Training Manual

on

Protocols for the Production of High Value Secondary Products from Industrial Fish and Shellfish Processing

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

Venue: ICAR-Central Institute of Fisheries Technology, Cochin, Kerala, India

Date: 26 November 2018 – 22 December 2018

Programme Director
Ravishankar C.N.

Course Director
Ashok Kumar K.

Course Coordinators
Bindu J.
Sreejith S.
Sarika K.

ICAR - Central Institute of Fisheries Technology
(ISO/IEC 17025 :2005 NABL Accredited & ISO 9001 :2015 Certified)
CIFT Junction, Matsyapuri P.O., Cochin - 682 029, Kerala, India
Ph: 091-0484-2412300, Fax: 091-484-2668212, E-mail: cift@ciftmail.org, directorcift@gmail.com
www.cift.res.in

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

Compiled and Edited by:

Bindu, J.
Sreejith, S.
Sarika, K.

For citing the book:
Bindu, J., Sreejith, S., and Sarika, K. (eds.) (2018) Protocols for the production of high value secondary products from industrial fish and shellfish processing, Central Institute of Fisheries Technology, Cochin, India. pp 278.

Example for citing a chapter in the book:
George Ninan (2018) Fish Processing and Value Addition – A Global Scenario *In*: Bindu, J., Sreejith, S., and Sarika, K. (eds.) (2018) Protocols for the production of high value secondary products from industrial fish and shellfish processing, Central Institute of Fisheries Technology, Cochin, India. pp 1- 17.

Design and Cover page: Razia Mohamed
Technical Assistance: Shyla N.C.

Printed at: Print Express, Kaloor, Ernakulam-17

FOREWORD

Fish and shell-fish processing industries generate a large amount of waste. These processing discards or secondary raw materials are sources of various biomolecules of pharmaceutical and food value and can be harnessed for the production of various high value products. ICAR-Central Institute of Fisheries Technology (CIFT) has developed various technologies to utilize these processing discards into high value compounds and transferred the know-how to several entrepreneurs. High value products from chitin and chitosan, collagen, squalene, fish calcium hydroxyapatite and other marine based nutraceuticals have been developed by the institute and commercialized.

The international training on '*Protocols for the Production of High Value Secondary Products from Industrial Fish and Shellfish Processing*' sponsored by Indian Technical & Economic Cooperation Programme (ITEC), Ministry of External Affairs, Government of India,assumes a greater importance as the technical expertise developed over many decades by the institute could be shared with researchers and officials from other countries. Over a duration of four weeks ten participants from eight countries were exposed to various technologies for the utilization of secondary raw material from industrial fish processing. The topics for the programme were selected to give a comprehensive knowledge on processing of fish and shellfish and conversion of the waste generated to high value products. This training manual consists of 33 chapters which cover different aspects of fish processing, waste utilization and value addition. The topics include harvest and processing of fish and shellfishes, waste generation profiling, fish meal and oil, chitin and its derivatives, collagen and its derivatives, protein hydrolysates, marine nutraceuticals, coated products, ready-to-eat products and other products fortified with functional ingredients. The training manual also covers topics like microbial quality and food safety, antimicrobial resistance issues, HACCP, entrepreneurship development and the role of extension in fish waste management. I am sure that this training manual will be very useful for the researchers and entrepreneurs working in the areas of seafood processing and fishery waste utilization. The conversion of fishery waste to high value products will minimize wastage and maximize utilization of a very precious natural resource.

Dr. Ravishankar, C. N.

Director
ICAR-Central Institute of Fisheries Technology

PREFACE

The shortage of fishery resources calls for the development and adoption of new technological processes for better utilization of waste and by-products from fisheries and fish processing activities. Most of these by-products are currently used as raw materials for animal feed, as fish meal or as fertilizer. It is estimated that their utilization in human foodstuffs, nutraceuticals, pharmaceuticals, or cosmetics would increase their value many fold. The strict environmental regulations for the disposal of fish processing waste add to the operational cost of seafood industry. The recovery of compounds from fish processing wastes would serve the dual purpose of obtaining these valuable biomolecules as well as controlling the environmental pollution problems associated with the disposal. This book, through its various chapters, discusses the opportunities for upgrading the fish discards by means of various technologies as well as better handling techniques. Also, it is an attempt to consolidate the research inputs in this field, particularly on the development of bioactive compounds, recovery of high value biomolecules; development of edible products; chitin, chitosan and its derivatives; collagen and its derivatives; protein hydrolysates and their applications in various field etc. The safety and quality issues in utilization of fish processing discards has been given due attention by including topics on HACCP, AMR in fisheries sector and quality issues in byproducts. There are also chapters on commercial production of various fishery byproducts. We hope that this publication will serve as a guide for researchers, academicians, technologists and entrepreneurs engaged in the area of development of high value products from fish and shell fish processing discards.

Dr. Bindu J.

Mr. Sreejith S.

Mrs. Sarika K.

CONTENTS

Fish Processing and Value Addition – A Global Scenario

George Ninan

Fish Processing Division

ICAR-Central Institute of Fisheries Technology, Cochin

Email: george66jiji@gmail.com

Value addition is defined as any activity along the supply chain that increases the usability, culinary attribute or economic viability of a food item. Processing of fish into a wide variety of value-added products is now common with the increase in demand for food products that are ready-to-eat or require little preparation before serving. Usually, value added fish products are perceived to be those that have added ingredients such as a coating (breaded/battered) or a sauce, are prepared neatly or in some way provide more convenience to the user. Actually it indicates a measure of factors added to the total worth of a product at each stage of the production. Value addition ties in with consumer convenience. For example, value addition can be a process for transforming fish fillets into products that are perceived by the customer as having added quality and interest.

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

Fish and fish products have presently emerged as the largest group in agricultural exports of India, with 10.51 lakh tonnes in terms of quantity and Rs. 33,442 crores in value. This accounts for around 10% of the total exports of the country and nearly 20% of the agricultural exports. More than 50 different types of fish and shellfish products are exported to 75 countries around the world. In 2016-17 India exported 11,34,948 MT of seafood, principally frozen shrimp and frozen fish, worth Rs 37,870.90 crores. Provisional export figures for April-November 2017 have shown an increase of 18.72% and 15.16% respectively, in volume and value (in $) of seafood exports. The increased production of Vannamei shrimp, increased productivity of Black Tiger shrimp and better price realization of major items like Cuttlefish, Shrimp and Squid helped India to achieve significant export turnover.

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

Chilling

Chilling is an effective way of reducing spoilage by cooling the fish as quickly as - possible without freezing. Immediate chilling of fish ensures high quality products (Connell, 1995: Huss, 1995). Chilling by use of ice is the most important method employed commercially. The storage life of fish kept in ice depends on a number factors which include species, size, method of capture, fat content, breeding conditions, feeding regime and the method of killing. In general, the keeping quality of non-fatty fish is better than fatty fish in ice storage. The quality and quantity of ice used are important factors in determining the shelf life of iced fish. In tropical countries, a 1:1 fish to ice ratio is ideal for ice storage. It is recommended to add about 12-20% extra ice to the fish in order to compensate for water loss from melting and bad handling (Zugarramurdi *et al*., 1995). It is generally accepted that some tropical fish species can keep for longer periods in comparison to fish from temperate or colder waters. Up to 35% yield of high value products can be expected from fish processed within 5 days of storage in ice, after which a progressive decrease in the utility was observed with increase in storage days (Venugopal and Shahidi, 1998).

Transportation of Chilled Fish

Land transportation of chilled fish is carried out in insulated or mechanically refrigerated vehicles. The refrigerated vehicle used for chilled fish transportation should have a minimum inside temperature of 7 ^{0}C (Venugopal, 2006). Air shipment of chilled fish requires a lightweight and protective container. Pads of nonwoven fabric encapsulating synthetic absorbent powder are used for chilling of air shipped fish. Special thermal barrier films are used in combination with the pads to protect fish containers from heat (Subasinghe, 1996).

Transportation of live fish and shellfish

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

Freezing

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

- Air freezing
- Indirect contact freezing
- Liquid Immersion freezing
- 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.

Air blast freezing

Circulating cold air at high speed enables freezing to proceed at a moderately rapid rate and this method is referred to as air-blast freezing. Air-blast freezing is usually accomplished by placing the products on a mesh belt and passing it slowly through an insulated tunnel containing air at-18 to -34°C or lower, moving counter current to the product at a speed of I to 20 meter/sec. Air at -29°C and at a speed of 10-12 meter/sec, is often satisfactory, although lower temperatures are preferred. Air blast freezing is economical and is capable of accommodating products of different sizes and shapes. It can result in (1) excessive dehydration of unpackaged products if conditions are not carefully controlled, and this in turn necessitates frequent defrosting of equipment and (2) undesirable bulging of packaged products which are not confined between flat rigid plates during freezing.

Spiral Belt Freezer

Modern designs of belt freezers are mostly based in the spiral belt freezer concept. In these freezers a conveyor 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 capacities 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. 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 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 disadvantage of fluidized-bed freezing is that large or non-uniform products cannot be fluidized at reasonable air velocities.

Contact Plate Freezing

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

Liquid Immersion Freezing

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

Cryogenic Freezing

Cryogenic freezing refers to very rapid freezing by exposing food products to an extremely cold freezant undergoing change of state. The fact that heat removal is accomplished during a change of state by the freezant is used to distinguish cryogenic freezing from liquid immersion freezing. The most common food grade cryogenic freezants are boiling nitrogen and boiling or subliming carbon dioxide. Boiling nitrous oxide also has been considered, but at present it is not being used commercially. 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. Freezing with carbon dioxide usually involves tumbling the product in the presence of powdered or liquid carbon dioxide. Carbon dioxide is absorbed or entrained by the product in this method. This entrapped CO_2 should be removed before it is packaged in an impervious material.

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 fluidized bed freezer without deformation or breakage.

Pre-freezing and Freezing Consideration

The quality of frozen-thawed cooked fish is influenced by a number of factors 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. Effective pre freezing 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 minimized by (1) eliminating oxygen (2) avoiding contamination with heavy metals (oxidative catalysts) (3) adding antioxidants and (4) by using low storage temperature. Dehydration can be avoided by applying glaze and suitable protective coatings.

Individually Quick Frozen Products (IQF)

Lobster, squid, cuttlefish, different varieties of finfish etc. are processed in the individually quick frozen style. IQF products fetch better price than conventional block frozen products. However, for the production of IQF products raw-materials of very high quality need to be used, as also the processing has to be carried out under strict hygienic conditions. The products have to be packed in attractive moisture-proof containers and stored at –30°C or below without fluctuation in storage temperature. Thermoform moulded trays have become accepted containers for IQF products in western countries. Utmost care is needed during the transportation of IQF products, as rise in temperature may cause surface melting of the individual pieces causing them to stick together forming lumps. Desiccation leading to weight loss and surface dehydration is other serious problem met with during storage of IQF products.

Some of the IQF products in demand are prawn in different forms such as whole, peeled and de-veined, cooked, headless shell-on, butterfly fan tail and round tail-on, whole cooked lobster, lobster tails, lobster meat, cuttlefish fillets, squid tubes, squid rings, boiled clam meat and skinless and boneless fillets of white lean fish. IQF products can be easily marketed as consumer packs, which is not possible with block frozen products. This is a distinct advantage in marketing.

Canning

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

However, the fish canning industry in India is declining due to the high cost of cans. Recent innovations like polymer coated Tin Free Steel (TFS) cans provide a cheaper alternative. Studies conducted at CIFT showed that polyester-coated TFS cans are used for processing ready to serve fish products, which can be stored at room temperature for long periods. The industry can utilize these cans for processing ready to eat fish and shell fish products for both domestic and export markets. This will help in reviving the canning industry in India (Mallick *et al.,* 2006; Sreenath *et al.,* 2007)

Unit Operations in a canning process are:

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

Retort Pouch Processing

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

The pouches are heat processed in an over pressure autoclave. Work carried out CIFT has shown that oil sardines packed in retort pouches having composition polyester / aluminium foil / cast polypropylene remained in excellent condition even after a period of 3 years (Ansar Ali et al., 2005). Mackerel in curry packed in indigenous retort pouch and processed to an F_0 value of 8.43 can be kept at room temperature for 18 months in acceptable condition (Gopal *et al.,* 2001). Seer fish in curry medium packed in locally manufactured retort pouches, having a three-layer configuration of thickness 12.5 µm polyester /12.5 µm aluminium foil / 80 µm cast polypropylene with a F_0 value of 11.5 remained in good condition for up to 24 months at room temperature(Ravi

Shankar *et al.,* 2002). The flexible pouches manufactured indigenously employing the configuration recommended by CIFT has opened the way for commercialization of fish curry in retortable pouches. The process relies on heat sterilization and in many respects is analogous to canning with the imported tin can being replaced by a cheaper indigenous heat resistant flexible pouch. In comparison with frozen foods, the retort pouch provides a longer shelf life and does not require refrigeration, energy, expensive methods of distribution and storage. No chemical additives are added as most of the bacteria are killed by heat sterilisation. Test marketing of mackerel curry conducted by MPEDA have shown that the product had good acceptability and there is good demand for fish curry in flexible pouches.

Curing

Traditional methods of processing fish by salting, drying, smoking and pickling are collectively known as curing. Cured fish consumption is more in areas where the availability of fresh fish is comparatively limited, namely interior markets and hilly areas. This is also the cheapest method of preservation, since no expensive technology is used. In India roughly 20 % of the fish caught is preserved by curing. Considerable quantities of cured fish are also exported, mainly to Singapore, Sri Lanka and to the Middle East. Simple sun drying was the widely practised traditional method of fish preservation. By this, preservation was achieved by lowering of water content in the fish, thereby retarding the activity of bacteria and fungi. The heat was able to destroy the bacteria to a certain extent. Later on, a combination of salting and drying or salting, smoking and then drying were developed.

Methods of Drying

There are basically two methods of drying fish. The common one is by utilizing the atmospheric conditions like temperature, humidity and airflow. This is traditional sun drying. The other is dehydration or artificial drying, by using artificial means like mechanical driers for removal of moisture from the fish under controlled conditions.

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

In mechanical driers, removal of water from the fish is achieved by an external input of thermal energy. This is an expensive method since there is need for fuel for heating and maintenance of the temperature. The drying chamber consists of a long tunnel in which the washed and cleaned fish is placed on trays or racks. A blast of hot air is passed over the material to be dried. After the required degree of drying the product is removed from drier and packed.

Salting

This is one of the oldest methods of preservation of fish. Salting is usually done as such or in combination with drying or as a pretreatment to smoking. During salting osmotic transfer of water out of the fish and salt into the fish takes place, which effect fish

preservation. It is based on different factors like diffusion and biochemical changes in various constituents of the fish. Salting amounts to a process of salt penetration into the fish flesh. Penetration ends when the salt concentration of the fish equals that of the surrounding medium. Loss of water during salting limits bacterial growth and enzyme activity, thus preserving the fish. The high salt content prevents the growth of normal spoilage microflora in the fish; but halophiles, which can survive 12-15% of salt, will survive.

Smoking

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

Irradiation

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

Radurization of fresh fish at 1 to 3 kGy reduces initial microbial loads by 1 to 3 log cycle, essentially reducing spoilage causing bacteria and extends their chilled storage life 2-3 fold. The treatment is effective for the extension of shelf life of most of the marine and freshwater fish species. Radicidation is sanitation of frozen products including fish minces by elimination of non-spore forming pathogenic bacteria such as Salmonella, Vibrio and other species at a dose of 4 to 6 kGy. The treatment, however, is limited in its ability to eliminate viruses and *Clostridium botulinum* type E spores, which jeopardize the safety of seafood through production of lethal botulinum toxin. Several studies have established the feasibility of low dose gamma irradiation at a dose of 1kGy for the disinfestations of dried fish. Irradiation at doses in the range of 0.1 to 1.0 kGy can prevent development of beetle larvae and adults in packaged, salted, dried fishery products (Rodrick, 1999; Venugopal 1990; Venugopal and Shahidi,1998; Venugopal *et al.,* 1999).

Battered and Breaded Products

The most prominent among the group of value added products is the battered and breaded products processed out of a variety of fish and shellfish. Battered and breaded products offer a 'convenience' food widely valued by the consumer. These are products, which receive a coat or two each of a batter followed by coating with breadcrumbs, thus increasing the bulk and reducing the cost element. The pick-up of coating can be increased by adjusting the consistency of the batter or by repeating the coating process. By convention, such products should have a minimum fish component of 50%. Coated products viz., fish fingers, squid rings, cuttlefish balls, fish balls and prawn burgers form one of the major fish and shellfish based items of trade by the ASEAN countries (Chang et al., 1996).

The production of battered and breaded fish products involves several stages. The method varies with the type of products and pickup desired. In most cases it involves seven steps. They are portioning/forming, pre-dusting, battering, breading, pre-frying, freezing and, packaging and cold storage.

The first commercially successful coated product is 'fish finger; or 'fish stick'. Later several other products particularly the coated fish fillet, fish portions, fish cakes, fish medallions, fish nuggets, breaded oysters and scallops, crab balls, fish balls, coated shrimp products, coated squid rings etc. became prominent in most of the developed countries with the advent of the fast food trade. The present day production of coated seafood items involve fully automated batter and breading lines which start from portioning and end with appropriate packaging of the product (Suderman & Cunningham, 1983; Dikhoof, 1990; Hutchison *et al.,* 1992; Joseph, 2003).A variety of battered and breaded products can be prepared from shrimp, squid, clams, fish fillets, minced meat from low cost fish etc. A brief profile of some important battered and breaded products is given below.

Fish Mince and Mince Based Products

Mechanically deboned fish meat is termed as fish mince. Fish mince is more susceptible to quality deterioration than the intact muscle tissue since mincing operation cause disruption of tissue and exposure of flesh to air, which accelerates lipid oxidation and autolysis. The quality of the mince is dependent on the species, season, handling and processing methods (Babitt, 1986). Also, low bone content in the mince (01-0.4%) is desirable for better functional and sensory properties (Grantham, 1981). Depending on the type of raw material, fish mince can have a frozen storage life up to 6 months without any appreciable quality deterioration (Ciarlo *et. al.,* 1985). Generally minced fish is frozen as 1-2 kg blocks at -40 ^{0} C in plate freezers and stored in cold store at -18 ^{0} C. Lipid oxidation and protein denaturation during frozen storage of mince can be prevented by the incorporation of spices, cryoprotectants and hydrocolloids (Joseph, *et.al.,* 1992; Jiang, *et. al*.,1986)

Fish mince is a major source of raw material for the preparation traditional products such as patties, balls, wafers, loaves, burgers, fish fingers, dehydrated fish minces, cutlets and pickled products (Regenstein,2004; Grantham,1981; Venugopal and Shahidi, 1995; Venugopal, e*t. al.*,1992; Joseph, *et.al*., 1984). The mince from different species could be combined to prepare composite fillets (Venugopal, 2006).

Surimi

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

Kneaded products

Several kneaded products like kamaboko, chikuwa, hampen, fish ham and sausage are processed using surimi incorporating other ingredients. The ingredients used in most of these preparations are identical; however, the classification is principally based on the

manufacturing process involved. The ingredients employed other than surimi include salt, monosodium glutamate, sugar, starch, egg white, polyphosphate and water. The method of processing all these products involves grinding together of the various ingredients to a fine paste and some sort of heat treatment at some stage.

Fibreized products

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

Fish sausage

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

Accelerated Freeze Drying

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

Extrusion technology

It is a technique used to form shapes by forcing a material through a region of high-temperature and/or pressure, and then through a die to form the desired shape. Food Extrusion is the process of cooking moistened, starchy, proteinaceous food material by the combined action of pressure, temperature and mechanical sheer. CIFT has worked on the production of extruded products by incorporating fish mince with cereal flours. The fish mince is mixed with cereal flours, spices and vegetable oil and extruded using a twin-screw extruded. The product obtained is finally coated with spice mix to provide a delicious snack that has been christened as "Fish Kure".

Hurdle technology

The concept of hurdle technology is based on the application of combined preservative factors to achieve microbiological safety and stability of foods (Leistner, 1978). The most important hurdles used in food preservation are temperature, water activity, acidity, redox potential, antimicrobials, and competitive microorganisms. A synergistic effect could be achieved if the hurdles hit at the same time at different targets that disturb the homeostasis of the microorganisms present in foods (Leistner, 2000).

For the fish products manufactured in industrialised countries, hurdle technology has been employed for two groups of products (Leroi, 2008). These are:

- Convenience products based on traditional products, like rehydrated salt-cured or dried fish. The raw material is a preserved semi-finished product but as the preservative is removed during processing, surviving pathogens in the raw material may recover. Minimising the survival of pathogens in this product is therefore, beside the hygienic process conditions, necessary to ensure product safety.
- Lightly preserved fish products which are uncooked or mildly cooked products, with low level of preservatives (NaCl < 6% WP, pH >5), such as cold-smoked salmon, carpaccio, slightly cooked shrimp. These products are usually produced from fresh seafood and further processing involves one or a few additional steps that increase risk of cross contamination. The treatments are usually not sufficient to destroy pathogens, and, as several of these products are eaten raw, minimising the presence and prevent growth of pathogens is essential for the food safety.

Innovative packaging Technologies

Modified Atmospheric Packaging (MAP)

Modified Atmospheric Packaging (MAP) is a process by which the shelf life of fish is increased by enclosing it in an altered atmosphere such that it slows down the degradation by microorganisms and development of oxidative rancidity. In practice fish/fish products are packed in an atmosphere of carbon dioxide and other gases like oxygen and nitrogen. MAP chilled fish is an attractive proposition both to the retailer and to the consumer. A number of authors have reported considerable increase of up to two or even three-fold in the shelf-life of fish packed in modified atmosphere compared to that of fish packed in air. (Shalini, 2000; 2001; Ozogul *et al*.,2000,2004; Jeya Shakila et al.,2003; Reddy *et al.*, 1995,1996; Yesudhason *et al.*, 2010).

Active Packaging

Active packaging refers to the incorporation of certain additives into packaging systems to alter the packaging atmosphere and to maintain it throughout the storage period

with the aim of maintaining or extending product quality and shelf-life. There are two types of active packaging systems viz., scavenging systems (O_2, CO_2, H_2O, ethylene, taints) and releasing systems (CO_2, H_2O, antimicrobials, antioxidants). Studies conducted at CIFT on the active packaging of fishery products have demonstrated a significant extension of shelf life over air packed samples (Mohan *et al.,* 2008; 2009a; 2009b; 2010)

Intelligent Packaging

Intelligent packaging systems monitor the condition of packaged foods and communicate information about quality of the packed food during transportation and storage (Brody *et al.,* 2001; Kerry *et al.,* 2006). This consists of an external or internal indicator which can indicate whether the quality of the packed food has decreased before the product has deteriorated. Examples are Time Temperature Indicators (TTI) and Freshness Indicators. Time Temperature Indicator is simple devices which can show an easily measurable, time-temperature dependent change that reflect the full time temperature history of a food product. It is attached to the packaging material externally and activated by adhesion of the two materials. Freshness indicators indicate the spoilage or lack or freshness of the product, in addition to temperature abuse or package leakage. This is based on the reaction with volatile metabolites produced during ageing of foods which gives a visible colour change as an indicator. The U.S. Food and Drug Administration (FDA) recognize TTI in the 3rd edition of the Fish and Fisheries Products Hazards and Control Guidance.

Future scenarios for the global seafood industry

The prevailing consumer attitude towards the benefits of seafood are:

Functional: The focus is on the benefit of seafood to be convenient. Seafood is perceived to be just another source of protein with little differentiation, as its status as a healthy food is under greater competition from other categories. As a result consumers want their seafood to convenient with enhanced health benefits, and affordable.

Experiential: Seafood is perceived to be a superior source of protein with its own distinct benefits and rituals of consumption compared to other proteins. As a result consumers want their seafood to be an experience and are willing to pay more for it. Consumers have relatively high levels of education and interest.

Positioning seafood on future market scenarios

The following factors can play a major role in determining the seafood demand in future market situations.

Reframing seafood as a healthier substitute to other protein sources

Targeting individual dietary needs and aspirations through seafood

Creating seafood experiences that deliver moments of healthy pleasure

Redefining long-life seafood as a smart shortcut for busy gourmands

Using preservation technologies to lock in and maximize the benefits of fresh seafood

Making new trends instantly accessible to all

Extracting the essence of seafood to reinvent recipes

Extracting the vital essence of seafood from offcuts
Unlocking the power of seafood by pairing with marine and land 'super foods'
Diversifying out-of-home and portable solutions by blurring meal and snack formats
Creating high engagement with seafood through no contact preparation
Providing universal building blocks that can be easily customized to local tastes

The drivers of change for the Global seafood industry will be:

Growing disposable incomes in emerging markets
Rising and more volatile fish prices
Increasing scope of fishing regulations
Growth of high-end and premium seafood
Rising consumer awareness about healthy nutrition
Growing demand for traceability and transparency
Increasing desire for convenience
Depleting wild fish stocks
Growing exports of cheaper farmed fish from emerging markets
Rising oil prices
Growing Population: 7 billion mouths to feed
Rapid aging of the global population
Growing concern about processed foods and additives
Stagnant to moderate growth in Europe
Growing concern about the negative environmental impact of consumption
Growth of climate change impacts and extreme weather
Rising levels of sea pollution
Rise of 'quantified self' and data driven diets
Development of unconventional protein sources
Growing development of aquaculture technologies
Development of marine biotechnology
Continuing challenge of cold chain distribution from Ireland
Growing consolidation of the global seafood industry
Growing resurgence of 'from scratch' cooking
Increasing sophistication of food culture
The rising popularity of supplements and nutraceuticals
Growing interest in 'mood food'
Continuing negative attitudes towards fish preparation
New retail models combining foodservice

Growing influence of NGOs

Growth of fast casual foodservice

Conclusion

Consumers want a healthy, trusted protein source they can rely on. In this world the seafood industry adds value through innovation that optimizes health and builds trust. As people became more conscious about the benefits of good health and wellbeing in the face of a growing 'lifestyle disease epidemic' globally, they looked for foods that demonstrated enhanced nutritional and wellness benefits. The perceived healthiness of seafood meant that consumers naturally gravitated to the category. Yet as concepts of 'health' evolved, so too did consumer expectations of the seafood category - people increasingly expected seafood to be managed in a way that 'optimizes' its health benefits. The result was that seafood marketed as 'healthy 'was no longer enough to keep consumers engaged – people wanted seafood that would reliably improve their brain health, their mood, their skin, their fitness etc. However the higher levels of standardization in seafood production and processing enabled by new technologies meant that seafood became increasingly perceived by consumers as a functional source of protein. Consequently seafood increasingly had to compete against new categories of alternative protein sources, such as supplements and vegetable-based proteins.

References:

Ali, A., Sudhir, B & T. K. S Gopal (2005) Effect of Heat Processing on the Texture Profile of Canned and Retort Pouch Packed Oil Sardine (*Sardinella longiceps*) in Oil Medium J. of Food Sci., 70 (5), pp S350–S354.

Babitt, K (1986) Suitability of the seafood species as raw materials, Food Technol. 40(3), p 97.

Balachandran, K.K (2001) Post-harvest Technology of Fish and fishery Products, Daya Publishing House, New Delhi, pp 308-323.

Brody A, Strupinsky ER & Kline LR. (2001). Odor removers. In: Brody A, Strupinsky ER, Kline LR, editors. Active packaging for food applications. Lancaster, Technomic Publishing Company, Inc. pp 107–117.

Chang, N.M., Hoon,C.G., & Kwang, L.H (1996) Southeast Asian Fish Products (3rd Ed.), Southeast Asian Fisheries Development Centre, Singapore.

Ciarlo, A.S, Boeri, R.L & Giannini,D.H (1985) Storage life of frozen blocks of Patagonian hake (*M.hubbsi*) filleted and minced, J.Food Sci., 50, p723.

Connel, J.J (1995) Control of Fish Quality. Fishing News Books, London, England 245 p.

Dikhoof,A (1990)Developments in equipment used for coating and frying, Infofish Int., 47, pp 3

Dunn, J., Clark, R. W., Asmus, J. F., Pearlman, J. S., Boyer, K., Pairchaud, F. & Hofmann, G. A (1991) Methods for preservation of foodstuffs. Maxwell Laboratories, Inc. US Patent 5,034,235.

Gopal, T.K.S , Vijayan P. K, Balachandran,K.K,, Madhavan, P & Iyer T.S.G (2001) Traditional Kerala style fish curry in Indigenous retort pouch Food Control 12 , pp 523-527.

Grantham,G.J (1981) Minced Fish Technology; A Review. Fisheries Technical Paper 216, FAO, Rome, Italy.

Hassan, F. & Mathew. S. (1999) A protein-rich base material for value added products from low-cost fishes. Sea Food Export Journal, 31, pp 4–5.

Huss, H.H (1995) Quality and quality changes in fresh fish, FAO Fisheries Technical Paper No. 348. Rome. 195 p.

Hutchison, J, Smith,T.H & Kulp, K (1992) Batter and Breading Process Equipment In: Batters and Breadings in Food Processing (Kulp,K & Loewe,R eds.), American Association of Cereal Chemists,Inc. St.Paul, Minnesota, USA, pp 163-176.

Jeya Shakila, R., Jeyasekaran, G. & Vijayalakshmi, S.K (2003) Effect of vacuum packaging on the quality characteristics of seer fish (*Scomberomorus commersonii*) chunks during refrigerated storage. J.Food Sci. Technol. 42, pp 438–443.

Jhingran, V.G. (1975) Fish and Fisheries of India. Hindustan Publishing Corporation (India), New Delhi.

Jiang, S.T., Lan,C.C & Tsau,C.Y (1986) New approach to improve the quality of minced fish products from freeze – thawed cod and mackerel ,J. Food Sci, 51, p 310.

Joseph, J., George,C & Perigreen ,P.A.(1992) Effect of spices on improving the stability of frozen stored mince. Fish. Technol. 29(1), pp 30 – 34.

Joseph, J., Perigreen, P.A & Thampuran, N (1984) Preparation & storage of cutlet from low priced fish. Fish. Technol. 21, pp 70 – 74.

Joseph,A.C (2003) Coated Fish Products for Export and Domestic market Markets In: Seafood Safety (Surendran,P.K *et al*. eds.), Society of Fisheries Technologists (India) , Cochin ,pp 1-12.

Kerry JP, O'Grady M.N & Hogan S.A (2006) Past, current and potential utilization of active and intelligent packaging systems for meat and muscle-based products: a review. Meat Sci 74, pp 113–130.

Lagunas-Solar M.C (1995) Radiation processing of foods: an overview of scientific principles and current status. J. Food Prot., 58, pp 186-92.

Leistner L. (1978). Hurdle effect and energy saving. In: Downey WK, editor. Food quality and nutrition. London: Applied Science Publishers. p 309–29.

Leistner L. (2000). Basic aspects of food preservation by hurdle technology. Int J.Food Microbiol 55, pp181–186.

Leroi, F., Jofftaud, J.J., Arboleya, J.C., Amarita, F., Cruz, Z., Izurieta, E., Lasagabaster, A., Martínez de Marañón, I., Miranda, I., Nuin, M., Olabarrieta, I., Lauzon, H.L., Lorentzen, G., Bjørkevoll, I., Olsen, R.L., Pilet, M.F., Prévost, H., Dousset, X., Matamoros, S., Skjerdal, T. (2008) Hurdle technology to ensure the safety of seafood products. In: Torger Børresen (ed.) "Improving seafood products for the consumer", Part IV Seafood from source to consumer products, Chapter 19, Woodhead Publishing Limited, pp. 399-425.

Mallick, A. K,. Srinivasa Gopal, T. K , Ravishankar, C. N & Vijayan, P. K (2006) Canning of Rohu (*Labeo rohita*) in North Indian Style Curry Medium Using Polyester-coated Tin Free Steel Cans , Food Sci. & Technol. Int. Vol. 12 (6), pp 539-545.

Mohan, C. O, Ravishankar C. N & Srinivasa Gopal T. K. (2010) Active Packaging of Fishery Products: A Review Fish.Technol., Vol. 47(1) pp: 1 – 18.

Mohan, C. O., Ravishankar, C. N& Srinivasa Gopal, T. K. (2008) Effect of O_2 scavenger on the Shelf-life of Catfish (*Pangasius sutchi*) Steaks during chilled storage. J. Sci. Food Agric. 88, pp 442-448.

Mohan, C. O., Ravishankar, C. N., Srinivasa Gopal, T. K & Ashok Kumar, K(2009a) Nucleotide Breakdown Products of Seer Fish (*Scomberomorus commerson*) Steaks Stored in O2 Scavenger Packs During Chilled Storage. Innovat. Food Sci. Emerg. Tech. 10, pp 272–278.

Mohan, C. O., Ravishankar, C. N., Srinivasa Gopal, T. K., Ashok Kumar, K &Lalitha, K. V. (2009b) Biogenic Amines Formation in Seer Fish (*Scomberomorus commerson*) Steaks Packed with O2 Scavenger During Chilled Storage. Food Res.Int. 42, pp 411-416.

Ozogul, F., Polata, A. & Ozogul, Y (2004) The effects of modified atmosphere packaging and vacuum packaging on chemical, sensory and microbiological changes of sardines (*Sardina pilchardus*). Food Chem. 85, pp 49–57.

Ozogul, F., Taylor, K.D.A., quantick, P. & Ozogul, Y (2000) Chemical, Microbiological and Sensory Evaluation of Atlantic herring (*Clupea harengus*) stored in ice, modified atmosphere and vacuum pack.Food Chem. 71, pp 267–273.

Park, J.W & Lin, J.T.M (2005) Surimi: Manufacturing and Evaluation, In: Surimi and Surimi Seafood 2nd Edn. (Park, J.W, Ed.), CRC press, Taylor & Francis Group, Boca Raton, FL, 923 p.

Poulose Yesudhason, Teralandur Krishnaswamy Srinivasa Gopal, Chandragiri Narayanarao Ravishankar, K.V. Lalitha & Ashok Kumar (2010) Effect of potassium sorbate and modified atmosphere packaging on the shelf-life extension Of seer fish (*Scomberomorus commerson*) steaks during iced storage, J. Food Biochem.34, pp 399–424.

Ravi Shankar, C. N., Srinivasa Gopal, T. K & Vijayan P. K. (2002) Studies on heat processing and storage of seer fish curry in retort pouches Packaging Technology and Science Vol. 15(1), pp 3–7.

Reddy, N.R., Paradis, A., Roman, M.G., Solomon, H.M. & Rhodehamel,E.J.(1996). Toxin development by Clostridium botulinum in modified atmosphere packaged fresh tilapia fillets during storage. J. Food Sci. 61, pp 632–635.

Reddy, N.R., Villanueva, M. & Kautter, D.A (1995). Shelf life of modified- atmosphere – packaged fresh Tilapia fillets stored under refrigeration and temperature abuse conditions. J. Food Protect. 58, pp 908–914.

Regenstein,J.M (2004) Total utilization of fish, Food Technol. 58(3), p25.

Rodrick G.E& Dixton, D. (1999) Code of Practice for the Irradiation of Fish, Shrimp and Frog Legs, , International Atomic Energy Agency, Vienna.

Shalini, R., Indra, J., Shanmugam, S.A. & Ramkumar, K (2001). Effect of Potassium sorbate dip-treatment in vacuum packaged *Lethrinus lentjan* fillets under refrigerated storage. J. Food Sci. Technol.38(1), pp 12–16.

Shalini, R., Indra, J., Shanmugam, S.A. & Ramkumar, K. (2000). Sodium acetate and vacuum packaging to improve shelf life of refrigerated *Lethrinus lentjan* fillets. Fish. Technol 37(1), pp 8–14.

Sini, T.K, Santhosh,S, Joseph A.C & Ravishankar,C.N (2008) changes in the characteristics of Rohu fish (*L. rohita*) sausage during storage at different temperatures , J. Food Process. Pres. 32(3), pp 429 -442.

Sreenath P. G., Martin Xavier K.A., Ravishankar C.N., Bindu J. & Srinivasa Gopal T.K. (2007) Standardisation of process parametres for ready-to-eat squid masala in indigenous polymer-coated tin-free steel cans. Int. J. Food. Sci. Technol. 42, pp 1148-1155.

Subsinghe,S (1996) Handling and marketing of aquacultured fish, Infofish Int.,3, 44 p.

Suderman, D.R & Cunningham,F.E (Eds.) (1983) Batter and Breading Technology, Ellis Horwood Ltd, Chichester, England.

Venugopal V& Shahidi F (1998) Traditional methods to process underutilized fish species for human consumption. Food Rev. Int., 14(1), pp35–97.

Venugopal, V (1990) Extracellular proteases of contaminant bacteria in fish spoilage J. Food Prot. 53, 341p.

Venugopal, V (2006) Freshwater and Aquacultured Fishery Products, In: Seafood Processing: Adding value through quick freezing, retortable packaging and quick chilling, CRC Press, Taylor & Francis Group, Boca Raton, FL., 485 p.

Venugopal, V (2006) Mince and Mince-based Products, In: Seafood Processing: Adding value through quick freezing, retortable packaging and quick chilling, CRC Press, Taylor & Francis Group, Boca Raton, FL., 485 p.

Venugopal, V., Doke, S.N. & Thomas, P (1999) Radiation processing to improve the quality of fishery products. Cri.Rev. Food Sci. Nutr. 39(5), pp 391-440.

Venugopal,V & Shahidi,F (1995) Value added products from under-utilized fish species, Crit.Rev.Food Sci. Nutr., 35, p 431.

Venugopal,V., Ghadi,S.V & Nair,P.M (1992) Value added products from fish mince , Asian Food J., 7, p3.

Yokoyama K, Nio N & Kikuchi Y (2004). Properties and applications of microbial transglutaminase. Appl. Microbiol. Biotechnol. 64 (4), pp 447–54.

Zugarramurdi,A, Parin,M.A & Lupin,H.M (1995) Economic engineering applied to the fishery industry. FAO Fisheries Technical Paper No.351, FAO, Rome, Italy.

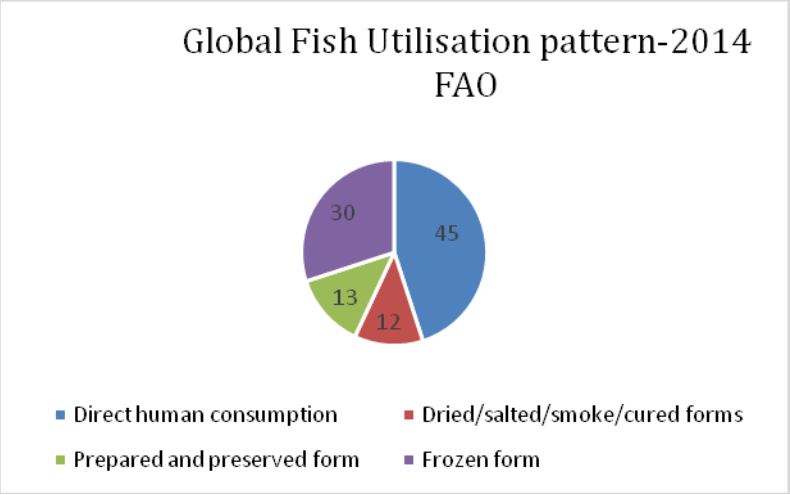
****

Overview of Waste Generation in Fish and Shellfish Processing Industry

Binsi P.K.
Fish Processing Division
ICAR-Central Institute of Fisheries Technology, Cochin
Email: binsipk@gmail.com

Introduction

Global fish production has witnessed a remarkable growth in recent past (excluding aquatic plants) reaching 167.2 million tonnes in 2014, with 93.4 million tonnes from capture and 73.8 million tonnes from aquaculture. A parallel development was observed in the share of world fish production utilised for direct human consumption from 67% in the 1960s to 87%, or more than 146 million tonnes, in 2014. Historically, fish has been considered as an important food source and even today it is one of the most traded commodities in international markets. Interestingly, about 15% of the world requirement for animal proteins is being met from fish alone which accounts to 4–5% of the calculated minimum requirements for protein (Guerardet al., 2005). The estimate in 2013, indicated a slightly higher value of 17% which account to 6.7% of all protein consumed (Seafish, 2014). However, there are growing concerns about the sustainability and management of seafood industry, in parallel with the increasing global demand for seafood. Recent reports project a figure of US$ 50 billion as the loss from seafood sector every year, due to poor management of available resources. Wastes are generated at different points in the value chain, viz. by-catch, onboard handling, landing centres, transportation, storage, retailers, and consumers. The waste generation begins with the practice of 'discard at sea' of unintentional catches. Subsequently, during processing operations, only the muscle parts are consumed and the rest is discarded. Global fish waste generation is estimated to be in excess of 100 mMT, and in the Indian scenario it is >4 mMT. It is estimated that fish processing waste after filleting accounts for approximately 75% of the total fish weight. This figure is too high before the challenging task of feeding the 9 billions of world population by the middle of this century.



Waste and By-products: Global Terminologies

In literature, quite often by-products, waste, discards etc are cited as alternate terms. However, a cleardistinction between by-products that can be used for human

consumption and waste / discards / viscera is made in regulatory papers (Rustad, 2003). The term 'waste' includes the remnants that cannot be recycled or converted to another high value products, and have to be composted, burned or destroyed (Bekkevold&Olafsen, 2007). On the other hand, the term 'by-products' refers to the left outs that are not generally regarded as conventional marketable products, but can be converted to industrial or edible products. Whereas, the EC regulation on animal by-products (ECNr 1774 / 2002, 2002), adopted on 3 October 2002, definesanimal by-products as whole carcasses or parts of animalsor products not intended for human consumption; by-products intended for human consumptionis not included in this definition. There are several other terms in usage to alternatively represent the by-products,such as waste via co-products or co-streams etc. Lately, as more and more research evidences were mounted on the potential biomolecules derived from marine sources, especially from fish other than meat part, there is a raising tendency to treat these as raw material rather than 'discards/waste'. Consequently, the term 'rest raw material' and 'secondary raw material' is the newly evolved expression today to highlight the importance of treating these materials as equivalent to 'targeted product'. For instance, fish skin is a rest raw material, whereas collagen is a by-product.

Global waste generation Profile

In seafood industry, the general understanding is that the edible meat part constitute forms the 'main product' and the remaining parts including head, trimmings, skin, viscera, scale, bone etc. are considered as 'left over', now as 'rest raw material/secondary raw material'. In a different angle, this perception is a bit ironical. This becomes more apparent, when a global estimate of waste generation profile is taken in to account. The amount of waste generated from seafood sector begins at the site of harvest itself. For the last few decades, the FAO estimate on postharvest losses in seafood sector remains to be 20-35% of the catch, at various stages of value chain. Approximately, 17.9 to 39.5 million tonnes of whole fish is discarded each year by commercial fishing operations. Apart from the quality losses in the supply chain, worldwide, around 130 million tonnes of fish waste is produced each year, which is approximated to more than 75% of total fish production. Normally in capture fisheries, a considerable portion of marine catch is dumped back to the sea, either as untargeted catch or as 'discards' in the case where on-board processing activities are carried out. Generally, bulk of demersal catch is processed on board. As the waste material is rarely landed onshore, a considerable proportion (11%) of the total capture biomass is disposed of at sea, mainly in the form of viscera and heads. This figure may be a bit less in the case of culture fisheries.

Table 1: Waste generation during industrial processing of fish in India

Products	Waste Generated (%; w/w)
Shrimp products	50
Fish fillets	65
Fish steaks	30
Whole and gutted fish	10
Surimi	70

Cuttle fish rings	50
Cuttle fish whole	30
Cuttle fish fillets	50
Squids whole cleaned	20
Squid tubes	50
Squid rings	55

Nature and composition of secondary raw materials from seafood industry

The nature and quantum of secondary raw materials generated in seafood industry depends on several factors, which may be broadly categorised into resource related factors and process related factors. The former category includes species, size, age, biological nature (including presence of toxins and allergens) and morphological features. Generally, 40-70% of original raw material is discarded in commercial processing operations depending on intended product, style of dressing, type of handling (manual/ mechanical), skill of handling person, intended use and to a greater extent on the quality of raw material. Largely, seafood processing operations generate both liquid and solid wastes; solid waste being the bulk ranging from 30% to 65% of the weight of the landed fish. Head, viscera, skin, fin, swim bladder, bone, frame meat, dark meat, scale, gills, shells (crustacean, mollusca), cephalopod pen, ink sac etc. are the major components of solid waste. The liquid effluents mainly consist of blood, slime, mucus, wash off and other soluble. In surimi processing, soluble proteins are washed off to a greater extent during repeated water washing steps

Table 2: Typical composition of secondary raw materials from fish processing operations

Waste Component	% of whole fish	Active component
Head	15 - 25	Protein, PUFA, Minerals, Plasmalogens, GAG
Frame Meat	~10% of frame	Protein
Skin	3 - 5	Protein
Scale	6 - 7	Protein, Minerals
Bone	8-10	Protein, Minerals, Chondroitin
Viscera	5 - 12	Protein, Enzyme, fat
Gill	4-5	Protein, Fe

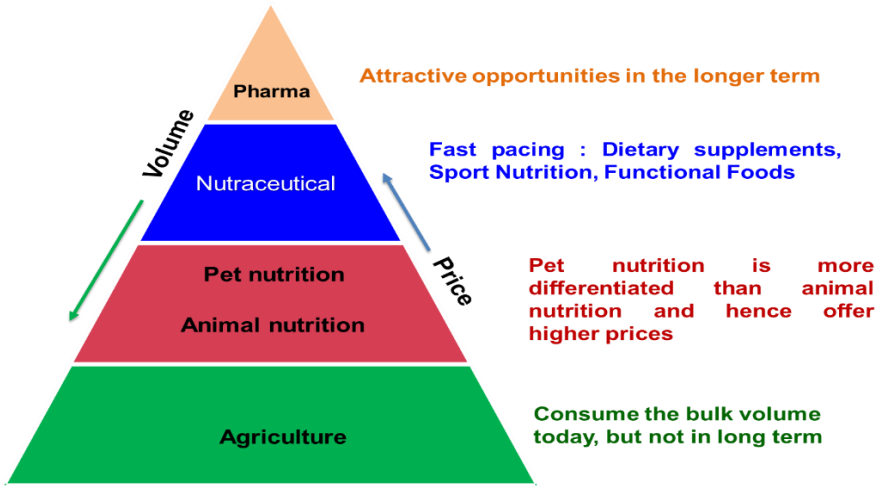
Global utilisation pattern of secondary raw materials

Presently, a major portion of the discards and low value catch, mainly pelagic varieties, are going for the production of fish meal and oil, which accounts to as much 30% of the world's total catch. A significant, but declining, proportion of world fisheries production

is processed into fishmeal and fish oil thereby contributing indirectly to human consumption when they are used as feed in aquaculture and livestock raising. As per FAO projection, by 2025, fish meal produced from fish waste will represent 38% of world fish meal production, compared with 29% for the 2013 to 2015 average level. Apart from fishmeal, a reasonable portion is going for fermented products such as fish sauce and silage. Norway is the main producer of fish silage that is used almost entirely for feed. A meagre portion is used for human consumption, to the tune of maximum 10%.

