection	Selection of beneficiaries
Group mobilization	Facilitation of group mobilization	Mobilization of beneficiaries
Technology training	Conducting training programmes for developing required skills for technology application	Facilitation of training
Infrastructure development	Guidance regarding required infrastructure Incubation support	Developing required infrastructure required for technology use
Capital requirement	Possible assistance under Government schemes and programmes Assistance in developing	Provision of loans and financial assistance for acquiring the technology; or Facilitation of access to credit, subsidy etc.. ,or

	project report, proposal etc.	Technology acquisition and provision of access on custom hiring basis
Liasoning with other agencies	Collaboration with other research institutes, Universities KVKs etc	Convergence of various schemes and programmes of governmental and non governmental agencies to develop the value chain. Cooperatives can act as platform for convergence.
Marketing	Facilitating role	Promotion of marketing through various avenues
Monitoring	Monitoring follow up of adherence to package of practices Collecting feedback and remedial measures	Monitoring financial feasibility

(Sajesh,2023)

Entrepreneur led extension

Entrepreneurs can provide extension services in varying areas of fisheries for the welfare of fisherfolks and other stakeholders in the fisheries value chain. Agriclinics And Agri Business Centres as well as AgriBusiness Incubation Centres are major initatives in this direction.

The Agriclinics and Agri Business Centres (ACABC) scheme is being implemented by Ministry of Agriculture and Farmers' Welfare, Government of India, with NABARD acting as subsidy channelising agency. Agri-Clinics are envisaged to provide expert advice and services to farmers on various aspects to enhance productivity of crops/animals and increase the incomes of farmers. Agri-Business Centres are commercial units of agri-ventures established by trained agriculture professionals. These ventures may include maintenance and custom hiring of farm equipment, sale of inputs and other services in agriculture and allied areas, including post-harvest management and market linkages for income generation and entrepreneurship development. In the same line, Aqua Clinics and Aquapreneurship Development Programme (AC&ADP)" conducted by National Institute of Agricultural Extension Management (MANAGE) in collaboration with National Fisheries Development Board (NFDB), Hyderabad since 2018. MANAGE has initiated this program to create self-employment opportunity and make more and more individuals self-reliant. With the aid of 19 Fisheries Nodal Training Institutes (NTIs) across the country MANAGE has

trained 766 participants to promote entrepreneurship development, support innovative technologies(MANAGE,2023).

Opening up of 22 Agribusiness Incubators by Indian Council of Agricultural Research (ICAR) through its World Bank funded National Agricultural Innovation Project (NAIP) in 2008-09 (10 Agribusiness Incubators) and 2013-14 (12 Agribusiness Incubators) has given a boost to technology based entrepreneurship in Agriculture. These Agribusiness Incubators were housed either in Agriculture Research Institutes or State Agricultural Universities which are generators of Agricultural technologies . The agribusiness incubators (ABIs) provide shared facilities and equipment, business development, market access, technology assessment services, financial services; as well as mentoring and networking (Sivakumar and Sivaraman, 2014).

Conclusion

Extension has major role to play in harnessing the potential of fisheries value chain for the welfare of various stake holders across the chain. Extension-plus approach including forging collective action, research institute cooperative linkage and entrepreneur led extension are some of the major strategies which can be deployed for the development of fisheries sector.

References

Ananth, P.N. (2010). Marine Fisheries Extension, Discovery Publishing House, New Delhi.

Cole, R. C. (1977). Fisheries extension services: Their role in rural development. Marine Policy, 1(2), 132142.

Ekboir, J. (2012) How to build innovation networks.Agricultural Innovation Systems. An Investment

Sourcebook, 44 p, World Bank

GFRAS (2012) The "New Extensionist": Roles, Strategies, and Capacities to Strengthen Extension and Advisory Services, Global Forum for Rural Advisory Services November 2012, gfras_newextensionist_position paper%20(3).pdf (Accessed on 02 May 2016)

Kumaran, M., Chandrasekharan, V. S and Kalaimani, N. (2003). Aquaculture Extension: The Neglected Aspect In Aquaculture Development. Fishing Chimes, 23(6), September, 2003.

MANAGE,(2023) https://www.manage.gov.in/managecia/ACADPProg.aspx accessed on 10/08/2023

Sajesh, V. K., Suresh, A., Mohanty, A. K., Sajeev, M. V., Ashaletha, S., Rejula, K., & Ravishankar, C. N. (2018). Trend and pattern of expenditure on fisheries extension in India: Implications for Policy. *Indian Journal of Extension Education*, *54*(2), 32-40.

Sajesh, V. K., Suresh, A., & Mohanty, A. K. (2023). Marine Fisheries in Kerala, India: An Extension Perspective. *Fishery Technology*, *60*, 1-7.

Sivakumar and Sivaraman (2014) Fostering entrepreneurship through Agribusiness Incubation: Role of extension professionals, AESA Blog 33,Agricultural Extension in South Asia http://www.aesa-gfras.net/Resources/file/Blog%2033%20ABI.pdf

Sulaiman, V. and Hall, A. (2004) Towards Extension-plus Opportunities and Challenges. Policy Brief 17, ICARNational Institute for Agricultural Economics and Policy Research, New Delhi-12

World Bank. (2012) Agricultural Innovation Systems: An Investment Sourcebook, 660 p, Washington DC

****

54

INPUT AND SERVICE DELIVERY SYSTEM IN FISHERIES

A. Suresh, Vijay Kiran and Neethu Mol Jacob
Extension, Information and Statistics division
Email: sureshcswri@gmail.com

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

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

Major inputs required for the fishery development are given below

1. Labour

Labour is the important element of any production system. Major labour market in the fishery sector constitutes by the fishermen community. The Government of India estimates that the

fisheries sector supports the livelihood of nearly 16 million people in India at the primary level (Table 1), and almost twice that number along the value chain (Van Anrooy et al.,2022). In terms of employment, the aquaculture supports the livelihood of over 28 mn people in India especially the marginalized and vulnerable communities. However, the sector witnessing a downward mobility or migration of labours from fishery to other sectors due to economically not viable and unprofitable especially after the modernisation.