Value addition options and opportunities

Generally, two different methods, mass transformation and sorting, have been developed to improve the economic value of fish wastes. Mass transformation involves the conversion of fish waste into a single product. Sorting enables the production of specialised products such as liver oil, gelatin, omega-3, protein containing sports food and drinks, calcium, cosmetics, and pharmaceuticals. Wider acceptance and adoption of both methods could lead to significant reductions in wastes going to landfill and reduce the damaging impact of fish wastes on the environment.



Pyramidal representation of opportunities in the utilisation of secondary raw materials

Legislatory framework and Regulatory norms

As secondary raw materials are heterogeneous mixtures of a number of biomolecules, there are several EU and national regulations and recommendations internationally concerning the norms for pathogens, toxins, allergens and biogenic amines in products, particularly for those intended for man and animal nutrition. The major ones are listed below.

- EC **Disposal, Processing and Placing on the Market of Animal By-products Regulations** (SI 257, 1994) that regulates the use, sale and disposal of high and low risk animal by-products which provides limited options for their use.
- EC Regulation No 1774/2002 of the European Parliament and of the Council of 3 October 2002 laying down **health rules concerning animal by-products not intended for human consumption** (amended by Commission Regulation (EC) No

808/2003 of 12 May 2003). Provides a mechanism for the reclassification of all animal by-products not intended for human consumption based on their potential risk - this will drive fish waste utilisation and disposal options in future years.

- Commission Regulation (EC) No. 811/2003 on the intra-species recycling ban for fish, the burial and burning of by-products and certain transitional measures provides additional clarification of Regulation (EC) No. 1774/2002 as follows:
  - ✓ Derogation to permit the feeding of fish with processed animal protein derived from bodies/parts of bodies of the same species. However this is academic, as a) it does not apply to feeding farmed fish with processed animal protein from farmed fish of the same species and b) doing this is already voluntarily banned by the feed industry.
  - ✓ Wild fish and by products from wild fish may be used for the production of fish feeds or directly as a feed.
  - ✓ Fish and animal by-products intended for feed for fish must:
    - Be handled and processed separately from other material
    - Originate from wild fish or non-mammal sea animals caught for the purpose of fish meal production or from fresh by-products from wild fish processed for human consumption
    - Be packaged after treatment and clearly identified appropriate for feeding of fish.
- A draft Commission Regulation SANCO/2153/2003 implementing EC Regulation No 1774/2002, approves six additional means of disposal or uses of animal by-products, including (i) alkaline hydrolysis, biodiesel production and combustion of animal fat in a thermal boiler for the treatment and disposal of Category 1 material, as well as (ii) the processes of alkaline hydrolysis, high pressure high temperature hydrolysis, high pressure hydrolysis biogas, biodiesel production, Brookes gasification, and combustion of animal fat in a thermal boiler for the treatment and use or disposal of Categories 2 or 3 material.Fish by-products do not arise in Category 1.

Table 3: Categorisation of Animal By-Product Materials

(Source: https://www2.gov.scot/Publications/2005/03/20717/52862)

Category	Raw material	Storage and disposal requirements
1	• All body parts affected by TSE, pet/zoo/circus animals, experimental animals. • Wild animals suspected of being infected with disease communicable to humans or animals, • Animals containing residues of	• Incineration • Processing in an approved Category 1 processing plants • For certain marked non-TSE material, may be buried in approved landfill sites

	environmental contaminants; • Animal material collected when treating waste water from Category 1 processing plants • Mixtures of Category 1 material with either Categories 2 or 3 materials or both.	
2	• Fish farming mortalities • Animal by-products containing digestive tract or manure components • Animal material collected from treating waste water from slaughter houses or Category 2 processing plants • Products containing residues of veterinary drugs and contaminants listed in Group B(1) and (2) of Annex I to Directive 96/23/EC • Non-Category 1 by-products from non-member States. • Animals or parts of animals that have been slaughtered for human consumption, inc those killed to eradicate an epizootic disease • Mixtures of Category 2 material with Category 3 material	• Incineration • Processing in an approved Category 2 processing plants • Certain marked material may be (i) used as an organic fertiliser, (ii) transformed in a biogas plant or (iii) buried in approved landfill sites • For material of fish origin, may be ensiled or composted (subject to approval). • Where authorised, used as a feed for zoo, circus, fur animal, hounds, maggot / worm (as bait)
3	• Parts of slaughtered animals for human consumption • Fish or other sea animals (exc. sea mammals) caught in the open sea for the purpose of reduction to fish meal • Fresh fish by-products from plants manufacturing fish products for human consumption.	• Incineration • Processing in an approved Category 3 processing plants • Used as a raw material in pet foods • Transformed in a biogas or composting plant • For material of fish origin, may be ensiled or composted • Where authorised, used as a feed for zoo, circus, fur animal, hounds, maggot / worm (as bait)

Table 4: Categorisation of Aquaculture By-products

Source: SEERAD, pers. comm., 2004

Source of Waste	Waste Category		
	1	2	3
On-farm mortalities			
- where no disease has been confirmed		✓	
- where controls have been applied because of the presence or suspected presence of notifiable disease		✓	
- as a result of jellyfish attack		✓	
- as a result of algal bloom		✓	
- as a result of adverse weather conditions		✓	
- due to a compulsory slaughter notice		✓	
Mortalities at the processor			
- where the fish are dead on arrival		✓	
- show clinical signs of disease and are not processed		✓	
Processing waste			
- where source is subject to disease controls (but fish show no clinical signs of disease)			✓
- where source is not subject to controls			✓

- The Animal By-Products Regulations, 2003 provides a recent (October 2003) enactment of Regulation (EC) 1774/2002 (and the subsequent Regulation (EC) 811/2003 mentioned above). This Regulation recognises the ability to utilise fish by-products (primarily Category 2) for zoo, circus, fur, certain dogs (e.g. hounds) and maggot farming under approved circumstances. In addition, the burning or burial of animal by-products is permitted in certain remote areas, so long these sites are monitored at regular intervals.
- EC 1999/31/EC Landfill Directive: requires Member States to reduce the quantities of biodegradable wastes to 35% of 1995 levels by 2020. This will inevitably encourage alternative disposal techniques, such as composting and incineration.

- UK Animal Protein Regulations (2001): prohibits the use of mammalian protein (with certain specified exceptions) to ruminants and the feeding for mammalian meat and bone meal to all farmed livestock.
- UK Environmental Protection Act 1990: prohibits the keeping, treatment or disposal of waste on land unless a waste management licence has been granted for that purpose.
- UK Food and Environment Protection Act (1985): controls the disposal at sea through strict licensing. This order allows the unlicensed disposal of fish wastes at sea, even after landing its catch. However the disposal at sea from processing onshore is not permitted without a licence.
- UK Food Hygiene (Fishery Products and Live Shellfish (Hygiene) Regulations 1998. Sets out the conditions under which fish and shellfish products must be produced in order to be placed on the market. Includes provision that:
  - ✓ Offal and viscera must be kept separate from products intended for human consumption
  - ✓ Onshore processing facilities must regularly remove waste from the processing area
  - ✓ Containers holding waste material must be water tight, corrosion-resistant and be designed to facilitate cleaning and disinfection
  - ✓ Waste material held overnight must be housed in a designated area
- The Fur Farming (Prohibition) Act 2002 prohibits the farming of animals solely or primarily for their fur
- UK Integrated Pollution Prevention and Control Regulations (2000): lay down measures to reduce the emissions to air, water and land from a range of activities including food processing. Affected business need to prove that the best available techniques have been introduced to reduce the environmental impact of its operation.
- UK Landfill Tax Regulations (1996): levy charges on waste disposed of in landfill sites and thus encourages waste minimisation and maximisation of recycling opportunities. Waste is either classified as inactive/inert and other - the latter attracts a higher tax rate per tonne.
- UK Waste Management Licensing Regulations (1994): permits a number of unlicensed exemptions for waste disposal, including the spreading of shell on agricultural land and the use of shell for land reclamation or improvement. Such unlicensed disposal must be registered.

Future market trends

The market for high-end by products from marine sources is fairly high, especially for nutraceutical and medical field. The market demand for high quality oil for functional foods alone is projected to be doubled in next five years (Skjævestad& Vogt, 2009).As of today, the actual market potential of marine biomolecules has not been fully realised. Even though the marine proteins are known to have superior nutritional quality index in terms of amino acid composition and bioavailability, meagre effort is put towards protein isolate or hydrolysate production, except for a few stakeholders in Western and

European markets. There is huge demand from health and sports nutrition industry for high quality proteins and peptides, where marine proteins could be ideally place in. The market for sports nutrition products is growing with 5–7% per year. Apart from marine oil and protein, several bioactive ingredients from process discards have entered beverage market as functional and medicinal supplements. These are primarily, chitins, pigments, taurine, squalene, proteoglycans, polyphenols, probiotics, polysaccharides, enzymes, vitamins and minerals. These bioactive molecules offer innumerable health benefits, including anti-oxidant, anti-arthritic, anti-hypertensive, anti-bacterial, anti-carcinogenic, anti-obese, and anti-inflammatory activities.

Challenges and way backwards

The key to successful seafood waste utilisation and management is to develop appropriate eco-friendly reprocessing technologies that can convert all the valuable components present in the waste into valuable products and reduce the amount of waste going to disposal route. However, there are many challenges that must be overcome to achieve this goal.

1. Consumer awareness and education is a major challenge. Without consumer acceptance of food waste reduction approaches, no sustainable eco-friendly food waste utilisation and management strategy can succeed. This demands proper extension efforts from the research and extension organizations.
2. Seafood sector is a poorly organised sector. Highly scattered nature of seafood processing operations (across domestic market and processing facilities) poses problems in collection and processing.
3. Seafoods are highly perishable in nature owing to its unique richness in terms of protein, peptides, enzymes and microbial flora. This quite often leads to the mass resistance from public in starting up a business venture in the vicinity.
4. Stringent legal and environmental restrictions from the regulatory bodies as seafood waste is not categorised as “inactive/inert” waste is a major discouraging for the entrepreneurs to invest upon this resource
5. Inappropriate cold chain management from the source of generation to the point of conversion as the processors are least interested to invest further on discards
6. There is no baseline data on the availability and economics of production collected over the past years, which poses uncertainty about economics and market demand of secondary products
7. Lack of clear legal classification of secondary products in the international market is yet another major challenge to the investors
8. Lack of unified protocols for quality assurance (such as HACCP) for secondary products leads to frequent rejections from the buyers

Strategies for future development

- Strengthening the baseline data (waste generation, local facilities, current disposal plan, major stakeholders etc)
- SWOT analysis accommodating regional disparities for the development of an economically and ecologically sustainable waste management plan

- Improve public awareness on fishery waste value addition options through effective extension efforts
- Establish locality-specific value chain routes covering waste generators (Market, peeling sheds etc), regional producers (SMEs, SHGs etc), and user groups (farmers, dealers etc.)
- Networking & establishing inter-industrial linkages between potential stakeholders (Timely follow-up and review of the efforts undertaken is a must)
- Develop mobile pilot technological platforms for testing and demonstrating different technologies
- Public-Private-organisational partnership (incubation centres for pilot production)
- Public policies and legislations against waste dumping
- Framing policies for better use of fishery wastes(such as coupling of licensing of markets and processing facilities with waste conversion measures taken at the source of generation)
- There are bigger challenges with regard to clinical testing, documentation, standardisation andquality, which need to be addressed in a greater way

Suggested Readings

- Rustad, Turid, IvarStorrø, and Rasa Slizyte. "Possibilities for the utilisation of marine by-products." *International Journal of Food Science & Technology* 46, no. 10 (2011): 2001-2014.
- Skjævestad, B. & Vogt, G. (2009). Omega-3 oljerfrafersktmarintra˚stoff. En muligkonkurransestrategi for den norske omega-3 industrien. Trondheim: Rubin.
- Venugopal, V. (1995). Methods for processing and utilization of low cost fishes: a critical appraisal. Journal of Food Science and Technology, 32, 1–12.
- Shahidi, F. (1994a). Proteins from seafood processing discards. In: Seafood Proteins (edited by Z.E. Sikorski, B. Sun Pan & F. Shahidi). Pp. 171–193. New York: Chapman and Hall.
- Shahidi, F. (1994b). Seafood processing by-products. In: Seafoods: Chemistry, Processing Technology and Quality (edited by F. Shahidi& J.R. Botta). Pp. 320–330. London: Blackie Academic & Professional.
- Kim, S.-K. &Mendis, E. (2006). Bioactive compounds from marine processing byproducts – a review. Food Research International, 39, 383–393.

****

High Value Products from Fish Processing Wastes

Zynudheen. A.A. and Binsi P.K.

Fish Processing Division

ICAR-Central Institute of Fisheries Technology, Cochin

Email: zynu@rediffmail.com

Processing of fish for human consumption results in enormous quantity of waste in the form of skin, head, viscera, scales, bones, trimmings and frames. The quantity of waste generated depend on the type and size of fish and the product manufactured out of it. Industrial fish processing for human consumption yields only 40% edible flesh and the remaining 60% is thrown away as waste. Annual discard from the world fisheries were estimated to be approximately 20 million tonnes (25%) per year which includes "waste" or by-products also. The immense scope for high end product from fishery waste has been realized and different technologies have been developed with a view to utilize processing waste, for converting them into products for human consumption, animal nutrients and products of pharmaceutical and nutraceutical significance. Among the most prominent current uses for fish waste are fishmeal production, extraction of collagen and antioxidants, isolation of cosmetics, biogas/biodiesel, production of chitin and chitosan, food packaging (gelatin, chitosan) and enzyme isolation. Fish waste is prone to faster spoilage since it contains easily digestible protein. The microbial population associated with the digestive process are the major reasons of spoilage. Since the processor does not bother to preserve the waste the problem of environmental pollution is enhanced. Accumulation of fishery waste results in nauseating and obnoxious smell due to the release of volatile nitrogenous compounds during decomposition.

Fish meal

Fish meal is highly concentrated nutritious feed supplement consisting of high quality protein, minerals, vitamins of B group and other vitamins and other unknown growth factors. Fishmeal is rich in essential amino acids. It is produced by cooking, pressing, drying and grinding the fish, by-catch fish, miscellaneous fish, filleting waste, waste from canneries and other processing operations. The composition of fishmeal differs considerably due to the variations in the raw material used and the processing methods and conditions employed.

Traditional fishmeal production in India was from the sun dried fish collected from various drying centers and the products were mainly used as manure. Better quality fish meal has been a prominent item of export from the very beginning of this industry. BIS has brought out the specification for fish meal as live stock feed for facilitating proper quality control.

The proximate composition of fish meal in general is given below:

Protein	-	50-57%
Fat	-	5-10%
Ash	-	12-33%
Moisture	-	6-10%

Manufacturing process

Fish can be reduced by two general process (1) Dry rendering (2) Wet rendering process.

Dry Rendering Process

Dry rendering or dry reduction process is suitable for only lean or non oil fish such as silver bellies, jew fish, sciaenids, ribbon fish, sole, anchoviella, carcasses of shark, fish offal and filleting waste. In this process, it is dried to moisture content of 10% and pulverized. If the quantity to be handled is sufficiently large a steam jacketed cooker dryer equipped with power devises for stirring is used. Sometimes, if the size of the fish is comparatively large a coarse grinding is also done before being fed into the cooker drier. The cooker dryer may be operated at atmospheric pressure or under partial vacuum. Being batch operation the process will have only limited capacity and labour cost is very high. Merit of this process is that the water-soluble materials are retained in the meal.

Wet rendering process

Wet rendering or wet reduction process is normally applied to fatty fish or offal where simultaneous production of fish meal and fish body oil is envisaged. The process consists of grinding, cooking to soften the flesh and bones and to release the oil, pressing to expel the liquor and oil, fluffing the press cake drying, grinding and packing the meal, The press liquor is centrifuged to remove the suspended particles and to separate oil. The stick water is concentrated. The process requires elaborate equipment and is normally a continuous one and therefore adaptable to the reduction of large quantities of fish.

In a continuous wet reduction process the coarsely ground fish or fresh raw fish or offal is passed through a stationary horizontal cylindrical cooker by means of a screw conveyor at a predetermined rate. Steam is admitted through a series of jets. The cooked mass is passed through a continuous screw press. The press cake is fluffed and dried to a moisture level of 8%. The suspended fish meal present in the press liquor is separated by centrifugal sedimentation and the oil by centrifugation or other conventional methods.

Fish body oil

The main source of fish body oil in India is oil sardine. A survey of the oil industry reveals that the extraction is done on a cottage scale in isolated places near the leading centers and is not well organized. The method of extraction followed is cooking the fish in iron vessels and pressing and separating the oil. Apart from sardine oil, fish body oil is also obtained from the fish meal plants operating in the country. In India oil sardine is a fishery which exhibited wide fluctuations from as low as 1% to as high as 32% of the total landings. The seasonal variation in oil content is predominant in Kerala and Karnataka coast. During the peak season fish has oil content of 17%. By the wet rendering process the fish will yield, on average 12% oil having analytical characteristics similar to other fish oils. Fatty acid composition of oil revealed that they contain high amounts of polyunsaturated fatty acids (PUFA). At present the medicinal values of fish oils are well known.

Fish liver oil

The therapeutic value of fish liver oil was discovered in 18th century and fish liver oil becomes a common medicinal product especially for Vitamin A and D. Cod, shark and haddock livers are the important sources of Vitamin A and D. The weight of liver, fat content and presence of vitamins are dependent on a number of factors like species, age, sex, nutritional status, stages of spawning, and area from where it is caught.

In cod (*Gadus collarius*), coal fish (*Pollahius vireus*) and haddock (*Melanggrammus aenglefinus*), the weight of liver normally amount to 4-9% of whole fish and livers contain about 45% to 67% oil. The species of shark such as dog fish (*Squalus acanthias*), Greenland shark (*Somniosus microcephalus*) and barking shark (*Certrohinus maximus*) have large fatty livers weighing up to 10-25% of the whole fish containing 60-75% oil. But halibut, tuna, and whale have 1% liver having 4 to 25% oil with high vitamin A & D content. Depending on the oil content and vitamin A potency fish livers are generally classified in to three groups.

Low oil content - high vitamin A potency

High oil content - low vitamin A potency

High oil content - medium vitamin A potency

Processing

The processing procedures of fish liver without affecting the quality of the oil extracted can be summarized as (1) steaming (2) solvent extraction and (3) alkali/enzyme/acid digestion. The process selected should depend on the vitamin and oil content of the livers.

Certain species of shark contain high oil content with high hydrocarbon content, viz. squalene. Squalene a highly unsaturated aliphatic hydrocarbon is present in shark liver oils, mainly of the family squalidae, cod and some vegetable oils like olive oil, wheat gum oil, and rice bran oil. Chemically it is known as 2,6,10,15,19,23 hexamethyl, 2,6,10,14,18,22 tetracosahexane having a molecular weight of 410.70, it is an isoprenoid compound containing six isoprene units.

Presentation and storage

Vitamin oils are stored in rust free, well washed and dried air tight drums. The head space should be kept minimum to avoid oxidation. It is advisable to fill head space with inert gas such as nitrogen. If properly processed and stored the oil will remain in satisfactory condition without the use of preservative. Small amounts of antioxidants like BHA, αtocopherol, BHT, NDGA can be used to preserve the oil for longer periods.

Fish hydrolysates

This is also liquefied fish product but it differs from silage. They are produced by a process employing commercially available proteolytic enzymes for isolation of protein from fish waste. By selection of suitable enzymes and controlling the conditions the properties of the end product can be selected. Hydrolysates find application as milk replacer and food flavouring agents. Enzymes like papain, nisin, trypsin, bromelein, pancreatin are used for hydrolysis of fish protein. The process consists of chopping, mincing, cooking, cooling to the desired temperature, hydrolysis, sieving, pasteurizing the liquid, concentrating and vacuum drying or spray drying of the product. This is deliquescent, so care should be taken to keep it in fine airtight bottles. It can be

incorporated in to beverages as a high energy drink for children and convalescent persons.

Fish maws and isinglass

The world isinglass is derived from the Dutch and German words, which have the meaning sturgeon's air bladder or swimming bladders. Not all air bladders are used for this preparation. The air bladder of deepwater hake is most suitable for production of isinglass. In India air bladders of eel and catfishes are used for the production of isinglass.

The air bladders are separated from fish and temporarily preserved in salt during transport. On reaching the shore they are split open, washed thoroughly, outer membrane is removed by scraping and then air dried. Cleaned, desalted, air dried and hardened swimming bladders (fish maws) are softened by immersing in chilled water for several hours. They are mechanically cut into small pieces and rolled or compressed between hollow iron rollers that are cooled by water and provided with scraper for the removal of any adhering dried material. The rolling process converts the isinglass into thin strips or sheets of 1/8 to ¼" thickness. There are processes for the production of isinglass in powder form also.

Isinglass dissolves readily in most dilute acids or alkalis, but is insoluble in alcohol. In hot water isinglass swells uniformly producing opalescent jelly with fibrous structure in contrast to gelatin. It is used as a clarifying agent for beverages like wine, beer, vinegar etc. by enmeshing the suspended impurities in the fibrous structure of the swollen isinglass.

India exports dried fish maws, which form the raw material for the production of isinglass and other such products. Process has been developed to produce the finished products from fish maws.

Fish Gelatin

Skin of fish constitute nearly 3% of the total weight and is suitable for the extraction of gelatin. Bones and scales can also be processed into gelatin. The process involves alternate washing of skin with alkali and acid and extracting gelatin with hot water. Gelatin finds applications in pharmaceutical products as encapsulation and in food industry as gelling agent. Fish gelatin has better release of a product's aroma and flavor with less inherent off-flavor and off-odor than a commercial pork gelatin.

Fish calcium

The recommended daily intake of calcium is 1000 mg for the adults, and 1300 mg for elderly women. Fish bones and scales are excellent source of calcium. Whole small fish or fish bone/scale can be used for calcium separation. The filleting frames of carps and other fishes can be used for extraction of calcium. The frames are washed and boiled to separate the adhering meat portions. It is washed again and treated with enzymes to remove the adhering connective tissue, washed, dried and powdered. Fish calcium is essentially dicalcium phosphate which has better nutritional qualities.

Hydroxyapatite

The hydroxyapatite extracted from the scale are having uses as bioceramic coatings and bone fillers. The coatings of hydroxyapatite are often applied to metallic implants to alter the surface properties so as to avoid rejection by the body. Similarly,

hydroxyapatite can be employed in forms such as powders, porous blocks or beads to fill bone defects or voids. For permanent filling of teeth hydroxyapatite is found to be a better option for import substitution.

Utilization of prawn shell waste

The head and shell of prawn and other crustaceans form the major fishery waste. The waste contains a good percentage of protein and chitin other than minerals. The protein can be extracted along with the flavour bearing compounds and converted into shrimp extract having potential use as a natural flavoring material. Chitosan, a deacetylated chitin, is one of such products, which has application in many fields. It is a modified natural carbohydrate polymer. It is a cationic polyelectrolyte, insoluble in water, organic solvents and alkaline solutions and is soluble in most organic acids, and dilute mineral acids except sulphuric acid. It can form ionic bonds and films. Chitosan finds applications in many industries.

Chitin

The residual shell waste obtained after extraction of protein with hot 0.5% caustic soda may contain small amounts of protein. This is then removed by boiling with 3% caustic soda for few minutes and filtering off the liquor. It should be washed free of alkali before demineralisation. The demineralization is done by treatment with dilute hydrochloric acid at room temperature. Demineralization reduces the volume of the shell considerably and therefore deproteiniser can hold more material if the demineralization is done initially.

Glucosamine hydrochloride

Chitin can be hydrolysed to glucosamine hydrochloride by adding concentrated hydrochloric acid and warming until the solution no longer gives opalescence and diluting with water. The excess acid can be distilled off under vacuum. The crude glucosamine hydrochloride is diluted with water and clarified with activated charcoal. The solution is filtered and evaporated under vacuum. The crude glucosamine hydrochloride can be separated by adding alcohol.

Chitosan

Chitin is dried or centrifuged or pressed to remove water. The deacetylation is done by heating at 90-95°C with 40% (w/w) caustic soda for 90-120 min. The water present in the chitin cake should also be taken in to account while preparing caustic soda solution. To achieve this 50% caustic soda is prepared and calculated quantity of it is added to the chitin cake. The reaction is followed by testing the solubility of the residue in 1% acetic acid. As soon as the dissolution is completed caustic soda is removed from the reaction mixture. The drained caustic soda can be reused for the next batch of deacetylation by fortification if necessary. The residue is washed with water free of alkali. It is then centrifuged and dried in the sun or an artificial drier at a temperature not exceeding 80°C and pulverized to coarse particles.

Chitosan is almost colourless, light in weight and soluble in dilute organic acids but soluble in water, alkali and organic solvents. It gives viscous solution when dissolved in dilute organic acids such as formic acid, acetic acid etc. Chitosan finds extensive applications in following areas viz; food industries, pharmaceutical applications, chemical industries, dental and surgical uses as a haemostatic agent, wound healing, biodegradable films as a substitute for artificial skins for removing toxic heavy metals,

wine clarification, Industrial effluent treatment, agriculture, photography, cosmetic applications and textiles, and in nano applications.

Conclusion.

Since the fresh water aquaculture is increasing every year the future utilization and development of high value items from this sector has high potential. Hence utilization of fishery waste for the development of high value products is gaining importance in recent years. A variety of by products can be developed which is found to have different applications in medical, food, and other fields. By simple cost effective techniques, valuable products can be developed which will enhance the revenue of the fishermen and allied industries. In fact, the materials which caused problems to the fish processing industry due to the environmental pollution has become raw materials for valuable products with versatile application

****

Chitins: Chitin and its Derivatives

Binsi P.K.

Fish Processing Division

ICAR-Central Institute of Fisheries Technology, Cochin

Email: binsipillai@gmail.com

Background

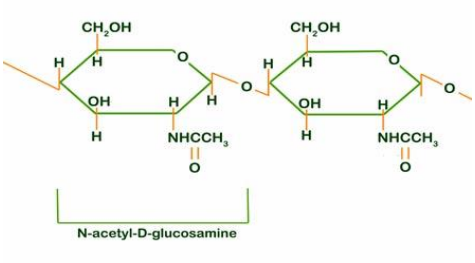
The growth of chitin and chitosan market has created good demand for the exoskeleton of shellfish through the world by the shrimp processing industry. Chitins is a relatively recent term used to collectively refer chitin and its derivatives such as chitosan and chitosan oligomers. Chitin was first isolated by Braconnot in 1811 from mushroom and was named 'fungine'. Subsequently, an identical material was isolated from insects in 1821 by Odier and named it as "chitine". He was the first person to observe remarkable similarity between cellulose and chitin. In 1859 chitosan, Rouget discovered a partial deacetylated chitin by boiling chitin in concentrated potassium hydroxide. It was finally named chitosan by Hoppe-Seiler (1894) but most information available today has been obtained since 1950. The book "The Integument of Arthropods" by Richards (1951) gave thrust on chitin while Tracey (1957) reviewed the structure and detection and quality analysis of chitin. Jeuniaux (1963) published a book on chitin and its enzymatic breakdown and in 1964 BrimaCombe and Webber wrote a monogram on chitin. In 1967, Rudall first addressed the concept of chitin-protein complex which opened the door for additional work on the subject. A bibliography on chitin and its derivatives was published by Pariser and Boch (1972).

Chitin

Chitin is the most abundant organic compound next to cellulose in the earth. Chitin represents 14-27% and 13-15% of the dry weight of shrimp and crab processing waste, respectively. Chitin is present as chitin-protein complex along with minerals mainly calcium carbonate. So the process of chitin production consists of deproteinisation with dilute alkali and demineralization with dilute acids. Chitin on deacetylation gives chitosan and on hydrolysis with concentrated HCl gives glucosamine hydrochloride. CIFT has developed technology for production of chitin, chitosan and glucosamine hydrochloride from prawn shell waste.

Structure of chitin

Chitin occurs in three polymorphic forms which differ in the arrangement of molecular chain within the crystal cell. The α- chitin is the tightly compacted most crystalline polymorphic form where the chains are arranged in an anti-parallel fashion, β- chitin is the form where the chains are parallel and γ- chitin is the form where the chains are "up" to everyone "down" (Muzzrelli, 1977a). Deacetylation of chitin with strong alkali yields chitosan, polymer of β- (1-4)-D glucosamine (Fig.2).



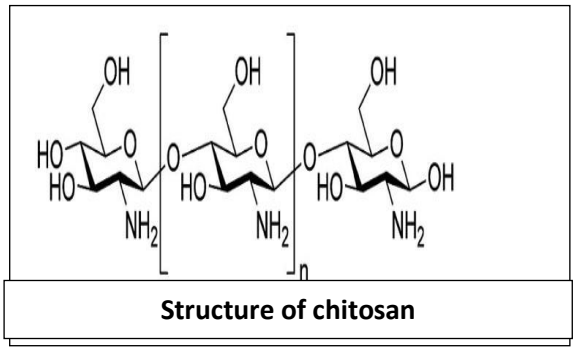
Structure of chitin

Carboxymethyl chitin

Carboxy methyl chitin is another high value derivative of chitin. It has successfully proved its use in the field of cosmetics as moisturizer, skin smoothener and a cleaner for face skin conditioning it is used for the preparation of food products also.

Chitosan

Chitosan is prepared by deacetylation of chitin. Chitosan is almost colorless, light in weight and soluble in dilute organic acids. Its uses are hindered due to its insoluble nature in water, alkali and organic solvents. It gives viscous solution when dissolved in dilute organic acids such as formic acid, acetic acid, citric acid etc. Pure chitosan is not hydrolyzed by lysozyme while chitin or partiallydeacetylated chitin is hydrolyzed. For hydrolysis to occur it is important that at least 6 contiguous acetamido side groups should be present in the substrate. The probability of having 6 contiguous residues with the necessary acetamido side groups decreases as the deacetylation increases. The molecular weight of chitosan derived from intrinsic viscosities of chitin from crab and shrimp generally falls in the range of 150-400 KDa. Depending on the extent of deacetylation chitin contains 5 to 8 % nitrogen, which in chitosan is in the form of primary aliphatic amino group. Chitosan undergoes the reactions typical of amines, of which N-acylation and schiff reactions are the most important. Chitosan derivatives are easily obtained under mild conditions and can be considered as substituted glucans. The properties of chitin and chitosan vary considerably depending on the source and production process. The quality requirements of chitosan and its derivatives vary with the end use.

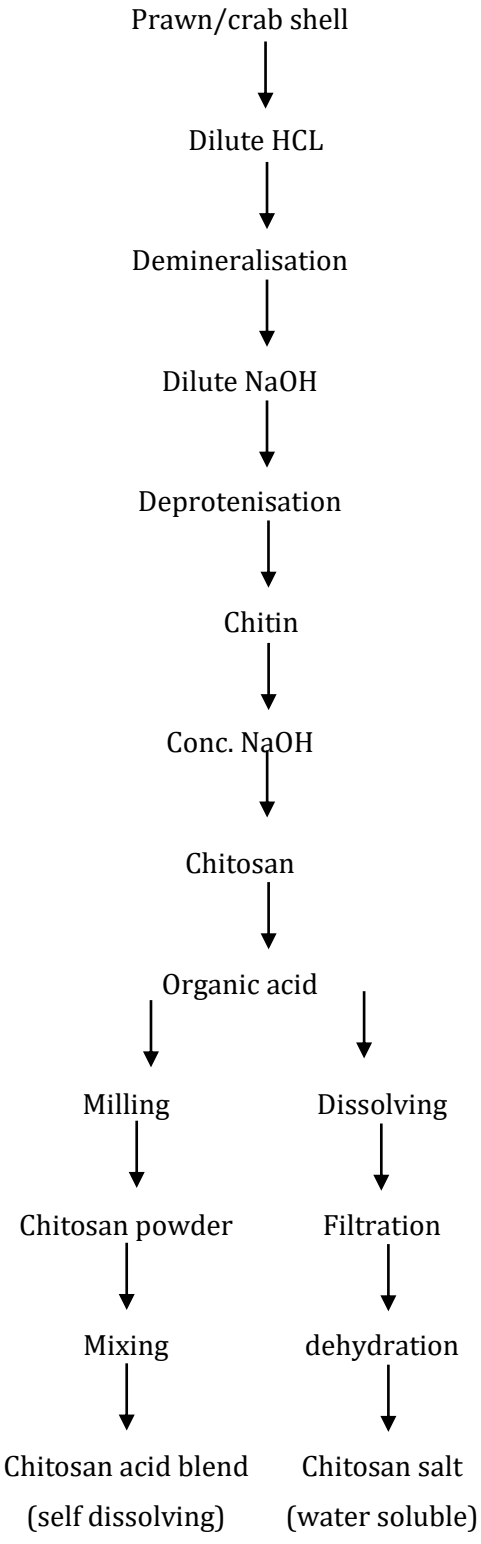


Structure of chitosan

Preparation of chitosan from prawn/crab shells

Chitin represents 14-27% and 13-15% of the dry weight of shrimp and crab processing waste respectively. Dry prawn waste contains 23% and dry squilla contains 15% chitin. Chitin is present as chitin protein complex along with minerals mainly calcium

carbonate. So the process of chitin production consists of deproteinisation with dilute alkali and demineralization with dilute acids. The chitin thus obtained is deacetylated to chitosan using conc. alkali.



Advantages of chitosan

Chitosan is soluble at acidic pH and becomes insoluble at pH above 6.5. Chitosan is a versatile polymer and interest in chitosan is because of its variety of useful forms that are commercially available or can be made available. Since chitosan is non-toxic and can be administered orally, it has mainly been studied as an oral delivery system in the form of tablets or particles (microspheres, beads, etc.). Its muco-adhesive and permeation enhancement properties have been used for transdermal and sustained gastrointestinal drug delivery. Its biodegradability and tissue compatibility make it a suitable compound for implantable delivery devices.

Applications of chitosan

Chitosan with its unique-combination of biological, physical and chemical properties is widely used in a variety of applications in both the industrial and medical fields. These properties produce a novel, versatile-biopolymer that can be tailor-made to suit a specific application with the required modes of function.Chitosan is used in dental and surgical appliances as a haemostatic agent, wound healing, biodegradable films as a replacement for artificial skins for removing toxic heavy metals, wine clarification, industrial effluent treatment, agriculture, photography, cosmetic applications and textiles and as an immobilizing agent for enzyme.The five international conferences on chitin and chitosan (1977, 1982, 1985, 1988 and 1991) have thrown light on various applications in different fields. These applications can be classified under the following heads.

1. Clarification and purification
2. Chromatography
3. Paper and textiles for photography
4. Food and nutrition
5. Medical and pharmaceuticals
6. Agriculture

Agriculture

Many products are marketed based on chitosan for plant protection, growth promotion, seed coating etc. But these applications have not put in to large scale adoption so far. The shelf life of vegetables and fruits also can be extended with the application of chitosan coating. With the growing awareness of the adverse effects of hazardous chemicals in agriculture and popularization of organic farming, chitosan products will find use. Antivirus, antibacterial, nematocidal, insecticidal and pesticidal properties of chitin and chitosan have to be taken to the field by researchers to ensure safety of agriculture products.

Medical and pharmaceuticals

The cholesterol lowering and weight management properties of chitosan has received much attention in recent years. Oral administration of chitin is generally recognized as safe (Harrison 2002). Sugano *et al* (1978) were the first to report the cholesterol lowering effect of chitosan and the report suggests that a diet containing 5% chitosan reduced liver cholesterol to half or more in cholesterol fed rats. In similar studies reduction of both liver and serum cholesterol in rats fed on diets containing 1% chitosan and 0.1% bile salts was observed. Chitin has also shown to reduce plasma cholesterol in cholesterol fed boiler chicken with diet containing 1.5 to 3% chitin. Thus the ability of reducing cholesterol in animals is well established (Razdan and Paterson,

1996).When adult males were fed on chitosan containing biscuits for 2weeks (3g/day for week1, 6g/day for week 2) experienced a significant decrease of 6% in total cholesterol. The reports of animal studies and human trials provide convincing evidence that chitosan is effective in lowering total and LDL cholesterol. Chitosan is also effective in lowering serum cholesterol and hypertension in human with restricted diet and is being used as food supplement in persons suffering from obesity.

Chitosan with its unique-combination of biological, physical and chemical properties is widely used in a variety of applications in both the industrial and medical fields. These properties produce a novel, versatile-biopolymer that can be tailor-made to suit a specific application with the required modes of function. Since chitosan is non-toxic and can be administered orally, it has mainly been studied as an oral delivery system in the form of tablets or particles (microspheres, beads, etc.). Its muco-adhesive and permeation enhancement properties have been used for transdermal and sustained gastrointestinal drug delivery. Its biodegradability and tissue compatibility make it a suitable compound for implantable delivery devices. Despite the outstanding scientific progress being made in terms of the application of chitosan in drug delivery systems, chitosan-based drug delivery products have not yet been launched in the market. Clinical trials involving chitosan-based drug delivery systems are underway for a wide-range of pharmaceutical formulations and some products may be expected in future. With a wide range of potential applications in medicine and pharmaceutics, there is tremendous scope for future research on chitosan and its derivatives. Chitosan certainly seems to be a carrier material of the 21st century in drug delivery devices.

Chitosan derivatives

Chitosan is not soluble in water but is soluble in dilute acid solutions like 1 % acetic acid. This has limited its applications in water soluble environments like human health and plant protection. Hence, the free amino and hydroxyl groups can be derivatized with new molecules to improve the functional properties of Chitosan.

Advantages of Chitosan derivatives

1. They are biodegradable and biocompatible
2. They are non-toxic and water soluble
3. They can be modified to impart special properties

Important derivatives

1. N-Trimethylene Chloride Chitosan: N-Trimethylene chloride Chitosan (TMC) is a quaternary derivative of Chitosan and it has a superior aqueous solubility, intestinal permeability as well as higher absorption over a wide pH range.
2. Chitosan Esters: Esters of chitosan with glutamate, succinate and phthalate have a differential solubility profile. These esteric forms are insoluble in acidic condition and provide sustained release of drugs in basic condition.
3. Chitosan Conjugates: Chitosan can be conjugated with a bioactive excepients for delivery of active ingredients such as Calcitonin. Chitosan conjugates such as 5-methylpyrrolidinone chitosan, chitosan-4-thiobutylamidine conjugate have exhibited enhanced absorption as well as mucoadhesives properties.
4. Carboxymethyl chitosan (CM-Chitosan): CM-Chitosan is prepared by reacting monochloroacetic acid with Chitosan. The derivative is soluble in water and gives

viscous solutions. An unique property of CMC is that it is more thermo-stable compared to the similar structural polymer carboxymethyl cellulose.

Major Applications

1. Controlled release and drug delivery
2. Scaffolds for biomedical applications like stents, organs
3. Tissue engineering, wound healing and regenerative medicine
4. Food supplements and natural preservatives
5. Anti-viral and anti-tumor applications
6. Bio-composite materials with functional properties

Glucosamine hydrochloride

Glucosamine is chemically glucose in which a hydroxyl group on the second carbon atom is substituted with an amino group. It crystalizes as glucosamine hydrochloride during purification under acidic conditions. It is one of the amino sugars used by biological systems for bringing modification to the functions of proteins (Ronda and Zynudheen, 2014).

Health claims of Glucosamine

Although glucosamine was discovered long back, the interest in nutraceutical use received great attention since last two decades.

- ***To treat joint pain***

  The rationale in using glucosamine for arthritis is that it is absorbed by the body and distributed to all organs. In the joint and synovial fluid this glucosamine will stimulate the synthesis of proteoglycans that help in repair of damaged cartilage. Although many clinical trials have shown benefits, the evidence is not equivocal. There are more than 100 generic preparations of glucosamine alone or in combination in the market.

- ***As stomach antacid***

  Research in mice has shown that, glucosamine is having good acid neutralization and peptic ulcer healing properties. Peptic ulcers is a major problem affecting adult human beings and oral administration of glucosamine will relieve pain associated with it and also helps in synthesis of gastric mucosa to repair the ulcer.

- ***Anti-aging property***

  Latest research has claimed that glucosamine supplementation mimics low calorie diet in rats and increased the life span compared to control animals. Calorie restriction was proven in animals to improve the life span in laboratory studies. Although the mechanism of action of glucosamine is not clear in this case, it was shown to reduce the amount of glucose metabolized through the glycolytic pathway thus mimics low calorie diet.

- ***Wound healing***

  Hyaluronic acid is highly hydrated and provides strength and elasticity to the skin. By binding and retaining some moisture in a wound re-epithelialization can proceed more quickly. This water-binding effect is also important for cosmetic uses of

glucosamine. It can increase the skin's content of hyaluronic acid to increase moisturization, leading to enhanced skin barrier properties and reduced dryness

- ***Cosmetics***

  Glucosamine has also been reported to have potential to inhibit skin melanin production. Glucosamine has been shown to inhibit glycosylation, the addition of polysaccharide units to proteins in in-vitro melanocyte cell culture. Glycosylation is a required step in the conversion of certain inactive pro-enzymes to their active forms. Active tyrosinase, a key enzyme in the pathway for melanin production, is glycosylated. Thus, glucosamine inhibits the production of melanin in melanocytes.

Suggested readings

1. Tracey, M. V. (1957) Rev. Pure Applied Chemistry. 7: 1-14
2. Pariser, E.R. and Boch, S. (1972). Derivatives – An annotated Bibliography of selected publications from 1965 to 1971. 142-146. MIT Cambridge, Mass.
3. Domard, A., Jeuniaux, C., Muzzarelli, R., Roberts, G. 1996. Advances in chitin science. Jacquez ANDRE Publisher, France

****

Collagen, Gelatin and its Derivatives from Fish Wastes

Mandakini Devi Hanjabam

Fish Processing Division

ICAR-Central Institute of Fisheries Technology

Email: mandakinicife@gmail.com

Processing of fish generate enormous amount of by-products (wastes). Fish processing waste is defined as secondary fish material generated during primary processing of the fish. The secondary fish material is in the form of scales, skin, head, viscera, bones, frames, fins etc. and comprised of around 50-80 % of the whole fish. Such waste generated in fish processing plant can become raw material for auxiliary industries or other industries. Some of the high value products which can be obtained from the solid waste of fish are oils, pigments, meals, minerals, enzymes and protein concentrate etc. Other possible utilization of processing waste would be manufacturing of valuable biomolecules such as collagen and gelatin. It is estimated that about 30% of the wastes generated in fish processing consists of skin and bone which are rich in collagen content. The global collagen market is anticipated to reach USD 6.63 billion by 2025. Collagen, gelatin and their hydrolyzed products are the key product segments in this market. The market is growing due to increasing demand for collagen-based products in healthcare applications (such as wound healing, tissue engineering, bone reconstruction etc), food & beverages and cosmetics industries.

Collagen and gelatin

Collagen is a structural protein having a characteristics triple helix structure. Collagen is insoluble in water and fibrous in nature. Approximate molecular weight of a collagen molecule is 300KDa. There are 19 genetically distinct collagen types which are characterized by considerable complexity and diversity in their structure, their splice variants, and the presence of additional, non-helical domains, their assembly and their function. Each of the three α-chains within the molecule forms an extended left-handed helix with a pitch of 18 amino acids per turn. The proper folding of each of these chains requires a glycine residue to be present in every third position in the polypeptide chain. For example, each α-chain is composed of multiple triplet sequences of repeating Gly-X-Y units where Gly is glycine, Y positions are mostly occupied by the imino acid proline and hydroxyproline and X is any of the other amino acids, account for 2% of the molecule and plays a vital role in fibril formation. The three chains are supercoiled around a central axis in a right-handed manner to form the triple helix known as tropocollagen. The triple-helix is approximately 300 nm in length and 1.5 nm in diameter, followed by short extra helical telopeptides. The telopeptides do not adopt triple-helical conformations due to the absence of repeating Gly-X-Y units. The triple helices are stabilized by inter-chain hydrogen bonds. β dimmers and γ trimmers are also found in collagen. The β component is due to the intermolecular crosslinking while that of γ component indicates intramolecular crosslinking. Collagen is rich in nonpolar amino acids such as glycine, proline and hydroxyproline. The stability of the collagen triple helix is related to the total content of proline and hydroxyproline. Collagen derived from fish is generally of Type I and Type III. Type I and Type III collagen are the building blocks for connective tissues, bones and skins.

Gelatin is soluble protein produced by controlled hydrolysis of fibrous insoluble collagen. Warm-water extraction process is generally used for hydrolysis of collagen into gelatin. Heat treatment cleaves the hydrogen and covalent bonds to destabilize the triple-helix, resulting in helix-to-coil transition and conversion into soluble gelatin.

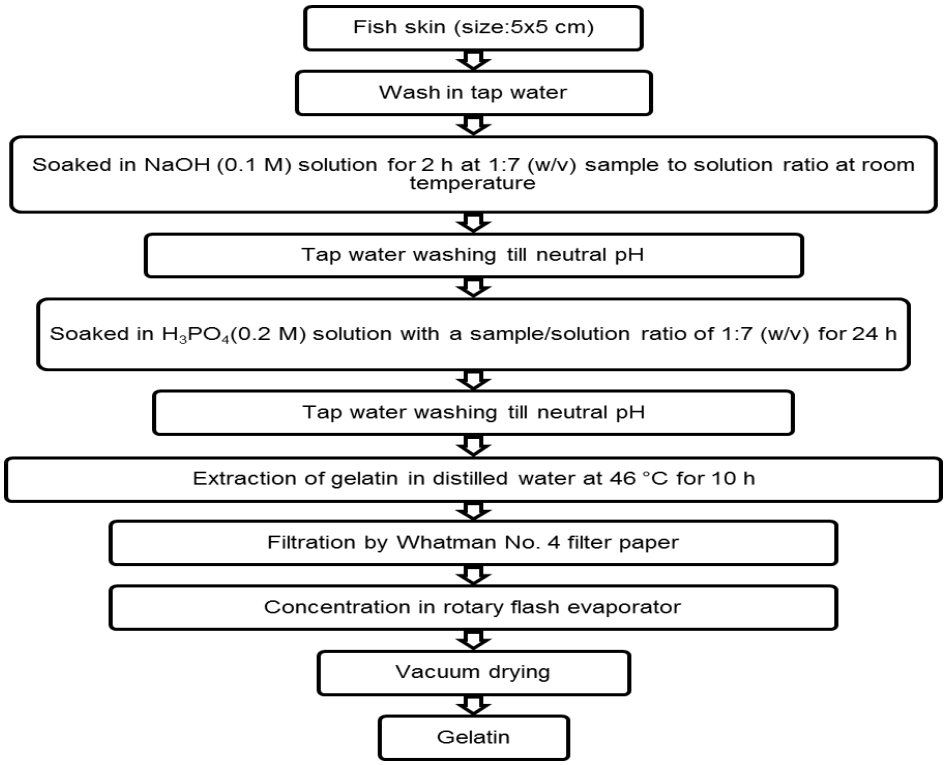


Figure 1: General steps for isolation of gelatin from fish skin. (Source: Hanjabam *et al.*, 2015)

Some of the functional properties of collagen and gelatin are discussed here. Gel strength/Bloom value is the test to measure the strength of gel. The gelation process for both collagen and gelatin is thermo-reversible, but in opposite directions: collagen gels melt by lowering the temperature, while gelatin gels melt by raising the temperature. The commercial value of gelatin is determined by its bloom value. Fish gelatin typically has a gel strength ranging from as low as 0 to 426 g. warm-water fish gelatin have been reported to exhibit high gel strengths than cold water fish gelatin.

The setting and melting points of gelatin are also considered important indices of the quality of gelatin preparations. Being thermo-reversible, gelatin gels will start melting when the temperature is increased above a specific point, the melting point, which is usually lower than the temperature of the human body. For gelatins from fish species, setting temperatures are in the range of 8–25°C, while the range of melting temperatures is 11–28°C. The melting and gelling temperatures of gelatin have been shown to correlate with the proportion of Proline and Hydroxyproline in the original collagen.

Collagen is not soluble in water. However, fish type I collagen is unique in its extremely high solubility in dilute acid compared to avian and mammalian collagen. Gelatin is only partially soluble in cold water; however dry gelatin swells or hydrates when stirred in water. On warming to about 40°C gelatin that has been allowed to hydrate for 30 minutes melts to give a uniform solution. The solubility of collagen is affected by the pH and NaCl concentration of the solution.

Collagen and gelatin hydrolysates

Although collagen/gelatin has several functional properties, its bioactivity is lower due to its high molecular weight. Hydrolyzing will enhance the bioactivities of the collagen/gelatin. Collagen or gelatin hydrolysates are produced by controlled hydrolysis of collagen or gelatin. Acid, alkali, enzyme or heat may be used for hydrolysis. During hydrolysis the peptide bonds are broken down producing low molecular weight peptides. The molecular weight of hydrolysate is generally in the range of 5.0-25 kDa. In case of gelatin, hydrolysate can be produced using two different processes. In the first process, hydrolysate could be manufactured after gelatin extraction from the source by enzymatic hydrolysis. In the second process, the hydrolysates derived peptides can be prepared without prior extraction of gelatin. The second process could shorten the processing time and production costs by eliminating the gelatin extraction step. Commonly used proteases and their reported optimal hydrolysis conditions (pH and temperature) for the production of hydrolysate protease are alcalase (8.0–9.5; 50–60), pepsin (2.0; 37-50), papain (6.5–7.0; 40–70), trypsin (7.0–8.0; 37–50), pancreatin (7.0–8.0; 37–50), bromelain (7.0; 40-50), flavourzyme (6.0–7.0; 50–60), protamex (7.0; 50), neutrase (6.5–7.0; 50–60) etc. Figure 2 shows the overall process for production of gelatin hydrolysate from fish skin using thermal and enzymatic hydrolysis. Thus hydrolysis of collagen or gelatin yields bioactive peptides that have great potential in processing industries as natural preservatives. Collagen and gelatin peptides are known to have excellent antioxidant properties unlike its parent molecules. Recently gelatin hydrolysate has been explored as plastisizer in protein film, identified as antihypertensive, cryoprotectant in additions to its wide known antioxidant activity.

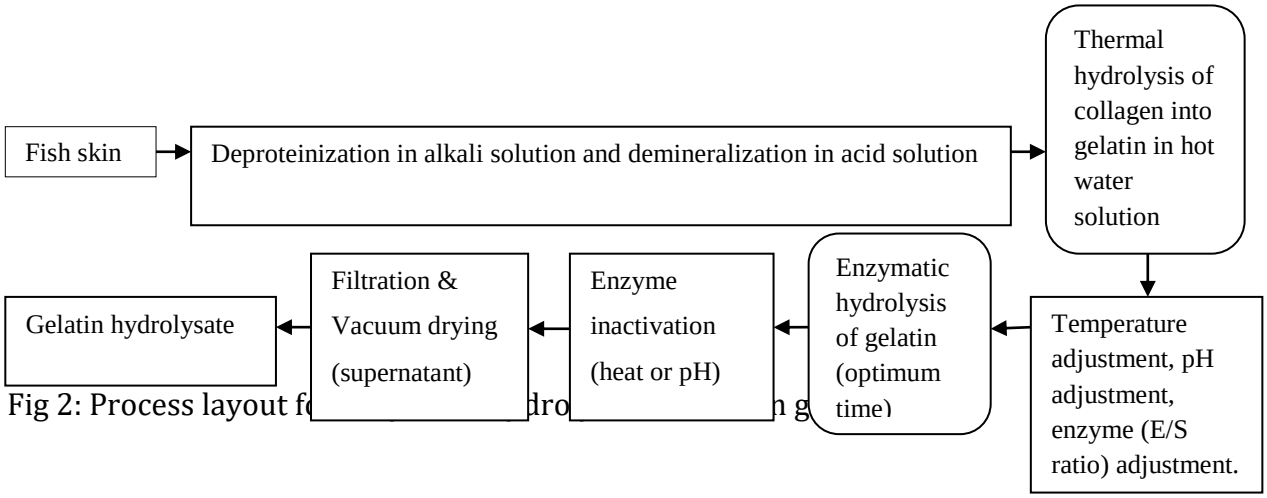


Fig 2: Process layout fo dro n g

The recovery of chemical components from seafood waste materials, which can be used in other segments of the food industry, is a promising area of research and development for the utilization of seafood by-products.

References:

Hanjabam, M. D., Kannaiyan, S. K., Kamei, G., Jakhar, J. K., Chouksey, M. K., & Gudipati, V. (2015). Optimisation of gelatin extraction from Unicorn leatherjacket (*Aluterus monoceros)* skin waste: response surface approach. *Journal of food science and technology*, *52*(2), 976-983.

****

Protein Hydrolysates from Fish Processing Waste: Health Benefits and their Potential Application

Elavarasan Krishnamoorthy
Fish Processing Division,
ICAR-Central Institute of Fisheries Technology,
Email: elafishes@gmail.com

Fish processing operations generate more than 60 % of the raw material. In developing country like India, these wastes are disposed or converted into animal feed, fish meal and fertilizer. This practice leads to underutilization of raw material and may affect the sustainable utilization of available resources. The disposal of fish processing waste is under strict regulations due to environmental issues and it adds to the operational cost of seafood industry (Elavarasan et al., 2016).Hence, the effective utilization of fish processing waste is gaining importance.Approximate quantity of waste generated during processing of major type of seafood products are presented in Table.1. There is no authenticated data on by-product generation from Indian fish processing sector. The amount of waste generated will vary with the size, style of product and species, nature of handling (machine/manual handling, skill of operating/handling person). The major wastes from crustaceans are shell waste which is utilized to some extent as a raw material in chitin industry. Wastes from fin fishes are containing considerable quantity of proteins which can be converted/recovered in to protein hydrolysates for improved utilization. From fish protein hydrolysate industry point of view, quantity of fin fish wastes / by-products is more important.

Global protein market

Global protein ingredient market analysis revealed that 43.2 % of revenues in the global health ingredients market contributed from protein (Fig. 3). Europe retains its big lead in the global protein market. Key products under animal protein ingredients include, gelatin, collagen, egg and dairy. There is a stable demand for animal protein ingredients. The global protein market is expected to reach the value of US $ 40.88 Billion (2.7843618 trillion Indian rupees) by 2022. The use of protein ingredients in infant formula reduced protein deficiencies. The use of protein ingredients in pharmaceutical and cosmetic industry is increasing continuously.Global protein ingredient market share is moderately consolidated with DuPont, Bunge, ADM, Cargill, and Mead Johnson being the major industry players.Raw material availability in China and India influence industry players to shift manufacturing base in the region.The protein supplements market in India is growing at 6%, and is currently valued at Rs. 252 crores annually.

Fish protein hydrolysate

Fish protein hydrolysate is a product prepared from proteins sourced from fish meat/fish processing by products via enzymatic or chemical process. Enzymatically produced hydrolysates are widely accepted which contain mixture of peptides of varying sizes and free amino acids.

Process for production of protein hydrolysate

Protein hydrolysates from fish processing discards can be prepared using four different process namely acid process, alkali process, enzymatic process and microbial fermentation. The basic mechanism of enzymatic hydrolysis and effect of different factors is discussed below.

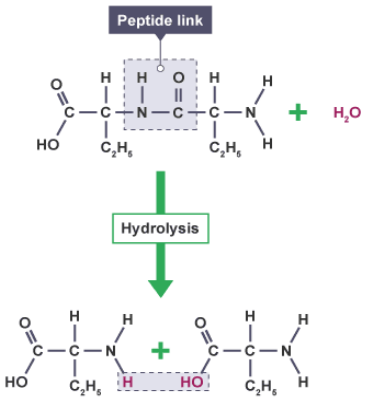


Fig. 4Hydrolysis of peptide bond

Enzymatic process

Enzymatic hydrolysis of fishery by products use either autolysis process or by adding exogenous protein. Autolysis process involves incubating ground fishery waste at optimum reaction conditions of endogenous enzymes and also uses the fish visceral waste (Kristinsson and Resco, 2000). The endogenous enzymes trigger the breaking down of biomolecules to smaller peptides through autolysis process. The autolysis is usually conducted at neutral or slightly alkaline pH, exploiting the presence of serine protease of intestine in alkaline or the carboxyl pro-tease of gastric juice in acidic pH (Pastoriza*et al.,* 2004).

Proteolysis is the enzymatic hydrolysis of the amide bond in peptides and proteins. The enzymes are exploited to perform desired functions in processing and analysis and to facilitate the conversions of raw materials into high quality more desirable foodstuffs (Richardson and Hyslop, 1984**)**. Enzymes used in the food industry and research are predominantly hydrolases. Proteolytic enzymes are economically the most important group of enzymes and their use is well established in the food industry (Godfrey and Reichelt, 1983**)**. Use of proteases in the preparation of fish protein hydrolysates has received a wide attention among the researchers as it is more economical and ease of process control. The nature of enzymes, substrate and hydrolysis will determine the properties of (Fish Protein Hydrolysate) FPH. The general flow line for the production of FPH by use of enzymes is depicted in Fig 1. The process involves homogenization of fish meat or fish waste with addition of water. The homogenate is brought to the optimum temperature and pH. The hydrolysis is initiated by the addition of enzyme at desired concentration. After a particular duration of incubation, the hydrolysis is terminated by applying heat or by adjusting the pH. The soluble fraction after removing the unhydrolysed portion is concentrated by freeze drying / oven drying / spray drying. The dried protein powder is referred as protein hydrolysate.

1. In order to produce the FPH with different and desired properties, it is important to know the mechanism of protein hydrolysis. Some proteases preferentially catalyze the hydrolysis of bonds adjacent to a particular amino acid residue,

while some are less specific. The catalysis by proteases occurs primarily as three consecutive reactions (Krsitinsson and Rasco, 2000): the formation of complex between the original peptide chain and the enzyme referred as the Michaelis complex

2. cleavage of the peptide bond to liberate one of the two peptides
3. nucleophilic attack on the remains of the complex to split off the other peptide and to reconstitute the free enzyme

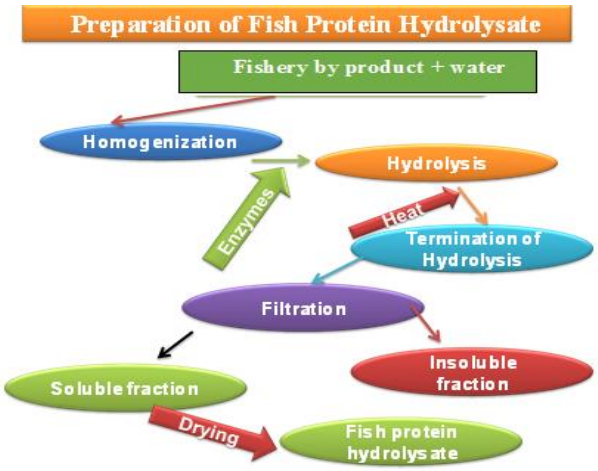
The hydrolysis of peptide bonds leads to an increase in the numbers of ionizable groups (NH_3^+ and COO^-), with a concomitant increase in hydrophobicity and net charge, decrease in molecular size of the polypeptide chain, and an alteration of the molecular structure leading to the exposure of the buried hydrophobic residues to the aqueous environment (Phillips and Beuchat, 1981; Kester and Richardson, 1984; Mahmoud *et al*., 1992). Upon addition of enzyme to the proteins, the enzyme-substrate complex will be formed. This complex is referred as Michaelis complex which may dissociate back to reactant substrate and free enzyme, or to free enzyme and product molecules (Adler Nissen, 1986). The generally accepted mechanism for proteases indicates that the second step that is dissociation of enzyme substratecomplex into free enzyme and product is the rate-determining step, which determines the overall rate of reaction.

Enzymatic hydrolysis of proteins is a complex process because of several peptide bonds and their specific accessibility to enzymatic reactions (Linder *et al*., 1995). The specificity of enzymes is not the only factor that affects the peptide profile of the final product and factors such as temperature and pH play an important role. The temperature and pH can greatly affect the enzyme reaction kinetics and theireffect is different for each enzyme. Generally, there is an optimum combination of both pH and temperature, where the enzyme is most active. Temperature and pH extremes deactivate the enzymes by denaturing them.

The factors involved in hydrolysis of proteins are most important both in terms of kinetics and nature of the end product. The most important factors influencing the properties of FPH are nature of substrate, nature of protease and degree of hydrolysis (DH) and drying method.

Protein hydrolysates from different fish processing –by products

Different wastes generated during fish processing like head, skin, roe, frame waste and bone have been used to produce the hydrolysate. Alternatively, the proteins isolated from the waste parts can also be used for this purpose. The protein content in different fish waste parts are presented in Table 1. Most of the studies have been carried out with reference to the hydrolysis process and their bioactive and functional properties.



General scheme used for enzymatic hydrolysis of fishery by-product

Table 1. Protein content in major fish waste parts

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

(Source: Rustard, 2007**)**

Scientific studies have been reported for the preparation of fish protein hydrolysate from fish head, viscera, roe, skin, frame and bone. Most of these studies have focused on their antioxidant properties and various antioxidant peptide molecules have been isolated and characterized. Chalamaih et al. (2012) has exhaustively reviewed the protein hydrolysates from various parts of fish waste. Fish head is a major fishery waste contains gills. Eyes, head frame and shoulder muscle. It is difficult to recover the protein due to its structural complexity. Enzymatic process will solubilize the protein by converting into peptide forms then facilitate easy recovery of proteins. Protein hydrolysatesfrom fish head by-product waste have been prepared from various species. The major protein present in fish head is collagen. Hence the peptides generated will have usually the sequence from collagen which are known for their anti-arthritis and anti-obesity properties. Fish skin is again a rich source of collagen. Attempts have been made to produce the hydrolysate either directly from the fish skin or after isolating the collagen or gelatin. Fish liver is an another by product usually goes for oil and meal production. Tuna liver has been used to prepare the hydrolysateusing protamex, flavorzyme, alacalase and neutrase (Je et al. 2009; Ahn et al, 2010). Fish viscera isalso a potential source ofprotein that can serve as a raw material for the preparation ofprotein hydrolysates. It is expected that visceral waste protein hydrolysate mayexhibitunique properties. In recent times, many attemptshave been performed for the utilization of fish visceral waste for protein hydrolysates production (Batista et al., 2010). Fish roe contains considerableamount of protein. In order to utilize this underutilizedprotein

source from fish roe, protein hydrolysates have been prepared. For example, roe protein hydrolysate from *Cirrhinusmrigala*using alcalase andpapain has been reported(Chalamaiah et al., 2010). Fish bone, which is separated after removal of muscle proteins on the frame, is another valuable source in identifying health-promoting components. The organic component of fish bone, which accounts for 30% of the material, is made out of collagen. Therefore, fish bone is considered as a source for protein hydrolysateparticularly collagen peptides and gelatin hydrolysates (Kim and Mendis, 2006).

Application of fish protein hydrolysates

Nutritional application

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

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

Table 2. Proximate composition of fish protein hydrolysate

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

(Source; Chalamaiah et al., 2010)

Nutraceutical applications

There are fish protein hydrolysate products/peptides specifically marketed as health supplements in developed countries (Table 3). These products are proven to have specific health role other than the nutritional benefit. Protein hyd5rolysates or peptides present in the hydrolysate have demonstrated to have antioxidant, anti-obesity, immune modulation, anti-coagulation, anti-microbial, anticancer and antihypertension etc. (Elavarasan et al., 2014; and Elavarasan et al., 2016).

Fish protein hydrolysate as a functional ingredient

Fish protein hydrolysates are soluble in wide range of pH which is an ideal characteristic helps to use in wide range of products. Protein hydrolysates have improved water holding, oil binding, emulsifying and foaming properties. However, the key factor which determine the functional properties is degree of hydrolysis. In general, extensive hydrolysis leads to loss of functionality. There is a critical degree of hydrolysis

at which protein hydrolysates should be prepared with reference to particular function to be used as a functional ingredient (Elavarasan et al., 2016; Gajanan et al., 2017).

Fish protein hydrolysate as a feed ingredient and other applications

Fish protein hydrolysates (FPHs) have been used in aquaculture feeds in order to enhance the growth and survival of fish. Studies have shown that FPH has boosted

Table 3. Commercially marketed fish protein hdyrolysate products as Nutraceuticals

Product brand name	Particulars	Nutraceutical applications	Country
PROTIZEN®	Produced by enzymatic hydrolysis of white fish proteins	It is “mood food” and dietary supplement to fight against stress and its symptoms (weight disorders<comma> work pressure<comma> sleep troubles<comma> concentration difficulties and mood troubles).	UK
Amizate®	Produced from Atlantic salmon fish proteins by autolysis	Sports nutrition (supports the body’s muscle anabolism and metabolic recovery).	North America
Seacure®	Prepared by hydrolyzing deep ocean white fish proteins	Dietary supplement helps to support the cells in the gastrointestinal tract and regulate bowel functions.	US and Canada
Vasotensin®	Produced from Bonito (*Sardaorientalis*) by thermolysin hydrolysis	It supports healthy vascular function for optimal blood flow and healthy blood pressure levels.	US and Japan
LIQUAMEN®	Prepared from *Molvamolva* by autolysis	Dietary supplement that helps in reducing oxidative stress<comma> lowering glycemic index and anti-stress.	UK
Stabilium® 200	Prepared from *Molvadypterygia* by autolysis	Supports the body’s response to stress and provides nutritional support for memory and cognitive function.	UK
PEPTACE®	Produced from Bonito (*Sardaorientalis*) by thermolysin hydrolysis	It lowers the blood pressure by inhibiting ACE enzyme.	US and Japan
MOLVAL®	Produced from North Atlantic fish *Molvamolva* by enzymatic hydrolysis	Dietary supplement recommended for cholesterol equilibrium<comma> stress control and promotes good cardiovascular health.	UK

(Source: Chalamaiah et al., 2010)

the growth performance and immunological status of many culture species. The amino acid composition and the peptides present in hydrolysate are responsible for the improved growth and immunological status. FPH is also being used as a source of protein in poultry feed formulation and in pet animal foods. Other applications include FPH as a plant booster, ingredient in microbiological media and as a cryo-protectant in fish mince/surimi.

Safety of protein hydrolysates in human nutrition

In general, food business operator should ensure the safety of products. The safety aspects of any food ingredient need to be documented before release in the market. Protein hydrolysates can be considered as safe when they are hydrolysed from proteins having a history of safe for consumption and they are produced using proteases which are of food-grade and used common food-processing methods. The safety of fractions and bioactive peptides, derived from safe hydrolysates, should be evaluated by the manufacture before market introduction. A review of the safety assessment of the company by an external independent committee and subsequent approval by the competent authorities according to novel food procedures is essential when the source of protein and process is novel and under unusual high intake of amino acids (Schaafsma, 2009).

Conclusion

The fish processing industry in India generate huge protein rich material which is untappedand can be utilized by converting in to protein hydrolysate. Depends on the properties and chemical composition, further FPH finds application in various industries ranging from nutraceutical to plant growth boosting ingredient. Recent interest of FPH as nutraceutical compound/bioactive peptide demands hygienic handling and proper preservation of fish processing waste. However, safety of FPH when produced from fishery waste, economic feasibility and business case are remains unaddressed worldwide.

References

Kristinsson HG, Rasco BA. Fish protein hydrolysates: production, biochemical, and functional properties. Critical reviews in food science and nutrition. 2000 Jan 1;40(1):43-81.

Pastoriza L, Sampedro G, Cabo ML, Herrera JJ, Bernárdez M. Solubilisation of proteins from rayfish residues by endogenous and commercial enzymes. Journal of the Science of Food and Agriculture. 2004 Jan 15;84(1):83-8.

Godfrey, T. and Reichelt, J., 1983. Industrial Enzymology. The Application of Enzymes in Industry, MacMillan, London, UK.

Richardson, T. and Hyslop, D.B., 1984. Enzymes. *In*: Food Chemistry Edt. Fennema, O.R. Edn. 2nd ., Marcel Dekker Inc., New York.

Kester, J.J. and Richardson, T. 1984. Modification of whey proteins to improve functionality. *J. Dairy Sci*., **67**: 2757-2774

Mahmoud, M.I., Malone, W.T. and Cordle, C.T., 1992. Enzymatic hydrolysis of casein: effect of degree ofhydrolysis on antigenicity and physical properties, *J. Food Sci.*, **57**: 1223-1229

Adler-Nissen, J.,1986. Enzymic Hydrolysis of Food Proteins, Elsevier Applied Science Publishers, Barking, UK.

Linder, M., Fanni, J., Parmentier, M., Sergent, M. and Phan-Tan-Luu, R., 1995, Protein recovery from veal bones by enzymatic hydrolysis. *J. Food Sci*., **60**: 949–952

Jemil I, Jridi M, Nasri R, Ktari N, Salem RB, Mehiri M, Hajji M, Nasri M. Functional, antioxidant and antibacterial properties of protein hydrolysates prepared from fish meat fermented by Bacillus subtilis A26. Process Biochemistry. 2014 Jun 30;49(6):963-72.

Venugopal, V. Production of fish protein hydrolysates by microorganisms. pp. 223 – 243, *in*: Martin, AM (Ed) 1994. Fisheries processing: biotechnological applications. *Chapman & Hall*, London, UK.

Godinho IS. *Production of fish protein hydrolysates by a marine proteolytic strain* (Doctoral dissertation, ISA).

Murthy PS, Rai AK, Bhaskar N. Fermentative recovery of lipids and proteins from freshwater fish head waste with reference to antimicrobial and antioxidant properties of protein hydrolysate. Journal of food science and technology. 2014 Sep 1;51(9):1884-92.

Rustad T. Physical and chemical properties of protein seafood by-products. Maximising the value of marine by-products. 2007:3-21.

Chalamaiah M, Hemalatha R, Jyothirmayi T. Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. Food Chemistry. 2012 Dec 15;135(4):3020-38.

Je JY, Lee KH, Lee MH, Ahn CB. Antioxidant and antihypertensive protein hydrolysates produced from tuna liver by enzymatic hydrolysis. Food Research International. 2009 Nov 30;42(9):1266-72.

Ahn CB, Lee KH, Je JY. Enzymatic production of bioactive protein hydrolysates from tuna liver: effects of enzymes and molecular weight on bioactivity. International journal of food science & technology. 2010 Mar 1;45(3):562-8.

Batista I, Ramos C, Coutinho J, Bandarra NM, Nunes ML. Characterization of protein hydrolysates and lipids obtained from black scabbardfish (Aphanopuscarbo) by-products and antioxidative activity of the hydrolysates produced. Process Biochemistry. 2010 Jan 31;45(1):18-24.

Chalamaiah M, Jyothirmayi T, Bhaskarachary K, Vajreswari A, Hemalatha R, Kumar BD. Chemical composition, molecular mass distribution and antioxidant capacity of rohu (Labeorohita) roe (egg) protein hydrolysates prepared by gastrointestinal proteases. Food research international. 2013 Jun 30;52(1):221-9.

Kim SK, Mendis E. Bioactive compounds from marine processing byproducts–a review. Food Research International. 2006 May 31;39(4):383-93.

Elavarasan K, Naveen Kumar V, Shamasundar BA. Antioxidant and functional properties of fish protein hydrolysates from fresh water carp (Catlacatla) as influenced by the nature of enzyme. Journal of Food Processing and Preservation. 2014 Jun 1;38(3):1207-14.

Elavarasan K, Shamasundar BA, Badii F, Howell N. Angiotensin I-converting enzyme (ACE) inhibitory activity and structural properties of oven-and freeze-dried protein hydrolysate from fresh water fish (Cirrhinusmrigala). Food chemistry. 2016 Sep 1;206:210-6.

Elavarasan K, Shamasundar BA. Effect of oven drying and freeze drying on the antioxidant and functional properties of protein hydrolysates derived from freshwater fish (Cirrhinusmrigala) using papain enzyme. Journal of food science and technology. 2016 Feb 1;53(2):1303-11.

Gajanan PG, Elavarasan K, Shamasundar BA. Bioactive and functional properties of protein hydrolysates from fish frame processing waste using plant proteases. Environmental Science and Pollution Research. 2016 Dec 1;23(24):24901-11.

Schaafsma G. Safety of protein hydrolysates, fractions thereof and bioactive peptides in human nutrition. European journal of clinical nutrition. 2009 Oct 1;63(10):1161-8.

****

Fish Meal and Oil from Fish Waste: An Industrial Perspective

Elavarasan Krishnamoorthy

Fish Processing Division

ICAR-Central Institute of Fisheries Technology, Cochin

Email: elafishes@gmail.com

Introduction:

In the year 2016, 88.5% of the world fish production used for human consumption and the rest around 11.5 MT diverted for non-food uses which includes meal and manure. Fish processing generates a huge amount of raw material as waste (45-55%). The processing waste includes skin, scale, visceral mass, head, fins, filleting frame waste etc. If these fish processing wastes are left unattended, they create pollution problems, civic problems and can cause spread of diseases such as cholera. Hence, there is a need to convert these waste into stable products such as fish meal and oil to overcome the problems.

WORLD FISHERIES AND AQUACULTURE PRODUCTION AND UTILIZATION (MILLION TONNES)[a]

Category	2011	2012	2013	2014	2015	2016
Production						
Capture						
Inland	10.7	11.2	11.2	11.3	11.4	11.6
Marine	81.5	78.4	79.4	79.9	81.2	79.3
Total capture	**92.2**	**89.5**	**90.6**	**91.2**	**92.7**	**90.9**
Aquaculture						
Inland	38.6	42.0	44.8	46.9	48.6	51.4
Marine	23.2	24.4	25.4	26.8	27.5	28.7
Total aquaculture	**61.8**	**66.4**	**70.2**	**73.7**	**76.1**	**80.0**
Total world fisheries and aquaculture	**154.0**	**156.0**	**160.7**	**164.9**	**168.7**	**170.9**
Utilization[b]						
Human consumption	130.0	136.4	140.1	144.8	148.4	151.2
Non-food uses	24.0	19.6	20.6	20.0	20.3	19.7
Population (*billions*)[c]	7.0	7.1	7.2	7.3	7.3	7.4
Per capita apparent consumption (*kg*)	18.5	19.2	19.5	19.9	20.2	20.3

[a] Excludes aquatic mammals, crocodiles, alligators and caimans, seaweeds and other aquatic plants.
[b] Utilization data for 2014–2016 are provisional estimates.
[c] Source of population figures: UN, 2015a.

(Courtesy: FAO, The state of world fisheries and aquaculture, 2018)

Fish meal

Fish meal is a dry product having brownish grey colour and milled to a fine to course powder. It is produced by removal of 90 to 95% of water and fat present in the raw material. Generally oily pelagic fish is being used all over the world for fish meal manufacture. It is a good source of major nutrients such as protein and contains fair amount of fat. It is rich in essential minerals, namely phosphorus, calcium and iron. It is also a good source of micro minerals, oil soluble vitamins and water soluble vitamins.

Fish oil

Fish oil is a by-product obtained during fish meal production and then subjected through various steps in order to yield the final product. The oils contain mainly triglycerides of fatty acids (glycerol combined with three similar or different acid molecules) with variable amounts of phospholipids, glycerol ethers and wax esters. It is characteristic of the oils that they contain a wide range of long-chain fatty acids with the number of carbon atoms ranging mainly from 14 to 22, and high degree of reactivity (unsaturation) ranging up to six double bonds per molecule (FAO, 1986).Fish oil is high in unsaturated fats and also aids in reducing blood cholesterol level. They are imparting positive effect in powerful metabolic and physiological regulators, which also influence the excessive fat deposition in the arteries.

- body oil of fish is more important as an industrial product besides its limited use in human consumption.
- body oils have recently won much attention
- Contains poly unsaturated fatty acids (PUFA), particularly n-3 PUFA
- n-3 PUFA used in the control of heart ailments in humans
- known to haveanticholesterolemic effect
- highly unsaturated fish body oils can be used as drying oils in paints and varnishes
- as a medium in fish canning.
- finds use in margarine
- used as carriers of fat soluble vitamins A and D. find use in the manufacture of linoleum, detergents, artificial rubber, lubricants, printing inks, soaps etc.

Manufacturing Process

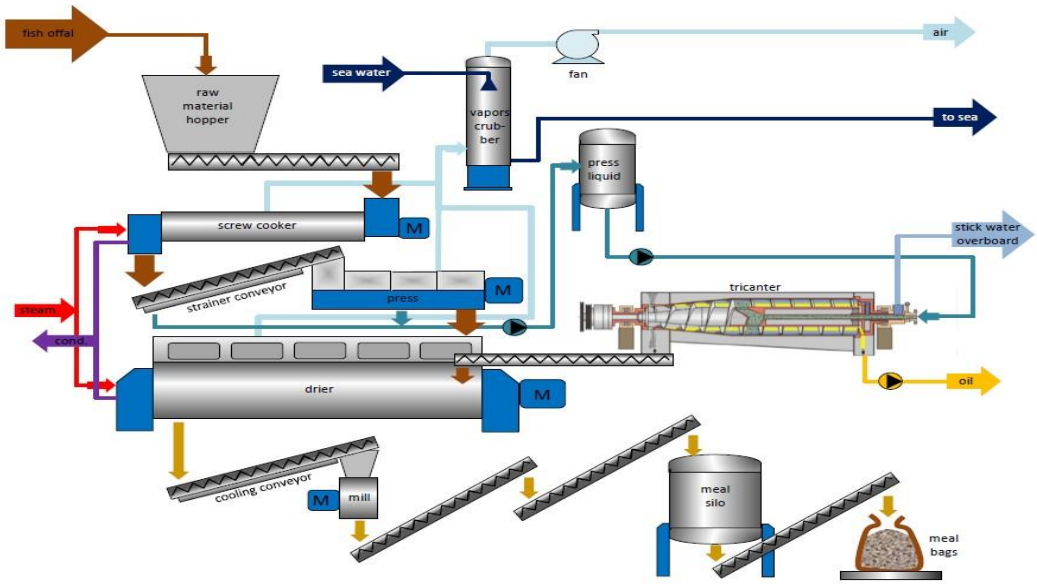
The main objective in the production of fish meal is to reduce the moisture content of fresh fish (70-80%) to about less than 10% in the meal. In other words, about 90-95% of moisture in fresh fish is to be removed. Oil content in the fish meal should not be more than 10%. Hence, 80 to 90% of oil present in fish has to be removed during fish meal production.

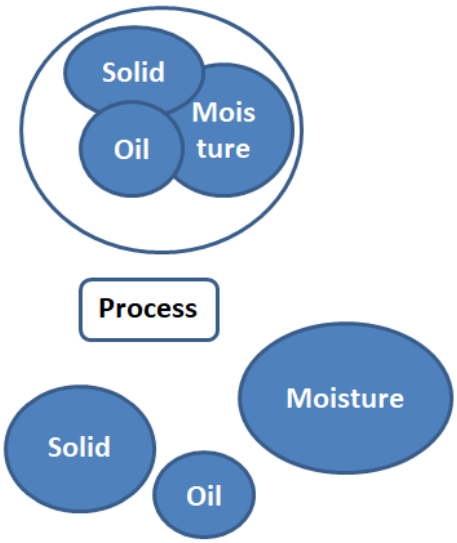
There are essentially two methods of fish meal production.

(1) Wet reduction method (suitable for low fat and high fat raw material)
(2) Dry reduction method (suitable for low fat raw material)

At present bulk of the fish meal is produced by wet reduction method all over the world including India. The main unit operations involved in fish meal production by wet reduction method is shown in the process flow.

Process flow in fish meal and oil production from processing waste





In India, oil sardine (*Sardinellalongiceps*) is extensively used for the production of fish meal and oil. Most of the pelagic fishes mentioned earlier are rich in body oil. Hence both fish meal and fish body oil are produced in the same industry. Freshness of fish is very important in getting good quality fish meal. If the fish has lost its freshness, it will have high TVBN content and consequently, the meal produced from it will also contain high TVBN which is unacceptable to shrimp feed industry. In addition to oil sardines, fish dressing waste or cutting wastes (head and viscera) of surimi industry are also used in fish meal manufacture in India. In this case also, the quality parameters regarding freshness of waste have to be maintained. Generally, fish meal produced from fish processing waste; contain low percentage of proteins and high proportion of ash/minerals. Hence, it is not possible to produce Grade I fish meal using only wastes from fish processing industry.

Process steps in reducing the fish waste to fish meal and oil

- heating, which coagulates the protein, ruptures the fat depots and liberates oil and physico-chemically bound water
- pressing (or occasional centrifugation), which removes a large fraction of the liquids from the mass
- separation of the liquid into oil and water (stickwater). This step may be omitted if the oil content of the fish is less than 3%
- evaporation of the stickwater into a concentrate (fish solubles)
- drying of the solid material (presscake) plus added solubles, which removes sufficient water from the wet material to form a stable meal
- grinding the dried material to the desired particle size

Unit operations in fish meal production

1. Receiving of Raw Material

2. Cooking

In general, cooking is done at a temperature of 95 to 100oC within 15-20 minutes. Most manufacturers operate cookers to heat the fish mass rapidly to 95oC. The purpose of cooking is to denature or coagulate the proteins of fish and to rupture the cell wall of tissues of fish so that it helps in separating oil and water present in fish.

3. Pressing

Pressing operation separates two distinct phases of cooked material. They are:

(1) Solid phase (press cake) and
(2) Liquid phase (press liquor).

Pressing is done using a screw press. The press may be a single screw press or a double screw press. At present, double screw press is preferred as it removes maximum quantity of oil and moisture from cooked fish.Usually press cake coming out of the screw press contains about 45-55% of water and 2 to 3% fat. Press liquor coming out of the press is saved for oil extraction.

4. Fluffing

Press cake coming out of the press is in the form of large lumps. In order to increase the efficiency of drying and to reduce drying time, the large lumps have to be broken down to small pieces of about 1cm size. In the present day fish meal plants, this step is eliminated as press cake is disintegrated by the screw conveyor which transfers press cake from the press to dryer.

5. Drying

In the drying process, fluffed press cake containing about 50% moisture is dried to a moisture content of less than 10%. Two types of driers are commercially used.

1. Direct driers or flame driers

2. Indirect driers or steam heated driers.

At present, direct dryers are seldom used by large scale fish meal manufacturers for several reasons.

It takes about 20 minutes for the press cake to travel from hopper to the exit of the drier. The moisture content of press cake coming out of the drier should be 10% or less. The dried press cake is then passed through a magnetic separator to remove any steel contaminants.

6. Cooling

The fish meal is cooled in coolers. Cooler is equipment which is similar to the drier except that instead of steam, cold water is passed. In the cooler dried fish meal is cooled to room temperature.

7. Sieving

Dried press cake is passed through a vibratory screen specially those who use trawl bycatch to separate extraneous materials such as wood, cloth, fishing hooks, shells and nails prior to milling.

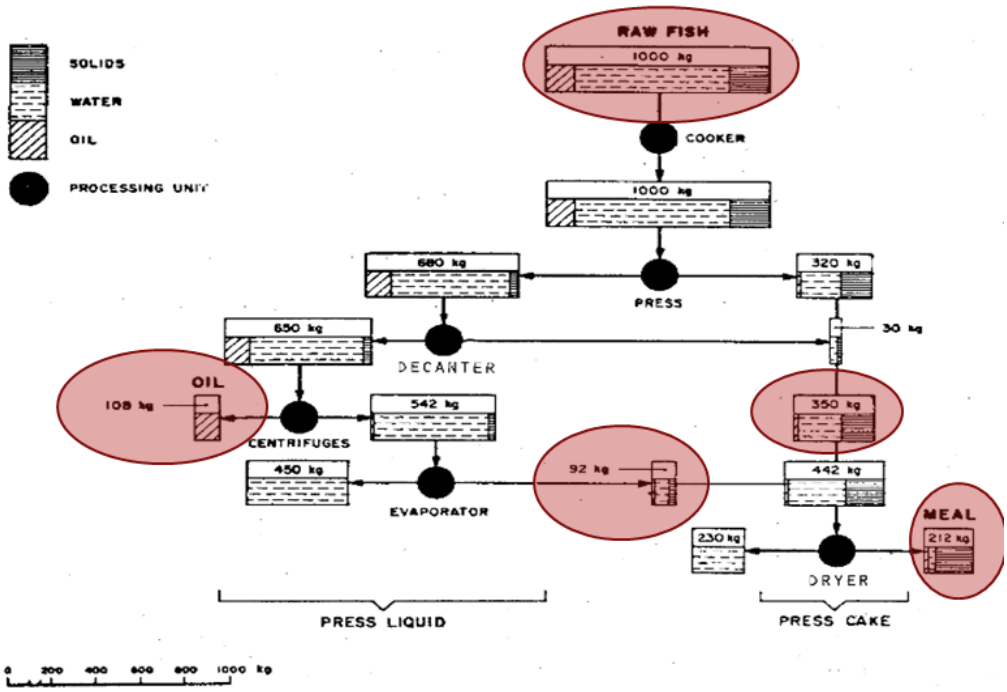
8. Milling

The important objective of milling is to produce fish meal with small particles averaging around No.40 mesh Tyler screen. The small sized particles thus produced pass through the sieve fixed at the bottom of the chamber. Normally the exit end of the hammer mill is connected to a cyclone separator to reduce the problem of dusting.

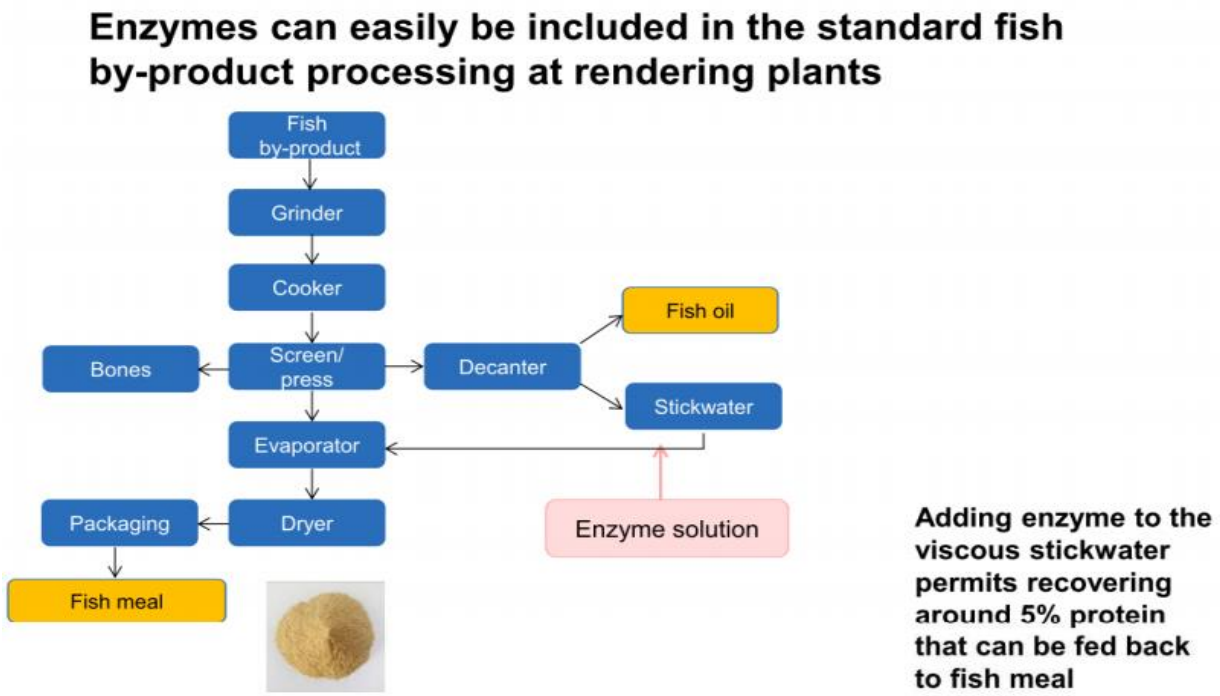
9. Packing, Labelling and Storage

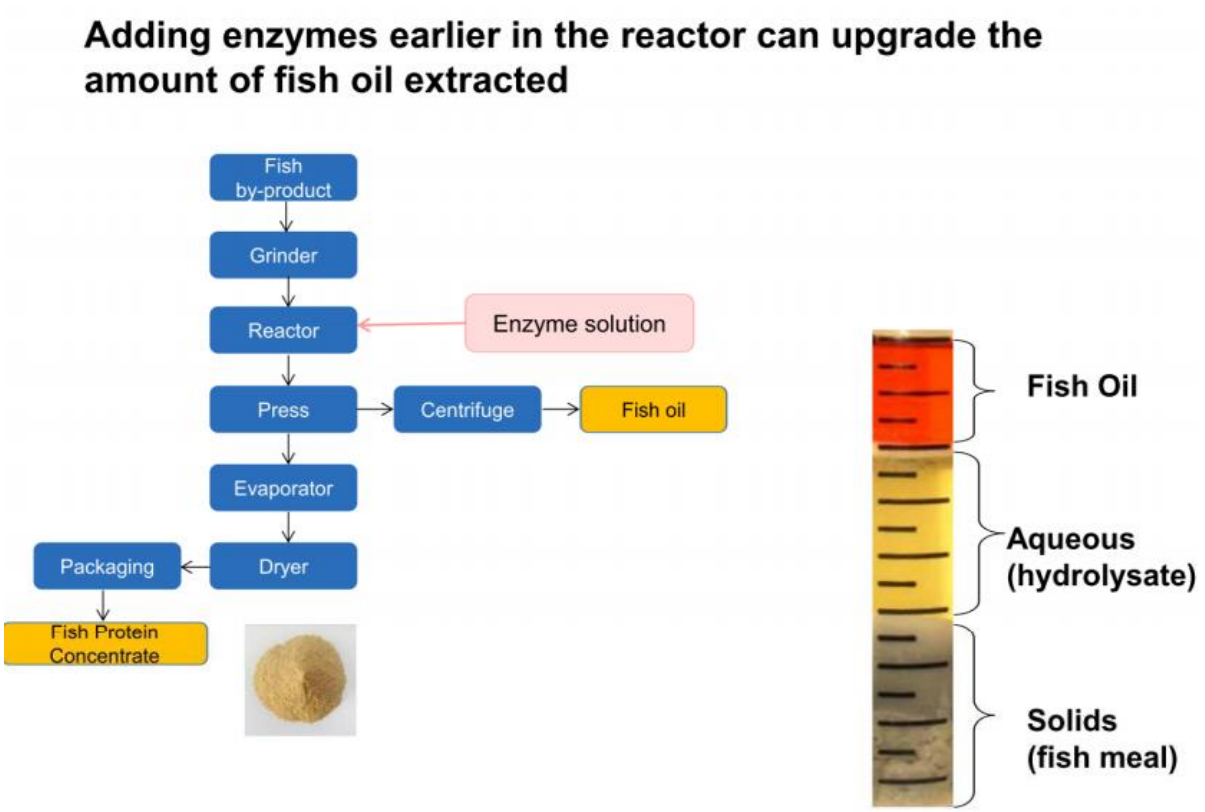
Fish meal is usually packed in polyethylene (PE) lined jute bags or PE lined paper bags or PE lined HDPE woven sacks. The outer packaging is then properly labeled.

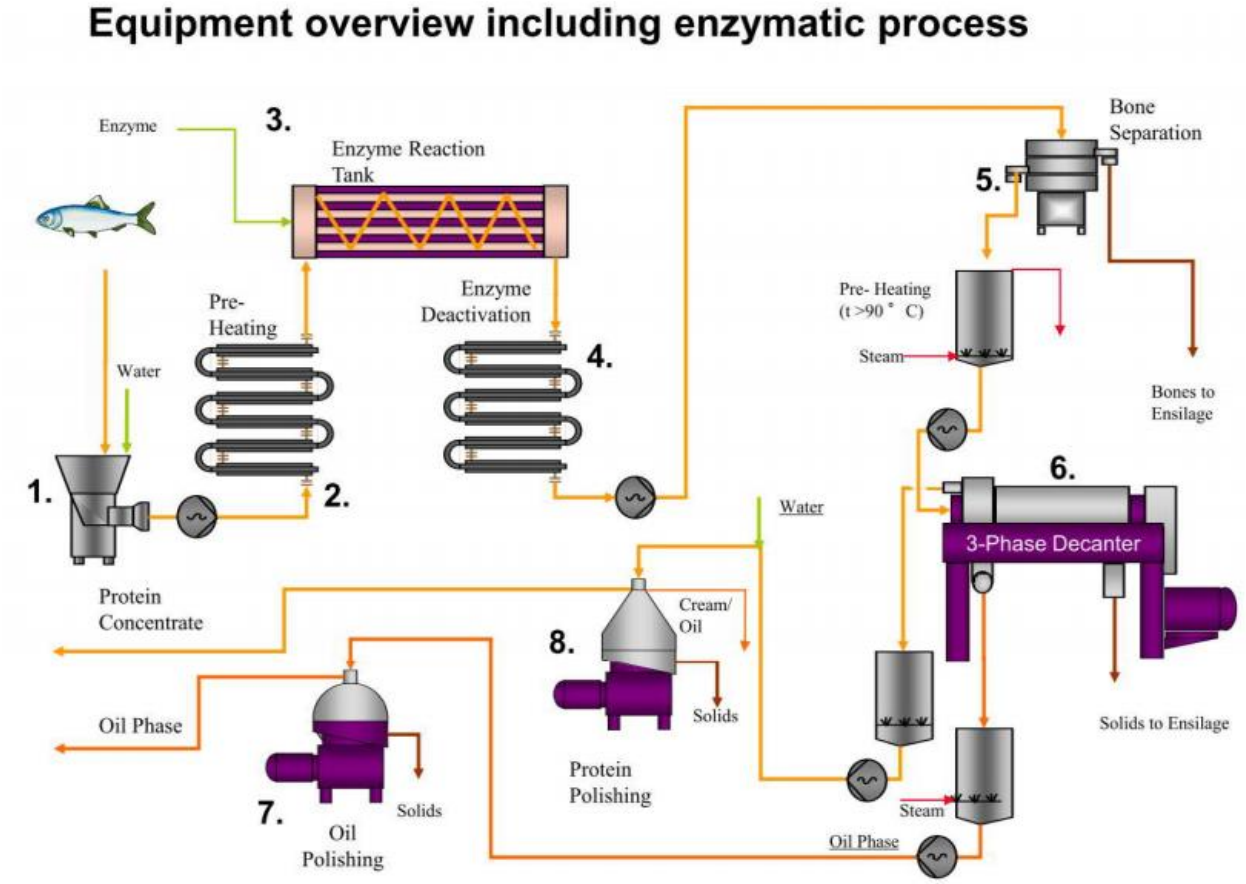
Mass balance



Enzymes in fish meal processing







Importance of Fish Meal

The nutritive components present in fish meal and their importance in animal, poultry and fish nutrition is discussed below.