Table 1: Number of fishermen engaged in fishery activities

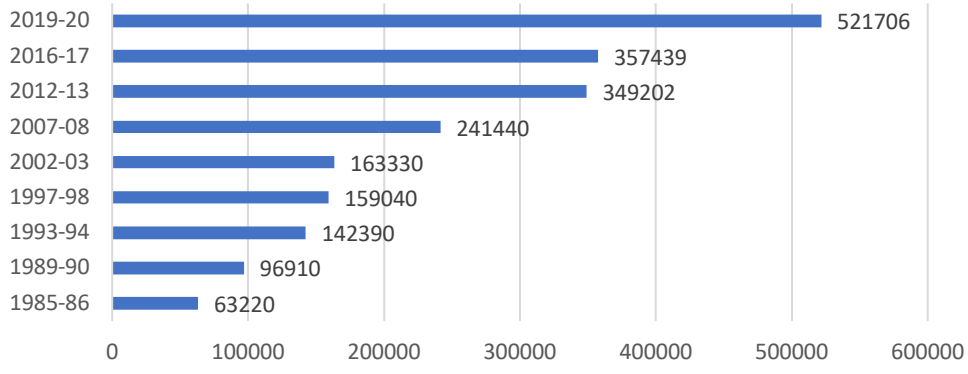
	Male	Female	total
Inland	1,3,0,13,978	10103842	2,31,17,820
Marine	26,51,652	22,99,065	49,45,717
	1,56,65,630	1,23,97,907	2,80,63,537

Source: Fishery statistics 2020

2. Fish seed and feeds

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

Figure 3. Fish seed Production (lakh fry)



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

3. Craft and gear

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

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

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

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

4. Ice and cold storage facility

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

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

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

Source: fishery statistics, 2020

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

Service delivery system in fishery sector

2.1 Credit delivery

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

2.1.1. Informal credit financers

a. Auctioneers / Commission agents

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

b. Third party

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

c. Money lenders

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

d. Fisherman to fisherman

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

2.1.2. Formal credit institution

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

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

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

2.2. Market system in fishery sector

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

2.2.1. Traditional fish marketing system

Traditionally fish marketing and distribution systems have involved collecting, processing and transporting fish from fishermen in remote landing areas to major consumption centers. Fresh fish is sold from the landing site to intermediate processors who smoke the fish (sometimes the smoking is done by family processors) and sell to wholesalers or middlemen at a distance, who pass through some middlemen and are finally sold to customers.Fish landing centres are the primary fish markets from where fishes are transported to the wholesale or retail markets and these centres had the maximum number of intermediaries like auctioneers, commission agents, retail traders and export agents (Aswathy*et al*, 2014). In the traditional marketing system, a large number of intermediaries are involved. Various marketing channels involved in the marine fish marketing system is given below, almost similar marketing channel exist in inland fisheries (CMFRI, 2020).**Marketing channel**is defined as a path traced in the direct or indirect transfer of title of a product as it moves from a producer to an ultimate consumer or industrial user. Thus, a channel of distribution of a product is the route taken by the ownership of goods as they move from the producer to the consumer or industrial user.Kohls and Uhl have defined marketing channel as alternative routes of product flows from producers to consumers. The number of

intermediaries between the fishermen and the final consumers varies in different marketing channels, based on the quantum of landings and the effort required to perform various marketing functions such as assembling, cleaning, grading, processing, storing and transportation (Sathiyadhas*et al*, 2011). Different market channels in the traditional marketing system given below.

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

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

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

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

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

Fish from the distant landing centres were able to reach, wholesale and retail markets due to the technological advancements in marine fish transport and processing. The perishable nature of fish, on the other hand, necessitated its prompt disposal at each point of transaction, resulting in the involvement of many intermediaries in the marketing channel, leading to high marketing costs and margins (Aswathy et al., 2014). Besidesauctioneers, market intermediaries in the traditional marketing system includes wholesalers, retailers, vendors, marine/ inland fishermencooperatives, contractors. They wereinvolved 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

2.2.2. Modern marketing system

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

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

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

Insurance system

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

Accident insurance: It is the most promising insurance product in the capture fishery sector and covers active fishermen's risk of life or disability while engaged in fishing activities. Among the insurance schemes, 'Group Accident Insurance Scheme for Active Fishermen' is the major scheme currently in operation, which covers the life and disability risks of the boat crew.