1. Depending on the raw material used in its manufacture, its protein content may vary from 40% to 60%. Hence, fish meal is considered as a concentrated source of protein and it is easy to incorporate with other feed ingredients so that the desired protein level is maintained.
2. Protein present in the meal is a good source of all essential amino acids and hence must be provided in the diet. As protein of fish meal contains all essential amino acids, its nutritive value is high.
3. Fish meal protein is a rich source of amino acid lysine which occurs in deficient quantities in most of the cereals and legumes.
4. Fish meal supplies water soluble B group vitamins such as riboflavin, niacin pantothenic acid, choline, vitamin B12 in addition to oil soluble vitamins such as vitamin A and D. As fish is a good source oil, the meal produced from it contains oil to the extent of 6 to 10%. Oil present in meal contributes towards energy for fish, animals and birds. This oil helps in growth and fattening of fish, animals and birds.
5. Fish meal made from whole fish containing bones is a rich source of essential minerals such on calcium, phosphorous and magnesium. Calcium is required for bone formation in animals and birds. Fish meal is also a good source of iron. In addition to major minerals, fish meal also supplies trace elements such as iodine,

molybdenum, copper, zinc and manganese, all of which are required for various biochemical processes going on in the body of fish animals and birds.

6. Crude fibre content in fish meal is very low. Birds require low content of fibre in their diet. For proper digestion & absorption of nutrients, the feed should be low in fibre content, specially for fish and poultry. Hence, fish meal is particularly suitable for poultry feed.
7. Fish meal contains certain growth factors such as protein utilization factor and animal protein factor which make the feed with fish meal to give maximum nutritive value as these factors ensure that the dietary proteins are utilized to a maximum extent in their body.

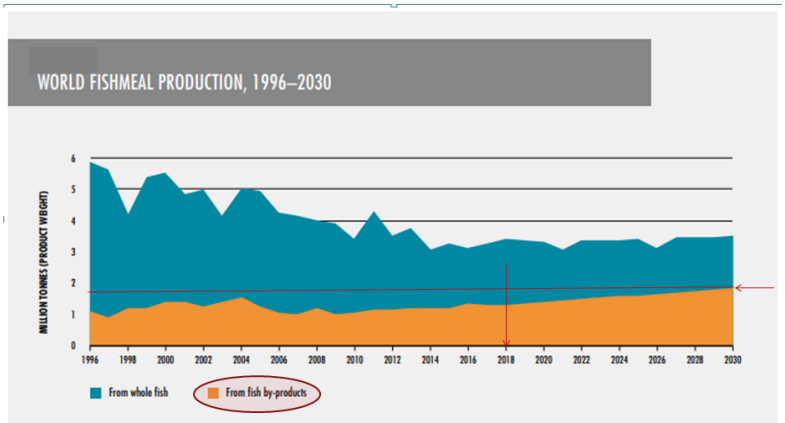
Fish meal-2030

- About 16 percent of capture fisheries yield will be used to produce fishmeal in 2030.
- The estimated fishmeal and fish oil production, in product weight, should reach 5.3 million tonnes and 1.0 million tonnes, respectively.
- In 2030, fishmeal production should be 19 percent higher than in 2016, but about 54 percent of the growth will derive from improved use of fish waste, cuttings and trimmings obtained from fish processing.
- Fishmeal produced from fish by-products will represent 34 percent of world fishmeal production in 2030, compared to 30 percent in 2016 (Figure 51).
- The fish model does not take into account the effects of the use of fish by-products on the composition and quality of the resulting fishmeal and/or fish oil.
- Possible effects include lower protein and increased ash (minerals) and small amino acids (e.g. glycine, proline, hydroxyproline) in comparison with products obtained from whole fish.

This difference in composition may hinder increased use of fishmeal and/or fish oil in feeds used in aquaculture and livestock farming.

(Ref: World bank report on Fish 2030)

According to FAO, in the year 2030, about 50% of world fish meal production will be manufactured from seafood processing waste.



References

- Windsor, M.L., 1971, Fish meal. TorryAdvis.Note, (49):12 p.
- FAO, 1986. The production of fish meal and oil.FAO fisheries technical paper – 142
- http://www.iffo.net/publications
- FAO. 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO
- World Bank (2013) Fish to 2030: prospects for fisheries and aquaculture. World Bank, Washington

****

Fish Oil: Health Benefits and Quality Issues

Anas K.K., Lekshmi R.G.K., Anandan, R.

Biochemistry & Nutrition Division,

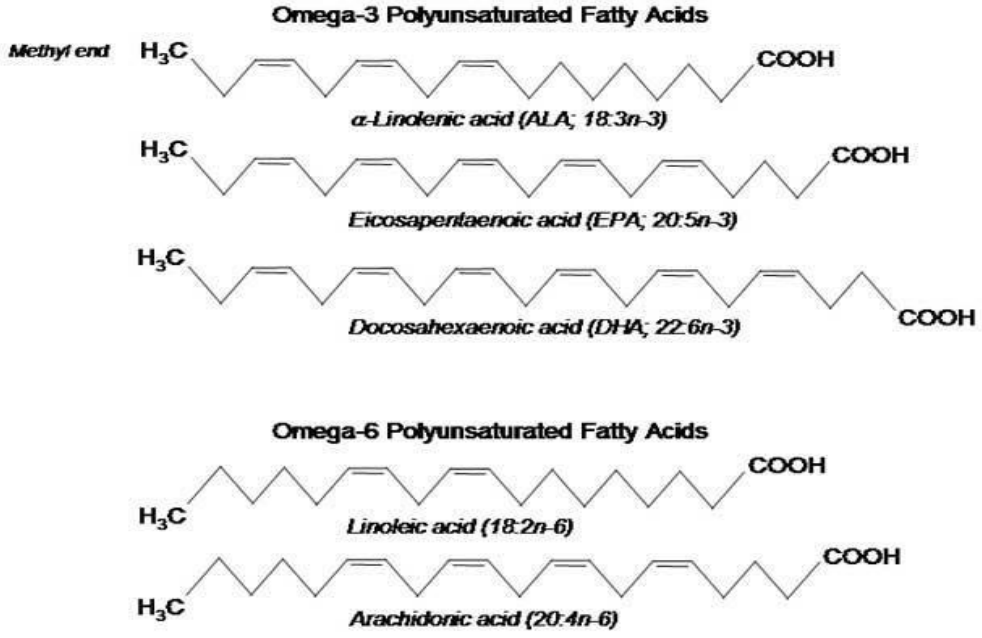
ICAR-Central Institute of Fisheries Technology

Email:anaskk40@gmail.com

Introduction

Long chain omega-3 polyunsaturated fatty acids (LC PUFA) are reported to have a positive effect on human health. The main omega-3 LC PUFA are eicosapentaenoic acid (EPA C20:5) and docosahexaenoic acid (DHA C22:6). Fish oil is the main dietary source of these long chain omega-3 (n-3) polyunsaturated fatty acids (PUFA). Dietary fatty acids are considered as a primary energy source in humans. Apart from being a major dietary source, it is also reported to have extensive nutritional and health benefits, especially, n-3 polyunsaturated fatty acids. Omega-3 fatty acids are long chain polyunsaturated fatty acids containing methylene-separated double bonds starting from the third carbon atom counted from the methyl-terminus (Kralovec*et al*., 2012). 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. There are several sources recognized for n-3 polyunsaturated fatty acids such as olive oil, rice bran and fish oil etc.

Fish oil is being widely recognized as an excellent dietary source of n-3 polyunsaturated fatty acids such as EPA and DHA. Researchers have shown that the fish oil supplementation is highly beneficial as it is having many health attributes such as the prevention of coronary heart disease, rheumatoid arthritis, hypertension, Crohn's disease, Type 2 diabetes etc.(Simopoulos, 1999, Tur,et.al,2012). Fatty fishes like sardine and mackerel are considered as better sources of n-3 fatty acids like EPA and DHA (Gunstone 1996). However, the fatty acid profile of fishes might vary according to the species, the environment in which it grows, season, diet, stage of sexual maturity and sex also. The structure of important omega-3 fatty acids are shown in fig.1



Health benefits of n-3 PUFA

1. Role in inflammation

Inflammation, which is body's response to infection and cellular injuries, is mainly manifested by the production of several inflammatory mediators such as cytokines, reactive oxygen species, expression of adhesion molecules and arachidonic acid derived eicosanoids. However, studies have shown that the increased consumption of n-3 polyunsaturated fatty acids inhibits the arachidonic acid metabolism by competing with arachidonic acid for the enzymes for eicosanoid production. This process results in an increased production of n-3 derived eicosanoids which is having anti-inflammatory effects. Apart from the production of anti-inflammatory eicosanoids, some studies have also reported the production of certain mediator compounds from EPA and DHA which is also having anti-inflammatory actions. For instance, E-series resolvins and D-series resolvins, docosatrienes and neuroprotectins formed from EPA and DHA respectively is reported to have anti-inflammatory properties. Bouwens, et al., (2009) have studied the effect of fish oil supplementation in inducing anti-inflammatory gene expression profiles in human blood mononuclear cells. The study has reported that the supplementation of EPA and DHA resulted in a decreased expression of genes which are mainly involved in inflammatory- and atherogenic-related pathways

Role in prevention of cardiovascular diseases

Several studies have reported the association of fish oil consumption and reduction in the risk of cardiovascular diseases. The relationship between weekly fish consumption and the reduced risk factors of cardiovascular diseases such as obesity, hypertension, glycohemoglobin has been reported by Mizushima *et al*. and Burr *et al*. have studied the effect of n-3 supplementation (either fish oil capsules of fatty fish twice in a week) on patients with a recent myocardial infarction for a period of 2 years. They have observed 29% reduction in total mortality and in deaths from coronary heart diseases in the group administered with an increased intake of n-3 PUFA. Taking into consideration the cardioprotective effects of fish oil, American Heart Association recommended that adults should eat fish at least two times per week (Kris-Etherton, 2003). The International Society for the Study of Fatty Acids and Lipids (ISSFAL) also recommended an adequate intake of 0.65 g of DHA plus EPA per person per day (0.22 g of each).

3. Role in prevention of thrombosis

The antithrombotic effect of fish oil was first reported in an epidemiological study of Greenland Eskimo by Dyerberg and Bang (1979) and Dyerberg (1986) suggested the relation between a low incidence of heart diseases and seafood consumption. It was later found that the consumption of fish resulted in increased levels of tissue plasminogen activator (TPA) and decreased concentrations of plasminogen activator inhibitor. One of the possible mechanisms of anti-thrombotic effect of omega-3 fatty acids is that it inhibits platelet TXA2 synthesis and acts as antagonists of the pro-aggregatory TXA2/PG H2 receptor in human platelets in vitro.

4. Role in prevention of Rheumatoid arthritis

Kremer et al. (1995) have studied the effect of fish oil supplementation on rheumatoid arthritis and found that patients taking dietary supplements of fish oil exhibited

significant improvements. Fish oil consumption resulted in a significant decrease levels of IL-1 beta from baseline. Even, some patients who take the fish oil on a daily basis were able to discontinue the non-steroidal drugs. Some patients who take fish oil are able to discontinue NSAIDs without experiencing a disease flare. Caughey, et al., 2010 studied the combined effect of fish oil and paracetamol on the anti-inflammatory effect in patients with rheumatoid arthritis and found out that there has been a significant suppression of COX-2 generated prostaglandin PGE2 synthesis.

5. Role in the treatment of ulcerative colitis

Ulcerative colitis is a disease condition which is characterized by the influx and accumulation of neutrophils in the colonic mucosa. The presence of leukotriene B4, a potent chemotactic factor, was observed in high levels in inflamed colonic mucosa and was reported to have a pivotal role in the accumulation of neutrophils in the affected region. Hence, treatments which will reduce the synthesis of leukotriene B4 will be beneficial in controlling the incidence of ulcerative colitis. Diets containing high levels of w-3 fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, are known to modify leukotriene production. Eicosapentaenoic acid levels in cell membranes rise, with an increase in eicosapentaenoic acid derived lipoxygenase products, such as leukotriene B56, which has markedly reduced chemotactic potency compared with leukotriene B4. In addition synthesis of lipoxygenase products derived from arachidonicacid is reduced as a result of diminished substrate (Barbosa et al 2003).

6. Role in the treatment of diabetes

In streptozotocin-diabetic rats, long-term ω-3 PUFA supplementation has been shown to prevent diabetic heart muscle disease. In neonatal cardiomyocytes cells, arrhythmia caused by agents such as high extra cellular calcium, ouabain, isoproterenol or lysophosphatidylcholine was prevented by exogenous EPA in the free form (Calder, 2004). As removal of free EPA with added bovine serum albumin quickly reversed this protective effect (Leaf et al 1999), it was suggested that the free carboxylic group of ω-3 PUFA modulates ion channels, especially the calcium and sodium channels on the cardiomyocyte membrane to prevent arrhythmia (Calder, 2004). It is possible that through similar mechanisms, EPA could prevent calcium overload in the diabetic heart, which is known to induce mitochondrial pore transition leading to cytochrome c release and cardiomyocyte apoptosis (Oliveira et al.,2003). Interestingly, exogenous DHA supplementation has also been demonstrated to correct calcium homeostasis and mitochondrial dysfunction in diabetic cardiomyocytes.

7. PUFA and cancer

Many trials using fish oil in diet shows promising results in the area of cancer treatment. In rats, linoleic acid, a precursor of arachidonic acid in tissues, increases the size and number of tumours whereas EPA and DHA decrease both. It is suggested that the potential of n-3 fatty acids to prevent recurrence and metastases of mammary cancer when used in adjuvant therapy is associated with a (n-6) to (n-3) ratio < 2:1(Cowing and Saker, 2001). Adding fish oil to a diet containing adequate polyunsaturated fatty acids enhances azaserine- induced carcinogenesis in rats and N- nitrosobis(2 oxopropyl)amine- induced carcinogenesis in hamsters (Woutersen and Appel, 1999). A meta-analysis of experimental animal studies found that n-6 fatty acids strongly enhanced carcinogenesis, monounsaturated fatty acids had no effect, and n-3 fatty acids weakly (but notsignificantly) inhibited carcinogenesis (Fay *et al.,* 1997).

8. PUFA and liver disease

Liver disease must be one of the major causes of PUFA deficiency because long chain PUFA biosynthesis mostly occurs in the liver. PUFAs are synthesized from their essential precursors in the smooth endoplasmic reticulum, especially in the liver, by successive desaturation (i.e., oxidation with double bond formation) and elongation (i.e., lengthening of the chain with two methylene groups) reactions. PUFA deficiency is a well established feature of advanced cirrhosis mainly in plasma, erythrocytes and platelets (Owen *et al.,* 1982; Wilcox *et al.,* 1978). PUFA deficiency may decrease the fluidity of cell membranes and hence impair their biological functions. Decrease in fluidity has been reported either in red blood cells (Owen *et al.,* 1982) or hepatocytes (Schuller*et al.,* 1986) of patients with cirrhosis as compared with healthy controls. Arachidonate deficiency may lead to impaired platelet aggregation often occurring in advanced cirrhosis (CabreandGassull, 1996). It has been reported that changes in membrane lipid composition hamper the insulin receptor function in the erythrocytes of cirrhotic patients (Peterson et al., 1992) and that the infusion of polyunsaturated lecithin improves such a derangement (Cantafora*et al.,* 1992). Eicosapentaenoic acid (EPA; 20:5n-3) up-regulates the metabolic action of insulin and inhibits cell proliferation (Murata *et al.,*2001). It has been found that fish-oil rich in EPA inhibit DEN-induced hepatocarcinogenesis in rats (Sasagawa*et al.,* 2002). On the other hand, some experimental studies have reported that, in alcohol fed rats, a PUFA enriched diet leads to more severe liver injury than a diet enriched in saturated fatty acids (Nanji*et al.,* 1989; Nanji*et al.,* 1995).

****

Fish Processing Waste: Valuable Raw Material Source for Silage, Foliar Spray and Animal Feed Preparation

Zynudheen A.A. and Binsi P.K.

Fish Processing Division

ICAR-Central Institute of Fisheries Technology

Email: zynu@rediffmail.com

Fishery waste which forms nearly 50% of the total weight of fish landed is an environmental issue in the present scenario. The recovery of biomolecules for the development of various products helps to eliminate harmful environmental aspects and improve quality in fish processing sector in addition to enhancing the profitability of the industry. In this respect the experiments conducted at CIFT has shown that, production of fish silage, foliar spray and feed from fishery waste has great potential as a high end product. However there are certain practical difficulties in the implementation of the techniques for utilisation. The problems in collection and processing is hindered due to highly scattered nature of availability i.e., On board, Fish markets, Preprocessing centres and processing centres. For e.g. Kerala has nearly 150 exporting companies and more than 2400 pre-processing centres. The highly perishable nature is also a major problem of handling the fishery by products.

Fish silage

Fish silage is defined as a product made from whole fish or parts of the fish to which no other material has been added other than acid and the liquefaction of the fish is brought about by enzymes present in the fish. The product is a stable liquid with a malty odour which has very good storage characteristics and contains all the water present in the original material. It is a simple process and it requires little capital equipment particularly if non oily fish are used. The use of oily fish requires oil separation. This involves expensive equipment and is suited to fairly large scale operation. Almost any species of fish can be used to make fish silage though cartilaginous species like shark and ray liquefy slowly. Fish waste, cuttle fish/squid waste can be used for the preparation of silage. The production of silage involves preferably organic acids like formic acid (35kg/tonne) to preserve the fish and then allow the enzymes already present in the fish to liquefy the protein. When 3.5% formic acid is added to the fish the pH will be nearly 4. Mineral acids like sulphuric acid also can be used for this purpose. But in this case pH would be about 2.5, which requires neutralization before formulating feeds to the poultry or cattle. There is an alternate method of production of silage by fermentation. The fish is mixed with a carbohydrate source like molasses and lactic acid is produced in the system to reduce the pH by introducing a lactic acid producing bacteria like *Lactobacillus plantarium.*

Foliar spray

Foliar spray is a technique of feeding plants by applying liquid fertilizer directly to their leaves by spraying. Plants are able to absorb essential elements and nutrients through their leaves and absorption takes place through the stomata of the leaves and also through the epidermis. Movement of elements is usually faster through the stomata and this result in faster growth and flowering. Some plants are also able to absorb nutrients

through their bark. The process of foliar spray preparation is by hydrolysing the fishery waste either by adding acid directly as in case of silage or by *in-situ* production of lactic acid by microorganisms. The clear upper portion of acid silage is decanted and suitable diluted and used as spray. In case of microbial process, the fish waste is mixed with a carbohydrate source like molasses and inoculated with lactic acid producing bacteria and the lactic acid produced will hydrolyse the protein partially. It will take 20-30 days for hydrolysis and the upper clear liquid can be used as foliar spray.

Feed from fish processing discards

Feed is considered as the major expense in fish farming, accounting for about 50–60% of the total variable costs. Preparation of feed for aquaculture and poultry is an important option for utilization of general, unsorted waste from industry as well as fish markets. There is a growing demand for pellet feeds, due to the increase in aquaculture activity. Feed is also a major input affecting water quality and subsequently effluent quality in culture ponds. Fish feed management includes several factors viz. choosing the right feed, using a correct feeding method, calculating the feeding cost and ensuring the cost effectiveness of fish farm. Currently, aquaculture accounts for 40.33% of the world's fish production. Fish frames and other discards contain significant amounts of muscle proteins. They have a better balance of the dietary essential amino acids compared to all other animal protein sources. About, 25% of the protein requirement for feed is met from fish waste

Table 1: Fat and protein content of discards from selected species

Fishmeal based Fish feed	Fat	Protein
Sardine fish	3.77	27.6
Sardine waste	7.62	27.70
Tilapia waste	3.58	28.18
Threadfin waste	3.67	28.81
Anchovy fish	5.89	27.10

Preparation of feed from fish waste

The proximate composition and characteristics of many processing wastes suggest that it can be converted directly into feed. Most of these protein sources can be converted to fish flesh, which in turn provides quality protein for man. Utilization of these wastes can be direct or indirect. In direct utilization, either the wastes can be used as such as in the case of meals; cakes etc. or it can be used with some simple processes like fermentation, silage preparation etc. In indirect utilization, the wastes can be utilized as a substrate for the growth of single cell proteins for example, and these secondary products can be included in feed with or without primary substrate.

Table 2: Nutritional composition of fish processing discards

Nutrient	Fish waste
Crude protein (%)	57.92 ± 5.26
Fat (%)	19.10 ± 6.06
Crude fiber (%)	1.19 ± 1.21
Ash (%)	21.79 ± 3.52
Calcium (%)	5.80 ± 1.35
Phosphorous (%)	2.04 ± 0.64
Potassium (%)	0.68 ± 0.11
Sodium (%)	0.61 ± 0.08
Magnesium (%)	0.17 ± 0.04
Iron (ppm)	100.00 ± 42.00
Zinc (ppm)	62.00 ± 12.00
Manganese (ppm)	6.00 ± 7.00
Copper (ppm)	1.00 ± 1.00

Values in % or mg/kg (ppm) on a dry matter basis.

Fish waste can be macerated into paste and prepared at farm site as meal and used for feed. Alternatively, fish waste may be initially converted to meal or silage, which later on can be made into feed after compounding with other essential nutrients like carbohydrate, fat, trace minerals and vitamins. A good amount of research work has focused on the replacement of fish meal in feeds by various processing wastes / byproducts. Fish soluble obtained as a byproduct of fish meal production also serves as an ideal protein source for animal feed. It is rich in B group vitamins and also contains unidentified growth factors. It also serves as an attractant in fish feed. Meals obtained from small prawns, prawn heads, mantis shrimp, crabs and krill is a potential ingredient for shrimp diet. It is reported that the crude protein level varies between 30-50% depending on species and the chitin content is 16%. Ash content ranges from 25- 40%. It is rich in cholesterol, carotenoid pigments, chitin, calcium, iron, manganese, choline, niacin, pantothenic acid and cyanocobalamine. The quality of the silage depends on the freshness of the raw material. Silage has chemo attractant properties due to the free amino acids. About 70 % of the total shrimp production ultimately gets transformed to waste. Replacement of fishmeal by shrimp meal is possible to the level of 10 -15%. Shrimp waste can also be ensiled. Crustacean silage has been found to exhibit feeding stimulatory properties in a variety of fish species. Squid waste which usually includes viscera may also contain head and tentacles, fin, skin and pen accounting for about 52%

of the total weight. Squid processing wastes are important feed ingredients particularly in shrimp diets. Protein content ranges from 70 to 90 %. Squid meal has a fat content of 4 - 7 % which contains high content of highly unsaturated fatty acids. Squid meal has chemo attractant and growth promoting properties. Inclusion in aquafeeds upto 30 % level is possible. Fish silage may be ideally seen as a source of protein and several minerals in feed preparations. Infact, it partially replaces fish meal in feeds (typically 5-15%). Silage contains comparatively, high level of free amino acids and peptides, which improve the growth performance and better disease resistance.

Quality of animal feed

Apart from nutritional composition, the quality of animal feed may be expressed in terms of physical quality and microbial quality. Physical evaluation is easy but tough in nature. One must be highly trained to identify the changes in the nature of the raw materials/ feeds. This primarily involves parameters such as such as bulk density, colour, odour, hardness (force at rupture), durability, pellet size and water stability. Handling practices followed presently for fish processing waste are not adequate and hence may harbour a number of microbial hazards including lethal toxins and metabolites. *Salmonella* is a major bacterial hazard in animal feed. *E. coli* also has been detected in animal feeds. Similarly, the contamination of foods and animal feeds with mycotoxins is a worldwide problem.Mycotoxins are fungal secondary metabolites that have been associated with severe toxic effects to vertebrates produced by many important phytopathogenic and food spoilage fungi including *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* species.

Environmental impacts

The utilization of fish waste derived feed for feeding livestock may clearly create a further range of potential environmental impacts, if proper measures are not taken. In the case of aquaculture feeds, leaching of protein and other nutrients into the pond can result in deterioration of water, if poor quality feed is used. Similarly, trash fish shreds may have greater loss rate (about 40%). Also, the feed residue deposited on the seabed or pond bottom will cause pollution, resulting in a heightened risk of anoxia and mortality rate.

****

Surimi and Surimi Products

Elavarasan, K., Sathish Kumar, K. and Mandakini Devi H.

Fish Processing Division

ICAR-Central Institute of Fisheries Technology

Email: elafishes@gmail.com

Surimi and Surimi Products

Surimi is stabilized mince made from deboned and washed fish meat. Surimi processing involves processing whole or gutted fish into mince, repeated washing of the mince (at mince and water ratio of 1:3 for 2-3 cycles), dewatering (done by manual press, nylon mesh bag method, Centrifugation and screw press till the moisture content of the meat, ranges between 80% and 84%) and refining. Refining is a screening mechanism, where the remaining scale, connective tissues and bones are separated from the mince. Surimi originated in Japan in 1115 and is used basically in Kamboko type products. Kamaboko is the term which often refers to all surimi seafood. Thus, surimi is minced and deboned fish meat that has been washed of lipids, water-soluble or sarcoplasmic proteins, and other impurities for use in the manufacture of intermediate products. These products are manufactured by manipulating the gel forming capacity of fish myofibrillar protein-myosin. Hence, the suitability to be raw material for surimi production is determined by the functionality of fish myofibrillar protein called 'gelation' which are generally greater in white-fleshed fish than in dark fleshed fish. Globally, Alaska Pollock is the main species used for the surimi production.

As mentioned earlier, surimi is an intermediate product and a large number of products can be developed from surimi depending on the creativity, innovation and knowledge of the one involved in this line. However, kamaboko, chikuwa, hanpan and satsum-age are the traditional surimi based Japanese products (Fig. 1). Of late, products like fish ball, fish sausage and fish ham has been introduced. Today, surimi is used mainly in analog or imitation products like crab stick, shrimp analog, lobster analog and scallop analogs.

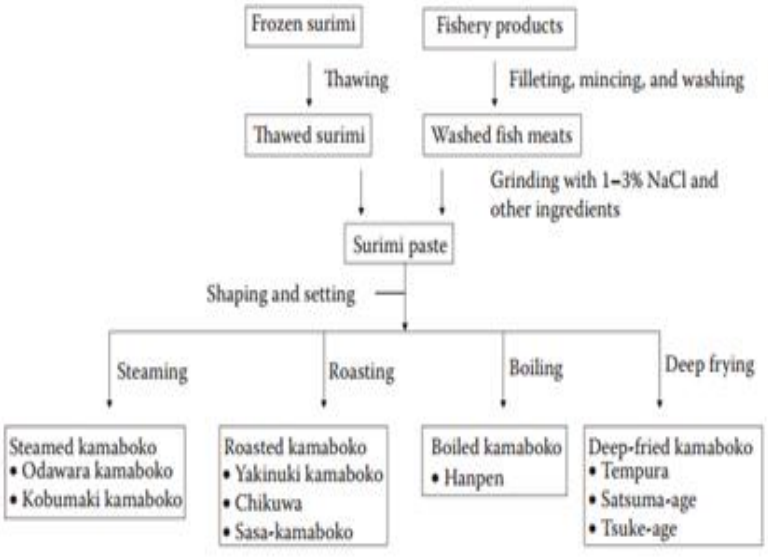


Fig. 1: Typical Kamaboko products

Surimi seafood products are defined as products prepared by heating (roasting, steaming, deep frying or smoking) of ground fish meats mixed with seasonings, stiffeners or other ingredients.

Fish sausage

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

Crab analog

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

Shrimp and lobster analog

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

Scallop analogs

The plant set up for the production of scallop analog is similar to crab analog. For the preparation of scallop analog, a wider and thicker surimi sheet is extruded compared to the surimi sheet extruded in crab analog preparation. After sheet formation, surimi sheet subjected to partial cooking for facilitating the gelation and subsequently subjected to slitting. After slitting, an uncooked layer of surimi paste is added on top of the gelled surimi sheet immediately. This additional layer of surimi paste is to enhance the binding of fibers. The gelled fibers are wrapped and cut into 2-foot lengths and heat processed. The cooked fiber bundles are cut into the desired dimension of scallops shapes using flaking machine.

****

Marine Nutraceuticals

Asha K.K. and Suseela Mathew

Biochemistry and Nutrition Division

ICAR-Central Institute of Fisheries Technology, Cochin

Email: asha.santhosh5@gmail.com

Increasing consumer awareness about the relation between diet, health, and disease prevention has triggered research and development of new functional foods over the last years. The ageing of population, decrease in quality of life due to stress, high incidence of lifestyle diseases (cardiovascular disease, obesity, cancer, diabetes, and allergies) represent the driving forces in the search for different foods and diets that promote healthy active ageing, improve well-being, and prevent the incidence of many diseases. Food is known to play an important role in prevention or onset and progression of chronic diseases such as atherosclerosis, obesity, diabetes, hypertension, osteoporosis, cancer, and cardiovascular disease. The marine environment is a huge source of healthy food, including seaweeds with several marine species containing a plethora of chemicals, many of them with biological properties referred to as bioactive compounds. These chemicals can be extracted and incorporated in several food matrices leading to development of new functional foods

According to Health Canada, a functional food is similar to a conventional food, which is consumed as part of an usual diet that either provides physiological benefits or reduces the risk of chronic disease beyond its basic nutritional functions. According to the Food Agriculture Organization (FAO), functional foods are those foods similar to conventional food in appearance, intended to be consumed as part of a normal diet containing biologically active compounds that offer potential for enhanced health or reduced risk of disease. Foods that besides their nutritional effects, have demonstrated that they improve the state of health or well-being, reduce the risk of disease, as well as benefit one or more functions of the human organism are considered as functional food. Functionality could be intrinsic to a feature introduced in the food matrix, improving health or reducing any adverse health effect, accomplished, for example, by

i) Elimination or promotion of a chemical change of a harmful ingredient.
ii) Addition of new health-promoting food ingredients or probiotic microorganisms in an effective concentration.
iii) Addition of an existing health-promoting food ingredient, increasing its concentration.
iv) Increasing the bioavailability or stability of the health-promoting food ingredient.

The characteristics of the marine environment such as temperature, salinity, light, pressure, and nutrients are of special importance, since due to their broad range of values marine organisms had to evolve some protective mechanisms and metabolites. Crustaceans, macro or microalgae, fish, and fish by-products, as well as bacteria and fungi are the most representative groups of organisms of potential interest as healthy food or as a source of functional ingredients, which include polysaccharides, chitin, proteins and peptides, lipids, pigments, vitamins, minerals, and phenolic compounds.

The term “nutraceutical” was first coined by Stephen DeFelice in 1989 which consists of two words nutrient (nurturing element) and pharmaceutical (medicinal component). It had gained importance in the recent years with increase in the field of health based research. The nutraceuticals are the substances which as a whole or as a part are delivered in the form of dietary supplements/ingredients that are clinically proven to hold health benefits (prevention and treatment of disease). Marine nutraceuticals refer to the compounds derived from sea. The potential of the marine nutraceuticals in human health had already been established

Types of marine nutraceuticals

Marine nutraceuticals can be broadly classified as follows: Marine lipids (animal origin and microalgal origin), Polysaccharides derived from macro algae, Marine probiotics, Marine natural pigments, Chitin and other related products, Bioactive marine peptides/enzymes and Vitamins.

I. Marine lipids:

a. Lipids of animal origin

Marine lipids are originated either from fish, crustaceans or other aquatic organisms. Phospholipids, sterols, triacyl glycerols, wax esters and their metabolic products form the main composition of marine lipids. Minor amounts of used lipids like glycerol esters, glycolipids, sulpholipids and hydrocarbons are also present in marine lipids. Marine lipids derived mainly from fatty fish flesh, lean fish liver and blubber of marine mammals. Fish oils and oils from marine mammals are rich sources of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). EPA and DHA have anti- inflammatory properties. Shrimp contains 1.8-2.6% of lipids based on wet weight basis. Crustaceans contain much sphingomyeline which is having anti-bacterial and anti-tumour property. Lobsters and crabs contain 08-2.0% of lipid. The lipid content of bivalves is below 1.0% but which is rich in polyunsaturated fatty acids (PUFA) i.e.50-64%. Lipids contain interesting health promoting compounds like sterols and alpha-tocopherols. Highly unsaturated fatty acids (HUFA) are found to reduce the effect of environmental change on the nervous system thereby reducing the stress in fish.

b. Lipids of microalgal origin

Lipids derived from marine microalgae have a wide range of applications in larval nutrition of aquaculture especially for enrichment of live feeds. They also exhibit various properties like anti-inflammatory, anti-allergic, anti-viral and therapeutic. The wide spectrum of the properties is due to the presence of various components like PUFA, HUFA and other substances. Various microalgal originated lipid/fatty acids and their activities are given in Table 1

Table.1

Lipid/Fatty acid	Activity
Eicosapentaenoic acid (EPA)	Nutraceutical; antimicrobial and anti-inflammatory
alpha-Linolenic acid (GLA)	Integrity of tissue and delay of aging
Arachidonic acid (ARA)	Aggregative and vasoconstrictive of platelets
Docosahexaenoic acid (DHA)	Nutraceutical and brain development

Brassicasterol and stingmasterol	Hypercholesterolemic
alpha-amino-butyric acid(GABA)	Neurotransmitter , antioxidant and anti-inflammatory
Okadaic acid	Antifungic, secretion of nerve growth factor (NGF)
Microcolin-A	Immunosuppressive

II. Polysaccharides derived from macro algae

Seaweeds contain higher amounts of the polysaccharides like agar, alginates and carrageenans. These act as food fiber and are collectively called phycocolloids or hydrocolloids. Being rich in fiber, seaweeds exhibit health benefits like reducing the absorption of toxins, anti-carcinogenic and antioxidant properties. In addition to the phycocollids, seaweeds are sources of biologically active phytochemicals like carotenoids, phycobilins, fatty acids, vitamins, sterols, tocopherol, phycocyanins and others. Some of the polysaccharides of the seaweeds and their properties are as follows:

Polysaccharide	Property
Fucoidan	Antioxidant, antiangiogenic and antitumor activities, anticoagulant, immunomodulating and Hypolipidemic, anti-inflammatory
Sphinganine amide and caulerpicin (green algae)	Antiviral activity
Carrageenan	Antibacterial, anti- tumour, antiviral and anti-inflammatory activities
Alginic acid and xylofucans	Antiviral activity
Hyperoxaluria	Potential blood anticoagulant agent
Sulfated polysaccharides	Antioxidant, antithrombin activity, antitumor, cell recognition and cell adhesion or regulation of receptor functions.
Alginate	Stimulates immune system, Reduces intestinal absorption, Modulates colonic microflora and elevates colonal barrier function

III. Marine probiotics

Microbial diversity of marine environments is very rich and can be helpful to develop safe and effective probiotics. Novel marine probiotics can be an effective alternative for fighting the antibiotic resistance. Lactobacillus and Bifidobacterium are found to possess anti- mutagenic and immunomodulatory activity in host animal. Different

strains of marine probiotic bacteria are Lactobacillus (*L. casei, L. acidophilus, L. rhamnosus* GG (ATCC53013), L. johnsonii La-1), Bifidobacterium(*B. bifidum, B. longum, B. infantis, B. breve, B. adolescentis*), Leuconostoc spp. (*Ln. lactis, Ln. mesenteroides* subsp*. Cremoris, Ln. mesenteroides* subsp*. dextranicum*) and Streptococcus spp. (*S. salivarius* subsp*. thermophiles*). The problem posed during the development of new marine probiotics is the isolation and identification of potential strain. Application of biotechnological and molecular biological tactics is necessary for the development of marine probiotic strains for use of aquatic industry.

IV. Marine natural pigments

Marine macro and micro algae provide various types of the bioactive compounds. The most important and the striking feature of the marine algae is their natural pigments. The natural pigments of the marine algae provide food by photosynthesis and also provide the pigmentation. In addition to these, the natural pigments are also found to exhibit health benefits which make them one of the important marine nutraceuticals. The marine natural pigments and their health benefits are given in Table 3.

Natural pigments	**Health benefits**
Chlorophyll a	Antioxidant and antimutagenic
Pheophytin a	Neuroprotective, Antimutagenic and anti-
Pheophorbide a	Antioxidant
Pyropheophytin a	Antioxidant
Phycoerythrobilin	Antioxidant
Lutein,zeaxanthin and canthaxanthin	Antimutagenic and antioxidant
alpha-Carotene	Antimutagenic and food additive
Fucoxanthin	Antioxidant, anticancer, anti- inflammatory, anti-obesity, anti- angiogenic and Neuroprotective
Siphonaxanthin	Anticancer and anti-angiogenic
Phycocyanin	Anti-inflammatory and antioxidant
Astaxanthin	Strong antioxidant, anti- inflammatory and dietary supplement

V. Chitosan and its derivatives

Chitosan is a natural polymer derived from chitin and it is the second most abundant polysaccharide after cellulose. Chitosan possesses special properties for use in pharmaceutical, biomedical, food industry, health, and agriculture due to its biocompatibility, biodegradability and nontoxic nature. Through encapsulation, it is being used as a vehicle for nutraceutical compounds and pharmacological compounds.

Antibacterial activity

Chitosan disrupts the barrier properties of the outer membrane of gram-negative bacteria due to ionic interaction between the cationic groups of the chitosan molecules and the anionic groups of the microbial cell membrane, which can rupture the cell membrane. Sulfuryl chitin, phosphoryl chitin and some chitin derivatives prepared by nitrous acid deamination of DAC, inhibited bacterial growth and increased cytotoxicity of a macrophage cell line. NTM-DAC had higher bacterial inhibition activity than carboxymethyl chitosan.

Antifungal activity

Chitosan can also function as an antifungal agent by forming gas-permeable coats, interference with fungal growth and stimulation of various defense processes like, buildup of chitinases, production of proteinase inhibitors and stimulators of callous synthesis.

Antioxidant activity

This property could be attributed to the ability of chitosan to chelate metals and combine with lipids. Derivatives of chitosan, namely, N,O-carboxymethyl chitosan, N,O- carboxymethyl chitosan lactate, N,O-carboxymethyl chitosan acetate and N,O-carboxymethyl chitosan pyrrolidine carboxylate had also exhibited the antioxidant activity.

VI. Bioactive marine peptides/enzymes

Peptides refer to the specific protein fragments. The bioactive peptides act as sources of biological compounds (nitrogen and amino acids) and also have numerous potential physiological functions within the body. Some of the peptides may exhibit multifunctional properties like opioid, immunomodulatory, antibacterial, antithrombotic and antihypertensive activity (Kim, 2012). Bio functional peptides have a size range of 2 to 20 amino acid residues and are encrypted within the sequence of the parent protein and are released during fish processing. They can be formed either by acid or alkaline hydrolysis. The type of bioactive peptides formed is dependent on two factors: (a) the primary sequence of the protein substrate and (b) the specificity of the enzyme(s) used to generate such peptides. The major bioactivities of peptides are as follows: antihypertensive (ACE inhibitory) activity, antioxidant activity, antimicrobial activity, antihypoallergenic activity, cell immunity

Peptide bioactivity	Marine resources
ACE inhibitory activity	Big eye tuna (muscle), Alaska pollock, sea bream, yellow fin sole, oyster, shrimp, clam and sea cucumber
Antioxidant activity	Big eye tuna (muscle), Alaska pollock, yellow fin sole, horse mackerel (skin), croaker (skin), conger eel, hoki fish (skin), squid, oyster, mussel,
Antimicrobial activity	Oyster, American lobster, shrimp and sea urchin
Antihypoallergenic activity	Big eye tuna (muscle), seaweed, pipe fish (muscle) and sea cucumber
Cell immunity	Oyster

Proteins isolated from Dunaliella, *Phaeodactylum tricornutum* and *Arthrospira platensis* are having potent antioxidant and anti-inflammatory activity which can be effectively used in aquaculture practices. Similarly, enzymes (Superoxide dismutase and Carbonic anhydrase) derived from Porphyridium, Anabaena*, I. galbana* and *Amphidinium carterae* can also play an important role in regulating the metabolite waste (CO2).

VII. Vitamins

Commercially produced cod liver oil is rich in vitamins A and D. Marine microalgae are also known to have good amount of alpha-carotene. Microalgae like, Arthrospira, *I. galbana, P. cruentum* and Tetraselmis are rich in vitamin C, K, A, E and alpha-carotene which possess strong antioxidant activity. Vitamin A (particularly provitamin A, alpha-carotene) and E (particularly alpha-tocopherol) serve as excellent antioxidants and free radical scavengers that protect cells from damage by oxidants. Research has shown that vitamin E has a number of extraordinary beneficial effects as a specific antioxidant, acting together with vitamin C and alpha- carotene, in improving antioxidant defenses in the body. Fat soluble vitamin K isolated from Pavlova helps in blood clotting or coagulation. The role of antioxidant vitamins in health and disease control has been well documented. These antioxidants may also be defined as substances which interfere with normal oxidation processes in oils and fats and delay their oxidation.

Marine Sources as Healthy Foods or Reservoirs of Functional Ingredients

Marine sources are known for their phenomenal biodiversity, which offers a strong basis for their use as a natural source of healthy food as well as of many novel functional food ingredients with biological properties. Crustaceans, macroalgae (seaweeds) or microalgae, fish, and fish by-products, as well as bacteria and fungi are the most representative groups of organisms of potential interest as healthy food or as a source of functional ingredients, which include polysaccharides, chitin, proteins and peptides, lipids, pigments, vitamins, minerals.

1. Seaweeds

Considering their great taxonomic diversity, algae or seaweeds, are a very interesting source of healthy food as well as a natural source of compounds with biological activity that could be used as functional ingredients. There are about 10,000 identified species of algae and about 5% of them are used as food especially in Asian countries as sea vegetables. Seaweeds when incorporated in diets are low in calories that can help in reducing obesity and blood pressure and also are known to help to overcome free radical stress. Seaweeds are rich in polysaccharides, minerals, vitamins, proteins, steroids, and dietary fibers in addition to possessing several biological properties such as antibacterial, antioxidant, anti- inflammatory, anticoagulant, antiviral and/or apoptotic activities. Presence of pigments such as carotenoids, phycobilins, chlorophylls, and phenolic compounds make them strongly antioxidant in nature. Some algae thrive in complex habitats exposed to extreme conditions. To adapt and survive, they produce a wide variety of biologically active secondary metabolites like acetogenins, terpenes, derivatives of aminoacids, phenols, and polyphenols, which are often halogenated. Algae are generally classified as: brownmacroalgae(phylum Ochrophyta), red macroalgae (phylum Rhodophyta), or green macroalgae (phylumChlorophyta). Brown algae owe their color to the presence of the carotenoid

fucoxanthin. Food reserves of brown algae are characteristically complex polysaccharides including laminarins, fucans, and cellulose, as well as higher alcohols; many bioactive metabolites with different pharmacological activities such as antioxidant, anti-inflammatory, antitumor, cytotoxic antifungal, and nematocidal activities, have been isolated from these algae. Green algae owe their color to the dominant presence of chlorophylls a and b, and the main polysaccharides present are normally ulvans. In turn, red algae, which are also considered an important source of many biologically active metabolites possess phycoerythrin and phycocyanin as the main pigments, and the primary polysaccharides are agars and carrageenans.

2. Microalgae

Microalgae or phytoplankton are microscopic marine organisms that can be found in benthic and littoral habitats in the ocean comprising blue–green algae (phylum Cyanobacteria, class Cyanophyceae), diatoms (phylum Ochrophyta class Bacillariophyceae), dinoflagellates (phylum Myzozoa, class Dinophyceae), as well as green and yellow–brown flagellates (chlorophyta, prasinophyta, prymnesiophyta, cryptophyta, and others). Microalgae play a key role in the productivity of oceans, constituting the basis of the marine food chain and are considered important producers of some highly bioactive compounds. Microalgae have abundance of PUFAs and pigments such as carotenoids and chlorophylls -chlorophyll a, phycocyanins, and phycoerythrin (phycobilins) are the pigments of interest found in blue–green algae. These compounds exhibit biological properties such as anticancer, antifungal, antibacterial, and immuno- suppressive properties. Diatoms are photosynthetic organisms that dominate the phytoplankton of cold and nutrient rich waters. They produce PUFAs such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and other omega-3 FAs, antioxidants fucoxanthin and chlorophyll. Dinoflagellates eukaryotic primary producers are rich in chlorophyll and carotenoids as well as PUFAs [42.38].

3. Fish and Fish By-Products

Several million tons of fish and fish by-products are discarded as waste, representing a large environmental problem. However fish and fish by-products are known sources of potential bioactive ingredients such as fish oils rich in PUFAs from fish livers, calcium from fish bones, protein hydrolysates of high biological value, peptides with biological properties such as antihypertensive activity, amino acids such as taurine, which have antioxidant activity and positive effects on cardiovascular system, as well as vitamins and minerals. Fish heads, viscera, skin tails, blood, and seafood shells possess a plethora of compounds with the potential to be used as functional food ingredients. Bioactive compounds from marine processing by-products can be obtained by extraction and purification procedures enabling the isolation of bioactive peptides, oligosaccharides, as well as FAs suitable for biotechnological applications

4. Crustaceans

Chitin is extractable from crustaceans shell, being the second most abundant natural polymer. Chitosan is a biodegradable and biocompatible polymer chitin derivative. Its ability to absorb fat is exploited in applications as an anticholesterol agent. Chitosan and chito oligasaccharides are reported to have several biological activities (antioxidant, antitumor, anticancer, hypocholesterolemic, immunity-enhancing, antimicrobial) and hence finds application in food and health industries.

5. Marine Fungi and Bacteria

Marine bacteria and fungi have drawn increasing attention from researchers from all over the world since they are considered as sources of new marine natural compounds. Marine extremophilic bacteria, for example, are of particular interest since they have metabolic pathways adapted to various extreme marine environments. Many microbial enzymes and exopolysaccharides from extremophiles have unique properties. Bacteria derived from intestinal tracts of marine organisms such as fish have also been researched with interest, since these strains may be new probiotics or have additional functions such as antibacterial activity. For example, it was observed that *Lactococcus lactis* isolates from the intestinal tract of freshwater fish possess different phenotypic properties, suggesting additional functions in comparison to those derived from a cheese starter. Much interest has also been focused on marine fungi, which have been studied for their metabolites. A unicellular marine fungus with high concentration of γ-amino-butyric acid (GABA), which is a promising functional and healthy food ingredient. In addition, marine fungi are a promising source of novel bioactive compounds with anticancer, antibacterial, antiplasmodial, anti-inflammatory, and antiviral properties.

Functional Foods Incorporating Marine-Derived Ingredients

Marine resources are a source of high value-added compounds with biological properties to be used as functional food ingredients. Several types of polysaccharides, such as sulfated polysaccharides, chitin or chitosan, proteins and protein hydrolysates, peptides, amino acids such as taurine, omega-3 oils, carotenoids, and other bioactive compounds are examples of compounds that can be added at different stages, from processing to storage, of the food production process. Since dairy products are widely accepted by consumers, the use of this type of product to deliver bioactive compounds has received attention from the food industry in the last years. Functional foods and natural health products are an emerging field in food science due to their increasing popularity with health-conscious consumers and are a source of new opportunities for the agri-food sector. Food products containing marine-derived chitin, chitosan, as well as oils rich in omega-3 fatty acids, are some food products that are being commercialized in several markets around the world including Japan, the USA, and some European countries.