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

Concerns over input -service delivery system for the fisheries development

a. Lack of formal institutional credit mechanism

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

b. Lack of market information

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

c. Lack of quality fish seeds

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

d. Inadequate infrastructure developments

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

e. Inadequate risk covering mechanism

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

f. Market intermediaries and inefficiency

About three-fourths of total marine fish landed in Kerala is marketed domestically. . The fish marketing system in the state is highly complex, involves multiple stakeholders, intermediaries and benefactors with high level of diversity in market structure and conduct. Though modern and innovative marketing models are emerging in recent years, marketing practices followed are predominantly old and traditional in many areas with inefficiencies pervasive across the value chain. The major market imperfection in fish supply chain emerges in the stage of auctioning. Fish auctioning is highly unorganized and is rooted in traditions. The market charges and operations are unregulated, and is characterized by monopoly elements. There is barriers to entry as a fish auctioneer (Kumar *et al*, 2008).Other than performing the function of auctioning, their activities are both horizontally and vertically integrated: they serve as a major agents for informal credit to the fishing sector, financing both capital requirements for acquiring fishing vessels and daily fishing operations, supplying of axillary inputs like ice, providing fuel (diesel, kerosene) on credit etc. The credit offered to the fishermen is tied with output marketing operations. The real interest rate charged by the auctioneers is much higher than the market interest rate. However, one useful function is that the auctioneers shoulder the risks in financing fishing operations as fish catch depends on an element of probability, and therefore the repayment is a risky affair. Further, there are several irregularities persist in the structure, conduct and performance of the marketing system, as is observed in case of price determination, weighing and quality checking, payment, large element of reduction in quantity of fish on several pretexts etc. In that sense the fish auctioning system has large element of imperfections and exploitative elements. On the other end, consumers are charged high for their fish purchase. Over a period of time, the retail price of fish has increased at a higher rate compared to several other food commodities, resulting in large price spread. Further, this renders several consumers inaccessible to fish.

References

Aswathy, N., Narayanakumar, R. and Harshan, N.K. (2014) Marketing costs, margins and efficiency of domestic marine fish marketing in Kerala, Indian Journal of Fisheries, 61(2): 97-102.

Baiju, K. K., Xavier, A. K., Ramachandran, C., & Shinoj, P. (2019). Some insights into the credit transactions of small-scale fishers along the Kerala coast, India.

CMFRI [Central Marine Fisheries Research Institute]. 2020. Marine Fish Landings in India 2020 [on-line].Available: http://eprints.cmfri.org.in/15781/1/Marine%20Landings%20in%20India%202020.pdf [04 April 2022].

FAO (2022) Fisheries and Aquaculture Division [online]. Rome. [Cited Tuesday, July 12th 2022]. https://www.fao.org/fishery/en/countrysector/naso_india

FAO. 2020. The State of World Fisheries and Aquaculture 2020: Sustainability in action. Rome. www.fao.org/3/ca9229en/CA9229EN.pdf

Fishery statistics (2020). Handbook of Fishery statistics. Department of Fisheries Ministry of Fisheries, Animal Husbandry & Dairying Government of India, New Delhi.

Government of Kerala.(2021). Economic review. State planning board. Trivandrum

Katiha, P. K., Jena, J. K., & Barik, N. K. (2003). Profile of key inland freshwater aquacultural technologies in India. *A Profile of People, Technologies and Policies in Fisheries Sector in India*, 59.

Kumar, B. G., Datta, K. K., Joshi, P. K., Katiha, P. K., Suresh, R., Ravisankar, T., ... & Menon, M. (2008). Domestic fish marketing in India–changing structure, conduct, performance and policies. *Agricultural Economics Research Review*, *21*(conf), 345-354.

Kumar, B. G., Datta, K. K., Joshi, P. K., Katiha, P. K., Suresh, R., Ravishankar, T., Ravindranath, K. and Menon, M. 2008. Domestic Fish Marketing in India – Changing Structure, Conduct, Performance and Policies**.** *Agric. Econ. Res. Rev.* 21 () : 345 - 354.

Narayanakumar, R., & Sathiadhas, R. (2006). Domestic fish marketing opportunities for marine fisheries sector in India. In *National Workshop on Post harvest Methods and Domestic Fish Marketing Opportunities* (pp. 59-67). State Insitute of Fisheries Technology.

Nyimbili , B. and Musuka, C.,G.(2017). Fish Seed Availability, Quality, Quantity and Its Significance to Aquaculture Development in Zambia, *Innovative Techniques in Agriculture.*

Parappurathu, S., Ramachandran, C., Gopalakrishnan, A., Kumar, D., Poddar, M. K., Choudhury, M., ... & Sunil, P. V. (2017). What ails fisheries insurance in India? An assessment of issues, challenges and future potential. *Marine Policy*, *86*, 144-155.

Salim, S. S. 2016. Fish consumption pattern in India: Paradigm shifts and Paradox of export trade (Fish consumption pattern in India, exports-Overview). Food and Beverage News. 25-28 p.

Salim, S. S., Monolisha, S. and Sunil, P. V. 2020. Fish Consumption: Gauging the determinants of consumption and buying patterns across Kerala markets. J. Mar. Biol. Ass. India, 62 (1) : 33 – 40.

Sathiadhas, R., Narayanakumar, R. and Aswathy, N. 2011. Efficiency of Domestic Marine Fish Marketing in India – A Macro Analysis. *Indian J. Fish.* 58 (4) : 125-131.

Sivagnanam, K. & Priya, T. & Pulikkamath, Ashraf. (2019). Problems of Inadequate Post-Harvest Infrastructure in Fisheries.

Upadhyay, A. D., Jagpal, P. D. and Roy. 2017. Structural performance of fish market and socio-economic status of market functionaries of Naveen Machhali Mandi Mahanva of Gorakhpur, Uttar Pradesh. Econ. Aff., 61:511 p.