The consumption of functional foods can provide various nutritional/health benefits, with diet controls and modulates many functions of the body, maintaining good health and homeostasis. Enhancement of immunity and antioxidant effect are most studied health benefits. Nowadays, marine- derived functional ingredients such as fish oils, fish proteins, and seaweeds themselves have found application in bakery, dairy, confectionary, and pasta products. They are added as fortificants and nutritional enrichments in food, to form functional foods. More concerted efforts in research and design of novel marine ingredients-based functional foods are needed to contribute to the reduction of health problems through diet. Despite the scientific progress in the use of marine-derived food ingredients, there still are various challenges ahead that have to be overcome:

i) Efficient extraction methods and purification steps, to obtain food grade validated extracts or purified compounds with biological properties (antioxidant, antibacterial, prebiotic, and others). Isolated functional ingredients should rely upon food methods compatible with economically viable yields. Hence, different extraction methods must

be applied in order to maximize the extraction efficiency of functional ingredients with biological properties.

ii) To design functional foods based on the incorporation of marine-derived functional ingredients upon biological validation. Consumers are more inclined to buy functional foods with physiological health claims.

iii) Foods should have good sensorial characteristics in order to be accepted by the consumer. In general consumers do not compromise taste for health. This is, in fact, one of the most important challenges to overcome in the use of some of the marine compounds, for example, fish oil.

Conclusions

In the present scenario people are very health cautious and prefer to consume organic food stuff which are free from antibiotics, pesticides, hormones and other contaminants. The ban on usage of antibiotics, pesticides and hormones in aquaculture industry improved the farmed fish quality but still it needs certain value addition to enhance health benefits of consumers. Similarly, to mitigate the stress in culture condition (present intensive farming practices), in addition to feed, certain compounds are desired by cultured fish. Now, the industry is looking for alternative products which are derived from nature (organic) and having the nutritional and health benefits. Marine nutraceuticals are naturally available organic substances which are having greater health promoting factors and are derived from seaweeds, marine micro algae, marine lipids, etc. So, directly or indirectly, marine nutraceuticals help in near future, to gratify everyone's (fish farmers and consumers) necessity of the aquaculture industry and overall enhance the aquaculture production.

****

Seaweeds as a Source of Micro and Macro Nutrients

Suseela Mathew and Ravishankar C.N.

Biochemistry and Nutrition Division

ICAR-Central Institute of Fisheries Technology, Cochin

Email: suseela1962@gmail.com

Introduction

The term seaweeds is used to refer to an assemblage of a diversegroup of multicellular marine macroalgae that usually grow along the coastlinesof oceans mostly as benthic forms (occasionally as floating forms) across different geo-climatic regions. Seaweeds are generallyregarded as marine plants since they are photosynthetic and thus, have similar ecological roles as that of other plants. Nevertheless, seaweeds are distinct from other marine plants such as seagrasses and mangroves in that they lack true roots, leaves, stem, flowers, vascular tissue etc. All marine plants obtain their nutrients including micronutrients and other compounds directly from the seawater for their growth, proliferation and development. Therefore, the growth of these plants is generally dependent on the availability of nutrients and sunlight, and lack of availability of any of these would limit the growth and overall productivity of seaweeds.

The typical structure of seaweed consists of the foliose blade (also called as the frond), the stem-like stipe and the holdfast that attaches the plant to a hard substrate. The entire plant, therefore, is called the thallus. Variation in the structureof the thallus provides a great degree of diversity in form. Seaweeds exhibit a wide diversity in their thallus morphology ranging from the simple crusts, leafy blade, tubular to filamentous form with a simple branchingto more complex forms. The size of seaweeds ranges from a few centimeters to several meters. The temperate giant kelps grow as tall as 60 meters in length. The seaweed beds provide excellent habitat and food for several economically important shellfish and crustaceans, while rendering invaluable ecosystem services that play a central role in maintaining the health of oceans.Seaweeds, based on photosynthetic pigments, are broadly divided into three groups: chlorophytes or green algae, rhodophytes or red algae, and phaeophytes or brown algae. The green algae like land plants contain *chlorophylla* and *b* and require good levels of light. Therefore, these forms are mostly foundin littoral waters. The red seaweeds contain *chlorophyll a* and *d*, and phycobilins (phycoerythrin and phycocyanin) which collectively imparts characteristic red colour to thallus. Contrary to the green seaweeds, red seaweeds can survive with the least light and indeed requires low levels of light and thus can be found even in deepest under waters where blue light alone penetrates. In brown algae, *chlorophyll a* and *c* is present along with fucoxanthin which gives a browncolour. Like green seaweed, it does require sunlight but less of it. Therefore, it can be found growing in deeper waters (but not as deep as the red seaweed). Thus, the vertical and horizontal distribution of seaweeds is determined in part by the availability of sunlight and consequently varies by depth, latitude andseason. Temperature also affects the distribution of seaweeds. The greatest number of seaweed species is in tropical waters as compared to the temperate waters. The species diversity decreases from the tropical waters to temperate waters. Also, the temperate species are quite different from that of

tropical flora and grow as perennials, i.e. growing longer duration for more than two years.

There are also several other distinct features in their evolutionary history, cell wall composition, cellular storage materials, reproductive strategies, life cycles distinguishing each major group of seaweeds. Contrary to land plants, seaweed cell walls are structurally complex and diverse in chemical composition. The amorphous matrix components dominate over crystalline skeletal components. As in higher plants, the most common skeletal polysaccharide is cellulose, a linear polymer of D-glucose with a β-1, 4 linkages. The matrix polysaccharides of seaweeds show great structural variability with significant eco-physiological and commercial interest. In chlorophyta, the matrix polysaccharides are either xylogalactoarabinans or glucurono xylo-rhamnans (ulvan) with varying sulphate contents. In rhodophytes, mainly linear sulphatedgalactans composed of two regularly repeated galactose units alternatively linked by β(1-4) and α (1-3) linkages or D-galactose alternatively linked by β (1-4) and α (1-3) linkages (agars and carrageenans) are found. The major matrix component in brown seaweeds (10-45% of the thallus dry wt) is a polyuronide made up of two β-1, 4-D-mannuronic acid and α-1, 4-L-guluronic acid (alginate).

The cellular storage materials -excess sugars synthesised as part of photosynthesis is converted into polymers and stored as food reserves in the cell as starches- also variesin terms of yield and composition among the groups of seaweeds. The chlorophycean members, like land plants, have starch consisting of amylose and amylopectin while rhodophycean with floridian starch (polymer of glucose, amylopectin, and glycogen) and phaeophycean with laminarian starch (polymer of glucose and mannitol). Some of these metabolites are found to have high bioactive functions while some (sugar alcohols) are known toprotect them from severe freezing climate.

Utilisation of Seaweeds

Seaweeds as Food

The food value of seaweeds depends on the proteins, minerals, trace elements and vitamins. Marine algae have almost all essential amino acids required in the human food. Genus *Acanthophora*, *Caulerpa, Codium, Enteromorpha, Eucheuma, Gracilaria, Laminaria, Laurencia, Macrocystis, Monostroma, Porphyra, Ulva* and *Undaria* constituted protein-rich algae and consumed as a salad, soup and curry in China, Indonesia, Japan, Korea, Malaysia, Philippines, and Thailand. Species of *Ulva*, *Enteromorpha*, *Monostroma* and *Porphyra* are added in soup in China, Japan, and Korea, while *Laminaria*and *Undaria*are eaten in dried form. In the Philippines, *Caulerpalentilliferai*s consumed as salad while *Codiumtomentosum, Eucheuma denticulatum* and *Kappaphycus alvarezii* in the form of curry. Several algal food products are in use in Southeast Asian countries that include jellies from *Gelidiella*a nd *Gracilaria*; jams from *Enteromorpha* and *Ulva*; pickle from *Acanthophora, Gracilaria*, *Hypnea* and *Laurencia* species. Agar is added in the preparation of foodstuffs such as tomato sauce, ice cream, jelly, lime jelly and marmalade.

Seaweeds as Nutrition Supplement

Seaweeds are more nutritional plants than land-based higher plants. Seaweeds do not have a circulatory system, leaves, roots, and stem,so it does not waste energy.It is the culinary delicacy in traditional Japanese and Chinese diet. Chinese utilizes highest

seaweed species in their diet than any other ethnic group in the world. The recent report released by the Ministry of Health, Labour and Welfare, Japan (2015) indicate that the life expectancy for men and women in 2014 is the highest ever recorded in the world. The average life expectancy for Japanese men is 80.5 years of age, while for women; life expectancy was calculated at 86.83 years of age. *(Source: Delaney et al., 2016)*. The Okinawa Prefecture of Japan has among the world's longest-lived population. The evidence of longevity comes from a combination of two important indices, namely average life expectancy, and that of the centenarian ratio (number of centenarians per one hundred thousand). They use kombu in large proportion in their daily diet.The interesting study revealed that maximum patenting (182 patents) being carried out under the category of food additives and beverages followed by their applications in nutraceuticals during 1836 to 2008

Seaweed Polysaccharides

Green algae (Chlorophyceae) contain sulphatedgalactans and acidicpolysaccharide. *Ulva* species contain a high amount of polysaccharides, i.e. 65% of dry weight. Brown algae (Phaeophyceae) yield alginic acid, fucoidanand laminarin. Red algae (Rhodophyceae) give agar, carrageenan, floridean and porphyran.

Dietary Fibres

Marine algae are a rich source of water-soluble (alginic acid, agars, furonan, laminarin and porphyran) and water-insolublefibres (cellulose, mannans andx ylan) which contain some valuable nutrients and also behave as functional foods. These fibres play an active role against obesity, cholesterol and large intestine cancer). In algae, high amount of dietary fibers (% dry weight) reported exceed those for wheat bran, ranging from 23.5% (*Codium reediae*) to 64.0% (*Gracilaria* spp) *Porphyra umbilicalis* contains slightly more fiber (3.8%) than bananas (3.1%)

Protein and Amino Acids

Seaweed protein contents differ greatly from phylum to phylum. Brown algae contain 5-16 % of protein while green algae have 10-30% of protein and red algae possess 15-20% of protein. Some red seaweeds such as *Palmaria palmata*and *Porphyra tenera* contain 36% and 48% of proteins respectively, are comparable with 35% content of soybeans. The protein in *Ulva* species is in the range of 15- 20%. The free amino acids are composed of alanine, amino butyric acid, citrulline, hydroxyproline, ornithine, and taurine. The edible algae have almost similar essential amino acid composition. Some have a high level of arginine e.g., *Porphyra tenera, Ulva pertusa* and *Undaria pinnatifida. Porphyra*species contain up to 70 % dry weight protein along with all of the essential amino acids that human cannot synthesize such as leucine, lysine, methionine, threonine, tryptophan, and valine compares well with egg albumin.

Lipids and Fatty Acids

Seaweed lipid contents are polyunsaturated fatty acids with omega-3 and omega -6 acids, which are important to prevent cardiovascular diseases, diabetes, and osteoarthritis. Green algae contain alpha-linolenic acid, while brown and red algae are rich in eicosapentaenoic acid and docosahexaenoic acid. Alpha-linolenic acid, eicosapentaenoic acid anddocosahexaenoic acid are omega-3 fatty acids, which are important for human physiology. Different types of sterols are reported from seaweeds. Green algae contain cholesterol, methylene cholesterol and β- sitosterol. Desmosterol, cholesterol, sitosterol, fucosterol and chalinasterol are common in red algae. Brown algae contain a high level of fucosterol. *Laminaria* and*Undaria* species contain 83-97%

of fucosterol of total sterol (0.66 - 2.32 mg/g dry weight). *Palmaria* and *Porphyria* species reported to possess 87-93% desmosterol of total sterol (0.08 - 0.33 mg/g dry weight).

Minerals

Seaweeds are also an important source of a variety of minerals of nutritional importance. Seaweeds have high calcium, iodine, iron, potassium, phosphorus and sodium.Genus *Porphyra* contains high Fe, ranges from (0.2–0.7 g/100 g). High manganese is reported from *Pseudofalla ciatenera,* i.e. 33.2–409 µg/g dry weight.Seaweeds are a good nutritional source for iodine, particularly in foods deficient regions. *Laminaria* and *Saccharina* species are traditionally used for treating thyroid goitre due to their high iodine contents.

Vitamins

Some red seaweed, e.g. *Palmaria palmate* and *Porphyra tenera* have a large quantity of vitamins A, B_1, B_2 and B_{12}. β-carotene (pro-vitamin A) found in *Codium fragile,* and*Gracilaria chilensis* exceeds those measured in carrots. *Ulva* and *Pyropia* sp. contain considerable amounts of vitamin B_{12}*Gracilaria changii*, *Himanthalia elongate,* and *Porphyra umbilicalis* contain same levels of vitamin C as of tomatoes and lettuce. The vitamin-C content of brown seaweed *Eisenia arborea* (34.4 mg/ 100 g dry wt) matches those reported for mandarin oranges. The Vitamin C contents of brown and green algae ranges from 50 to 300 mg/100 g dry weight, are comparable to *Petroselinum crispum* (Fuss. i.e. parsley 2009).Brown algae contain vitamin E higher than green and red seaweeds. *Ascophyllum* and *Fucus* sp. contain 200 - 600 mg of tocopherols/kg of dry weight. *Macrocystis pyrifera* contain similar levels of α-tocopherol (vitamin E) as compared with vitamin E rich plant oils such as *Elaeis guineensis*J acq. (palm oil), *Helianthus annuus* L. (sun flower seed oil) and *Glycine max* (L.) Merr. (soybean oil).

Seaweeds as Medicine

Seaweeds are rich in the antioxidant vitamins C and E, in higher concentrations than land plants. Vitamin C prevents from scurvy, while vitamin E helps to manage neurological problems due to poor nerve conduction and anaemia due to oxidative damage to red blood cells. Algae iron is more readily absorbed by the human body as compared with higher land plants due to its blue pigment, phycocyanin, which forms soluble complexes with iron and other minerals during digestion. The phenolic-rich extracts obtained from *Alaria, Ascophyllum, Palmaria, Ulva* species are not only natural antioxidants but also inhibit digestive enzymes and achieve anti-diabetic effects. *Laminaria* species (kelp) contain up to 13 times more calcium than milk and powerful antioxidants. *Fucoxanthin* and *fucoidan* kelps are rich in vitamin B, C and K1 with high mineral contents of magnesium, potassium and iron.

Seaweeds in Industrial Uses

Agar, algin and carrageenan are the three major polysaccharides obtained from seaweeds. Agar is extracted from red algae such as *Gracilaria, Gelidiella, Gelidium,* and *Pterocladia*; while carrageenan from *Eucheuma, Gigartina* and *Hypnea.* Algin is obtained from brown algae like *Sargassum* and *Turbinaria, Ascophyllum, Cystoseira, Lallinaria, Macrocystis.* These polysaccharides have more than 200 industrial uses.

The physical properties and texture of agar gel determine its application, especially in the food and pharmaceutical industries. Agar with gel strength of about 400 $g.cm^2$ is generally used in various food products. Agar with about 800 $g.cm^2$gel strength is being used in biotechnological and pharmaceutical industries as a substrate for bacteriologic

culture and tissue culture eukaryotic cell research. Carrageenan is having major industrial use as ingredients for gelling, thickening and stabilizing food, pharmaceuticals, cosmetics, hand and body lotions, shampoo, soap, toothpaste, gel fresheners and many other consumer products.Alginates, extracted from brown seaweeds, are used by the textile industry as thickeners for the paste containing dye. The chemical and pharmaceutical industries utilize alginate and carrageenan as immobilizing agents for various biocatalysts in commercial synthesis and conversion reactions. In the paper industry, alginate is used for surface sizing applications to give a continuous film surface. Xanthophyll has a large application in the colouration of cosmetic and drugs.

Biofuels from Seaweeds

The carbohydrate content of seaweed, about 50% of dry mass, can be used in biofuel production. Production of energy inseaweed can be as simple as microbial anaerobicdigestion to produce methane or as complex asa microbial breakdown of lignins and other complex carbohydrates into simple sugars for use in ethanolproduction. An annual harvest of 500million dry tons of seaweeds with 50% carbohydratecontent could produce about 1.25 billion megawatt-hours worth of methane or liquid fuel. The worldused about 85 billion megawatt-hours of energy fromfossil fuels in 2012, so energy production from theseseaweed products would equate roughly to 1.5% ofcurrent energy use from fossil fuels (IEA 2014). In terms of gas yield per tonnage,the largest size seaweed *Laminaria* sp. to biogas achieves yields of 22 m^3 per tonne of seaweed.In terms of energy yield per hectare -Current state-of-the-art seaweed-to-methane achieves a yield of 171 GJ/ha.

Seaweed-based Crop Bio stimulants

The use of sap of different seaweeds as plant growth stimulant is gaining momentum for sustainable agricultural productivity. Most of the seaweed-basedagri products available in the market (mostly imported) are manufactured from brown algae.

Global Market Value of Seaweeds

Global aquaculture production of aquatic plants in 2015 was 29.4 million tonnes with an estimated commercial value of US$4.8 billion. Seaweeds dominate the production of aquatic plants. In 2015, aquatic plants farming counted for 27.7% in the total production volume of global aquaculture in the world. Initially, seaweeds were most often used for domestic purposes as food and feed, whereas later, industrial uses (gels, fertilizers) emerged (Delaney et al., 2016).The global seaweed processing industry is estimated to use some 27 million tonnes of seaweeds annually, collected as 'wild harvest' or cultivated in offshore and onshore farms. The bulk of seaweed produced globally is from aquaculture, categorized as cultivated production (FAO, 2016). Wild harvest of seaweeds only accounted for about less than five percent of total seaweed production in the year 2015. Wild harvest includes harvesting of seaweeds by hand or collection of beach cast/drift algae. There is a large and diverse array of applications and uses of macroalgal products. The seaweed hydrocolloid (agar, alginate, and carrageenan) industry showed 2–3% growth per year with the Asia-Pacific region increasingly dominating the raw material and manufacturing aspects of the industry. The seaweed industry is estimated to have an annual value of US$4.8 billion, the largest share of which is human food products (FAO, 2016). The remaining US$1.058 billion is largely based on seaweed hydrocolloids (Porse and Rudolph, 2017), At least 221 species of seaweeds are exploited globally, with 145 species for food and 101 species for

phycocolloid production. These include 32 chlorophytes, 125 rhodophytes and 64 phaeophytes.

Indian Scenario

Wild harvest

India has a long coastline and more than 7,500 km coastline is rich in algal diversity and resources. There are about 840species of seaweeds have been reported. Indian coastline is bestowedwith larger stretches of suitable areas for cultivation and communities of traditional fisherfolk who have interest in seaweed cultivation. In spite of all these advantages, commercial cultivation has not been taking place as being practised in South-East Asian countries. Seaweed raw material for seaweed-basedphycocolloid industries are mainly gathered from wild stocks.

In India, *G. acerosa* and *G. edulis*are being commercially harvested for agar production and *Sargassum,* and *Turbinaria*are harvested for alginate production since the early 1950s. These algae are harvested from 20 islands and the mainland coast of Gulf of Mannar and Palk Bay, Southeast coast of India. A broad (approximately 300 meters wide) and long stretch (approximately 140 km long) of coralreef runningparallel to the islands and reef in the coastalregion of Gulf of Mannar from Kilakkarai to Valinokkamsupports the growth of *Sargassum*spp, *Turbinaria* spp., *G. acerosa* and *G.edulis*.

Similarly, the coastline of Palk Bay from Mallipattinam (in Pudukkottai District) to Devipattinam (in Ramanathapuram District)harbours rich resources of *Gracilariasalicornia* and *Gracilariaedulis*. Commercial harvest of these seaweeds from the reef of Gulf of Mannar islands and mainland coast is donemostly during spring tides of the full moon and new moon days. About 5000 women in the age group of 18-35 with primary level education are harvesting the seaweeds from the reef. Seaweeds harvested from the Gulf of Mannar island reef and the mainland are collected in the boat and brought to the shore. Agents or middle men are procuring the seaweeds from the harvesters on the shore itself and selling the seaweeds to the industry.

Landings of Agar yielding seaweeds viz. *Gelidiella acerosa* and *Gracilaria edulis* during 2003 to 2016 showed gradual depletion of their resources. *G. acerosa*is firmly attached to the hard substrata and difficult for uprooting the alga while *G*. *edulis* is attached to the muddy and sandy bottom which can be uprooted easily. Landings of alginate yielding seaweeds viz. *Sargassum* spp. And *Turbinaria* spp during 2003 to 2006 showed heavy fluctuations.Nevertheless, hundreds of thousands of tonnes of *Sargassum* and *Turbinaria* are available in the resources, only few thousand tonnes are being harvested annually.

Reference

- Delaney, A.K. Frangoudes, S.A. Ii (2016) Society and Seaweed: Understanding the Past and Present. In Seaweed in Health and Disease Prevention (J. Fleurence, I. Levin,eds), pp. 7–38. Academic Press.
- Food and Agriculture Organization of the United Nations Rome 2016, 'The State of World Fisheries and Aquaculture', Contributing to Food Security and Nutrition for All'.
- Policy paper made by TIFAC on seaweeds.

****

Thermal Processing of Fishery Products

C.O. Mohan

Fish Processing Division

ICAR-Central Institute of Fisheries Technology

Email: comohan@gmail.com

Preservation is the process achieved to store food for storing longer duration. Human beings are dependent on products of plant and animal origin for food. As most of these products are readily available only during certain seasons of the year and fresh food spoils quickly, methods have been developed to preserve foods. Preserved foods can be eaten long after the fresh products would normally have spoiled. Preservation must be seen as a way of storing excess foods that are abundantly available at certain times of the year, so that they can be consumed in times when food is scarce.

Fish and shellfishes pass through a number of processing stages immediately after catch before it is consumed or sold for consumption. These processes can be divided into primary processing and secondary processing. Primary processing includes the steps that enable fish to be stored or sold for further processing, packaging and distribution. Examples include washing, cleaning, heading, gilling, scaling, gutting, grading, filleting, de-boning, skinning, chilling and freezing, whereas secondary processing includes the production of 'value-added products'. Examples are salting, drying, smoking, canning, marinating and packaged ready to eat foods. There are number of reasons for processing fish and shellfish which are given below.

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

Fresh fish will spoil very quickly due to its internal and external factors. Once the fish has been caught, spoilage progresses rapidly. In the high ambient temperatures of the tropics, fish will spoil within few hours. The storage life of fishery products can be increased by adopting good fishing techniques (to minimize fish damage) and cooling the fish immediately to minimize the spoilage of caused by enzymatic, bacterial action and oxidation process. Fish spoilage can be effectively minimized if the effects of enzymes, bacteria and oxidation are controlled properly. This can be achieved by understanding the optimum conditions that enzymes, bacteria and oxidation processes prefer and modifying these conditions. Many processing techniques aim to alter these conditions to achieve preservation. Some of the approaches are given in Table 1.

Table 1. Possible preservation approaches

Approaches	***Examples of process***
Low temperature	Chilling, Refrigeration, Freezing
High temperature	Pasteurization, Thermal processing,

	smoking
Reduced water availability	Drying, salt curing, spray drying, freeze drying
Chemical based preservation	Organic acids, natural extracts from plants
Microbial product based	Bacteriocins
Radiation	Ionizing (Gamma rays) and non-ionizing (UV rays) radiation
Hurdle technology	Altered atmosphere (vacuum and modified atmosphere with CO_2, O_2, N_2 and other gases); active packaging; high pressure treatment; smoking etc

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

a) type and heat resistance of the target microorganism, spore, or enzyme present in the food
b) pH of the food
c) heating conditions
d) thermo-physical properties of the food and the container shape and size
e) storage conditions

Thermal processing is designed to destroy different microorganisms and enzymes present in the food. Normally in thermal processing, exhausting step is carried out to before sealing the containers. In some cases, food is vacuum packed in hermetically sealed containers. In such cases very low levels of oxygen is intentionally achieved.

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

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

Table 2. Approximate pH range of different food

Food	pH	Food	pH
Lemon juice	2.0 - 2.6	Sweet potato	5.3 – 5.6
Apples	3.1 - 4.0	Onion	5.3 – 5.8
Blueberries	3.1 – 3.3	Spinach	5.5 – 6.8
Sauerkraut	3.3 – 3.6	Beans	5.6 – 6.5
Orange juice	3.3 – 4.2	Soybeans	6.0 – 6.6
Apricot	3.3 – 4.0	Mushroom	6.0 – 6.7
Bananas	4.5 – 5.2	Clams	6.0 – 7.1
Beef	5.1 – 7.0	Salmon	6.1 – 6.3
Carrot	4.9 – 5.2	Coconut milk	6.1 – 7.0
Green pepper	5.2 – 5.9	Milk	6.4 – 6.8
Papaya	5.2 – 6.0	Chicken	6.5 – 6.7
Tuna	5.2 – 6.1	Whole egg	7.1 – 7.9

The acidity of the substrate or medium in which micro-organisms are present is an important factor in determining the extent of heat treatment required. With reference to thermal processing of food products, special attention should be devoted to *Clostridium botulinum* which is a highly heat resistant mesophilic gram positive, rod shaped spore-forming anaerobic pathogen that produces the toxin *botulin*. It has been

generally accepted that *C. botulinum* and other spore forming, human pathogens does not grow and produce toxins below a pH of 4.6. The organisms that can grow in such acid conditions are destroyed by relatively mild heat treatments. For food with pH values greater than 4.5, which are known as low-acid products which includes fishery products, it is necessary to apply a time–temperature regime sufficient to inactivate spores of *C. botulinum* which is commonly referred to as a *botulinum cook* in the industry. Thermal processes are calibrated in terms of the equivalent time the thermal centre of the product, i.e. the point of the product in the container most distant from the heat source or cold spot, spends at 121.1°C, and this thermal process lethality time is termed the F_0 value. Although there are other microorganisms, for example *Bacillus stearothermophilus*, *B. thermoacidurans,*and *C. thermosaccolyaticum*, which are *thermophilic* in nature (optimal growth temperature ~ 50–55°C) and are more heat resistant than *C. botulinum a* compromise on the practical impossibility of achieving full sterility in the contents of a hermetically sealed container during commercial heat processing, whereby the initial bacterial load is destroyed through sufficient decimal reductions to reduce the possibility of a single organism surviving to an acceptably low level. This level depends on the organism, usually *Clostridium botulinum*, which the process is designed to destroy. The time required to reduce the number of spores of this organism (or any other micro-organism) by a factor of 10 at a specific reference temperature (121.1°C) is the decimal reduction time, or *D* value, denoted D_0. The D_0 value for *Clostridium botulinum* spores can be taken as 0.25 minutes. To achieve a reduction by a factor of 10^{12}, regarded as an acceptably low level, requires 3 minutes at 121.1°C, and is known as the process value, or *F* value, designated F_0 so, in this case, F_0 = 3, which is known as a botulinum cook which is the basis of commercial sterility.

Thermal resistance of microorganisms

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

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

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

From the survivor curve, as the graph is known, it can be seen that the time interval required to bring about one decimal reduction, i.e. 90% reduction in the number of survivors is constant. This means that the time to reduce the spore population from 10,000 to 1000 is the same as the time required to reduce the spore population from 1000 to 100. This time interval is known as the decimal reduction time or the 'D' value.

The D value for bacterial spores is independent of initial numbers, but it is affected by the temperature of the heating medium. The higher the temperature, faster the rate of thermal destruction and lower the D value. The unit of measurement for D is 'minute'. An important feature of the survivor curve is that no matter how many decimal reductions in spore numbers are brought about by a thermal process, there will always be some probability of spore survival. Different micro-organisms and their spores have different D values as shown in Table–3.

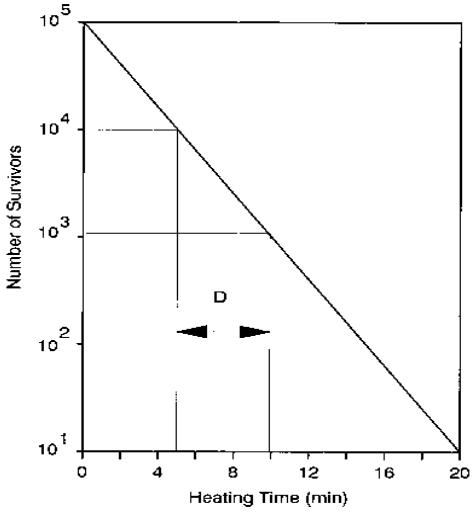


Fig 1. Survivor curve

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

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

The thermal death time may be defined as the time required at any specified temperature to inactivate an arbitrarily chosen proportion of the spores, the higher the proportion the greater will be the margin of safety. TDT is the heating time required to cause complete destruction of a microbial population. Such data are obtained by subjecting a microbial population to a series of heat treatments at a given temperature and testing for survivors. The thermal death time curve is obtained by plotting the thermal death time on logarithmic scale against temperature of heating on linear scale on a semilogarithmic graph paper (Fig. 2). Comparing TDT approach with the decimal reduction approach, one can easily recognize that the TDT value depends on the initial microbial load (while D value does not). Further, if TDT is always measured with reference to a standard initial load or load reduction, it simply represents a certain multiple of D value. For example, if TDT represents the time to reduce the population from 10^0 to 10^{-12}, then TDT is a measure of 12 D values. i.e., TDT = nD, where n is the number of decimal reductions. The extent of inactivation in the case of pathogenic microorganisms (*C. botulinum*) is equivalent to a 12 D process. The slope of the TDT curve is defined as 'z' value, which is the number of degrees for the TDT curve to traverse one log cycle. The temperature sensitivity indicator is defined as z, a value which represents a temperature range which results in a ten-fold change in D values or, on a semilog graph, it represents the temperature range between which the D value curve passes through one logarithmic cycle. The 'z' value which is also known as the temperature sensitivity indicator is usually taken as 10°C in the case of *C.botulinum.*

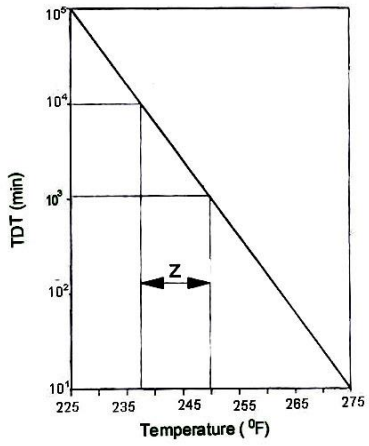


Fig. 2 TDT Curve

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

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

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

Thermal Process Severity or F_0 value

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

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

where, t = time required to achieve commercial sterility

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

$$F_0 = mD_{121.1°C}$$

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

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

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

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

Commercial sterility

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

Mechanism of heat transfer

Understanding the mechanism of heat transfer is very important for thermal processing. Normally, there are three different modes of heat transfer: conduction, convection and radiation. Conduction is the transfer of heat by molecular motion in solid bodies. Convection is the transfer of heat by fluid flow, created by density differences and buoyancy effects, in fluid products. Radiation is the transfer of electromagnetic energy between two bodies at different temperatures. In thermal processed foods, the mechanism of heat transfer is either by conduction, convection or by broken heating (combination of conduction and convection). The factors which determine the mode of

heat transfer are nature or consistency of a food product, the presence of particles, and the use of thickening agents and sugars. The heating modes in the thermal processing are first by heat transfer to the container or packaging material from heating and cooling media, second through the container wall and third is into the product from container wall. Convective-heat transfer rates depend largely on the velocity of flow of the media over the container, and this is an important factor to be controlled in all processing operations. In conduction heading method, energy transfer takes place when different parts of a solid body are at different temperatures. The slowest heating point or cold point in cylindrical metal containers is at its geometric centre for food products heated by conduction method. Convection heat transfer involves the transfer of heat from one location to the other through the actual movement or flow of a fluid. The slowest heating point for convection heated products in cylindrical metal container is approximately $1/10^{th}$ up from the base of the container. Packaging material forms the most important component of thermal processed foods. It should be able to withstand the severe process conditions and should prevent recontamination of the product.

Containers for thermal processing

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

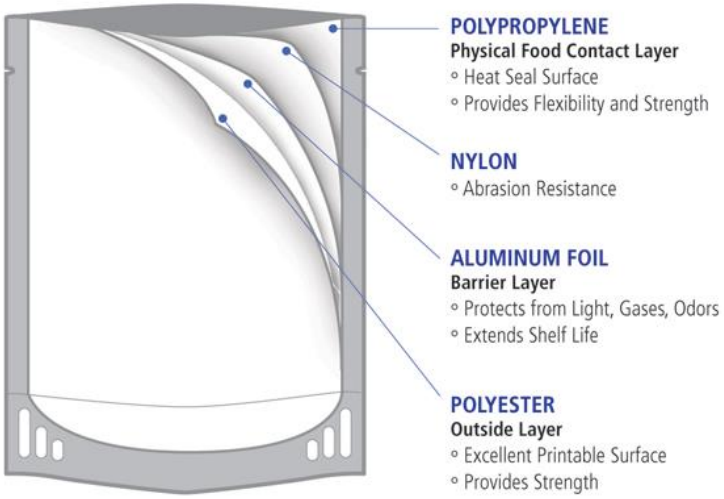
Table 1. Cans used in fish canning industry

Trade Name	Dimension	Over-seam dimension
41/2 OZ prawn cans	301 x 203	77 x 56
8 oz prawn cans	301 x 206	77 x 60
1 lb. jam can	301 x 309	77 x 90
No.1 tall can	301 x 409	77 x 116
8 oz. tuna can	307 x 113	87 x 43

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:

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

Containers used for thermal processing



Composition of Retortable pouch

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

The thermal processing is carried out for achieving two objectives; the first is consumer safety from botulism and the second is non-pathogenic spoilage which is deemed commercially acceptable to a certain extent. If heat processing is inadequate the

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

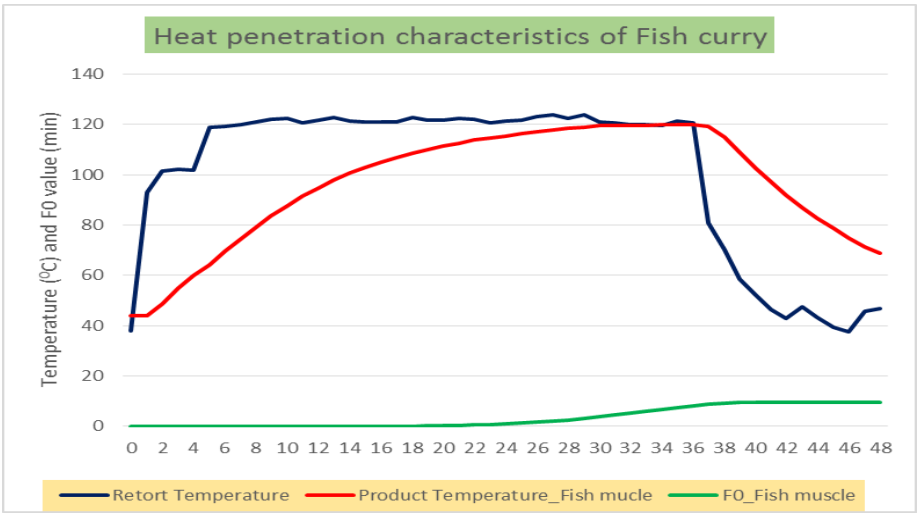
Fishery products, being categorized as low acid foods require heat processing severity with respect to *C botulinum* and F_0 value recommended is 5-20 min. Thermal processing of fishery products include various steps. These steps include, preparations like washing, beheading, gutting, removing scales / fins, cutting into required size, blanching (hot / cold), pre-cooking, filling fish pieces into containers, filling content or medium, exhausting to remove air, sealing, loading into the retort or autoclave, sterilization, washing and storing. Various packaging materials have been used from historically starting from glass container to metal container, flexible retortable pouches and rigid plastic containers. The sterilization process in the canned product can be subdivided into three phases. First one is heating phase, in which the product temperature is increased from ambient to the required sterilization temperature by means of a heating medium (water or steam). This temperature is maintained for a defined time (phase 2 = holding phasing). In (phase 3 = cooling phase) the temperature in the container is decreased by introduction of cold water into the autoclave. In order to reach temperatures above 100°C (sterilization), the thermal treatment has to be performed under pressure in pressure cookers, also called autoclaves or retorts. Simple autoclaves are generally vertical ones with the lid on top. Through the opened lid, the goods to be sterilized are loaded into the autoclave. The cans are normally placed in metal baskets. The autoclave and lid are designed to withstand higher pressures up to 5.0 bar. These types of autoclaves are best suited for smaller operations as they do not require complicated supply lines and should be available at affordable prices. Larger autoclaves are usually horizontal and loaded through a front lid. Horizontal autoclaves can be built as single or double vessel system. The double vessel systems have the advantage that the water is heated up in the upper vessel to the sterilization temperature and released into the lower (processing) vessel, when it is loaded and hermetically closed. Using the two–vessel system, the heat treatment can begin immediately without lengthy heating up of the processing vessel and the hot water can be recycled afterwards for immediate use in the following sterilization cycle. In rotary autoclaves, the basket containing the cans rotates during sterilization which enhances the heat penetration resulting in reduced process time. This technique is useful for cans with liquid or semi-liquid content as it achieves a mixing effect of the liquid/semi-liquid goods. Water immersion

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

The important steps in canning process are:

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

Steam retort and water immersion retort



Typical heat penetration curve of fish curry in retortable pouches

****

An Overview of Non-Thermal Preservation Techniques in Food

Sarika K. and Bindu J.

Fish Processing Division

ICAR-Central Institute of Fisheries Technology, Cochin

Email: sarikacift@gmail.com

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

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

1. High pressure Processing(HPP)

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

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

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

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

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

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

Mechanism of Pressure Treatment

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

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

Major Advantages of the Technology

1. HPP does not involve in breaking covalent bonds which prevents the development of unpleasant flavours to the product and maintains the natural freshness and quality.
2. High pressure is able to modify the palatability and functional properties by inducing denaturation and muscle protein gelation.
3. Process can be carried out at ambient temperatures, that helps in reducing the thermal energy used during conventional processing.
4. High pressure processing is isostatic in nature, equally applied to all particles of food, with no particle escapes.
5. Since high pressure is not time-mass dependent, pressure acts instantaneously thereby reducing the processing time.
6. This non thermal technology is independent of size and geometry of the food.
7. The process is ecofriendly, with no waste and requires only electric energy.

Application in marine Products

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

High Pressure Processing Facility at ICAR-CIFT

A Research model of 2 litre capacity High Pressure machine from M/s Stansted Fluid Power Ltd, United Kingdom at Central Institute of Fisheries Technology, Cochin.

2. Pulse electric field

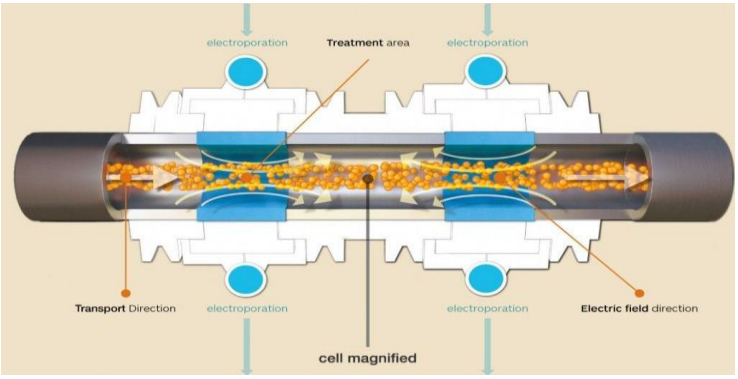
Pulsed electric field processing is a non-thermal food preservation technique used mainly for inactivation of microbes. PEF technology is the application of short pulses of high electric fields with duration of micro- to milliseconds and intensity in the order of 10-80 kV/cm in order to preserve the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

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

Working

PEF technology is based on a pulsing power delivered to the product placed between a set of electrodes confining the treatment gap of the PEF chamber. The equipment consists of a high voltage pulse generator and a treatment chamber with a suitable fluid handling system and necessary monitoring and controlling devices. Food product is placed in the treatment chamber, either in a static or continuous design, where two electrodes are connected together with a nonconductive material to avoid electrical flow from one to the other. Generated high voltage electrical pulses are applied to the electrodes, which then conduct the high intensity electrical pulse to the product placed

between the two electrodes. The food product experiences a force per unit charge, the so-called electric field, which is responsible for the irreversible cell membrane breakdown in microorganisms. This leads to dielectric breakdown of the microbial cell membranes and to interaction with the charged molecules of food. Hence, PEF technology has been suggested for the pasteurization of foods such as juices, soups, and other liquid based products.



(Source i³ foods)

3. **Pulsed Electric Field Preservation**

Pulsed electric field can be applied in fishes fresh and frozen fish dried, brined or marinated fish. Mass transport processes, such as moisture transport and removal, are improved by the electroporation of fish tissue, resulting in enhanced drying, brining and marinating of fish. The required field strength for cell disintegration of fish is 1,0 – 3,0 kV/cm and the energy delivery is 3 – 10 kJ/kg. The applied pulsed electric field leads to cell disintegration in tissue, enhancing product quality and production processes. It also helps in inactivation of parasites such as nematodes. PEF processing enhances mass transport, processes during extraction, pressing, drying, brining and marinating processes. PEF technology speeds up drying of food products, minimizing processing times and energy consumption. The process can be applied to fruits, vegetables, potatoes and meat. Enhancement of extraction processes is also an advantage of electroporation. Extraction and pressing yields are increased, for example for fruit juice, vegetable oil and algae oil and protein. PEF technology speeds up freezing of food products, allowing a reduction of processing times and energy consumption. The cell disintegration increases the freezing rates. Cellular water flows easily out of the cell and ice nucleation outside the cell starts. As smaller ice molecules are formed, product quality of frozen food is improved. (www. pulsemaster).

4. **Pulse Light technology**

Pulse light technology is one such explored Non thermal technology in the food industry, especially for decontamination of food surfaces and food packages. This technique works by applying high-voltage, high-current short electrical pulse to the inert gas in the lamp, which results in strong collision between electrons and gas molecules cause excitation of the latter, which then emit an intense, very short light pulse to decontaminate and sterilize foods (Palmieri & Cacace, 2005). Usually short pulses of light one to twenty flashes per second is used in food industry. The term light is generally used to mean radiations having wavelength ranging from 180 to 1100 nm, which includes ultraviolet rays (UV 180–400 nm, roughly subdivided into UV-A, 315–400 nm; UV-B, 280–315 nm; UV-C, 180–280 nm); visible light (400–700 nm) and

infrared rays (IR 700–1100 nm) (Palmieri and Cacace, 2005). This technology can be used for the rapid inactivation of microorganisms on food surfaces, equipment's and food packaging materials (Dunn et al., 1995). The effect on microorganisms is mostly due to the photochemical action of the ultra violet part of the light spectrum that causes thymine dimerization in the DNA chain preventing replication and ultimately leading to cell death (Gomez-Lopez et al., 2007).

The principle involved in generating high intensity light is that a gradual increase of low to moderate power energy can be released in highly concentrated bursts of more powerful energy. The key component of a Pulse Light unit is a flash lamp filled with an inert gas. A high-voltage, high-current electrical pulse is applied to the inert gas in the lamp, and the strong collision between electrons and gas molecules cause excitation of the latter, which then emit an intense, very short light pulse. It is generally accepted that UV plays a critical role in microbial inactivation. So pulsed light is a modified and claimed improved version of delivering UV-C to bodies. The classical UV-C treatment works in a continuous mode, called continuous-wave (CW) UV light. Inactivation of microorganisms with CW-UV systems is achieved by using low-pressure mercury lamps designed to produce energy at 254 nm (monochromatic light), called germicidal light (Bintsiset al.,2000). More recently, medium-pressure UV lamps have been used because of their much higher germicidal UV power per unit length. Medium-pressure UV lamps emit a polychromatic output, including germicidal wavelengths from 200 to 300 nm (Bolton & Linden, 2003). Pulse Light treatment of foods has been approved by the FDA (1996) under the code 21CFR179.41. The treatment is most effective on smooth, non-reflecting surfaces or in liquids that are free of suspended particulates. In surface treatments, rough surfaces hinder inactivation due to cell hiding.

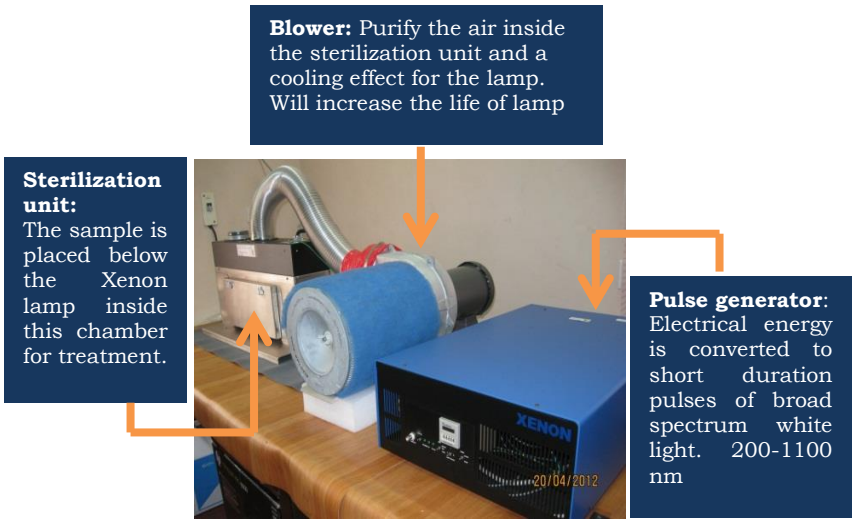
Generation of Pulsed Light

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

Merits and Demerits

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

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



Pulsed Light Equipment at CIFT

5. Ultrasound processing

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

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

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

- *Low power ultrasound:* Uses a small power level that the waves cause no physical and chemical alteration in the properties of the material through which it passes. This property is being utilized for non-invasive analysis and

monitoring of various food materials during processing and storage, to ensure quality and safety.

- *High power ultrasound*: Uses high energy [high power, high intensity] ultrasound of 20 and 500 kHz. It causes disruptive and enforce effect on the physical, mechanical, or biochemical properties of foods. These effects are promising in food processing, preservation and safety.

6. IRRADIATION

Irradiation is the process of applying low levels of radiation to any food material to sterilize or extend its shelf life. It is a physical method that involves exposing the prepackaged or bulk foodstuffs to gamma rays, x-rays, or electrons. Foods is generally irradiated with gamma radiation from a radioisotope source, or with electrons or x-rays generated using an electron accelerator (Barbosa-Canovas et al., 1998). These rays have high penetration power and thus can treat foods for the purpose of preservation and quality improvement. During exposure of food the amount of ionizing radiation absorbed is termed 'radiation absorbed dose' (rad) and is measured in units of rads or Grays. A strictly regulated process of dosimetry is used to measure the exact dose of radiation absorbed by the food. One Gray is equal to one joule of energy absorption per kilogram of a material. Irradiation has been approved for the microbial disinfestations of various food products in the US (USFDA, 1998). A number of countries have marketed irradiated products worldwide. Irradiation has the potential to enhance food safety for fresh foods that will be consumed raw and for raw foods that require further processing. Food irradiation mainly is done by the radioactive element cobalt-60 as the source of high energy gamma rays. Gamma rays are electromagnetic waves or photons emitted from the nucleus of an atom. These gamma rays have energy to dislodge electrons from food molecules, and to convert them into ions which are electrically charged. However, the rays do not have enough energy to dislodge the neutrons in the nuclei of these molecules and hence they are not capable of inducing radioactivity in the treated food. The radiation dose varies depending on the thickness moisture, and characteristics of the foods. External factors, such as temperature, the presence or absence of oxygen, and subsequent storage conditions, also influence the effectiveness of radiation (Doyle, 1990).

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

Table I gives details of irradiation processes for seafood.

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

Treatment and storage temperature	Radiation process	Benefits
-10° to -20°C Packaged, frozen, ready-to-export fish can be treated before shipment. Frozen storage	Radicidation (Radiation hygienization) Dose required: 4-6 kGy Elimination of non-spore forming pathogens such as *Salmonella, Vibrio, Listeria* etc.	Improvement of hygienic quality of frozen, materials for export such as frozen shrimp, cuttlefish, squid, finfish, fillets, and IQF items.
15° to 30°C Ambient storage	Radiation disinfestation Dose required< 1 kGy Elimination of eggs and larvae of insects.	Dry products free from spoilage due to insects, from dried fishery products including fish meal and feed for aquaculture. Inactivation of *Salmonella* spp. and other pathogens
-1°to +3°C (Post-irradiation storage: under ice).	Radurization (Radiation pasteurization for shelf life extension) Dose: 1-3 kGy Reduction of initial microbial content by 1 to 2 log cycles. Specific reduction of spoilage causing organisms.	Extends chilled shelf life of fresh marine and freshwater fishery products two to three times. Additional benefit includes reduction of non-spore forming pathogens

References

Barbosa-Canovas, G. V and Bermudez-Aguirre, D. (2011). In Zang, H. Q., Barbosa-Canovas, G.V., balasubramaniam, V. M., Dunne, C.P., Farkas, D.F and Yuan, J.T.C (Ed.) Nonthermal Processing Technologies for Food. IFT press, Wiley- Blackwell Publishers

Barbosa-Cánovas, G. V., Pothakamury, U. R., Palou, E., Swanson, B. G., eds. 1998. Food irradiation. Non thermal preservation of foods, pp.161-213. New York: Marcel Dekker.

Bintsis, T., Litopoulou-Tzanetaki, E. and Robinson, R. K. (2000). Existing and potential application of ultraviolet light in the food industry-a critical review. J. Sci. Food Agr. 90: 637-645

Bolton, J. R. and Linden, K. G. (2003). Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. J. Environ. Eng. 129: 209-215

Dunn, J., Ott, T. and Clark, W. (1995). Pulsed light treatment of food and packaging. Food Technologist. 49(9): 95–98

Fine, F. and Gervais, P. (2004). Efficiency of pulsed UV light for microbial decontamination of food powders. J. Food Protect. 67: 787–792

Gomez-Lopez, V.M., Ragaerta, P., Debeverea, J. and Devlieghere, F. (2007). Pulsed light for food decontamination: A review. Trends Food Sci. Technol. 18: 464-473

Hoover, D.G., Metrick, C., Papineau, A.M., Farkas, D.F., Knorr, D., (1989). Biological effects of high hydrostatic pressure on food microorganisms. Food Technology 43(3), 99-107.

https://www.pulsemaster.us/pef-pulsemaster/product-process-improvement

Knorr, D., (1999). Novel approaches in food-processing technology: new technologies for preserving foods and modifying function. Current Opinion in Biotechnology 10(5), 485-491.

Lopez-Malo, A.; Palou, E.; Barbosa- Canovas, G. V Swanson, B. G.; Welti-Chanes, J.,(2000). Minimally processed foods and high hydrostatic pressure. In Advances in food Engineering; Lozano, J, Anon, M. C., Parada-Arias, E., Barbosa-Canovas, G. V., Eds.; Technomic Publishing Co.; Lancaster, P A. 267-286

Mohd. Adzahan, N. and Benchamaporn, P. (2007). Potential of Non-Thermal Processing for Food Preservation in southeast Asian countries. ASEAN Food Journal 14(3):141-152

National Center for Food Safety and Technology (2009). NFSCT receives regulatory acceptance of novel food sterilization process. Press release, February 27, 2009. Summit-Argo, IL

Palmieri L and Cacace, D (2005). High Intensity pulsed light technology. In: Emerging Technologies for food processing (Da-Wen Sun., Ed), pp 279-306, Elsevier Academic Press, UK

Smelt, J.P.P.M. (1998). Recent advances in the microbiology of high pressure processing. Trends in Food Science & Technology, 9, 152-158

Suzuki, A., (2002). High pressure-processed foods in Japan and the world, in: Rikimaru, H. (Ed.), Progress in Biotechnology. Elsevier, pp. 365-374.

Toepfl S, Mathys A, Heinz V, Knorr D. Review: potential of high hydrostatic pressure and pulsed electric fields for energy efficient and environmentally friendly food processing. Food Rev Int. 2006;22: 405–423. doi: 10.1080/87559120600865164

U.S.FDA, (2011) Fish and Fishery Products Hazards and Controls Guidance (contains 21 chapters) Fourth Edition, Food and Drug Administration.

Urrutia, G.; Arabas, J.; Autio, K.; Brul, S.; Hendrickx, M.; Ka¸kolewski, A.; Knorr, D.; Le Bail, A.; Lille, M.; Molina-Garcı´a, A. D.; Ousegui, A.; Sanz, P. D.; Shen, T. & Van Buggenhout, S. (2007). SAFE ICE: Low-temperature pressure processing of foods: Safety and quality aspects, process parameters and consumer acceptance. Journal of Food Engineering, 83, 293–315

Venugopal, V., Doke, S.N. and Thomas, P., (1999) Radiation processing to improve the quality of fishery products, Critical Reviews in Food Science and Nutrition, 39, 391-440,

Wan J., Coventry, J., Swiergon, P., Sanguansri, P., and Versteeg, C. 2009. Advances in innovative processing technologies for microbial inactivation and enhancement of food safety – pulsed electric field and low-temperature plasma. Trends in Food Science and Technology. doi:10.1016/j.tifs.2009.01.050

****

Extruded Products from Fish Frame Meat

Sreejith S.

Fish Processing Division

ICAR-Central Institute of Fisheries Technology, Cochin

Email: ssreejith1985@gmail.com

Extrusion technology is a size enlargement process where in small granular food or powdered particles are reinforced into larger pieces with different shapes, texture, colour etc.Extrusion cooking or thermoplastic extrusion is a common extrusion technology which is considered a HTST (High-Temperature, Short-Time) process for the preparation of snack foods. It permits, with little or no modification of the basic equipments and appropriate process control, the production of a great variety of food products. Extrusion cooking is used for starchy and proteinaceous materials for the preparation of nutritious foods. Generally such products are rich in calories and the protein content is comparatively low. Considerable amounts of frame meat aregenerated in fish filleting industry which currently impose a cost burden on the processing industry. Frame meat can be effectively utilized in combination with cereal flours to develop extruded products. Alternatively they can be converted to fish protein hydrolysate powder. The incorporation of protein powder derived from the fish frame meat and processing wastes can improve the nutritional value of extruded products.

Principle of Extrusion cooking:

Raw materials (minced frame meat/fish protein hydrolysate powders and cereal flours) are fed into the extruder barrel through a feeder and the screws convey along it. Towards the barrel end, smaller flights restrict the volume and resistance to movement of the food is increased. As a result, it fills the barrel and the spaces between the screw flights become more compressed. As it moves further along the barrel, the screw kneads the material into a semi-solid, plasticized mass. The food is heated above 100˚C and the process is known as extrusion cooking (or hot extrusion). Here, frictional heat and the additional heating that is used cause the temperature to rise rapidly. The food is then passed to the section of the barrel having the smallest flights, where pressure and shearing is further increased. Finally, it is forced through dies (restricted openings) at the end of the barrel. As the food emerges under pressure from the die to normal atmospheric pressure and temperature, it expands to the final shape, gets characteristic texture and cools rapidly as moisture is flashed off as steam.

Extruders:

Extruders are the tools used to introduce mechanical shear and thermal energy to food ingredients.Extruders are classified into two according to operation: Hot and cold extruders. Based on type of construction extruders are classified into: Single screw and twin screw extruder. Twin-screw extruders are used for high-moisture extrusion, products that include higher quantities of components such as fibers, fats, etc. and for the production of more sophisticated products. Twin screw extruders are again classified as co-rotating and counter-rotating types based on the direction of rotation of

the screws. In the counter-rotating position the extruder screw rotates in the opposite direction, whereas in the co-rotating position the screw rotates in the same direction.

Extruders are composed of five main parts:

(i) Pre-conditioning system

(ii) Feeding system

(iii) Screw

(iv) Barrel

(v) Die and cutting mechanism

Pre-conditioning is not applied to all extrusion processes. It is applied when moisture contents around 20 to 30% and long residence times are required for of the material. Pre-conditioning favours uniform particle hydration, reduces retention times within the extruder and increases throughput and increasing the life of the equipment, due to a reduction in the wearing of barrel and screw components. It also reduces the cost of energy involved in the process.

The feeding system is normally composed of a holding bin where the material is loaded and the discharge of the material can occur through a vertical or horizontal feeding screw. It ensures a constant and non-interrupted feeding of the raw materials into the extruder for an efficient and uniform functioning of the extrusion process.

The screw of the extruder is its most important component. It determines the cooking degree, gelatinization and dextrinization of starch and protein denaturation and also ensures final product quality. Screws can be mono-piece or multi-piece. Screw elements can vary in number and shapes, each segment is designed for a specific purpose. Some elements only convey raw or pre-conditioned material into the extruder barrel, while other segments compress and degas the feed. Others promote kneading, backflow and shear.

Barrels or sleeve surrounds the screw and are often jacketed to permit circulation of steam or superheated oil for heating or water or air for cooling, thus enabling the precise adjustment of the temperature in the various zones of the extruder. Generally barrels are equipped with pressure and temperature sensing and temperature control mechanisms. The barrel is divided into feeding, kneading and high pressure zones.

The die has two main functions: to give shape to the final product and to promote resistance to the material flow within the extruder permitting an increase in internal pressure. The die can be in various designs and number of orifices. Dies are usually designed to be highly restrictive, giving increased barrel fill, residence time and energy input.

The cutting mechanism is necessary for obtaining final products with uniform size. Product size is determined by the rotation speed of the cutting blades. This mechanism can be horizontal or vertical.

Coating:

The flavouring of extruded products follows a similar pattern to colouring. A product with fish incorporated has characteristic fishy flavour and it may develop further flavours by thermal reactions between flavour precursors in the mix or be flavoured by adding synthetic or natural flavorings. The addition of flavouring is usually carried out

on the dry extrudate by spraying or dusting, because of the changes caused by the losses of volatiles during extrusion.This can be performed with simple rotating drums with electric heaters installed or with a gas operated hot air installation.

Packaging:

One of the major properties of snacks is the crispness, which is achieved during the manufacture of the product. Retention of desirable texture (crispness) is directly related to the moisture level in the product.The moisture content of snack is very low, and any increase due to the hygroscopic nature of the product may lead to loss of crispness of the product.Moisture also accelerates other biochemical changes such as oxidative rancidity.Oxygen inside the package may be replaced by an inert gas like Nitrogen. Low water vapourand gas permeability of the package is, therefore, a very critical requirement.Also the packaging material must be physically strong enough to withstand the processes of vacuumising/gas flushing.Metalized Polyester-Polyethylene laminated pouches with Nitrogen flushing are used for the packaging of extruded products.

Storage:

Extruded product can be stored at ambient temperature. Nitrogen flushed pouches can be bulk packed in carton box and stacked inside the store.

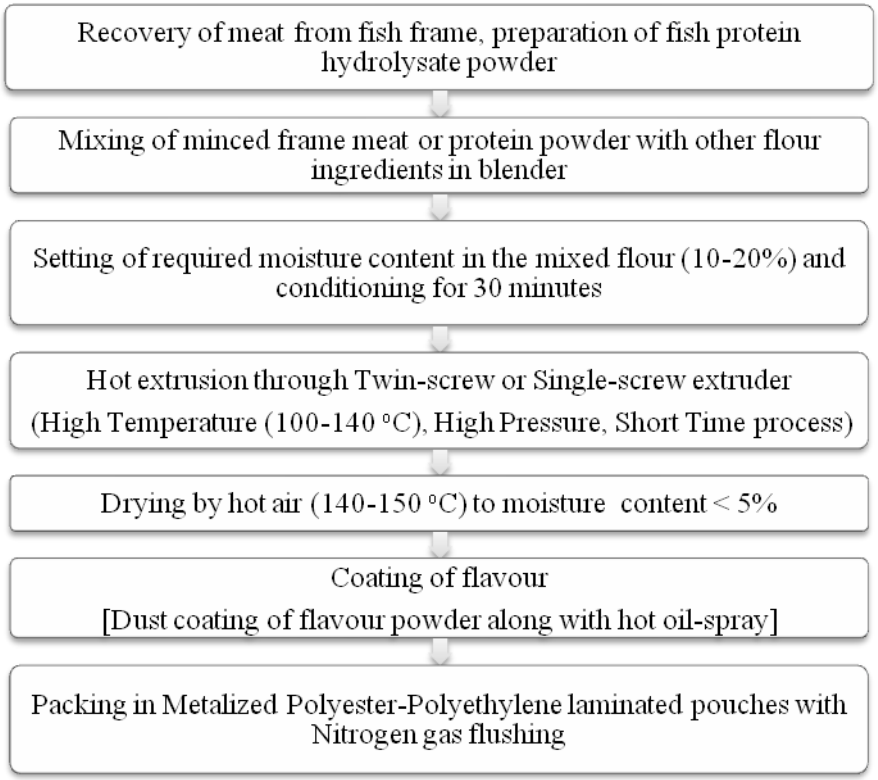
Advantagesof thermoplastic extrusion:

Versatility, low costs, high production yields, good quality nutrient enriched products and no effluents.

Rice-based extruded product incorporated with protein hydrolysate powder derived from the frame meat of Milk fish (*Chanoschanos*)

A work was conducted on preparation of extruded product incorporated with fish hydrolysate powder (FPH) derived from the frame meat of Milk fish (*Chanos chanos*). 5% level of fortification with FPH was found to have similar physical properties as control sample like higher expansion ratio, low bulk density. The protein and lipid content (15% and 9% respectively) in the product increased significantly with the incorporation of FPH at 5% level in comparison with the control (7% and 4.5% respectively). The product hardness remained similar while in colour, the lightness (L*) values showed a slight decrease with the incorporation of hydrolysate powder.

Preparation of extruded snacks enriched with protein from fish frame meat– flow chart:



****

Smoke-Drying Technology in Fish Preservation

Sathish Kumar K

Fish Processing Division

ICAR-Central Institute of Fisheries Technology, Cochin

Email: 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 fish 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.

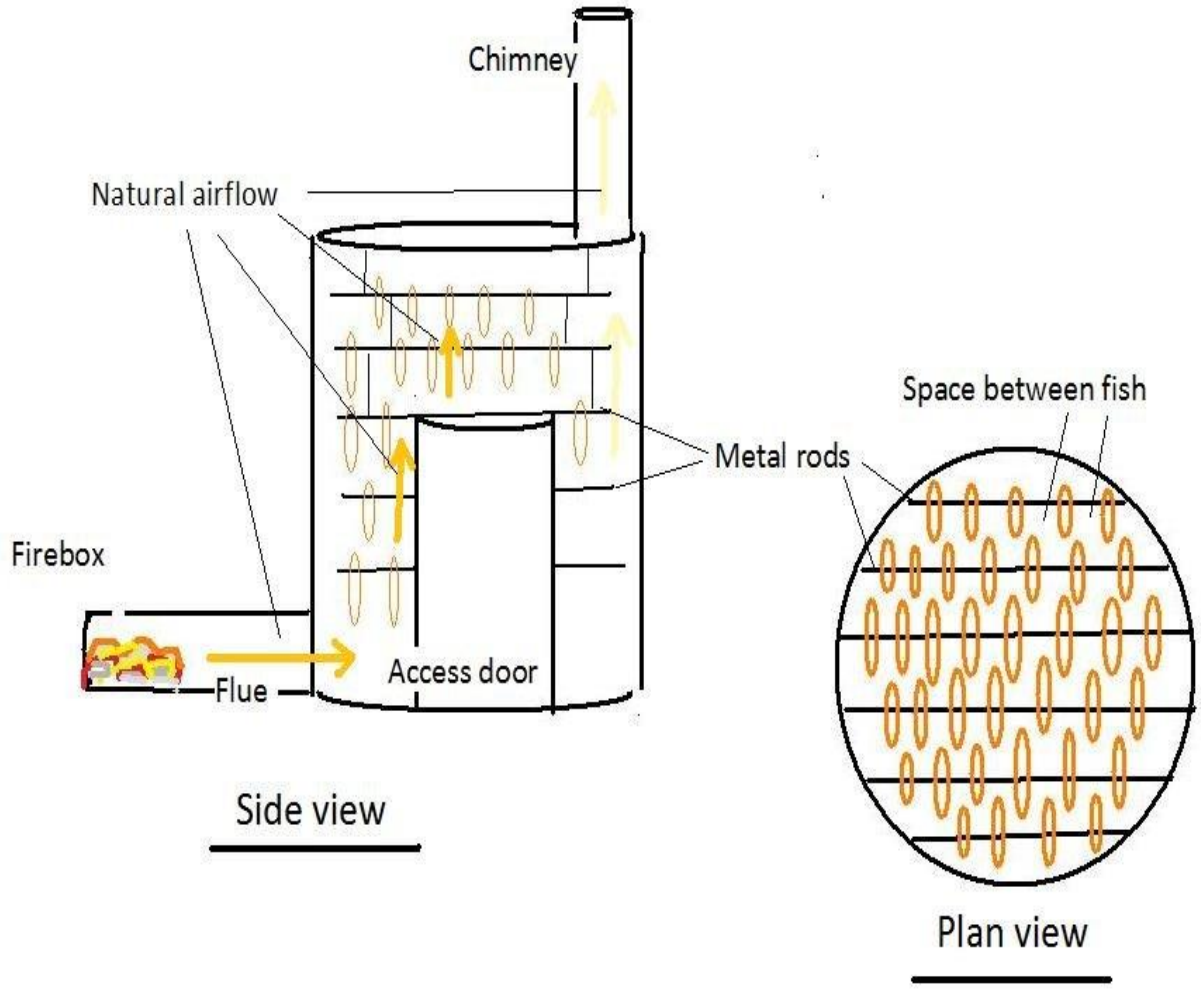


Fig. Illustration of the hot smoke airflow in the smoking chamber

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 ahigher 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.

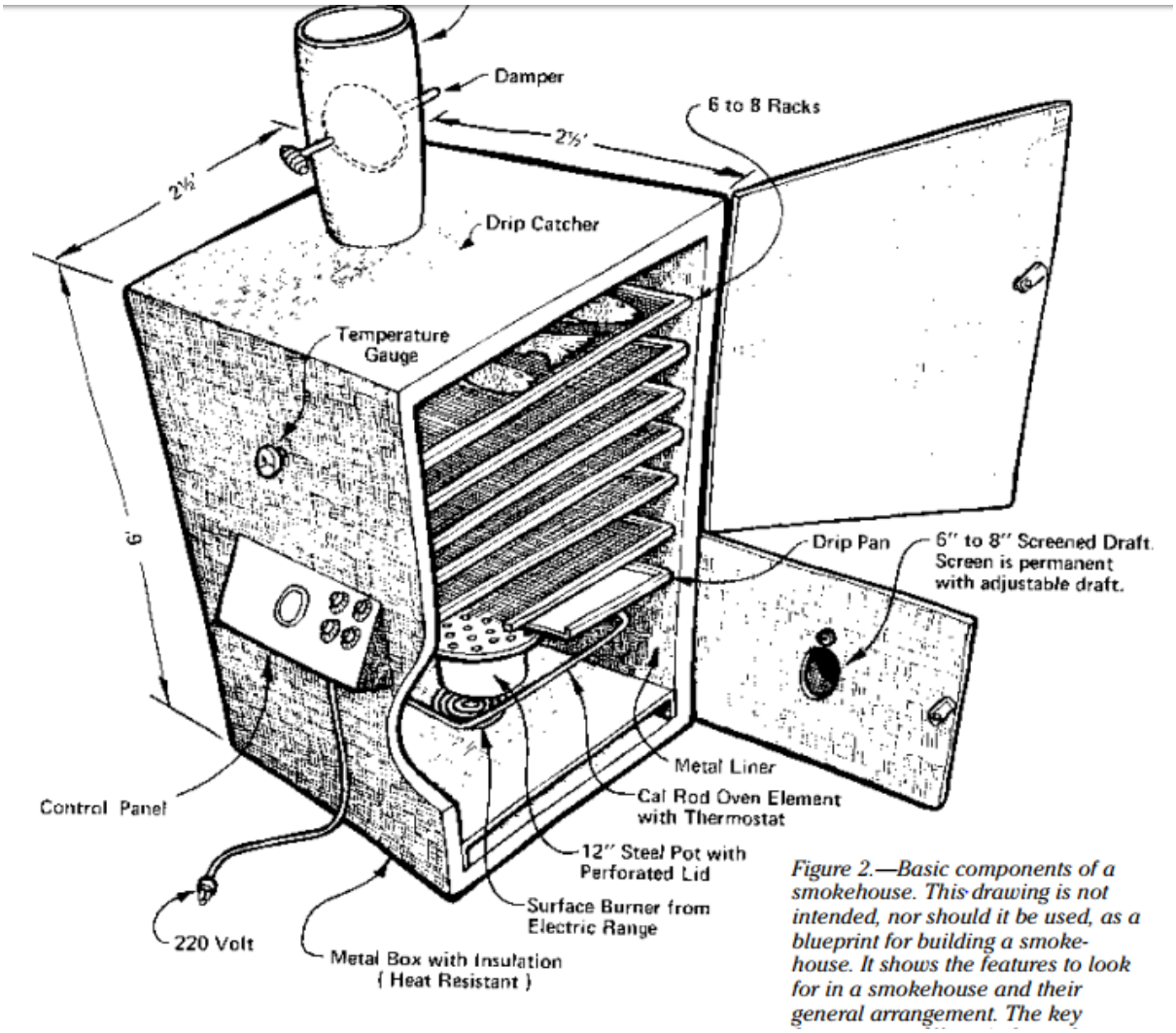


Figure 2.—Basic components of a smokehouse. This drawing is not intended, nor should it be used, as a blueprint for building a smoke-house. It shows the features to look for in a smokehouse and their general arrangement. The key

Fig. Schematic diagram of smoke house 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 alsoreduces the water activity (aw) 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 hotsmoked 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 isthe only major humectant in the cured food, the relationship between the aw and WPS canbe 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 saltingis 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 thelayers 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. Abetter 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 lowto 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 becarefully 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 byseveral 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 surfacebecause 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 solutionsat 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 thefish tissue and also the relatively high solute concentration involved in cured fish. Dryingof the fish can still be simulated with the formula in a way that drying the fish will causea 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 flowis 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 penetratinginto 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 offinished 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 isthe 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 thedispersing smoke phase is complicated, many of which have yet been identified. More than 200 components have been identified. The most abundant chemicals found in smokeare 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 becharred 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 eitheridentified 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 intemperature range 400 to 1000 °C (Eyo, 2001). Indirect smoking like liquid andelectrostatic smoking also significantly reduces the PAHs amount.

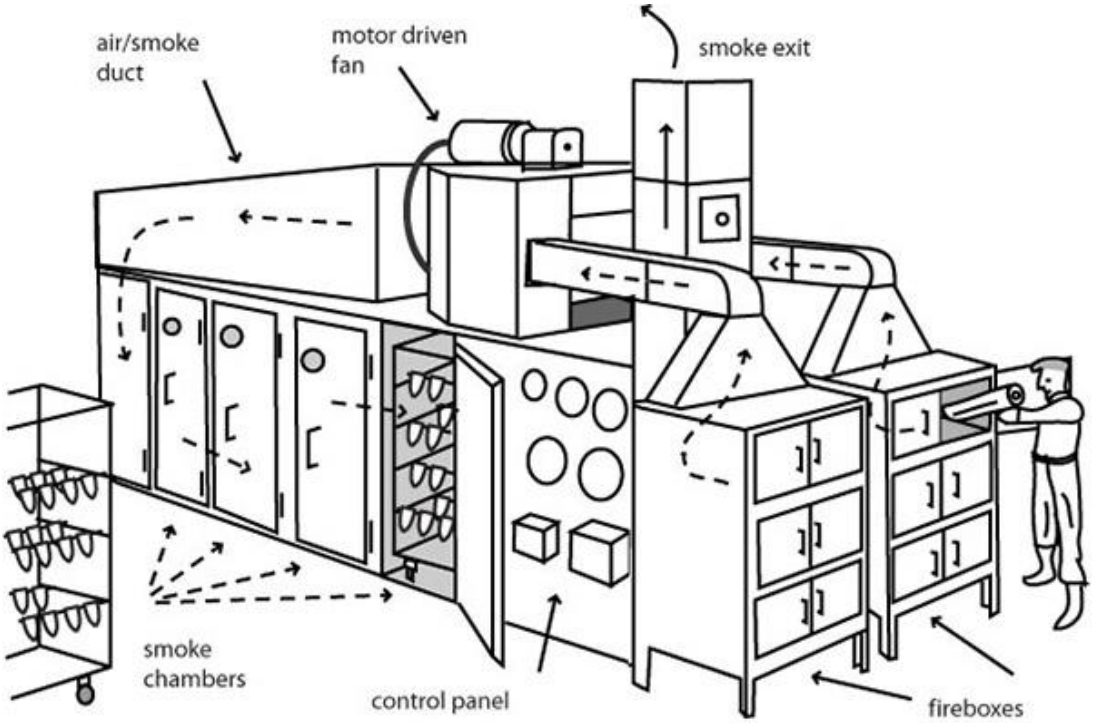


Fig. Smoking kiln

Potential hazards associated with smoking of fish

I. 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 22-28˚C. However, this temperature is not sufficient to eliminate the risk from *Listeria monocytogens,* a gram positive, facultative anaerobic, psychrotropicbacteria 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.

II. Chemical hazards

1. Polycyclic Aromatic Hydrocarbons (PAHs)

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

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

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

2. Histamine:

   Histamine poisoning is associated with Scombroid fishes and other dark meat fishes. The fishes showing potential treats of histamine poisoning are tunas, bonitos, mackerel, mahimahi, carangids, herring etc. These fishes having high content of free histidine, which during spoilage are converted to histamine by bacteria like *Morganellamorgani, Klebsiellapnuemoniae* and *Hafniaalvei*. 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 Codexstandards, the maximum allowable histamine content in smoked fishes is 200 mg/Kg for species like *Scombridae, Clupeidae, Engraulidae, Coryphaenidae, Pomatomidae,* and*Scomberesocidae.* Low temperature storage of fishes right from catch can effectively reduce the production of histamine in fishes.

3. Biotoxins:

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

III. 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.

IV. 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.

****

Fermentation Technology for Fish

Devananda Uchoi
Quality Assurance and Management Division
ICAR-Central Institute of Fisheries Technology, Cochin
Email: **uchoidev514@gmail.com**

Introduction

Fermentation of fish is practiced in different parts of the world and is most popular in Southeast Asian countries, including India. Fish and fishery products have been associated with the socio-economic life of the people since many years. Fermentation is one of the ancient and most economical curing methods adopted for preserving fish. In times when there were no modern preservation techniques such as canning, refrigeration, freeze drying, etc. fermentation played an important role for preserving foods. Some of the popular fermented fish products from Southeast Asian countries are *nam-pla* and *pla-ra* in Thailand, *phu quoc*, *shiokara* and *narezushi* in Japan, *budu* and *belacan* in Malaysia, *patis* and *buro* in Philippines, *nuoc-mam* and *mams-ca* in Vietnam, *makassar* and *trassi* in Indonesia, *ngapi* in Myanmar etc. Most of these products are either in sauce or paste form.

Some major fermented fish products from India are *ngari* and *hentak* in Manipur, *tungtap* in Meghalaya, *puthi shidal, lona ilish* &*phasa shidal* in Tripura, *nghaum*, *nghathu* &*dang pui thu* in Mizoram, *ngyii papi* in Arunachal Pradesh, *seedal* in Assam, etc. Most of these products are almost similar in manyaspects; however, the names vary due to different locality. In earlier days fermentation was used to preserve foods, and later came to be valued for medicinal and nourishing properties. Some of the fermented fish products are marketed in the name of functional foods by various companies. Eg. Intestive, Seacure, Seavive, etc.

Why to ferment fish?

Fish should be consumed in fresh condition, but fish due to its rich nutrients composition deteriorates very quickly. Therefore, there is a need to delay their degradation by application of some preservation technique. Fermentation is one such technique which is applied for the following reasons.

1. Preservation of fish/ to handle surplus catch/ prevent spoilage
2. To overcome fishing off-season
3. Flavour development
4. Nutrient enhancement
5. Value addition
6. To develop product variety
7. To develop unique taste (savory/umami)
8. Fish fermentation is still existing because the consumers enjoy the taste

How does fermentation preserve fish?

Fermentationis an ideal example of hurdle technology. It works as preservative technique by lowering the pH, redox potential (Eh) andwater activity (a_w) of the substrate.In modern technique, fermentation is sometime referred to as bio-preservation by addition of lactic

acid bacteria (LAB) to the fish to be fermented. LAB produces antimicrobials such as lactic, acetic acid, antimicrobial nisin, hydrogen peroxide and peptide bacteriocins. These active substances prevent pathogenic and spoilage bacteria to proliferate and thus helps to preserve the fish.

Table 1.Countries producing fermented fish product

Countries	Sauce	Paste	Retain original form
Japan	Phu Quoc	Nukazuke, Shiokara,	Narezushi, Funazushi
Thailand	Nam-pla, pla-ra,		Plasom, som-fug
Indonesia	Makassar,	trassi	
Malaysia	Budu, pekasam, belacan		
Philippines	Patis, buro,	bagoong (shrimp)	
Vietnam	Nuoc-mam,	Mams-ca	
Norway			Fermented salmon, saithe
Taiwan	Fish sauce		
Korea			Jeotgal (shrimp, oyster, fish)
Myanmar	Ngapi	Ngapi	
Bangladesh			Shutki, Lona ilish
India			Seedhal, ngari, Hentak, Lona ilis etc.
Greece	Garam		
Egypt			Feseekh (gray mullet)
Iceland			Hakarl (shark)
Sweden			Surstromming (herring)
China			Fermented silver carp
Brazil			Fermented sardine

(**Source:** Tamang and Kailastapathy, 2010)

Types of fermented fish products

1) Products retaining original shape:

Examples: Pedah siam (Thailand), makassar (Indonesia), Burong Isda (Philippines), shidal (India), Perkasam (Malaysia), Surstromming (Sweden)

Fig 1. Shidal **Fig 2.** Perkasam **Fig 3.** Surstromming

2) Products in the form of a paste:

Examples: Ngapi (Myanmar), mams (Vietnam), prahoc (Kampuchea), belachan (Malaysia), trassi (Indonesia), bagoong (Philippines).

Fig 4. Trassi **Fig 5**. Ngapi

3) Product in a liquid form:

Examples: Budu (Malaysia), patis (Philippines), nuoc-mam (Vietnam), nam-pla, pla-ra (Thailand).

Fig 6. Pla-ra

Fig 7. Major fermented fish products in India

Puthi shidal (Tripura), Phasa shidal (Tripura), Lona ilish (Tripura), Ngari (Manipur), Hentak (Manipur, Tungtap (Meghalaya), Seedal (Assam), Dang pui thu (Mizoram), Nagaland phasa shidal (Nagaland), Ngyii papi (Arunachal Pradesh) [Names of product start from left to right]

Steps for shidal preparation

1. Raw materials (dry puti fish)
2. Sorting by hands
3. Sun drying in open space
4. Water washing and overnight partial drying at room temperature
5. Packing of oil smeared matkas with partially dried fish and filled up to neck portion
6. Sealing of mouth portion with cover paste
7. Covering of the paste with paper or banana leaves and keep it undisturbed for 3-4 days
8. Removal of the cover-leaf and application of thick layer of mud on the mouth
9. Keeping the matkas undisturbed for 3-4 months for fermentation at ambient temperature
10. Final product shidal after 3-4 months by removing the mud and putrefied paste

Benefits of fermented fish

1) Beneficial bacteria in fermented fish compete and eliminate all the nasty bacteria and help to maintain good gut micro-flora.

2) Fermented fish has strong antioxidant scavenging capability against free radicals and reactive oxygen species
3) Fermented fish are rich in protein hydrolysates, improving our body's ability to utilize amino acids in the production of muscle and in tissue repair
4) Easier to digest and nutrients are easily assimilated.
5) Retains enzymes, vitamins, and other nutrients as no heat is applied.
6) Improve appetite.
7) Fermentation causes cleavage of food proteins by microbial or indigenous proteases which yield bioactive peptides, leading to substantial increase in the biological properties of the food substrates with protein, essential amino acids along with essential fatty acids, vitamins, minerals, etc.(Steinkraus, 2002).
8) Many peptides released during fermentation of food proteins exhibit biological activities, such as antimicrobial properties, blood-pressure lowering effects, cholesterol lowering ability, antithrombotic and antioxidative activities (Hartmann and Meisel, 2007).

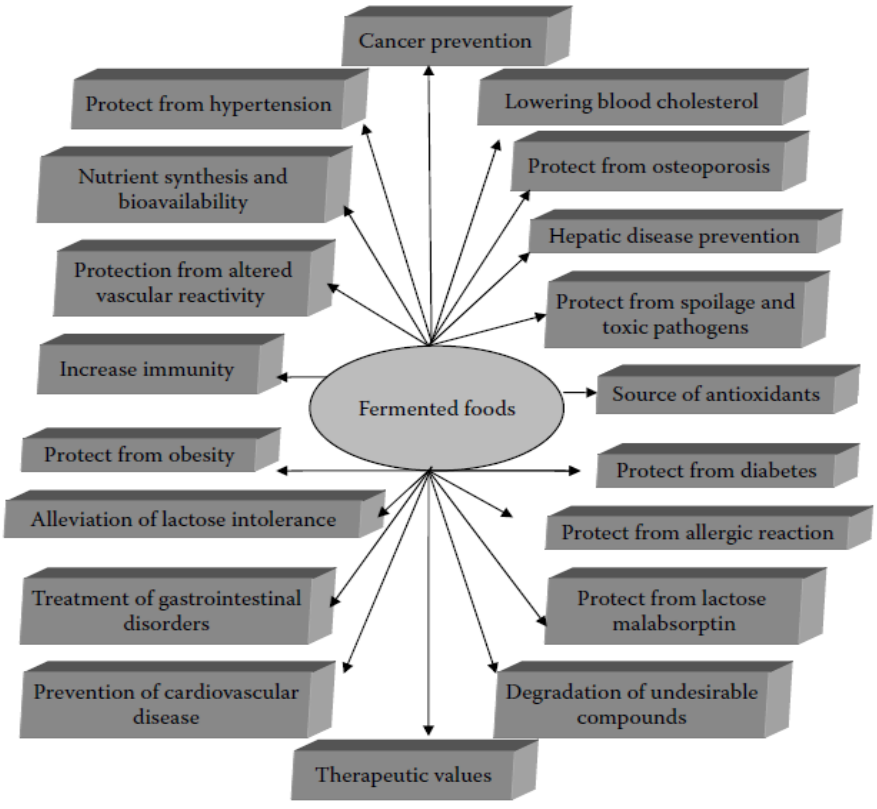


Fig 8. Various health benefits of fermented food

(**Source:** Tamang and Kailastapathy, 2010)

What can fermented fish present to us?

1. Nutrition
2. Health

3. Wealth
4. Beauty
5. Strength

1) NUTRITION

Table 2. Free amino acids (mg/100 ml) composition of fish sauces

Amino Acid	*Nam pla*	*Nuoc mam*
Aspartic acid	760	1150
Threonine	460	700
Serine	360	610
Glutamic acid	950	1370
Proline	230	330
Glycine	340	360
Alanine	700	1010
Valine	590	830
Cysteine	0	0
Methionine	230	270
Isoleucine	360	390
Leucine	450	490
Tyrosine	50	60
Phenyl alanine	310	420
Tryptophan	90	90
Lysine	890	1360
Histidine	320	460
Arginine	0	80

Source: From Ninomiya, K. 2002. *Food Review International* 18: 23–38; Yoshida, Y. 1998. *Food Reviews International* 14: 213–246.

Table 3.Nutritional composition of ethnic fish products of eastern Himalayas

		Percent (%)			Food value	Minerals (mg/100 g)				
Product	pH	Moisture	Protein	Fat	(kcal/100 g)	Ca	Fe	Mg	Mn	Zn
Suka ko maacha	6.4	10.4	35.0	12.0	395.2	38.7	0.8	5.0	1.0	5.2
Gnuchi	6.3	14.3	21.3	14.5	404.9	37.0	1.1	8.8	1.1	7.5
Sidra	6.5	15.3	25.5	12.2	394.6	25.8	0.9	1.6	0.8	2.4
Sukuti	6.4	12.7	36.8	11.4	402.6	17.7	0.3	1.4	0.2	1.3
Ngari	6.2	33.5	34.1	13.2	381.6	41.7	0.9	0.8	0.6	1.7
Hentak	6.5	40.0	32.7	13.6	408.0	38.2	1.0	1.1	1.4	3.1
Tungtap	6.2	35.4	32.0	12.0	384.4	25.8	0.9	1.6	0.8	2.4
Karati	6.3	11.8	35.0	12.4	404.0	ND	ND	ND	ND	ND
Bordia	6.4	12.0	24.5	12.3	400.3	ND	ND	ND	ND	ND
Lashim	6.4	9.6	28.3	11.8	407.8	ND	ND	ND	ND	ND

Note: Data represent the means of five samples. ND, not determined.

(**Source:** Jyoti Prakash Tamang, 2009)

2) **HEALTH**

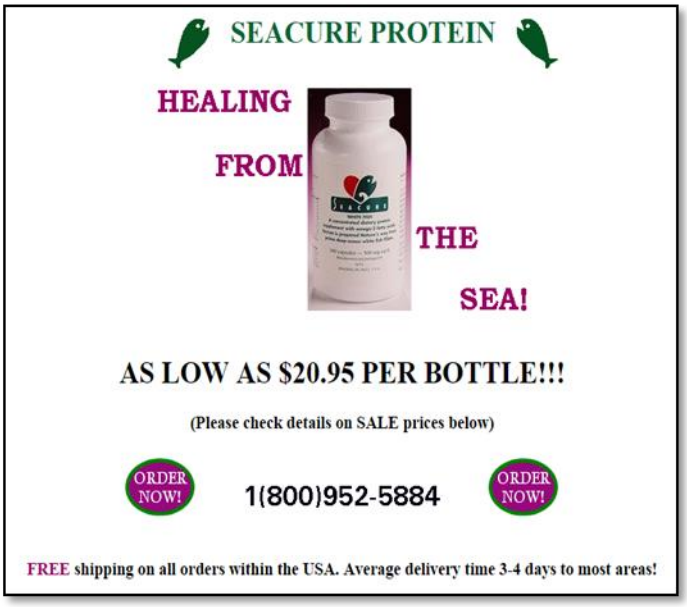
Antioxidant bioactive peptides inhibits angiotensin-I-converting enzyme (ACE) which lower blood pressure.The bioactive peptide with sequence Leu-Gly-Leu-Asn-Gly-Asp-Asp-Val-Asn, exhibited high levels of antioxidant activity (Ranathunga S., 2006). Boost immune system-by protecting cell damage (WBC) from free radicals.Anti cancer peptide from anchovy sauce have apoptosis inducing activities in human carcinoma cells which could be potentially useful in preventing the spread of cancer (Lee et al. 2004, Ngo et al. 2012). The product is reported to prevent arthritis, psoriasis caused by compromised immune.

3) WEALTH

Apart from fermented fish product, different supplementproducts from fermented fish have been commercialized and marketed which fetch good price. These products are beneficial in many ways. These products are 'Intestive', 'Seacure', 'Seavive',

'Seacure'are product prepared by the help of marine micro-organism which digest whole fish fillets into protein fragments mostly 2 and 3 AA long (di-peptides and tri-peptides). 'Seacure' protein supplement does not involved heat and chemicals having smaller fragments and made easy for the human body to absorb.'Seacure'help to maintain memory.It helps to speed the healing of wounds after surgery, car accidents, sports injuries and falls. It helps the digestive tract repair itself. Helps prevent irritable bowel syndrome, ulcerative colitis, Crohn's disease, ulcers and leaky-gut syndrome. Reduce the side effects of chemotherapy. Help premature babies to gain ideal body weight faster.Helps individuals with HIV/AIDS to maintain their weight and avoid muscle loss and diarrhoea.'Intestive' strengthen immune system, stimulate body to repair itself as well as burn fat and build lean muscle.It is useful when there is inflammation and pain coming from the digestive tract.

'SeaVive' increases-number of circulating WBC, stimulates phagocytes and elevates levels of non-specific antibodies.



4)BEAUTY

Fermented fish products slow down the aging process as the antioxidant peptides prevent immature cell death leading to longer life span.The antioxidant peptides prevent skin cancer, wrinkle formation, etc. (F. Domenico, 2007)which is the considered to maintain beauty of a person.

5)STRENGTH

'Seacure' also helps the elderly to maintain their strength and stamina. The nutrients are quickly digested, assimilated and thus produce energy. The microfloracontained in the product fight against pathogen thereby boosting our immune system and helps maintain healthy body which keep a person strong and fit.

Why some people avoid fermented fish products?

People avoid fermented fish products generally due to intense strong flavour, unfamiliar taste, physical appearance, lack of knowledge about its benefits and cultural barrier.

Risks in fermented fish

The risk associated with fermented fish product is botulism. Alaska has more cases of botulism than any other state in the United States of America. This is caused by the traditional Eskimo practice of allowing animal products such as whole fish, fish heads, sea lion and whale flippers, birds, etc. to ferment for an extended period of time before being consumed. The risk occurs when a plastic container is used for this purpose instead of the old-fashioned, traditional method, a grass-lined hole, as the botulinum bacteria thrive in the anaerobic conditions created by the air-tight enclosure in plastic.

To avoid such risk, the pH of the product must be maintained below 4.5 because *Clostridium botulinum* cannot produce toxin at pH below 4.5.

Conclusion

Replace artificial industrial foods with natural food such as fermented foods. Consume proper balanced diet in order to remain healthy. Prefer fish over meat if possible. And numerous research scopesare left under this field which can be explored.

References

- Ababouch, Lahsen (2005) "Preservation techniques" FAO Fisheries and Aquaculture topics. Rome. Updated 27 May 2005.
- Alzamora, Stella; Tapia, Maria Soledad; López-Malo, Aurelio (2000). Minimally Processed Fruits and Vegetables: Fundamental aspects and applications. Springer. p. 266. ISBN 978-0-8342-1672-3.
- Hartmann, R., & Meisel, H. (2007). Food-derived peptides with biological activity: from research to food applications. Current opinion in biotechnology, 18(2), 163-169.
- Ninomiya, K. (2002). Umami: a universal taste. Food reviews international, 18(1), 23-38.
- Steinkraus, K. H. (2002). Fermentations in world food processing. Comprehensive Reviews in Food Science and Food Safety, 1(1), 23-32.
- Tamang JP, Kailasapathy K, editors. Fermented foods and beverages of the world. CRC press; 2010; 460 p.
- Tamang, J. P. (2009). Himalayan fermented foods: microbiology, nutrition, and ethnic values. CRC Press.
- 'Why does Alaska have more botulism'. Centers for Disease Control and Prevention (U.S. federal agency). Archived from the original on 7 August 2006. Retrieved 18 July2011.
- Yoshida, Y. (1998). Umami taste and traditional seasonings. Food Reviews International, 14(2-3), 213-246.

****

Drying and Salting of Fish

Parvathy U.

Fish Processing Division
ICAR-Central Institute of Fisheries Technology, Cochin

Email: p.pillai2012@gmail.com

Fish is regarded as an excellent source of nutrients with its richness in health beneficial omega-3 fatty acids, quality and easily digestible proteins with balanced aminoacid profile, essential minerals and vitamins. These nutritional advantages offer considerable benefits to fish as a means to achieve nutritional as well as social security. Better awareness regarding this biomass as a potential source of nutrients has created increased interest in effective exploitation of these resources. However their richness in nutrients as well as high moisture content increases its perishability, necessitating the processing and preservation of fish mandatory soon after harvesting. Different processing and preservation methods like salting, drying, smoking, chilling, freezing, chemical treatments, as well as combination of these two or more methods (referred to as hurdle technology) are used for the preservation of fish.

The traditional methods of processing fish by salting, drying, smoking, pickling, marination and fermentation are collectively known as Curing. It is the oldest and cheapest method of fish preservation and is still widely practiced in many parts of the World. This can be done either by any single method or a combination of these methods. In the current market situation both wet and dry cured fishery products have commercial importance. Cured fish consumption is more in areas where the availability of fresh fish is comparatively limited viz., interior markets as well as hilly areas. This method is also practiced in the coastal areas when an excess catch is to be preserved for later utilization during the lean season or for marketing to other areas.

Drying

In general, the term 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. 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, electrical heating etc. The thermal energy can also be supplied directly to the fish tissue by microwave electromagnetic radiation or ultrasonic heating.

Drying Phases

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

brought to the surface of the fish. As the concentration of moisture in the fish falls, the rate of movement of moisture to the surface is reduced and the drying rate becomes slower; this phase is called the 'falling rate period'.

Constant rate drying phase

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

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

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

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

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

Falling rate drying phase

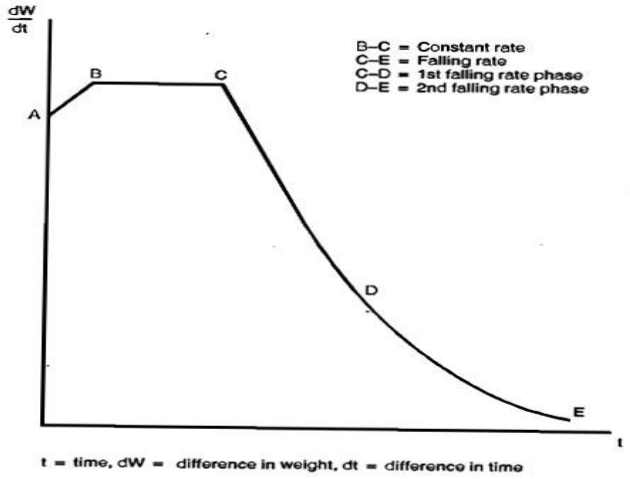
Once the free surface moisture has been removed, the rate of drying depends on the movement of moisture from interior to the surface of the fish. Several factors influence the rate of drying:

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, slowing down the drying rate.

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

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



Drying rate curve.

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

Methods of Drying

There are basically two methods of drying fish. The common and traditional method being sun drying which is done by utilizing the atmospheric conditions viz., temperature, humidity and airflow. 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 or sun drying:

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

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

Artificial / Mechanical Dryers

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

Sun drying on racks

Solar driers

Cabinet driers

Salting

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 is placed in a strong solution of salt (brine) which is stronger than the solution of salt in the fish tissue. Penetration ends when the salt concentration of the fish equals that of the surrounding medium. This phenomenon is known as osmosis. It is based on different factors like diffusion and biochemical changes in various constituents of the fish. This process facilitates preservation of fish by reducing the water activity. A concentration of between 6–10 % salt in the tissue together with the removal of some water from the tissue during the salting process will prevent the activity of most spoilage bacteria. If fish are salted before drying, less water needs to be removed to achieve preservation. A water content of 35–45%, depending on the amount of salt present, will often prevent, or drastically reduce, the action of bacteria.