Van Anrooy, R., Espinoza Córdova, F., Japp, D., Valderrama, D., Gopal Karmakar, K., Lengyel, P., ... & Zhang, Z. (2022). *World review of capture fisheries and aquaculture insurance 2022* (Vol. 682). Food & Agriculture Org..

****

55

TRAINING NEED ASSESSMENT AND PROBLEM ANALYSIS

Dr. Rejula K.
Extension, Information and Statistics division
Email: rejulak@gmail.com

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). Absence of the need analysis in training invite risk of overdoing training, doing too little training, or missing the point completely (Brown, 2002). 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 TN

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 Recent advances in harvest and post-harvest technologies in fisheries ICAR-Central Institute of Fisheries Technology (CIFT) 379 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 behaviour. 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.

References

Andipamu, Raahalya. 2020.Training need analysis of Agricultural Officers (AOs) of Department of Agriculture Development and Farmers' Welfare Kerala" Unpublished

MSc thesis submitted to Department of Agricultural Extension, College of Agriculture, Vellayani, Kerala Agricultural University

Assessment of training needs. Development of e-Courses for B.Sc (Agriculture). http://ecoursesonline.iasri.res.in/Courses/Extension%20Methodologies%20for%20Transfer%20of%20ag.%20Tech/Data%20Files/lect18.html

Brown, J. (2002). Training needs assessment: A must for developing an effective training program. Public personnel management, 31(4), 569-578. Retrieved from https://journals.sagepub.com/doi/abs/10.1177/009102600203100412

Rajwinder Singh.2011. Development of need-based training module for farmers regarding farm forestry. Unpublished MSc thesis submitted to Department of Extension Education, College of Agriculture, Punjab Agricultural University, Ludhiana.

****

56

POST-HARVEST LOSSES IN THE FISH VALUE CHAIN: TYPES, CAUSES AND METHOD OF ASSESSMENT

Dr. Pe. Jeyya Jeyanthi
Extension, Information and Statistics division
tvjeyanthi@gmail.com

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.

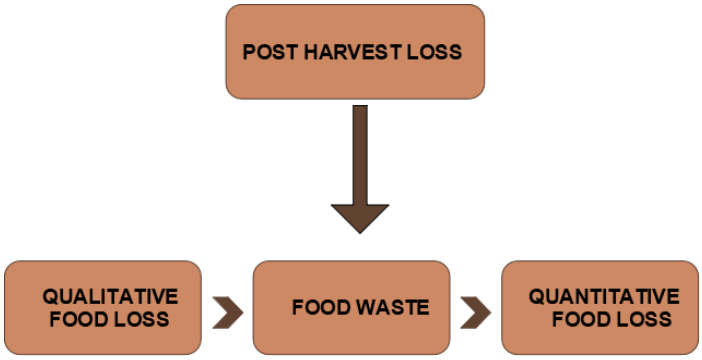


Fig. 1 Components of post-harvest loss

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.

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.

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.

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.

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.

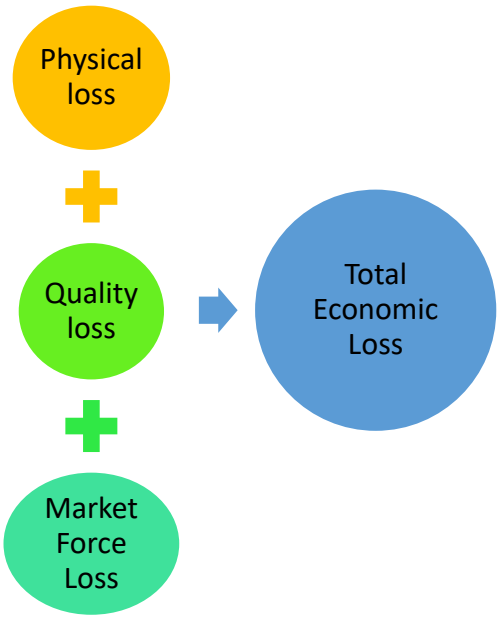


Fig. 2. Total Economic Loss

Total Economic Loss (TEL) is the summation of economic loss at landing centre, processing and marketing nodes (FAO, 2011; FAO 2018 & Torell et al., 2020). T

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 sta

ges of fish supply chain are 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)

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).

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.

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.

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).

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.

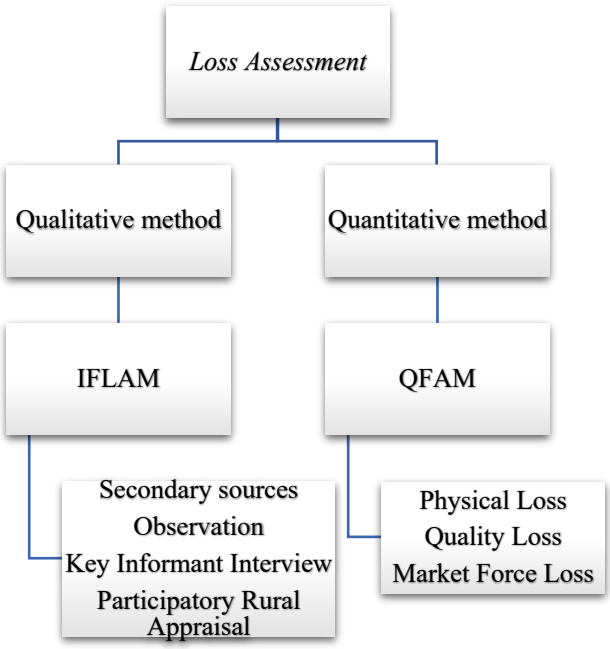


Fig. 2. Fish Loss Assessment Method (FLAM) - FAO

Load Tracking (LT)

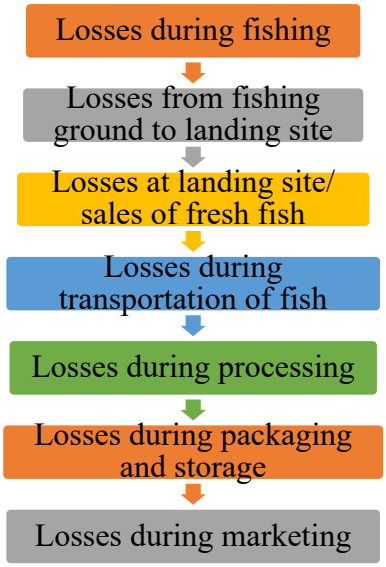
LT is an experimental method that produces statistically valid results for the calculation of loss between stages in a distribution chain involving the loads at different stages. This method is most robust because of the experimental and replicable nature of the procedure.

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.

Under the QLAM method, the three losses in fish value chain will be assessed viz., physical loss, quality loss and market force loss.

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. 3 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.

References

Akande, G.; Diei-Ouadi, Y. (2010) Post-harvest losses in small-scale fisheries: case studies in five sub-Saharan African countries. FAO Fisheries and Aquaculture Technical Paper. No. 550. Rome, FAO. 72p.

Kader, A.A. (2002) Postharvest technology of horticultural crops. 3rd ed. Univ. Calif. Agr. Nat. Resources, Oakland, Publ. 3311.

Kitinoja, L. and Gorny, J.R. (999). Postharvest technology for small-scale produce marketers: economic opportunities, quality and food safety. Univ. Calif. Postharvest Hort. Series No. 21.

FAO (2011) Post -harvest fish loss assessment in small-scale fisheries: A guide for the extension officer, FAO technical paper. No. 559. Rome, 93p.

Torell, E.C., Jamu, D.M., Kanyerere, G.Z., Chiwaula, L., Nagoli, J., Kambewa, P., Brooks, A. and Freeman, P. (2020) Assessing the economic impacts of post-harvest fisheries losses in Malawi, World Development Perspectives, 19.

****

57

WOMEN IN FISH-PRENEURSHIP

Dr. Ashaletha S.
Extension, Information and Statistics division
ashalethasuresh@gmail.com

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 nondominant)

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 Recent advances in harvest and post-harvest technologies in fisheries ICAR-Central Institute of Fisheries Technology (CIFT) 402 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: Passion 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.

****

58

APPLICATION OF QUANTITATIVE STATISTICS IN FISHERIES RESEARCH

Geethalakshmi V.
Extension, Information and Statistics Division
Email: geethasankar@gmail.com

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 have 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 postharvest 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 analysing the season wise data and pooled data.

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.

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	Sun-dried, processed smoked fish, salting

processing	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 customerservice, manage logistics, analyse medical records, or even write news stories.

****

59

INTRODUCTION TO QUALITY CONTROL IN FISH AND FISHERY PRODUCTS

Zynudheen. A. A, Priya E.R., Laly S.J
Quality Assurance and Management Division
Email : zynucift@gmail.com

Quality is the driving force in any industry. The desire for quality is the major factor providing a boost towards the operational efficiency. Most often quality refers to the aesthetic appearance and freshness of the fish. The term quality may also involve the safety aspects also. Quality is a subjective concept. As per the International Organization for Standardization (ISO) the term quality is defined as "degree *to which a set of inherent characteristics that fulfills requirements*".

Food safety can be termed as the assurance that the food will not cause an adverse health effect for the consumer when it is prepared and/or consumed in accordance with its intended use. Due to the ever-growing global population and raising demand for food to meet the requirements, food safety became a very important aspect. In the manufacturing process it is vital to ensure that the products delivered to consumers do not interfere with the consumers' health adversely. If the production system fails to comply with the food safety regulations, that will lead to the transmission of foodborne illness. According to World Health organization reports, about 2 million deaths occur every year from contaminated food or drinking water. Around 600 million cases are caused by 22 different enteric diseases (disease caused by intestinal infection) and among that about 52000 deaths are caused by enteric disease caused by Salmonella typhi. Over 40% people suffering from enteric diseases caused by consumption of contaminated food were children under the age of 5 years.

Quality Assurance and Quality Control

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

feedback, committed leadership, strategic planning, cross functional training, and employee involvement.

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

Quality and safety issues in fish products:

Quality issues	Safety issues
Live/fresh/chilled/frozen fishes	
Belly bursting Discoloration Blackening/ melanosis in crustaceans Pink discoloration in squid and cuttlefish Freezer burn/ dehydration Off flavors	Pesticide residues and Other Persistent organic pollutants Residues of veterinary drugs and extra label chemicals Unapproved additives Presence of adulterants Growth of pathogenic bacteria Allergens
Dried fish	
Shrinkage Casehardening Protein denaturation and rehydration Maillard reaction Rancidity Dun, Pink/Red Insect infestation Fragmentation	Growth of pathogenic bacteria Clostridium botulinum toxin production (for uneviscerated products) Staphylococcus aureus toxin Pesticide residues Unapproved additives Allergens
Fish mince and surimi	
Dehydration Presence of foreign matter Denaturation of protein	Parasites Growth of pathogenic bacteria Pathogenic bacteria survival Heavy metals Natural toxins Allergens and Food intolerance substances Metal inclusion
Smoked fish	
Presence of pathogens Decomposition Parasites	Growth of pathogenic bacteria Clostridium botulinum toxin production Pathogenic bacteria survival Allergens and Food intolerance substances Metal inclusion Natural toxin Polyaromatic hydrocarbons
Canned fish	