Salt: Source 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 upto 99.9 % sodium chloride, whereas low quality salt may only contain 80 % sodium chloride. The main chemical impurities of commercial salts include calcium and magnesium chlorides and sulphates, sodium sulphate and carbonate, and traces of copper and iron. Apart from these, contaminants such as dust, sand and water may also be present in salt. Presence of calcium and magnesium chlorides even in small quantities tends to slow down the penetration of salt into the flesh and hence their presence may lead to increase in the rate of spoilage. Further magnesium chloride is hygroscopic and tends to absorb water, making the fish more difficult to dry and to keep dry. Calcium and magnesium salts give a whiter colour but tend to impart a bitter taste. Very often the consumer demands a whitish colour in salted fish products and small quantities of calcium and magnesium compounds in the salt are usually considered desirable. Excessive quantities, however lead to a bitter flavour and the dried product tends to be brittle which can cause problems during packaging and distribution. Trace quantities of copper in salt can cause the surface of salted fish to turn brown affecting the appeal of dried fish.

Microbiological purity

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

Physical properties

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

Types of Salting

- ***Dry salting:*** This is the most widely used method of fish curing. Dry salting is advisable for fishes of any size, except fatty fishes. The fish is gutted, beheaded or ventrally split open and the viscera removed followed by washing. Scoring is also practiced if the flesh portion is thick for facilitating better salt penetration. Salt is then applied in the ratio 1:3 to 1: 10 (salt to fish) depending upon the size of the fish. The fish is then stacked in clean cement tanks or other good containers layered with salt and weight is applied from top for better salt penetration. The fish is kept in this condition for 24-48 hours. After salting period, the fish is taken out, washed in brine to remove adhering salt and drained. It is then hygienically dried to a moisture content of about 25%. Yield of the product by this method is about 35-40% with a storage stability of upto three months under ambient conditions.
- ***Wet salting:***The initial stages of processing and salting are the same as for dry curing. However the fish kept in tank is allowed to remain in self brine till marketing without further drying. For marketing, as per the demand the wet salted fish is drained and packed in palmyrah leaf baskets or coconut leaf baskets. This method is particularly suitable for fatty fishes like oil sardine, mackerel etc. Wet salted fishes have shortshelf stability with a moisture content of 50-55% and a salt content of around 25%.
- ***Pickle salting:***Pickle curing is a type of wet salting where the fish is layered by granular salt which, dissolves in the surface moisture of the fish forming solution which penetrates into the fish removing moisture from the fish. The fish is allowed to remain in this self brine. If the self brine is not sufficient, saturated brine is added to immerse the fish.
- ***Kench salting:***In this method, salt is rubbed on to the surface of the fish and stacked in layers of salt and fish. The self-brine formed is allowed to drain away. This method cannot be recommended for general use in the tropics as the fish are not covered by the brine or pickle and are therefore more susceptible to spoilage and insect attack. Exposure to the air and the presence of salt also encourages the rate of fat oxidation which gives rise to discoloration and the characteristic rancid flavours.
- ***Mona curing:*** Mona curing is mainly adopted for medium to small size fishes. Before salting, the intestine and entrails are removed by pulling out through the gill region without split opening the fish. The flesh is not exposed during salting thereby causing less contamination and the product has a shelf stability of about two months. The yield obtained by this method is about 70%.
- ***Pit curing:*** In this method, fish is mixed with salt (4:1) and placed in pits dug on beaches. The pits may be lined with palymrah / coconut leaves. After 2-3 days of maturation, the fish is taken out for marketing in wet condition and packed in bamboo baskets and transported to markets without drying. The quality of fish cured by this technique is poor with a shelf stability of upto three weeks only.
- ***Colombo Curing:***Colombo curing is similar to pickling process which is widely practiced in Sri Lanka. A piece of dried malabar tamarind (*Garginia cambogea*) is kept in the abdomen portion of the gutted and cleaned fish which is further

stacked in airtight wooden barrels filled with brine. Fishes cured by this method has a shelf life for upto 6 months.

Quality issues in dried and salted fish

- ***Pink/Red:*** Salt content prevents the growth of normal spoilage microflora in the fish but halophiles, which can survive at 12-15% of salt concentration, will survive. Halophilic bacteria are present in most of the commercial salt. A particular group of halophiles called Red / Pink cause reddening of wet or partially dried salted fish. These do not grow in brine or in fully dried fish. They are aerobic and proteolytic in nature, grows best at 36ºC by decomposing protein and giving out an ammoniacal odour. Spoilage appears on the surface as slimy pink patches. However these bacteria are not harmful in nature. Usage of good quality salt is recommended to avoid this condition. This spoilage is mostly found in heavily salted fish and absent in unsalted fish.
- ***Dun:*** In salted fish, brownish black or yellow brown spots are seen on the fleshy parts, referred to as "dun". This is mainly caused by growth of halophilic mould called *Sporendonema epizoum*. This gives the fish a very bad appearance. Moulds usually grow at relative humidity above 75%. The optimum temperature for growth is 30-35 ºC. During the initial stages of appearance of moulds on the fish, it is possible to remove them manually. In advanced stages it penetrates into the flesh. To avoid the mould growth it is necessary that the fish be dried, packed and stored properly to avoid uptake of moisture. Chemical method of prevention includes dipping the fish in a 5% solution of calcium propionate in saturated brine for 3-5 minutes depending upon the size of the fish.
- ***Salt Burn***: A mixture of large and small grain sizes is recommended for dry salting of fish. If fine grain is used directly on the fish, salt burn may occur due to the rapid removal of water from the surface with no penetration of salt to the interior of the fish.
- ***Case hardening:*** Under certain conditions, where the constant rate drying is very rapid due to high temperature and low relative humidity, the surface of the fish can become 'case hardened' and the movement of moisture from the deeper layers to the surface is prevented. This can result in a fish which is dry at surface. However the centre remains wet and hence spoils quickly.
- ***Rancidity:***This is caused by the oxidation of fat, which is more pronounced in oil rich fishes like mackerel, sardine etc. The unsaturated fat in the fish reacts with the oxygen in the atmosphere forming peroxides, which are further broken down into simple and odoriferous compounds like aldehydes, ketones and hydroxy acids, which impart the characteristic odors. At this stage the colour of the fish changes from yellowish to brown referred to as rust. This change results in an unpleasant flavour and odour to the product, leading to consumer rejection.
- ***Insect Infestation:***Spoilage due to insect infestation occurs during initial drying stages as well as during storage of the dried samples. The flies which attack the fish during the initial drying stage are mainly blowflies belonging to the family Calliphoridae and Sarcophagidae. These flies are attracted by the smell of decaying matter and odours emitted from the deteriorating fishes. During the glut season when the fish is in plenty and some are left to rot, these flies come and lay their eggs. These eggs develop into maggots, which bury within the gill

region and sand for protection from extreme heat. and develop mainly when conditions are favourable. The most commonly found pests during storage are beetles belonging to the family Dermestidae. Beetles attack when the moisture content is low and especially when the storage is for a long time. The commonly found beetles are *Dermestes ater, D frischii, D maculates, D carnivorous* and *Necrobia rufipes*. The larva does most of the damage by consuming dried flesh until the bones only remain. Mites are also an important pest, which are found infesting dried and smoked products. *Lardoglyphus konoi* is the commonly found mite in fish products. Infestation can be reduced by proper hygiene and sanitation, disposal of wastes and decaying matter, use of physical barriers like screens, covers for curing tanks etc, and use of heat to physically drive away the insects and kill them at 45 º C.

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

Conclusion

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. However a major drawback with this traditional processing is the lack of standard operating procedures being followed which affects the quality of cured products. Moreover, there is a general conception that drying/salting is a secondary method for preservation applicable for low value as well as inferior quality varieties. Efforts towards effective and hygienic handling practices in the process chain, popularization of improved drying and packaging practices, and adequate extension services can facilitate better adoption of cured fishery products in the seafood sector.

****

Microencapsulation and Spray Drying Technology

Parvathy U. and Jeyakumari A.
Fish Processing Division
ICAR-Central Institute of Fisheries Technology, Cochin
Email: p.pillai2012@gmail.com

Introduction

Till recent, food was analysed only based on sensory flavor and texture as well as its nutritional value. However, on account of the growing evidence the other bioactive components play in linking food and health, an increased interest has been evident among consumer regarding their health benefits. This has further resulted in accounting for food beyond the basic nutritional benefits to the disease prevention and health enhancing aspects. Nutrients and dietary supplements are major bioactive constituents in functional foods as well as nutraceuticals which make them instrumental in maintaining health, act against various disease conditions and thus promote the quality of life. Bioactive ingredients include proteins, vitamins, minerals, lipids, antioxidants, phytochemicals probiotic bacteria etc. These bioactives are very sensitive and their application in food is a great challenge to the industry without affecting their properties. Microencapsulation technique has proved to be one of the quality preservation techniques for sensitive substances and a method for production of novel food materials with new valuable properties. Spray drying is one of the most commonly used microencapsulation and drying technologies in food and pharmaceutical industries which produces microcapsules in micrometer to millimeter range.

Microencapsulation

Microencapsulation can be defined as a technology wherein solids, liquid or gaseous material (core particle) are compactly packed with thin polymeric coatings (matrix) to form small particles referred to as microcapsules in micrometer to millimeter range (2-5000 μm) (Gibbs et al. 1999). The polymer acts as a protective film, isolating and protecting the core material of interest. On exposure to specific stimulus, this wall membrane dissolves itself facilitating the release of core material at the appropriate place and time for effective utilization. The active agent that is encapsulated is referred to as core material, the active agent, internal phase, or payload phase. The material that is used for encapsulating is called as coating, membrane, shell, carrier material, wall material, external phase or matrix. Generally, the term microcapsule is used for a reservoir-like structure with a well-defined core and envelope/coat. There exist a variety of microcapsules which differ in size, composition, and function. The characteristics of the microcapsules ultimately depend on the final goal of the encapsulated product. In general, there are two forms of encapsulates viz., reservoir type; and matrix type (Fig. 1). In reservoir type, the active agent is surrounded by an inert diffusion barrier. It is also called single-core or mono-core or core-shell type. In matrix type, the active agent is dispersed or dissolved in an inert polymer.

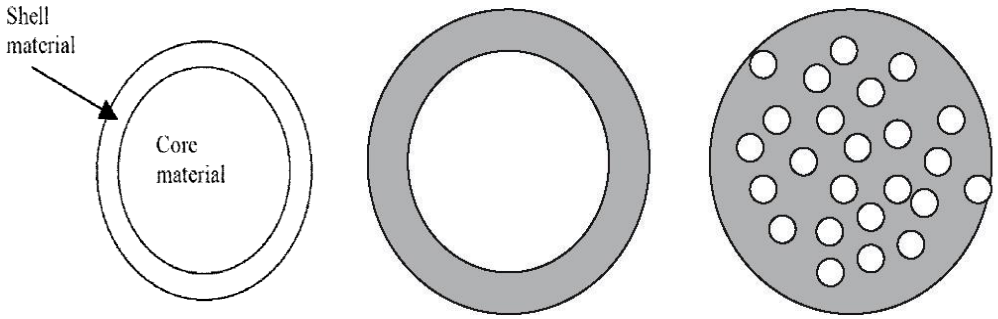


Fig. 1 M a) Reservoir type **:apsule** b) Matrix type

Purpose of Microencapsulation

Microencapsulation can be used to achieve a number of objectives, which in general include structural integrity of the material, protection of the enclosed product/core material, and controlled release of the encapsulated contents. Microcapsules can provide structuration to compounds that are normally difficult to administer on account of various factors viz., insolubility of material, volatility, reactivity, hygroscopicity as well as physical state. Microcapsules also facilitate the role of core content protection preventing product degradation due to external environmental factors. Stability of microcapsules should also be ensured during oral administration for therapeutic purposes, due to exposure to harsh conditions in the upper gastrointestinal tract.

In brief, the purpose of microencapsulation includes the following (Desai and Park 2005):

To protect the core material from degradation and to reduce the evaporation rate of the core material to the surrounding environment.

To modify the nature of the original material for easier handling.

To ensure slow, regulated and targeted release of active ingredient

To mask unwanted flavor or taste of the core material.

To reduce nutrient interaction with other ingredients

To ensure uniform mixing due to dilution with the matrix and in powder form

To improve the bioavailability, stability and efficacy of product

MICROENCAPSULATION METHODS

Numerous techniques can be adopted to fabricate microcapsules, depending on the desired characteristics and application of the final product. The method of preparation and the techniques employed for microencapsulation overlap considerably. In general, the various microencapsulation processes can be divided into chemical, physical and physiochemical methods.

Table 1 Methods for microencapsulation

Chemical methods
Solvent evaporation
Interfacial cross-linking
Interfacial polycondensation/interfacial condensation polymerization
polymerization
Matrix polymerization
Physical methods
Spray drying
Pan coating
Fluid-bed coating
Centrifugal extrusion
Vibrating nozzle/vibrating-jet
Spinning disk/rotational suspension separation
Physicochemical methods
Ionotropic gelation
Polyelectrolyte complexation
Phase separation/coacervation (simple and complex)
Supercritical fluid technology

Source: Tomaro-Duchesneau et al. (2012)

Spray Drying

Spray drying is one of the most commonly used microencapsulation and drying technologies in food and pharmaceutical industries on being flexible, economical, efficient, easy to scale-up, easily available equipment and produces good quality powder (Desobry et al. 1997). It has been extensively used for decades in the encapsulation of bioactive food ingredients such as proteins, fats, vitamins, enzyme, pigments and flavours. But its use in thermo-sensitive products, such as microorganisms and essential oils is limited because the required high temperature causes volatilization and/or destruction of the product (Gharsallaoui et al. 2007). Microencapsulation by spray drying involves the formation of an emulsion, solution or suspension containing the

core and wall material, followed by nebulization/atomization in a drying chamber with circulating hot air (Fig. 2). The water evaporates instantly in contact with the hot air, and the matrix encapsulates the core material (Laohasongkram et al. 2011).

Fig. 2 Microencapsulation process by spray drying

Preparation of Emulsion: For encapsulation of any bioactive compounds, preparation of stable emulsion is the primary step (Desobry et al. 1997). Emulsion is a mixture of two or more liquids that are normally immiscible. To aid the process, the addition of emulsifiers is required wherein emulsifier stabilizes the emulsion by reducing the interfacial tension between the two phases by forming a rigid interfacial film which serve as mechanical barrier to coalescence. Once the wall or coating material is selected for encapsulation of active ingredient, it must be hydrated. After solubilization of wall material, the active ingredient to be encapsulated viz., flavors, vitamins, minerals, oil etc is added to wall material solution. This is followed by homogenization of the mixture to create small droplets of active ingredient within the wall material or encapsulating solution. A typical ratio of encapsulating agent to core material is 4:1 to 5:1. Emulsion can be prepared either as two layer or multilayer system (Fig. 3) for improved stability (Bortnowska 2015).

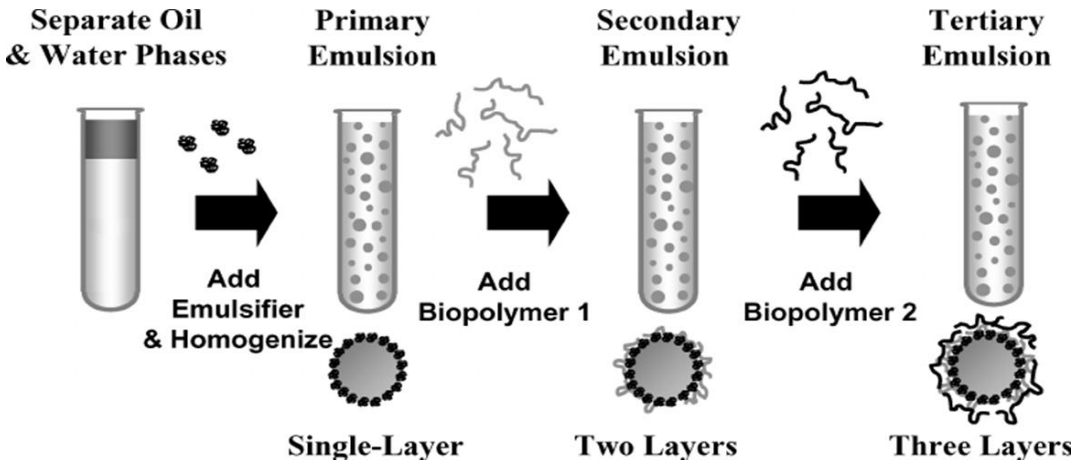


Fig. 3 Preparation of Multilayer emulsion (Source: McClements et al. (2009))

Atomization of the in feed Emulsion: The core- wall material mixture or emulsion is fed into a spray dryer where it is atomized through a nozzle or spinning wheel. The major components of a standard spray dryer include an air heater, atomizer, main spray chamber, blower or fan, cyclone and product collector (Fig. 4).

Dehydration of the atomized particle: When the atomized particle contacts hot air flowing in either a concurrent or countercurrent direction, water in the particle gets evaporated and a dried encapsulated product is produced. Morphology of microencapsulated product obtained by spray drying will be matrix type with the particle size in the range of 10-400 µm.

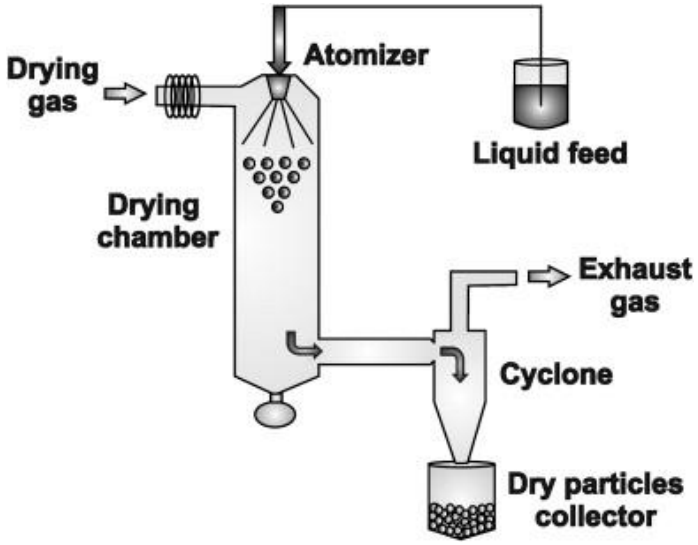


Fig. 4 Schematic diagram of Spray drying (Source: Sosnik and Seremeta (2015))

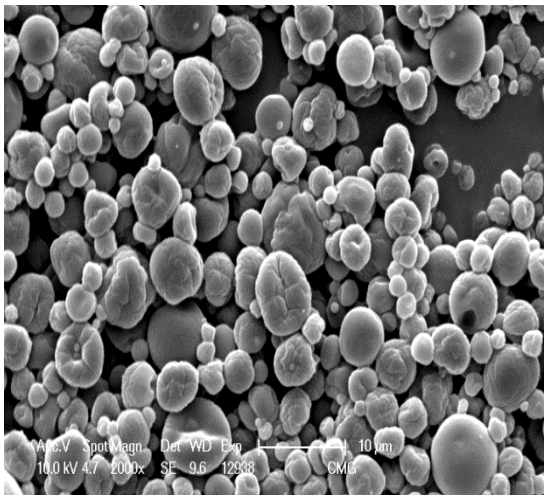


Fig. 5 SEM image of microencapsulated fish oil

Coating/wall materials used for microencapsulation of food ingredients by spray drying

The correct choice of a wall material for microencapsulation of food ingredients by spray-drying is very important to achieve better encapsulation efficiency and microcapsule stability. The ideal wall material should have the following characteristics viz., not reactive with the core; ability to maintain core integrity and stability; lack an unpleasant taste in the case of food applicability and economic viability (Gharsallaoui et al. 2007; Nazzaro et al. 2012). The criteria for selecting a wall material are mainly based

on the physico-chemical properties such as solubility, viscosity, molecular weight, glass/melting transition, film forming, and emulsifying properties etc. Hence, the selection of wall material or encapsulating material according to the desired application is an important task. Most wall materials do not offer all the desired properties and hence to fulfill all the requirements, generally a combination of wall materials is employed. Wall materials can be selected from a wide variety of natural and synthetic polymers, including the following: Carbohydrates: starch, modified starches, dextrins, sucrose, cellulose etc.; Gums viz., gum Arabic, alginate and carrageenan; Lipids: wax, paraffin, monoglycerides and diglycerides, etc.; Inorganic materials including calcium sulfate and silicates; Proteins viz., gluten, casein, gelatin and albumin. Wall materials used for microencapsulation of various food ingredients by spray drying are given in Table 2.

Table 2 Wall materials used for microencapsulation of food ingredients by Spray drying

Food ingredients	**Coating material used**
Fish oil	Gelatin, maltodextrin, casein, lactose, sodium caseinate, dextrose equivalence, highly branched cyclic dextrin, methylcellulose, hydroxypropyl methylcellulose, n-octenylsuccinate, derivatized starch/glucose syrup or trehalose, sugar beet pectin, gum arabic, corn syrup solids, egg white powder
Poly phenols: Black carrot extracts (anthocyanins), procyanidins, olive leaf extract, *Hibiscus sabdariffa* L. extract (anthocyanins), soybean extract, grape seed extract, apple polyphenol extract, olive leaf extract, oregano essential oil, mint oil, cardamom oleoresin, black pepper oleo resin , cumin oleo resin, turmeric oleo resin	Maltodextrin, gum arabic, chitosan, citrus fruit fiber, colloidal silicon dioxide, maltodextrin and starch, sodium caseinate, soy lecithin, skimmed milk powder, whey protein concentrate, gelatin
Vitamin C, vitamin A	Tripolyphosphate, cross-linked chitosan, starch, β-cyclodextrin, maltodextrin, gum arabic,
β-Galactosidase, lipase from Y. lipolytica	Chitosan, modified chitosan (water soluble), alginate, calcium alginate and arabic gum, α- amalase, gum, α- amalase,
Hydrolysate and peptide	Soy protein isolate, gelatin, whey protein concentrate, alginate, maltodextrin, gum Arabic, carboxymethylated gum

(Source: Shahidi and Han 1993; Desobry 1997; Schrooyen et al. 2001; Desai and Park 2005; Jeyakumari et al. 2014; Bortnowska 2015; Mohan et al. 2015)

Advantages and Disadvantages of Spray Drying Process

Advantages

- ✓ Relatively simple, fast and easy to scale-up, equipment is readily available
- ✓ The cost of spray-drying method is 30–50 times cheaper than other encapsulation method with low process cost and reduced storage and transportation costs
- ✓ Possibility of employing a wide variety of encapsulating agents viz., both hydrophilic and hydrophobic polymer
- ✓ Ideal for production of sterile materials
- ✓ Rapid solubility of the capsules
- ✓ It increases stability and shelf-life of food product
- ✓ It improves handling of the viscous and sticky food materials.

Disadvantages

- ➢ Considerable amounts of the material can be lost during the process due to sticking of the microparticles to the wall of the drying chamber.
- ➢ Process variables that should be optimized for encapsulation
- ➢ Non uniformity of microcapsule size
- ➢ Limitation in the choice of coating material
- ➢ Produce very fine powder which needs further processing
- ➢ Not good for heat sensitive material

Challenges

Microencapsulation has been applied widely in a variety of food and pharmaceutical products. Studies have shown its enormous potential to provide superiorly featured core, resulting in advanced quality products applicable in the food and pharmaceutical industry. It provides an effective protection for active agent against oxidation, evaporation or migration in food as well as facilitate conversion of liquids to powders. In spite of recent developments of spray drying technique, the process remains far from completely being controlled for microencapsulation of active food ingredients. Spray drying technology is yet to become a conventional tool for food and pharmaceutical industry to produce encapsulated ingredients. To produce effective encapsulated products, the appropriate selection of coating material is a great challenge which can be achieved by multidisciplinary based research approach and consideration of industrial requirements and constraints.

References

- Bortnowska, G. (2015). Multilayer oil-in-water emulsions: formation, characteristics and application as the carriers for lipophilic bioactive food components–a review. Polish Journal of Food and Nutrition Sciences, 65(3), 157-166.
- Desai, K. G. H., & Park, H. J. (2005). Drying Technology. Recent Developments in microencapsulation of food ingredients, 23, 1361-1394.

- Desobry, S. A., Netto, F. M., & Labuza, T. P. (1997). Comparison of spray-drying, drum-drying and freeze-drying for β-carotene encapsulation and preservation. Journal of Food Science, 62(6), 1158-1162.
- Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. Food research international, 40(9), 1107-1121.
- Gibbs, F., Selim Kermasha, Inteaz Alli, Catherine N., & Mulligan, B. (1999). Encapsulation in the food industry: a review. International journal of food sciences and nutrition, 50(3), 213-224.
- Jeyakumari, A., Kothari, D. C., & Venkateshwarlu, G. (2014). Microencapsulation of fish oil-milk based emulsion by spray drying: Impact on Oxidative Stability. Fishery Technology, 51, 31 – 37.
- Laohasongkram, K., Mahamaktudsanee, T., & Chaiwanichsiri, S. (2011). Microencapsulation of Macadamia oil by spray drying. Procedia Food Science, 1, 1660-1665.
- McClements, D. J., Decker, E. A., Park, Y., & Weiss, J. (2009). Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. Critical reviews in food science and nutrition, 49(6), 577-606.
- Mohan, A., Rajendran, S. R., He, Q. S., Bazinet, L., & Udenigwe, C. C. (2015). Encapsulation of food protein hydrolysates and peptides: a review. RSC Advances, 5(97), 79270-79278.
- Nazzaro, F., Orlando, P., Fratianni, F., & Coppola, R. (2012). Microencapsulation in food science and biotechnology. Current opinion in biotechnology, 23(2), 182-186.
- Schrooyen, P. M., van der Meer, R., & De Kruif, C. G. (2001). Microencapsulation: its application in nutrition. Proceedings of the Nutrition Society, 60(4), 475-479.
- Shahidi, F., & Han, X. Q. (1993). Encapsulation of food ingredients. Critical Reviews in Food Science & Nutrition, 33(6), 501-547.
- Sosnik, A., & Seremeta, K. P. (2015). Advantages and challenges of the spray-drying technology for the production of pure drug particles and drug-loaded polymeric carriers. Advances in colloid and interface science, 223, 40-54.
- Tomaro-Duchesneau, C., Saha, S., Malhotra, M., Kahouli, I., & Prakash, S. (2012). Microencapsulation for the therapeutic delivery of drugs, live mammalian and bacterial cells, and other biopharmaceutics: current status and future directions. Journal of pharmaceutics, 2013.

****

Coated Products from Fish Meat

Sreelakshmi. K.R. and George Ninan

Fish Processing Division

ICAR- Central Institute of Fisheries Technology, Cochin

E-mail: sreecift@gmail.com

The present day consumers' particularly urban consumers are showing more and more interest in food products which are available as ready to eat or ready to cook. These food items are called convenient products and the global demand for such products is increasing rapidly. This has led to the development of several fishery products varied in taste, texture and appearance. One group among them getting high consumer appeal is battered and breaded products popularly known as coated products. Battering and breading techniques have contributed significantly to value addition of fish and fishery products.

In essence, a coated food product is one that is coated with another foodstuff. Coating by battering and breading enhances a food product's characteristics such as appearance, flavour and texture. 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.

There are several ingredients used in the formulation of coatings. Each ingredient performs its functions to contribute to the unique characteristics and functionality of coatings. The commonly used ingredients fall under five categories. They are polysaccharides, proteins, fats, seasonings and water. Besides small quantities of leavening agents, gums, spices, colour etc. may be added to provide specific functional effects. The major ingredients used for the production of batter mix and breadcrumbs are more or less same but the manufacturing techniques employed are different. The preparation of coated products includes seven major steps.

1. **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.
2. **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.
3. **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. Typical formulation of a batter system is given in Table. 1. The ingredients are classified as critical and optional based on the functions.

Table.1 Formulation of batter

Ingredients	Addition range%
Critical	
Wheat flour	30-50
Corn flour	30-50
Sodium bicarbonate	Upto 3
Acid phosphate	Adjust based on neutralizing value
Optional	
Flours from rice, soy, barley	0-5
Shortening oil	0-10
Dairy powders	0-3
Starches	0-5
Gums, emulsifiers, colours	Less than 1
Salt	Upto 5
Sugars, dextrins	0-3
Flavourings, seasonings etc.	As required

Ingredients of batter mix formulated at CIFT

An adhesive type quick setting batter is usually used. A typical adhesive batter formulated at CIFT, Kochi is given in Table 2.

Table 2. Batter Ingredients

Maida	2000 g
Corn flour	200 g
Bengal gram	200 g
Salt	30 g

Guar gum	5 g
Turmeric powder	5 g
Sodium tripolyphosphate	10g

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

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

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

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

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

The ingredients are mixed evenly and one part of batter powder is mixed with two parts of water to get the required consistency.

4. ***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.

   Preparation of Bread Crumbs

   - Remove the outer brown layer of bread
   - Grind in mixer grinder
   - Spread over aluminium tray
   - Keep for drying for 2 ½ hrs at 70^0C in dryer (smoker)
   - Store in appropriate packages

Commercial bread crumbs like planko bread crumbs (flake like), extruded bread crumbs (float in oil) etc. can also be used.

5. **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.

6. **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.
7. **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 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. Fresh water fish fillet of table size and having minimum fin bones can be used for this purpose. Various stages in the production of coated fish fillet are:

- Filleting
- Cold blanching
- Pre-dusting
- Coating with batter
- Coating with bread crumbs
- Pre-frying
- Freezing
- Packaging
- Storage

Filleting: 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.

Cold Blanching: 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.

Pre-dusting: The 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.

Battering: The pre-dusted fillets are then coated with batter uniformly.

Breading: 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.

Pre-frying: 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°C.

Freezing: 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.

Packaging: 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.

Storage: 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.

Preparation of Fish Fingers

I. Fish Fingers from Fillet

Ingredients

Fish fillet	1 kg
Salt	3%

Procedure: Fillet the fish and cut into small pieces (about 10 cm in size) and blanch in 3% salt solution for 10-15 minutes. Drain off and pre-dust with batter powder and coat with batter and breadcrumbs and fry.

II. Fish Fingers from Mince

Ingredients

Fish fillet	1 kg
Tri-sodium polyphosphate	0.1 %
Salt	0.6 %

Procedure: Dress and fillet the fish and mince in a meat mincer. Add 0.1 % tri-sodium polyphosphate, 0.6% salt, mix, spread the mince in a tray uniformly and freeze. Cut into small pieces (about 10 cm in size) in the frozen condition itself. Pre-dust the finger with batter powder and coat with batter and breadcrumbs using a bamboo stick. 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 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. The removal of fin 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 fin bones using a fish meat strainer

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.

Ingredients

Cooked fish meat	:	1000 g
Salt	:	25 g (approx.)
Oil	:	125 ml
Green chilli	:	20 g

Ginger	:	25 g
Onion	:	250g
Potato (cooked)	:	500g
Curry leaves	:	20 g
Mint leaves	:	20 g
Pepper (powder)	:	3 g
Clove (powdered)	:	2 g
Cinnamon (powdered)	:	2 g
Turmeric	:	2 g
Batter mix	:	250 g
Bread crumb	:	300 g

Method of preparation

- Cook the dressed fish /skeletal frame/mince 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
- Freeze the cutlets preferably in an IQF machine.
- Pack in thermoformed trays/pouches and store at –20 °C.

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.

Ingredients

Fish mince	:	1000g Corn
starch	:	50g

Ginger	:	20g
Garlic	:	20g
Pepper	:	2g
Salt	:	10g (1%)
Batter	:	250 g
Bread crumbs	:	350 g

Process

- Allow the frozen fish mince to thaw. Wash the mince and drain.
- Add corn starch and salt to fish mince and mix thoroughly.
- Add ginger and garlic made into a paste along with pepper powder and mix thoroughly.
- Prepare balls of size 2-3 cm diameter.
- Cook in 1% boiling brine for 10 minutes.
- Take out, drain and cool.
- Pre-dust the balls with the dry batter mix
- Using a bamboo skewer dip in batter prepared in the ratio 1:2 with water
- Apply bread crumbs
- Flash fry in vegetable oil
- Pack the balls in thermoformed trays
- Freeze at -40°C (Blast Freezer or IQF machine) and store at -20°C

Preparation of Specialty Products from Shrimp

1. 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^{o}C & storage below -18°C in master carton.

2. 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.

3. **Easy-peel shrimp**

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

Process: Wash the whole shrimp in potable water and remove the head. 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. Cut the cuticle, up to end of the last segment dorsally or laterally leaving it intact, just to make the cooked shrimp easy to peel. Arrange in PVC/polystyrene trays and vacuum pack in laminated pouches. Blast freezing at –40°C and storage below -18°C in master carton.

4. Cooked easy-peel shrimp

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

Process: Wash the whole shrimp in potable water and remove the head. 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. Cut the cuticle, up to the end of the last segment dorsally or laterally leaving it intact. Arrange in PVC/polystyrene trays and vacuum pack in laminated pouches. Blast freezing at –40°C and storage below -18°C in master carton.

5. 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

6. 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.

7. 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.

8. 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.

9. 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.

10. 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.

11. 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

12. 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.

13. 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

14. 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

****

Biscuits with Aquatic Bioactive Ingredients

Anuj Kumar

Fish Processing Division

ICAR- Central Institute of Fisheries Technology

Cochin -682 029, India

Email: anuj19.ak@gmail.com

In modern-day societies, health is one of the central values, which are primarily focused upon. Healthy attributes of foods are prime theme in various organizational or governmental initiatives. The fast moving lifestyle of today's environment has burdened us with huge amount of stress, which is taking a toll on our mind and body. Due to upsurge in various diseases like coronary heart disease, diabetes, ageing etc., there is a continual demand for foods with enhanced functionality, which can be handy against many diseases either through preventive or recuperative effect. Due to consumers' demand for healthier foods, the food industry is directing new product development towards the area of functional foods and ingredients. Functional products are a new variety of foods that promise targeted improvement in physiological functions in the body. In these foods, particular components are directly connected with well-defined physiological effects and the health benefit is linked to a single product. Functionality creates a novelty in the food without necessarily changing the sensory quality of the product. The importance of functional foods, nutraceuticals and other natural health products has been well recognized in connection with health promotion, disease risk reduction and reduction in health care costs.

Bakery products are a suitable carrier for utilization of seaweed functionality because of their wider reach. Biscuits are favourite food widely consumed mostly due to their pleasant taste, ready to eat nature, accessible cost and availability and longer shelf time and they can contribute significantly to daily cereal intake. However, in the era of increasing popularity of functional foods and nutraceuticals, new demands have been set for different categories of snack foods including biscuits maintaining traditional nutritional aspects of foods and exhibiting additional health benefits. The development of new functional ingredients has the advantage that food manufacturers can add extra value to products the consumer is already familiar with. The main factors that have to be considered are the variations affecting the processing conditions, the sensory properties, and the nutritional value of the final product. Biscuits represent a potential choice for the addition of functional ingredients.

Collagen peptide is a major component of skin, scale, bone and visceral mass. Collagen is one of the most abundant animal proteins. It is fibrous in nature and forms the basis of mechanical/ structural support in living tissues. Collagen is having unique amino acid composition and it possess distinct bioactive propertiesCollagen peptide are considered as a functional ingredient for its health beneficial effects. Collagen peptides are hydrolyzed forms of collagen i.e. short chains of amino acids. Hydrolysis of collagen produces small molecular weight peptides having increased biological activities. Collagen peptides also promote the absorption of vitamins and minerals. Small peptides

are desirable for nutraceutical and pharmaceutical applications. The collagen peptides are water soluble and their bioavailability is relatively higher than native collagen. Collagen peptide consumption increases the bone mineral density and supports healthy joints. The peptide also provides better inflammatory response against inflammation arising from training and exercise Elderly/aged people suffer from various age-associated degenerative diseases particularly, bone-linked problems. Ageing is associated with inflammation and higher risk of osteoporosis due to changes in bone density. Age- related bone loss can be effectively prevented by the dietary supplementation of collagen peptide.

Biscuits are important and commonly eaten baked products like cakes, pastries, bread etc. obtained from wheat flour. Biscuits are energy dense foods consumed either as breakfast items or snacks. Biscuits can be classified into three categories: sweet, semi-sweet and salted. Sweet biscuits are produced from soft dough and have higher sugar and fat content than semi-sweet and salted biscuits. Semi-sweet biscuits are produced from hard dough and have lower fat and sugar content. Salted varieties also called crackers have low fat and sugar. They are also produced from fermented doughs. Refined wheat flour is the base material required for biscuit preparation and acts as main structural component. Protein content of wheat flour governs the quality of biscuits produced. Generally, flour having protein content < 9% are preferred for biscuit making. Sugar imparts sweetness to the biscuit. It also improves the colour and flavour of the biscuits. Higher sugar causes hardening of biscuit texture. Fat gives a shortening effect to the dough and makes the dough more extensible. Moreover, fat gives the palatability to the biscuit. Salt also imparts taste to the biscuits. The leavening agents are added to biscuits for porous and crisp texture. Ammonium and sodium bicarbonate are generally used for leavening in biscuits. Water is added to maintain consistency of dough. It also helps in uniformly distributing the salts and leavening agents. Steam generated also gives some leavening effect to biscuits. Skim milk powder or milk are added for flavour and colour development. Emulsifier help in uniform dispersion of fat throughout the dough. Lecithin, glyceryl monostearate and sodium steroyl lactate are added as emulsifier in biscuits.

Preparation of Collagen Peptide Supplemented Biscuits

The formula for biscuit is presented in table 1. The stages for biscuit preparation are presented in Figure 1. Initially, fat and powdered sugar are creamed for 3-4 min in a planetary mixer. The mixing is carried out further after adding lecithin, flavour, skim milk powder and invert syrup. Salts and leavening chemical are dissolved in water and then added to creamed mix. Then, collagen peptide and refined wheat flour mix is added to the mixer and mixing is continued. Water may be added to achieve desired consistency dough. The dough produced is soft in nature and breaks very easily. The dough is allowed to rest for 30 min. The dough is then sheeted into desired thickness (4 mm) and shaped. The shaped biscuits are placed over a baking tray and baked at appropriate time-temperature combination (180°C for 14-15 min) to get optimum quality biscuits. Biscuit are then allowed to cool at room temperature and packed in metallized polyester pouches.

Table 1. Recipe for collagen peptide biscuits

Refined wheat flour	:	90 g
Collagen peptide	:	10 g
Powdered sugar	:	30 g
Biscuit fat	:	40 g
Lecithin	:	0.5 g
Salt	:	0.5 g
Sodium bicarbonate	:	0.5 g
Ammonium bicarbonate	:	1.0 g
Skim milk powder	:	2 g
Invert syrup	:	2 g
Vanilla flavour	:	0.1 g

Fig. 1 Collagen peptide supplemented biscuits

Fig. 2 Different stage in preparation of collagen peptide enriched biscuits

****

Modern Practices in Seafood Packaging

C.O. Mohan

Fish Processing Division

ICAR-Central Institute of Fisheries Technology

Email: comohan@gmail.com

Introduction

There is ever increase in the demand for good quality food product with improved quality and shelf life. Over the years, packaging have brought out a revolution in the marketing and distribution of food products including fish. Among the food categories, seafood ranks 3rd with respect to consumption which explains the importance of fish. Fish is a vital source of food for people. It is the most important single source of high-quality protein, providing approximately 16% of the animal protein consumed by the world's population (Food and Agriculture Organisation (FAO), United Nations, 1997). By any measure, fishes are among the world's most important natural resources. Fisheries production was 171 mmt in 2016 of which capture and culture fisheries accounted to 90.9 and 80 mmt, respectively (FAO, 2018). The total first sale value of fisheries was USD 362 billion. Additionally, with over 25000 known species, the biodiversity and ecological roles of fish are being increasingly recognized in aquatic conservation, ecosystem management, restoration and aquatic environmental regulation.

Like any other food commodities, fish is one of the highly perishable items which undergoes spoilage if sufficient care is not taken. Various preservation methods have been in place to overcome the spoilage of fish. Chilling and refrigeration is the most preferred preservation method as it helps in preserving fresh like quality. Chilling or icing is reducing the temperature of fish so as to prolong the lag phase of bacteria and helps in reducing the spoilage rate. Fish being one of the most perishable foods, its freshness is rapidly lost even when stored under chilled conditions. Further, consumers demands to have fish in as fresh a state as possible so that the characteristics flavours are retained. Bulk transportation of fresh fish in ice has several limitations like limited extension of shelf life, unnecessary expenditure on freight due to ice, difficulty in handling and maintaining hygienic conditions due to leaching of ice melt water with leaching losses of soluble nutrients and flavouring compounds. Proper packaging will help in improving the keeping quality of fish. Packaging is an important aspect for improving the shelf life and marketability. Packaging enhances the consumer acceptability and hence saleability of the product. Traditionally, food packaging is meant for protection, communication, convenience and containment. The package is used to protect the product from the deteriorative effects of the external environmental conditionals like heat, light, presence or absence of moisture, pressure, microorganisms, and gaseous emissions and so on. Packaging is an integral part of the food processing and plays an important role in preventing or reducing the generation of waste in the supply of food. Packaging assists the preservation of the world's resources through the prevention of product spoilage and wastage, and by protecting products until they have performed their function. Basic requirements of a package are good marketing properties, reasonable price, and technical feasibility, utility for food contact, low

environmental stress, and suitability for recycling. Simply packing fish is suitable packaging material will enhance the shelf life of chilled and refrigerated fish to 7 to 15 days depending on fish species (Stammen et al. 1990; Mohan et al. 2008, 2009a,b, 2010, 2012, 2017, 2018; Ozogul et al. 2006a; Pons-Sanchez-Cascado et al. 2006; Ruiz-Capillas and Moral, 2005; Ozogul et al. 2006b; Ozugul et al. 2007; Losada et al. 2005; Campos et al. 2006; Remya et al., 2016, 2017).. However, in the normal packaging the spoilage process will be accelerated due to presence of O_2 in the normal air packing. Alteration in the package atmosphere will help in overcoming the problem of shelf life, which can be achieved by vacuum packaging or modified atmosphere packaging.

Vacuum packaging

Important properties by which consumers judge fish and shell fish products are appearance, texture and flavour. Appearance, specifically colour, is an important quality attribute influencing the consumer's decision to purchase. In fresh red meat fishes, myoglobin can exist in one of three chemical forms. Deoxymyoglobin, which is purple, is rapidly oxygenated to cherry red oxymyoglobin on exposure to air. Over time, oxymyoglobin is oxidised to metmyoglobin which results in a brown discoloration associated with a lack of freshness. Low oxygen concentrations favour oxidation of oxymyoglobin to metmyoglobin. Therefore, in order to minimize metmyoglobin formation in fresh red meats, oxygen must be excluded from the packaging environment to below 0.05% or present at saturating levels. Lipid oxidation is another major quality deteriorative process in muscle foods resulting in a variety of breakdown products which produce undesirable off-odours and flavours. Hence O_2 may cause off-flavours (e.g. rancidity as a result of lipid oxidation), colour changes (e.g. discolouration of pigments such as carotenoids, oxidation), nutrient losses (e.g. oxidation of vitamin E, β-carotene, ascorbic acid) and accelerates microbial spoilage thereby causing significant reduction in the shelf life of foods. Therefore, control of oxygen levels in food package is important to limit the rate of such deteriorative and spoilage reactions in foods. Oxygen level in the package can be controlled by using the vacuum packaging technique in which, the air present in the pack is completely evacuated by applying vacuum and then package is sealed. Vacuum packaging, which is also referred as skin packaging involves removal of air inside the pack completely and maintaining food material under vacuum conditions, so that oxygen available for the growth of microbes and oxidation will be limited. This will help in doubling the shelf life of fish under chilled conditions. This technique is particularly useful in fatty fishes, where the development of undesirable odour due to the oxidation of fat is the major problem. Vacuum packaging for chilled and refrigerated fishes doubles the shelf life compared to normal air packaging (Mohan et al., 2008). Application of this to frozen fishes is also commonly followed as it helps in reducing problem of freezer burn. This technique can be applied to fresh meat and fishes, processed meat and fishes, cheese, coffee, cut vegetables etc. One of the important aspect in the vacuum packaging is the use of packaging material with good barrier properties. Normally polyester-polyethylene or nylon-polyethylene laminates are used. Polyester and nylon provides good strength and acts as good barrier to oxygen. Polyethylene proves good heat sealing property and is resistant to water transmission. The advantages of vacuum packaging include reduction in fat oxidation, growth of aerobic microorganisms, reduction in evaporation thereby dryness and freezer burn in frozen products, extends shelf life and reduces volume for bulk packs containing lighter materials. Disadvantages include difficulty in use for sensitive crispy products and products with sharp edges, requires high barrier packaging material to

maintain vacuum, creates anaerobic condition, which may trigger the growth and toxin production of *Clostridium botulinum* and the growth of *Listeria monocytogenes.* Additional barriers / hurdles are needed to control these microorganisms and also it is capital intensive. Alternative to vacuum packaging, reduced oxygen level in the package can be achieved by using active packaging system like oxygen scavenger. Use of oxygen scavenger is very effective in reducing the oxygen level to <0.01% within 24 h, which helps in preserving the quality of food (Mohan, 2008). This is not capital intensive and can be applied to any products including crispy and products with sharp edges.

Modified atmosphere packaging (map)

Marketing of modified atmosphere packaged (MAP) foods have increased, as food manufacturers have attempted to meet consumer demands for fresh, refrigerated foods with extended shelf-life. It is also used widely, as a supplement to ice or refrigeration to delay spoilage and extend the shelf life of fresh fishery products while maintaining a high-quality end product. A modified atmosphere can be defined as one that is created by altering the normal composition of air (78% nitrogen, 21% oxygen, 0.03% carbon dioxide and traces of noble gases) to provide an optimum atmosphere for increasing the storage length and quality of food/produce (Moleyar and Narasimham 1994; Phillips, 1996). Oxygen, CO2, and N2, are most often used in MAP (Parry 1993; Phillips 1996). Other gases such as, nitrous and nitric oxides, sulphur dioxide, ethylene, chlorine (Phillips 1996), as well as ozone and propylene oxide (Parry 1993) have been suggested for a variety of products and investigated experimentally. However, due to safety, regulatory and cost considerations, they have not been applied commercially. These gases are combined in three ways for use in modified atmospheres: inert blanketing using N_2, semi-reactive blanketing using CO_2 : N_2 or O_2 : CO_2 : N_2 or fully reactive blanketing using CO_2 or CO_2 : O_2.