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

The most important factors deciding the quality and safety of fish are the time temperature tolerance. The rigor period starts immediately after death depend on various factors such as temperature, stress and species. If the fish is properly iced and kept at 0°C, the rigor can last up to 2-4 days. Most of the consumers, except those who are in proximity to fish landing centers/harbors or fishermen, prefer taste and texture of post-rigor fish only. So, this pre-rigor and rigor period can be used for transportation purpose, so that high quality fish can be served

to consumers. Along with that if there is an effective quality assurance system in practice, the safety of the product also can be assured.

References:

- Safety and quality issues in fish processing (2002). Woodhead publishing limited
- ISO 22000: 2018- Food Safety Management Systems- Requirements for any organization in food chain
- FDA Fish and Fishery Products Hazards and Controls Guidance, Fourth edition 2021
- Zynudheen, A. A., Mohanty A K., Satyen Kumar Panda, Laly S J., Pankaj Kishore., Anuj Kumar., Devananda Uchoi., Pe. Jeyya Jeyanthi and Chandrasekhar V. (eds) (2021) Quality assurance of fish and fishery products, Central Institute of Fisheries Technology, Cochin, India. pp398.

60

QUALITY ISSUES IN FROZEN FISH AND FISHERY PRODUCTS

K.A. Martin Xavier
Quality Assurance and Management Division
Email : martinxavierkochery@gmail.com

Fish that has been frozen will degrade depending on the pace of freezing, storage temperature, oxygen content, temperature swings, and stages of transport. Freezing is predicated on the development of larger, slower-growing ice crystals that denaturate proteins, breach cell membranes with fluid loss upon thawing, and produce low-quality products. A high-quality product is produced when the freezing process is completed fast because the smaller crystals minimise fluid losses during defrosting. However, temperature changes cause recrystallization, which lowers the product's quality and makes it comparable to a product that slowly freezes. As a result, deterioration at the food surface, chemical, and biological processes result in changes in colour, weight loss (caused by the formation of ice crystals), increased enzymatic activity, and fat oxidation. Freezing is a popular method for preserving seafood. It can extend the shelf life of seafood by several months or even years (up to 2 years). However, freezing does not prevent all quality changes from occurring. Frozen seafood is a popular and convenient way to enjoy seafood. However, it is important to be aware of the quality changes that can occur during frozen storage. Over time, frozen seafood can still lose moisture, develop off-flavors, and become tough. The rate of quality changes in frozen seafood depends on several factors, including the type of seafood, the freezing method, the storage temperature, and the packaging. These changes can affect the taste, texture, color, and nutritional value of the seafood. The rate of these quality changes will depend on several factors, including the type of seafood, the freezing method, the storage temperature, and the packaging.

- **Types of seafood**

Some types of seafood are more susceptible to quality changes during freezing than others. For example, shrimp and other shellfish are more likely to lose moisture and become tough than fish. This is because shrimp have a high water content and a delicate texture.

- **Freezing method and storage conditions of product**

The freezing method can also affect the quality of frozen seafood. Rapid freezing is generally better than slow freezing because it minimizes the formation of large ice crystals. Large ice crystals can damage the cell membranes of seafood, leading to loss of moisture and flavor. The storage temperature is also important for maintaining the quality of frozen seafood.

Seafood should be stored at a temperature of -18°C (0°F) or lower. At this temperature, the quality changes will be slow.

- **Packaging**

The packaging can also affect the quality of frozen seafood. Seafood should be packaged in a way that prevents moisture loss and oxidation. Vacuum packaging or modified atmosphere packaging are effective ways to prevent these quality changes. Some of the most common quality changes that occur in frozen seafood include:

Denaturation of proteins

The proteins in seafood can denature during frozen storage, which can result in a loss of texture and flavor. This is caused by the exposure of the proteins to high temperatures during the freezing process. Protein denaturation in frozen seafood can occur due to various factors, and it can lead to changes in texture, flavor, and overall quality. Here are some of the main causes of protein denaturation in frozen seafood and potential solutions to mitigate these issues

Causes of Protein Denaturation

- **Temperature Fluctuations**: Rapid temperature fluctuations during freezing and thawing can cause proteins to denature. Ice crystals can form within the seafood, leading to mechanical damage to the protein structure.
- **Freezing and Thawing**: Incorrect freezing and thawing methods can result in denaturation. Slow freezing or improper thawing can lead to large ice crystals forming within the tissue, damaging the protein structure.
- **Storage Conditions**: Prolonged storage at temperatures above the recommended freezer temperature (-18°C or 0°F) can cause protein denaturation over time.
- **Oxidation**: Exposure to oxygen can lead to oxidative damage to proteins, altering their structure and causing denaturation. This can occur during storage or processing.

Solutions to Prevent Protein Denaturation in Frozen Seafood

1) **Proper Freezing Techniques**: Use rapid freezing methods such as blast freezing to minimize ice crystal formation and preserve the integrity of proteins.
2) **Vacuum Packaging**: Vacuum packaging removes air and reduces the risk of oxidation during storage, helping to maintain protein structure.
3) **Controlled Thawing**: Thaw seafood slowly in a controlled environment, ideally in a refrigerator, to prevent temperature fluctuations that can damage proteins.
4) **Low-Temperature Storage**: Maintain a consistent freezer temperature of -18°C (-0.4°F) or lower to prevent protein denaturation during storage.
5) **Protective Coatings**: Some seafood can be coated with protective layers, such as ice glaze or edible films, to shield proteins from freezer burn and reduce denaturation.

6) **Antioxidants**: The addition of natural antioxidants like ascorbic acid (vitamin C) or tocopherols can help minimize oxidative damage to proteins during processing and storage.
7) **Quality Control**: Implement stringent quality control measures to ensure that seafood products are handled, processed, and stored correctly to minimize protein denaturation.
8) **Proper Packaging Materials**: Use packaging materials designed for frozen seafood to prevent moisture loss and maintain product quality.
9) **Educating Consumers**: Provide consumers with instructions on how to properly thaw and cook frozen seafood to minimize protein denaturation during home preparation.

It's essential to follow industry best practices and quality standards to prevent protein denaturation in frozen seafood, as this will help maintain the quality, flavor, and texture of the product for consumers.

Freezer burn

Freezer burn is a common issue that can affect frozen seafood, as well as other frozen foods. It occurs when food is improperly stored in the freezer and results in the deterioration of the food's quality and texture. Freezer burn doesn't make the food unsafe to eat, but it can lead to a less pleasant eating experience.

The main mechanism behind freezer burn in frozen seafood (and other frozen foods) involves a combination of two processes: dehydration and oxidation.

Dehydration: Frozen seafood can lose moisture during storage, which can result in a dry, tough texture. This is caused by the evaporation of water from the surface of the seafood. Freezer burn begins when the moisture within the seafood starts to evaporate, even at freezing temperatures. This occurs because the freezer's air is extremely dry. When the moisture in the seafood begins to sublimate (change directly from a solid to a gas), it leaves the food's surface. As a result, the seafood becomes dehydrated, leading to dry and shriveled areas. This dehydration process can make the seafood lose its juiciness and become tough.

Oxidation: The second part of the freezer burn process involves oxidation. Oxygen can penetrate packaging materials over time, and when it comes into contact with the seafood, it can cause oxidative reactions. These reactions can lead to changes in the seafood's flavor and color. You might notice that freezer-burned seafood often has a grayish or brownish appearance, which is a result of these oxidation reactions.

To prevent freezer burn in seafood and other frozen foods, consider the following tips:

1) **Proper Packaging**: Use airtight, moisture-resistant packaging to prevent air and moisture from reaching the food. Vacuum-sealed bags or a double layer of plastic wrap can help create a better barrier.

2) **Remove Air:** When packaging seafood for freezing, try to remove as much air as possible from the container to minimize moisture loss.
3) **Use Quality Freezer Bags**: Invest in quality freezer-safe bags or containers designed for long-term storage in the freezer.
4) **Label and Date**: Clearly label packages with the date of freezing to help you keep track of storage times.
5) **Maintain Freezer Temperature**: Ensure that your freezer maintains a consistent and appropriate temperature (usually around 0°F or -18°C) to slow down the dehydration and oxidation processes.
6) **Rotate Stock**: Use a first-in, first-out (FIFO) approach to ensure you consume older frozen seafood before newer additions, reducing the chances of freezer burn.
7) **Avoid Over packing**: Do not overpack your freezer, as overcrowding can hinder proper air circulation and temperature maintenance.
8) **Quick Freeze**: Freeze seafood as quickly as possible after purchase or preparation to minimize the time it spends at warmer temperatures.

By following these tips, you can reduce the likelihood of freezer burn in your frozen seafood and maintain its quality for a longer period.

Lipid oxidation

Oxidation is a chemical reaction that can cause seafood to develop off-flavors and rancidity. This is caused by the interaction of oxygen with the fat in the seafood. Lipid oxidation in frozen seafood occurs when the fats or lipids present in the seafood react with oxygen in the air or dissolved in the moisture of the product. This process leads to the degradation of the lipids, resulting in undesirable changes in the flavor, odor, texture, and nutritional quality of the seafood. Understanding the mechanism of lipid oxidation in frozen seafood is crucial for maintaining product quality and shelf life. Here's an overview of the key steps in the lipid oxidation mechanism

- **Initiation**: Lipid oxidation typically begins with the initiation step, where oxygen molecules (O2) are absorbed by unsaturated fatty acids present in the seafood. Seafood lipids are rich in polyunsaturated fatty acids (PUFAs), which are more susceptible to oxidation due to their multiple double bonds.
- **Formation of Free Radicals:** The absorbed oxygen molecules can be converted into free radicals, such as hydroperoxyl radicals (HO•), through various processes. This is often facilitated by factors like temperature, light, and the presence of transition metals (e.g., iron or copper), which act as catalysts in generating free radicals.
- **Propagation**: Free radicals initiate a chain reaction of lipid oxidation. They react with neighboring unsaturated fatty acids in a process called autoxidation. This chain reaction involves three main stages: initiation, propagation, and termination. In the

propagation stage, a free radical abstracts a hydrogen atom from a neighboring fatty acid molecule, leading to the formation of a new fatty acid radical and a lipid hydroperoxide (LOOH).

- **Lipid Hydroperoxide Formation**: Lipid hydroperoxides (LOOH) are unstable and can further decompose into secondary products, such as aldehydes and ketones, which are responsible for the off-flavors and odors associated with rancid seafood.
- **Termination**: The chain reaction can be terminated by antioxidants naturally present in seafood, such as tocopherols (vitamin E), or added antioxidants, which can neutralize free radicals and prevent further propagation of lipid oxidation.
- **Synergistic Reactions**: Lipid oxidation can be accelerated by synergistic reactions involving proteins, pigments, and other components in seafood. For example, iron or copper ions can catalyze lipid oxidation by promoting the formation of free radicals.
- **Storage Conditions**: The rate of lipid oxidation in frozen seafood depends on storage conditions. Factors such as temperature, packaging, and the presence of oxygen all play a role. Vacuum packaging or using gas flushing to remove oxygen from packaging can help slow down lipid oxidation.

Control Measures

To prevent lipid oxidation in frozen seafood, it's important to minimize the exposure to oxygen during processing, packaging, and storage. Proper freezing techniques, the use of antioxidants, and maintaining low temperatures can extend the shelf life and maintain the quality of frozen seafood products. Additionally, monitoring for signs of oxidation, such as off-flavors and rancidity, is essential for quality control in the seafood industry.

Microbial spoilage:

Microbial spoilage in frozen seafood can occur when microorganisms, such as bacteria, yeasts, and molds, are not effectively controlled during processing, storage, or handling. While freezing seafood is a common method to extend its shelf life by slowing down microbial growth, it is not a complete guarantee against spoilage. If frozen seafood is not stored properly, it can become contaminated with bacteria. This can lead to spoilage, which can cause off-flavors, discoloration, and the growth of harmful bacteria. Here are some factors and types of microbial spoilage to consider:

- Temperature Fluctuations: Maintaining consistent and sufficiently low temperatures is crucial for preventing microbial growth in frozen seafood. If the temperature fluctuates above the recommended storage temperature (usually -18°C or 0°F), microorganisms can become active and cause spoilage over time.
- Contamination during Processing: Microbial contamination can occur during the processing of seafood before freezing. It's essential to have strict hygiene practices in place to minimize the introduction of harmful microorganisms.

- Packaging: Proper packaging is essential to prevent contamination and freezer burn. Inadequate packaging can lead to moisture loss, which can encourage microbial growth and affect the quality of the seafood.
- Thawing and Refreezing: Repeated thawing and refreezing can create conditions conducive to microbial spoilage. When seafood is thawed, any microorganisms present can become active, and refreezing may not completely kill them.
- Storage Time: Even when frozen at the correct temperature, seafood has a limited shelf life. Over time, microorganisms can slowly degrade the quality of the product.
- Inadequate Cooking: While not directly related to frozen seafood, improper cooking practices can lead to foodborne illnesses caused by pathogens. Cooking seafood to the recommended internal temperature is essential to kill harmful microorganisms.
- Microbial Types: Various microorganisms can spoil frozen seafood, including psychrotrophic bacteria (able to grow at low temperatures), yeast, and molds. These microorganisms can produce off-flavors, odors, and changes in texture and appearance.

To prevent microbial spoilage in frozen seafood, follow these practices:

1) Store seafood at or below the recommended freezing temperature (-18°C or 0°F) to maintain quality and safety.
2) Use proper packaging materials designed for freezing to prevent freezer burn and moisture loss.
3) Practice good hygiene during processing and handling to minimize contamination.
4) Avoid thawing and refreezing seafood whenever possible.
5) Follow recommended storage times and discard seafood past its shelf life.
6) Ensure seafood is cooked to the recommended internal temperature to kill any harmful microorganisms.

By following these guidelines and maintaining a cold chain throughout the seafood's lifecycle, you can minimize the risk of microbial spoilage in frozen seafood and ensure its safety and quality.

Signs of quality loss

There are a few signs that can indicate that frozen seafood has lost quality. These include:

- Dehydration: Frozen seafood that has lost moisture will appear dry and shriveled.
- Off-flavors: Frozen seafood can develop off-flavors, such as a fishy or ammonia smell.
- Toughness: Frozen seafood can become tough and rubbery.
- Discoloration: Frozen seafood can discolor, especially if it has been exposed to air.

To prevent quality loss in frozen seafood, it is important to:

- Choose fresh, high-quality seafood.

- Freeze the seafood as soon as possible after it is caught. Use a fast freezing method, such as blast freezing for this purpose.
- Store the seafood at a low temperature (-18°C or below).
- Package seafood in a way that prevents moisture loss and oxidation. In the case of frozen blocks use wax coated duplex cartons
- Use seafood within its recommended shelf life.

By following these tips, you can help to ensure that your frozen seafood retains its quality and freshness for as long as possible. In addition to the factors mentioned above, there are a few other things that can affect the quality of frozen seafood. These include:

- The time and conditions of storage before freezing.
- Initial microbial load of seafood sample.
- The amount of exposure to air.
- The use of additives.

By understanding the factors that can affect the quality of frozen seafood, you can make informed choices about the seafood you buy and how you store it. This will help you to enjoy fresh, delicious seafood for longer. By following these tips, you can help to ensure that your frozen seafood retains its quality and freshness for as long as possible. Here are some additional tips for choosing and storing frozen seafood:

- Look for seafood that is bright in color and has firm flesh.
- Avoid seafood that has any signs of freezer burn, such as brown or black spots.
- Store frozen seafood in the coldest part of your freezer.
- Do not refreeze thawed seafood.