Development of modified atmosphere packaging

Kolbe was the first to investigate and discover the preservative effect of carbon dioxide on meat in 18th century and Coyne was the first to apply modified atmospheres to fishery products as early as 1930's. Modified atmosphere packaging (MAP) is the removal and/or replacement of the atmosphere surrounding the product before sealing in vapor-barrier materials. While technically different many forms of map are also case ready packaging, where meat is cut and packaged at a centralized location for transport to and display at a retail store. Most of the shelf life properties of meat are extended by use of map, but anoxic forms of MAP without carbon monoxide do not provide bloomed red meat color and MAP without oxygen may promote oxidation of lipids and pigments. Advances in plastic materials and equipment have propelled advances in MAP, but other technological and logistical considerations are needed for successful MAP systems for raw chilled fresh meat. The growth inhibition of microorganisms in MA is determined by the concentration of dissolved CO_2 in the product. The preservation effect of MAP is due to the drop in surface pH in MA products because of the acidic effect of dissolved CO_2, but this could not entirely explain all of CO_2's bacteriostatic effect. The possibility of intracellular accumulation of CO_2 would upset the normal physiological equilibrium by slowing down enzymatic processes. Thus, the effect of CO_2 on bacterial growth is complex and four mechanisms of CO_2 on micro-organisms has been identified (Parkin and Brown, 1982; Daniels et al. 1985; Dixon and Kell, 1989; Farber, 1991):

1. Alteration of cell membrane functions including effects on nutrient uptake and absorption

2. Direct inhibition of enzymes or decrease in the rate of enzyme reactions
3. Penetration of bacterial membranes, leading to intracellular pH changes
4. Direct changes in the physico-chemical properties of proteins.

Probably a combination of all these activities account for the bacteriostatic effect. A certain amount (depending on the foodstuff) of CO_2 has to dissolve into the product to inhibit bacterial growth. The ratio between the volume of gas and volume of food product (G/P ratio) should be usually 2 : 1 or 3 : 1 (gas : food product). This high G/P ratio is also necessary to prevent package collapse because of the CO_2 solubility in wet foods. The CO_2 solubility could also alter the food-water holding capacity and thus increase drip.

The major function of carbon dioxide in MAP is to inhibit growth of spoilage microbes. Carbon dioxide (CO_2) is soluble in both water and lipid it has a bacteriostatic and fungistatic properties. Carbon dioxide lowers the intra and extra cellular pH of tissue including that of microorganisms. It affects the membrane potential and influence the equilibrium of decarboxylating enzymes of microorganisms. CO_2 increases the lag phase and a slower rate of growth of microbes during logarithmic phase. This bacteriostatic effect is influenced by the concentration of CO_2, the partial pressure of CO_2, volume of headspace gas, the type of micro organism, the age and load of the initial bacterial population, the microbial growth phase, the growth medium used, the storage temperature, acidity, water activity, and the type of the product being packaged. Pathogens like *Clostridium perfringens* and *Clostridium botulinum* are not affected by the presence of carbon dioxide and their growth is encouraged by anaerobic conditions. In general, carbon dioxide is most effective in foods where the normal spoilage organisms consist of aerobic, Gram negative psychrotropicbacteria. The CO_2 is flushed into the modified atmosphere package by evacuating the air and flushing the appropriate gas mixture into the package prior to sealing. Another method to create a modified atmosphere for a product is either to generate the CO_2 and/or remove O_2 inside the package after packaging or to dissolve the CO_2 into the product prior to packaging. Both methods can give appropriate packages with smaller gas/product ratio to the package. The solubility of CO_2 decreases with increasing temperature, hence MAP products should be stored at lower temperatures to get the maximum antimicrobial effect. Also the temperature fluctuations will usually eliminate the beneficial effects of CO_2. The rate of absorption of CO_2 depends on the moisture and fat content of the product. If product absorbs excess CO_2, the total volume inside the package will be reduced, giving a vacuum package look known as “pack collapse”. Excess CO_2 absorption along with “pack collapse” results in the reduction of water holding capacity and further drip loss to the products.

The major function of oxygen is to avoid anaerobic condition which favours the growth and toxin production of *C botulinum* and growth of *L monocytogenes*. Oxygen in the MAP is also useful to maintain the muscle pigment myoglobin in its oxygenated form, oxymyoglobin. In fresh red meats, myoglobin can exist in one of three chemical forms. Deoxymyoglobin, which is purple, is rapidly oxygenated to cherry red oxymyoglobin on exposure to air. Over time, oxymyoglobin is oxidised to metmyoglobin which results in a brown discoloration associated with a lack of freshness. Low oxygen concentrations favour oxidation of oxymyoglobin to metmyoglobin. Therefore, in order to minimize metmyoglobin formation in fresh red meats, oxygen must be excluded from the packaging environment to below 0.05% or present at saturating levels. High oxygen

levels within MAP also promote oxidation of muscle lipids over time with deleterious effect on fresh meat colour. O_2 in MA-packages of fresh fish will also inhibit reduction of TMAO to TMA. Nitrogen (N_2) is an inert and tasteless gas, and is mostly used as a filler gas in MAP, either to reduce the proportions of the other gases or to maintain pack shape by preventing packaging collapse due to dissolution of CO_2 into the product. Nitrogen is used to prevent package collapse because of its low solubility in water and fat. Nitrogen is used to replace O_2 in packages to delay oxidative rancidity and to inhibit the growth of aerobic microorganisms. The exact combination to be sued depends on many factors such as the type of the product, packaging materials and storage temperature. The gas ratio normally used are 60% CO_2 and 40% N_2, for fatty fishes and 40% CO_2, 30% O_2 and 30% N_2 for lean variety fishes. Shelf life of different fishes packed under vacuum and MAP at different storage conditions are given in Table 1.

Advantages of MAP

- The natural colour of the product is preserved
- The product retains its form and texture
- Reduces the growth of microogranisms
- Product retains its vitamins, taste and reduces fat oxidation
- The need to use preserving agents is reduced
- Helps in marketing products to distant locations
- Improved presentation –clear view of product
- Hygienic stackable pack, sealed and free from product drip
- Longer durability of perishable food / decrease of spoilage
- Extends the shelf life of fish in chilled / refrigerated storage by 2 – 3 times
- Helps in reducing post-harvest loss

Disadvantages of MAP

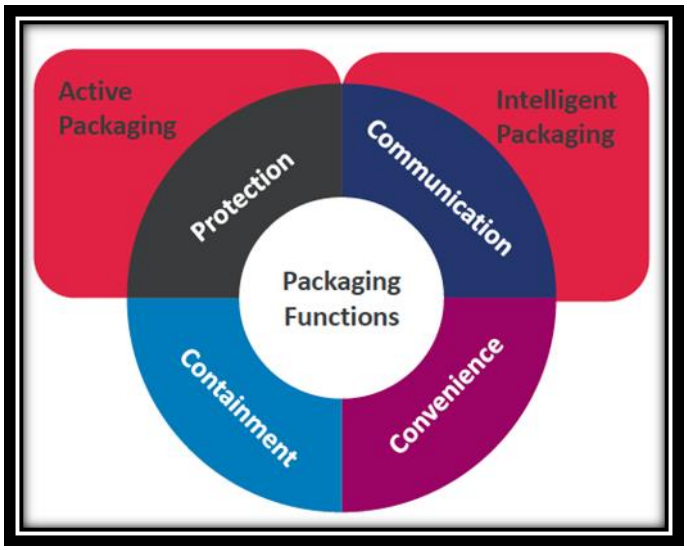
- Capital intensive due to high cost of machinery
- Cost of gases and packaging materials
- Additional cost of gas analyser to ensure adequate gas composition
- No control over the gas composition after packing
- Increase of pack volume which will adversely affect transportation cost and retail display space
- Benefits of MAP are lost once the pack is opened or leaks
- High concentration of CO_2 may favour anaerobiosis
- Strict maintenance of temperature has to be ensured to avoid the risks of *C botulinum* and *L monocytogenes.*

Smart Packaging Technologies

Traditional packaging concepts are limited in their ability to prolong the shelf-life of fish products. This can be overcome by adopting vacuum and modified atmosphere packaging technologies. However, these require capital investment apart from requirement of fresh food grade gas in case of MAP. This promoted the researchers to develop new and improved methods for maintaining food quality and for extending shelf life. Active and intelligent packaging, which are regarded as smart packaging technologies, is one such advanced packaging technique which is finding its way in the preservation of various food systems including fish and shellfish. The market for active and intelligent packaging systems are fast growing and their demand is projected to reach $10.5 billion by 2021, fuelled by the development of new generations of products and more cost competitive prices, which will spur greater market acceptance for many product types.

Basis of Smart Packaging

Packaging has four basic functions, viz., Containment, convenience, protection and communication. Conventional packaging systems offer limited protection and communicates only through the labelling. It will not provide any information about the quality and safety of the product. Active and intelligent packaging enhances the protection and communication functions, respectively. The following graphics explains how this enhanced functionality works.



Source: AWA Alexander Watson Associates

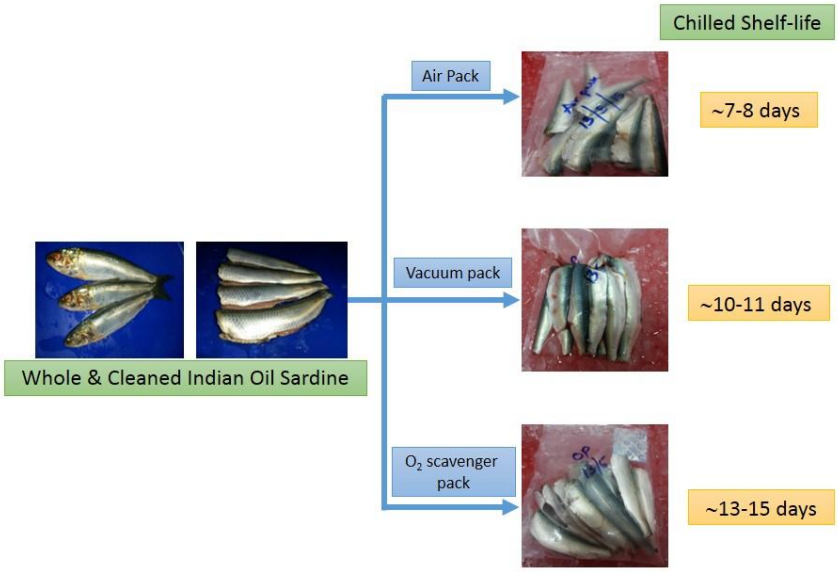
Active Packaging

Active packaging is an innovative concept that can be defined as '*a type of packaging that changes the condition of the packaging and maintains these conditions throughout the storage period to extend shelf-life or to improve safety or sensory properties while maintaining the quality of packaged food*' (Vermeiren et al. 1999; Rooney, 1992; Ahvenainen, 2003). Active packaging (AP) performs some desired role other than providing an inert barrier between the product and external conditions and combines advances in food technology, bio-technology, packaging and material science, in an effort to comply with consumer demands for 'fresh like' products. This involves incorporation of certain additives into the packaging film or within packaging containers with the aim of maintaining and extending product shelf life. Active

packaging technique is either scavenging or emitting systems added to emit (e.g., N_2, CO_2, ethanol, antimicrobials, antioxidants) and/or to remove (e.g., O_2, CO_2, odour, ethylene) gases during packaging, storage and distribution. In case of a gas-scavenging or emitting system, reactive compounds are either contained in individual sachets or stickers associated to the packaging material or, more recently, directly incorporated into the packaging material. Major active packaging techniques are concerned with substances that absorb oxygen, ethylene, moisture, carbon dioxide, flavours/odours and those which release carbon dioxide, antimicrobial agents, antioxidants and flavours. The most important active packaging concepts for fishery products include O_2 scavenging, CO_2 emitters, moisture regulators, antimicrobial packaging concepts, antioxidant release are discussed here.

O2- scavenger

Fish products are highly susceptible to oxygen as it leads to the growth of aerobic microorganisms and oxidation which causes undesirable colour changes (e.g. discolouration of pigments such as myoglobin, carotenoids), off-odours and flavours (e.g. rancidity as a result of lipid oxidation) and leads to loss of nutrients (e.g. oxidation of vitamin E, β-carotene, ascorbic acid) which adversely affects the quality. Therefore, control of oxygen levels in food package is important to limit the rate of such deteriorative and spoilage reactions in foods. Although O_2-sensitive foods can be packed appropriately using modified atmosphere packaging (MAP) or vacuum packaging, these technologies do not always remove O_2 completely. Moreover, the O_2 that permeates through the packaging film cannot be removed by these techniques. By use of an O_2-scavenger, which absorbs the residual O_2 after packaging, quality changes of O_2-sensitive foods associated with low residual oxygen levels can be minimized. O_2 scavengers were first commercialized in the late 1970s by Japan's Mitsubishi Gas Chemical Company (Ageless®). O_2 scavengers are able to eliminate oxygen contained in the packaging headspace and in the product or permeating through the packaging material during storage. O_2 scavengers are efficient in preventing discolouration of fresh and cured fish, rancidity problems, mould spoilage of intermediate and high moisture products or oxidative flavour changes. O_2 scavenging concepts are mainly based on iron powder oxidation, ascorbic acid oxidation, photosensitive dye oxidation, enzymatic oxidation (e.g. glucose oxidase and alcohol oxidase), unsaturated fatty acids (e.g. oleic or linolenic acid), rice extract or immobilized yeast on a solid substrate. Structurally, the oxygen scavenging component of a package can take the form of a sachet, label or film (incorporation of scavenging agent into the packaging film, which avoids the accidental consumption of sachet), card, closure liner or concentrate.



Schematic representation of Influence of active packaging on the shelf life of Indian Oil sardine

CO2- emitter

The method of preserving food products using CO_2 is not new. Modified atmosphere packaging which mainly employs the gases like CO_2, N_2 and O_2 has been in use for extending the freshness of fish products since many decades. The high CO_2-levels (10-80%) are desirable for moist food products like fish, shellfish and meat products which inhibit surface microbial growth and thereby extend shelf-life. The overall effect of CO_2 is to increase both the lag phase and the generation time of spoilage microorganisms. Over the years this has been achieved by modified atmosphere packaging, in which a package is flushed with a mixture of gases including carbon dioxide at sufficient levels. However the concentration of CO_2 within the package will change due to the partial dissolution of CO_2 in to the product and permeability through the packaging film. Normally, the permeability of carbon dioxide is 3–5 times higher than that of oxygen in most plastic films, so it must be continuously produced to maintain the desired concentration within the package. A carbon dioxide generating system can be viewed as a technique complimentary to MAP to overcome the drawbacks. The potential of CO_2 in MAP and more recently generation of CO_2 inside the packaging system have been explored in relation to a number of commodities for their successful preservation. Such systems are based on sodium bicarbonate, ferrous carbonate, ascorbate, citric acid etc. Sodium bicarbonate, when used together with ascorbic acid or citric acid in the presence of sufficient moisture generates CO_2. This technique is very simple and economical as it does not require any costly equipment and pure gases.

Moisture regulator

Wet food has a high vapour pressure, and hence the humidity in the food package increases. Apart from this a certain amount of moisture will be trapped in the packaging due to temperature fluctuations in high equilibrium relative humidity food packages or the drip of tissue fluid from cut fish and fish products. If it is not removed, this moisture will be absorbed by the product or condense on the surface, which cause microbial

spoilage and/or low consumer appeal. An excessive level of water causes softening of dry crispy products. On the other hand, excessive water evaporation through the packaging material might result in desiccation of the packed foodstuffs. It may also favour rancidity of lipids. The controlling of this excess moisture in food package is important to lower the water activity of the product, thereby suppressing microbial growth and preventing foggy film formation. Apart from this, removal of drip from chilled fish and melting water from frozen fish and shellfish makes the package more attractive to the consumer. An effective way of controlling excess water accumulation in a food package is the use of high barrier film material with the appropriate water vapour permeability and use of moisture scavenger, such as silica gel, molecular sieves, natural clays, calcium oxide, calcium chloride and modified starch etc. Among these, silica gel is the most widely used desiccant because it is not toxic and non-corrosive. Drip-absorbent sheets for liquid water control in high a_w foods such as fresh fish and shellfish basically consist of a super absorbent polymer in between two layers. Large sheets are also used for absorption of melted ice in packages of seafood during air transportation. The preferred polymers for absorbing water are polyacrylate salts and graft copolymers of starch. For dried fish applications, desiccants such as silica gel, molecular sieves, CaO and natural clays (e.g. montmorillonite) packed in sachets can be used.

Antimicrobial packaging

Major part of the fish spoilage is attributed to the microbial contamination and subsequent growth which reduces the shelf life of foods and increases the risk of food borne illness. Traditional methods of preserving fish from the effect of microbial growth include thermal processing, drying, freezing, refrigeration, irradiation, MAP and addition of antimicrobial agents or salts. However, some of these techniques cannot be applied to fresh fish products as they alter its fresh nature. Antimicrobial packaging is a fast developing active packaging especially for fish and meat products. Since microbial contamination of these products occurs primarily at the surface, due to post-processing handling the use of antimicrobials either by spray or dip treatment and more recently using antimicrobial packaging can be advantageous to improve safety and to delay spoilage. The principle action of antimicrobial films is based on the release of antimicrobial entities into the food which extends the lag phase and reduce the growth phase of microorganisms in order to prolong shelf life and to maintain product quality and safety. To confer antimicrobial activity, antimicrobial agents may be coated, incorporated, immobilised or surface modified onto package materials. Promising active packaging systems are based on the incorporation of antimicrobial substances in food packaging materials in order to control undesirable growth of microorganisms on the surface of food. The antimicrobial compound embedded into the polymer acts by two different kinds of mechanisms. In the first method, the preservative is covalently immobilized into the polymer matrix and acts directly from the film when the food is brought in contact with the active material. Regarding the latter, the preservative is embedded into the matrix in the dry state. When the active material is brought in contact with a moist food or a liquid-like food, the preservative is released from the material and acts directly. In both cases the aim of the system is to extend the shelf life of the packaged foodstuff, inhibiting the microbial growth and preserving its properties. The classes of antimicrobials range from acid anhydride, alcohol, bacteriocins, chelators, enzymes, organic acids and polysaccharides. Apart from these, various plant derivatives

and derivatives from fishery waste like chitosan can be incorporated into the packaging system as antimicrobials.

Antioxidant release

Antioxidants are widely used as food additives to improve oxidation stability of lipids and to prolong shelf-life, mainly for dried products and O_2-sensitive foods such as fishes as they contain highly unsaturated fatty acids. Antioxidants can also be incorporated into plastic films for polymer stabilization in order to protect the films from degradation. Incorporation of butylated hydroxytoluene (BHT) into the packaging film as an antioxidant is widely practiced. However, there has been some concern regarding the physiological effects of consuming BHT due to its tendency to accumulate in human adipose tissue. Hence, the use of synthetic antioxidants in contact with foods is decreasing. It is therefore desirable to use natural and harmless antioxidants. Vitamins E and C are the common natural antioxidants, and their incorporation in polymer films to exert antioxidative effects is still at the experimental stage. Vitamin E is stable under processing conditions and has an excellent solubility in polyolefins. Apart from these, natural antioxidants extracted from plant and animal substances and their use as antioxidant packaging is under experimental stages.

Active packaging systems with dual functionality

A more sophisticated way of extending the shelf life of packaged foods with active packaging systems is to use multiple function active systems. For example, the combination of oxygen scavengers with carbon dioxide and/or antimicrobial / antioxidant releasing systems significantly improves the storage stability of packaged foods. In the packages with O_2 scavenger alone, the removal of oxygen from the package creates a partial vacuum, which may result in the collapse of flexible packaging. Also, when a package is flushed with a mixture of gases including carbon dioxide, the carbon dioxide dissolves in the product creating a partial vacuum and certain amount of CO_2 permeates through the packaging film. But relatively high CO_2 levels are necessary in order to inhibit surface microbial growth and to extend the shelf life. In such cases, the self-working systems, which absorb O_2 and generate sufficient volume of CO_2 will be promising in extending the shelf life of foods particularly fishery products. ICAR-CIFT has developed the technologies for these active packaging systems to be adopted in different food systems to enhance the shelf-life.

Intelligent Packaging

Intelligent packaging senses some properties of the food it encloses or the environment in which it is kept and inform the manufacturer, retailer and consumer of the state of these properties. Although it is distinctly different from the active packaging concept, features of intelligent packaging can be used to check the effectiveness and integrity of active packaging systems. Intelligent packaging has been defined as '*packaging systems which monitor the condition of packaged foods to provide information about the quality of the packaged food during transport and storage*'. Smart packaging devices, which may be an integral component or inherent property of a foodstuff's packaging, can be used to monitor a plethora of food pack attributes. A variety of indicators such as temperature, time-temperature, pack integrity, microbial growth, product authenticity and freshness are of interest to the fish packaging industry.

Time-temperature indicators

The basic idea behind this indicator is that the quality of food deteriorates more rapidly at higher temperature due to biochemical and microbial reactions. Operation of TTIs is based on mechanical, chemical, electrochemical, enzymatic or microbiological change usually expressed as a visible response in the form of a mechanical deformation, colour development or colour movement. The visible response thus gives a cumulative indication of the storage temperature to which the TTI has been exposed. Essentially TTIs are small tags or labels that keep track of time-temperature histories to which a perishable product like fish is exposed from the point of production / manufacture to the retail outlet or end-consumer. Their use in fish and shellfish products offers enormous potential where monitoring of the cold distribution chain, microbial safety and quality are of paramount importance. Hence, a time-temperature indicator or integrator (TTI) may be defined as a small measuring device that shows a time and temperature dependent, easily, accurately and precisely measurable irreversible change that reflects the full or partial temperature history of a food product to which it is attached.

Leakage indicator

The development of improved methods to determine food quality such as freshness, microbial spoilage, oxidative rancidity or oxygen and/or heat induced deterioration is extremely important to food manufacturers. In order to maximise the quality and safety of foodstuffs, prediction of shelf-life, based on standard quality control procedures is normally undertaken. Replacement of such time-consuming and expensive quality measurements with rapid, reliable and inexpensive alternatives has lead to greater efforts being made to identify and measure chemical or physical indicators of food quality. Determination of indicator headspace gases provides a means by which the quality of a fish and meat product and the integrity of the packaging in which it is held can be established rapidly and inexpensively. One means of doing so is through the intelligent packaging incorporating gas sensor technology for sensing the oxygen and CO_2, as these two are the most commonly used gases. The monitoring of these gases in the package helps in establishing the food quality. The profiles of oxygen and carbon dioxide can change over time and are influenced by product type, respiration, packaging material, pack size, volume ratios, storage conditions, package integrity etc. A number of analytical techniques are available to monitor gas phases in MAP products. Instrumental techniques such as GC and GC/MS require breakage of package integrity and are time-consuming and expensive. Portable headspace oxygen and/or carbon dioxide gas analysers use 'minimally destructive' techniques (packages can be re-sealed) but tend not to be applicable to real-time, on-line control of packaging processes or large scale usage. An optical sensor approach offers a realistic alternative to such conventional methods. They can be used as a leak indicator or to verify the efficiency of O_2-scavenger, CO_2 emitter or MAP systems. Most of these indicators assume a colour change as a result of a chemical or enzymatic reaction. The most common redox dye used for leak indicators is methylene blue.

Freshness indicators

An ideal indicator for the quality control of packaged food products should indicate the spoilage or lack of freshness of the product, in addition to temperature abuse or package leak. The information provided by intelligent packaging systems on the quality of food products may be either indirect (e.g deviation from storage temperature and

changes in packaging O_2/CO_2 concentration may imply quality deterioration through established correlation) or direct. These freshness indicators are based on the detection of volatile metabolites produced during ageing of foods, such as CO_2, diacetyl, amines, ammonia and hydrogen sulphide. Freshness indicators provide direct product quality information resulting from microbial growth or chemical changes within a food product. Microbiological quality may be determined through reactions between indicators included within the package and microbial growth metabolites. The chemical detection of spoilage of fish and the chemical changes in fish during storage provide the basis for which freshness indicators may be developed based on target metabolites. Total volatile nitrogenous compounds and biogenic amines such as histamine, putrescine, tyramine and cadaverine have been implicated as indicators of fish product decomposition. As the biogenic amines are toxic compounds and they cannot be detected sensorily, the development of effective amine indicators would be beneficial. Hydrogen sulphide, a breakdown product of cysteine, with intense off-flavours and low threshold levels is produced during the spoilage of fish and shellfish by a number of bacterial species. It forms a green pigment, sulphmyocin, when bound to myoglobin and this pigment can be used as a basis for the development of a freshness indicator in red meat fishes. Normally, the freshness indicators are incorporated into the packaging film, which reacts with volatile amines and other indicating agents produced during the storage of fish and other seafoods, and the freshness is indicated by a colour change.

Future prospects

Smart packaging systems contribute to the improvement of food safety and extend the shelf-life of the packaged foods. However these are evolving technologies in the seafood area and many of these systems are in the developmental stage. Continued innovations in active and intelligent packaging are expected to lead to further improvements in food quality, safety and stability.

*****

Packaging of Fishery Byproducts

Bindu J.

Fish Processing Division

ICAR-Central Institute of Fisheries Technology, Cochin

Email: bindujaganath@gmail.com

Introduction

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

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

Types of Packaging Material

Glass

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

Cans

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

desirable properties like acid resistance and sulphur resistance. But care has to be taken to avoid tainting of the lacquer.

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

Paper

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

Paper board

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

Polymer Packaging

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

Low Density Polyethylene (LDPE)

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

High Density Polyethylene (HDPE)

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

Linear Low Density Polythene (LLDPE)

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

Polypropylene (PP)

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

Cast polypropylene (CPP)

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

Oriented, Heat set Polypropylene (OPP):

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

Polystyrene

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

and as a breathing film for packaging fresh produce. These materials have low heat sealability and often tend to stick to the jaws of heat sealer.

Polyester

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

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

Polyamides (Nylon)

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

Polyvinyl Chloride (PVC)

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

Copolymers

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

Aluminium foil

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

Packaging of fishery products

Packaging materials have to be designed based on the nature of the product that is to be packed. Fish products require packaging that retards spoilage and extend its shelf life over a period. A few fish products and suitable packaging material is given below.

Fish pickles

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

Fish soup powder

Fish soup powder is a speciality product containing partially hydrolysed fish, protein, carbohydrates, fat and several other seasonings including salt. The product is hygroscopic and hence the selection of the package assumes great significance. Appropriate package developed for such products are 12 micron plain polyester laminated with LDPE-HDPE co-extruded film or 90-100 micron LD/BA/Nylon/BA/Primacore multilayer films which ensure a safe storage of the product up to six months.

Extruded products

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

Surimi and surimi based products

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

Fish Sausage

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

Glucosamine hydrochloride

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

Chitin and Chitosan

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

Fish Hydrolysate

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

Fish Meal

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

Fish oils

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

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

Fish silage

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

Shark fin rays

Dried shark fin is a traditionally exported item from India. Significant value addition is possible if the rays from the shark fins are extracted and exported in place of shark fins. With the indigenous development of inexpensive and simple technology for extraction of fin rays, export of fin rays have picked up. Moisture resistant packaging having good puncture resistance and sufficient mechanical strength to withstand the hazards of transportation are the major requirements in the packaging employed for shark fin

rays. Polyester / polythene laminates or Nylon based co-extruded films having good puncture resistance are appropriate for shark fin rays. Traditionally dried shark fins are packed as bulk pack in jute sacks. The improved bulk pack consists of high-density polythene woven sack or polypropylene woven sack.

Squalene

Squalene is a natural 30-carbon organic compound obtained from shark liver oil (*Squalus* spp.) which is considered as the richest source of squalene. Since it is highly oxidisable and unstable it is packed in glass bottles preferably amber coloured and under inert conditions. The head space of the bottle should be filled with an inert gas. It is also packed in the capsulated form.

Fish Gelatin

Fish gelatin is odorless, colorless (pale yellow), translucent substance which is hygroscopic in nature. It is prepared from the skin of fishes. Edible fish gelatin is packed in 25kg/bag which has an outer package made of Kraft paper bag or woven poly bag with an inner liner which is water-proof plastic film.

Fish protein concentrate

Fish protein concentrate (FPC) is a substance in which the protein is more concentrated than in the original fish. The product is processed into three different grades based on its purity. The product is spray dried and marketed in the encapsulated form or as powder in woven sacks or glass bottles. Fish Protein concentrate is also marketed in the liquid form in poly bottles.

****

Quality Issues in Fishery Byproducts

Satyen Kumar Panda

Quality Assurance and Management Division

ICAR- Central Institute of Fisheries Technology, Cochin – 29

Email: satyenpanda@gmail.com

Introduction

Fish and fishery products constitute an important component of human diet. Contribution of seafood in the average animal protein consumed worldwide is around 16.6 %. Due to the increasing focus on nutritional significance of fish, the per capita consumption has increased to 18.6 kg in 2010. Seafood has become one of the most internationally traded commodity (109 billion US$ in 2010), the safety concerns related to seafood consumption has also become global in nature.

Quality issues related to seafood sector have been diverse, which include progressive changes associated with loss in freshness quality as well as those introduced by poor post-harvest handling practices. The much discussed about issue is presence of biological hazards, which includes human pathogenic bacteria and their toxins. Fish and fishery products are known vectors for disease causing pathogens like *Vibrio cholerae*, *Salmonella*, V*ibrio parahaemolyticus*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridium botulinum.*

In absence of a foodborne disease surveillance mechanism, it becomes difficult to ascertain the magnitude of disease. In USA alone, there are 48 million cases of food-borne diseases, 128,000 hospitalizations and 3000 deaths, are reported to occur each year (CDC, 2011). WHO estimates of 2005 shows that 1.8 million people die every year due to contaminated food and drinking water. The burden of foodborne disease will continue and it is highly probable that new food-borne pathogens of zoonotic origin and twice likely to cause new and emerging disease will be discovered in the 21st century.

Like other food sectors, seafood industry is not immune to emergence of new pathogens, which are becoming inherently unpredictable. As value-added seafood products incorporating plant based ingredients or other animal meat are not uncommon across countries, seafood mediated transmission of emerging pathogens like *Escherichia coli* O157:H7 and *Campylobacter jejuni* cannot be ignored. Further, it requires a national surveillance network to source-track pathogens and design suitable control measures.

Microbial Safety with respect to seafood

Developed countries like USA and Japan are biggest importers of seafood. In USA, 85% of the seafood consumed domestically is imported from third world countries. As per USFDA, during 1996-2006, seafoods contributed 21.6% of total outbreaks due to foodborne diseases and 11.3% of total foodborne illnesses. A glance on the actual causes of foodborne disease outbreaks in USA reveals that 87% are bacterial origin, 8.5% are viral origin and <1% by parasites. In India there is no database on disease outbreaks related to seafood consumption. The figures of import refusals and rejection of export consignment by importing countries like USA, EU and Japan provide an indirect estimate of the current level of hazards in Indian seafood. For example, during 2010-2011, out of total 65 import refusals of Indian seafood by USFDA, 37 were due to

presence of Salmonella. During the same period, there were 2 RASFF (Rapid Alert System for Food and Feed) alert notifications on *Salmonella* and 1 on *Vibrio cholerae* for seafoods exported to European Union.

Phenomenon of emergence of microbial pathogens

Genetic diversity among bacterial species as evidenced by whole genome sequence information provides basis of emergence and re-emergence of pathogens. With this flurry of genetic information, the distinction between pathogen and non-pathogens, virulence factor and colonization factor is getting gradually blurred. As revealed through genome sequence data, biological function of 30-50% of the predicted genes of food borne pathogens is still unknown. Apart from this an array of mechanisms exist which contribute to evolution of pathogenic bacteria such as extensive lateral gene transfer, genetic recombination and duplication, antigenic variation by slipped strand miss-pairing, genome decay or change in fidelity of proof-reading enzymes.

Mechanism like gene duplication although introduces additional genetic element, helps the pathogen to survive extreme environments due to expansion in functional characteristics. Multiple paralogous groups resulting from 260 gene duplications are observed in *Vibrio vulnificus*, which is implicated in foodborne diseases associated with consumption of bivalves like mussel and oysters. Mutations are shown to occur frequently in these duplicated sequences leading to changes in pathogenicity and survival of this emerging pathogen.

Acquisition of genetic elements from other microorganisms through lateral/horizontal transfer remains the most potent source of genetic variation and speciation in pathogenic bacteria. The lateral gene transfer is mediated by mobile genetic elements that include conjugative plasmids, bacteriophages, transposons and pathogenicity islands. These mobile genetic elements contain genes that encode virulence factors. For example, the SpvR, SpvA, SpvB, SpvC and SpvD virulence factors in *Salmonella typhimurium* are carried by the plasmid. Similarly, promiscuous plasmids that move freely between cells are pathogenic determinants in enterotoxigenic *Escherichia coli*.

Bacteriophages are mostly implicated in lateral gene transfer in foodborne pathogens. The recent emergence of antibiotic resistant strains of Salmonella i.e. *Salmonella typhimurium* DT 104 has been attributed to *Salmonella* genomic island I (SGI-I), containing phage- and plasmid-related genes, and five antibiotic resistance genes to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline. Similarly, the prophage CTXϕ that encodes for cholera toxin genes (ctxA and ctxB) plays a significant role in the life cycle of *Vibrio cholerae*. These toxin genes are acquired by *V. cholerae* after getting infected with the phage CTXϕ with the entire phage genome getting integrated into the *V. cholerae* genome. The emergence of toxigenic *E. coli* O157:H7 from its nontoxigenic, less virulent ancestor, *E. coli* O55:H7 has also been ascribed to bacteriophage that codes for Shiga toxins 1 and 2 (Stx1 and Stx2).

Another class mobile genetic element involved in horizontal gene transfer is "Pathogenicity Island", which are linked blocks of genes (5-100 kb) that impart virulence to the recipient organism. They are generally associated with t-RNA gene loci and have a G+C content different from that of the host bacterial genome. Many type III and type IV secretion systems from foodborne pathogens such as *E. coli* and Salmonellae and Yersinia are products of the pathogenicity islands. In *S. typhimurium* two type III secretion systems (Spi-1 and Spi-2) play an important role in their invasion and

colonization. Pathogenicity islands can be both insertion and deletion in different lineages, indicating series of gene acquisition and erosion during the emergence.

Slipped strand mispairing (SSM) is a mutation process that takes place during DNA replication. The DNA strands gets denatured and displaced, resulting in mispairing of the complementary bases. Changes in length of these tracts within or immediately upstream of genes causes alteration in translation and synthesis of proteins. This results in phenotypic variation of a species such as variation in antigenic characteristics of a pathogen which is used to elude immune system of the host. Identification of such genes with variation in repeat sequences helps in investigation of host adaptation and pathogenesis of foodborne pathogen.

Another mechanism of emergence of foodborne pathogens is progressive purging of unnecessary genes from the genome, called as genome decay. Downsizing of the genome content takes place as these genes no longer provides selective advantage to the organism. In *Salmonella typhi* which is only restricted to human host, many non-functional genes called as pseudogenes are observed in comparison to Salmonella Typhimurium. The accumulation of pseudogenes in *Salmonella typhi* is indicative of host-adaptation or genetic drift associated with population pressure following adaptation. Positive selection for gene loss i.e. pathoadaptation also helps in improving fitness of the pathogen and makes it more virulent. In Shigella, loss of cadA gene (which encodes for lysine decarboxylase enzyme) makes it more virulent as cadaverine (product of lysine decarboxylase activity) inhibits the plasmid-encoded virulence factors.

Due to defective DNA replication and mismatch repair system, sometimes mutator strains are more predominant in foodborne pathogens like Salmonella and *Escherichia coli*. These mutator strains have altered response in host microcosm and lead to emergence of new pathogenic groups.

Threat perceptions

Vibrios

Among the different species of vibrios, *Vibrio cholerae, V. parahaemolyticus and V. vulnificus* contribute to most cases of foodborne illness. Other species of vibrios emerging as pathogen due to consumption of seafood include *V. alginolyticus, V. mimicus, V. damsel, V. hollisae, V. cholerae* non-O1 non-O139 and *V. fluvialis*. Highest case fatality rate is observed in *Vibrio vulnificus*. There are already seven pandemics caused due to *Vibrio cholerae*. The pandemic strain of *Vibrio parahaemolyticus* O3:K6 emerged in Kolkata and rapidly spread throughout Asian continent. The pandemic O3:K6 strain carries the tdh gene but not the trh genes and do not produce urease.

Listeria monocytogenes

As *Listeria monocytogenes* can survive high salt concentration (up to 20% w/v), low water activity (aw: 0.91), broad pH range (4.3-9.8) and temperature (0.5-45oC) (Lado and Yousef, 2007), this pathogen is reported from different spectrum of fishery products. Compared to other food processing environments, prevalence of this pathogen in fish processing environments is relatively low. Seafoods are implicated in outbreaks of Listeriosis in North America, Europe and New Zealand in crustaceans, smoked fishes and ready to eat products. Although the prevalence of Listeriosis is reported to be below 0.7 per 100000 populations, the fatality rate in outbreaks is quite high

In India prevalence rate of *Listeria monocytogenes* in seafood has been reported as 0-17%. Although earlier reports till 1992 denied presence of this pathogen in Indian fish and fishery products, there has been a surge of reports citing the incidence of this pathogen in fresh finfish and shellfish samples in Goa, Mangalore and Mysore

Salmonella

Salmonella continues to be the leading cause of seafood related disease outbreaks throughout the world (EFSA, 2010; CSPI, 2009). The threat posed by Salmonella is compounded by growing consumption of seafood, preference for minimally processed and RTE products, expansion of international trade and growing aquaculture contribution to the fish trade (Amagliani et al., 2012). The prevalence of Salmonella is very high in tropical seafood compared to temperate waters. In India Kumar et al (2008) reported 30.5% prevalence in fish, 29% in shrimps and 34.1% in clam species. The serovars of Salmonella reported from Indian seafood are *S. worthington, S. weltevreden, S. typhimurium, S. enteritidis, S. bareilly, S. gallinarum, S. rissen, S. derby and S. infantis.*

Foodborne viruses

As viruses do not grow on food and have rapid spread other pathogens, there is no systematic surveillance for foodborne viral disease. Noroviruses which are now recognized as one of the most common causes of gastro-enteritis are transmitted through seafood. Noroviruses and hepatitis A are mostly detected in bivalve molluscan shellfish such as mussels, oysters and clams and as the RASFF portal indicates there are large number of rejections of imported seafood in EU.

Foodborne parasitic zoonotic agents in seafood

Changes in dietary practices including food preferences and eating patterns have influenced emergence of parasitic organisms in fishery products. Parasites like *Anisakis simplex* and *Pseudoterranova decipens* transmit due to ingestion of raw fish in European countries, USA and Japan, where there is growing demand for raw or lightly cooked fish. Consumption of various raw preparations of fish such as Sushi, Sashimi, marinated anchovies, proliferation of these parasites of public health importance. Similarly, consumption of raw freshwater fish is linked to trematode (*Clonorchis sinensis*) linked infections in China, S. Korea and other Asian countries, cestode (*Diphyllobothrium* spp.) in northern Europe. Seafood trade across continents in recent years have also influenced emergence of hitherto unreported parasites. Transmission of lung fluke (*Paragonimus* spp.) from Vietnam to EU countries through export of fishery products has brought to the forefront the significance of this parasite. The zoonotic transmission of major emerging protozoan parasites like Cryptosporidium and Giardia are linked to bivalve molluscs, which are eaten raw in many parts of the world.

Surveillance and Monitoring

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

genes, transposons, plasmids and phages and their spread across different species, specially designed microarray would be quite useful.

Quality Issues of Fisheries Byproducts

Seafood industry generates a large amount of byproducts which comprises of several bioactive substances, such as proteins, enzymes, fatty acids, and biopolymers. Seafood by-products are mostly used for human health purposes with wide potential for biotechnological, nutritional, pharmaceutical, and biomedical applications. Considering wide use of these byproducts as human food, the safety aspects cannot be ignored. Microbial pathogens and their toxins as well as chemical contaminants can pose severe health hazard if present in these by-products.

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

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

Quality issues with feed material obtained from processing of fish or other marine animals

Animal feed (both for terrestrial and aquatic animals) prepared from fish based ingredients are used in large quantity throughout the world. Out of the total fish production in the world, 14 percent (21.7 million tonnes) is destined to non-food uses, of which 75 percent (16.3 million tonnes) is reduced to fishmeal and fish oil (SOFIA, 2014). The average fishmeal production during 2011-13 is reported to be 5.189 million tonnes. Around 73% of global fishmeal production is being used in aquaculture sector alone. The quality of fishmeal and fish oil ultimately influences the aquacultured fish; hence safety standards are also applied to these commodities.

Quality issues with Fish Oil

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

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

Undesirable substance	**Product**	**Maximum content in mg/ kg (ppm) relative to a feeding stuff with a moisture content of 12 %**
Arsenic	Feeding stuffs obtained from the processing of fish or other marine animals	15

Lead	Feeding stuffs obtained from the processing of fish or other marine animals	10
Fluorine	Feeding stuffs of animal origin with the exception of marine crustaceans such as marine krill	500
	marine crustaceans such as marine krill	3000
	calcareaous marine algae	1000
Mercury	Feeding stuffs produced by the processing of fish or other marine animals	0.5
Nitrite	Fishmeal	60 (expressed as sodium nitrite)
Cadmium	Feed materials of animal origin	2
Aflatoxin	complete feeding stuff	0.01
Dieldrin	Fish Feed	0.02
Camphechlor (toxaphene) — sum of indicator congeners CHB 26, 50 and 62	Fish, other aquatic animals, their products and by-products with the exception of fish oil	0.02
	Fish oil	0.2
	Feeding stuffs for fish	0.05
Chlordane	All feeding stuff	0.02
DDT (sum of DDT-, TDE- and DDE isomers, expressed as DDT)	All feeding stuff	0.05
Endosulfan	complete feeding stuffs for fish	0.005
Endrin	All feeding stuff	0.01
Heptachlor	All feeding stuff	0.01
HCB	All feeding stuff	0.01
HCH	All feeding stuff	
Alpha isomer		0.02
Beta isomer		0.01
gamma isomer		0.2

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

Food stuffs	**Maximum level**		
	Sum of dioxins (WHO-PCDD/F-TEQ)	Sum of dioxins and dioxin-like PCBS (WHO-PCDD/F-PCB-TEQ)	Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6)
Marine oils (fish body oil, fish liver oil and oils of other marine organisms intended for human consumption)	1.75 pg/g fat	6.0 pg/g fat	200 ng/g fat
Fish liver and derived products thereof with the exception of marine oils		20.0 pg/g wet weight	200 ng/g wet weight

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

****

Microbial Quality and Safety of Fish and Fishery Waste

Prasad M. M. and Murugadas V.

Microbiology, Fermentation and Biotechnology Division

ICAR-Central Institute of Fisheries Technology, Cochin

E-mail: prasadmm@hotmail.com

Introduction

Fisheries contributes immensely to global food and nutritional security. FAO estimates that fisheries and aquaculture provide livelihoods to over 10-12% of global population. About 75% of fish resources are used for human consumption globally of the world fish production and substantial part of the fish produced were lost as fish waste discards and various other uses (over 25% rough estimate). Whole waste fish, fish head, viscera, skin, bones, blood, frame liver, gonads, guts, some muscle tissue are the major discard materials and anticipated to generate 32 million tons of waste in the solid waste and wastewater from the processing. Solid waste only represents 20–60 % of the initial raw material.

Internationally enormous efforts have been taken to convert fish waste to wealth considering the facts fish wastes are important source of proteins, lipids and minerals with high biological value i.e., otherwise as secondary raw material for the production of the wealth viz., anti-hypertensers, immuno-modulators, antioxidants, anticoagulants, osteoporosis, arthritis, diabetes, obesity, etc. This can be used in various sectors viz., human nutrition, cosmetology, aquaculture, microbiology, etc., In this diction utilization of fish waste is an important step and reduces the incineration or dumping at sea causing environmental problems.

In the recent past three important strategies are being implemented for the reduction of waste and recovery of marketable products from fish wastes viz., production of fishmeal/oil, hydrolyzed fish wastes (heat, enzymatic and chemical treatments) for fish or pig meal as well as organic fertilizer components/ compost or silage or fermentations. In addition to this due to biotechnological interventions other utilizations are also taken viz., fish oil with higher level of polyunsaturated fatty acids for human consumption, fish skin or cartilage for production of gelatin or chondroitin sulphate useful in food, cosmetic and pharmaceutical sectors.

Among the discards collagen is a rich source found in skin, scales, and bones over 7% of the total body weight of fish. Collagen is a major structural protein of animal origin and constitutes about 30% of total protein which is an insoluble fibrous protein contributes to the unique physiological function of connective tissue. Among the enzymes highly marketed commercially, proteases take the maximum share and collagen or collagen peptide or collagenase has several applications including the food industry. Collagen hydrolysate prepared enzymatically using collagenase enzyme from microbial sources were comparatively better than thermochemically produced with strong alkali and high temperature.

Hence, the fish by-products provide an excellent source for microbial growth used for various metabolites production such as lysine, enzymes (protease, lipase, etc.) having

high biotechnological interest (food processing, detergent, textile, pharmaceutical products, medical therapy, etc.).

Products from the fish and fishery waste.

What is equally important is the microbial quality and safety pertaining to the use of the fish and fishery waste as secondary raw material for production of by-products.

1. Fish collagen
2. Fish gelatin
3. Fish hydro chondroitin sulphate
4. Fish silage
5. Fish meal
6. Fish compost
7. Chitin
8. Chitosan
9. Fish media

Heads and viscera are utilized for preparing microbial growth media. Protein hydrolysates obtained by acid, alkali, or enzymatic treatments of raw or defatted by-products were also used as a nitrogen source for protease production.

Pseudomonas aeruginosa MN7 and *Bacillus subtilis* are cultivated in media containing combined heads and viscera powder allowing an acceptable level of protease production.

Rainbow trout "Oncorhynchus mykiss", swordfish "*Xiphias gladius*", squid "*Loligo vulgaris*" and yellowfin tuna "*Thunnus albacares* for vibrio species *Vibrio anguillarum* and Vibrio splendidus, tuna waste by Bacillus cereus, lipase production by Rhizopus oryzae, alkaline protease production by Bacillus mojavensisA21 was obtained using Sardinella peptone.

Shrimp shell powder (SSP), squid pen powder (SPP), chitin flake of shrimp shell (CFSS), chitin flake of crab shell (CFCS), shrimp and crab shell powder (SCSP) were used as raw material in industries. *Chryseobacterium* sp. TKU014, Bacillus subtilis TKU007, Bacillus sp. TKU004, *Lactobacillus paracasei* subsp *paracasei* TKU010, *Lactobacillus paracasei* subsp paracasei TKU012, *Serratia ureilytica* TKU013, *Bacillus cereus* TKU006, Serratia sp. TKU016 are few species of bacteria used for production of secondary raw material. Ligninolytic enzymes were also produced from bacteria using the waste.

Similarly, wastewater from fish processing industry supplemented with cuttlefish by-products powder was also tested as growth media for microbial growth and protease production by five bacterial species (*Bacillus licheniformis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Bacillus cereus* BG1, and *Vibrio parahaemolyticus*).

Silage obtained by lactic acid fermentation of shrimp head wastes containing chitin, proteins lipids and minerals was also used as substrate and inducer of β-N-acetylhexosaminidases of *Verticillium lecanii* in submerged fermentations (SF) and solid-state fermentations (SSF). The addition of sucrose or sugar cane pith bagasse reduces the growth time of *V. lecanii*. Interestingly, a mixture of shrimp waste silage and sugar cane pith bagasse in SSF improved significantly the enzyme yield. Chitinous

materials from marine sources can be considered as good inducers for microbial chitinase production.

Microbial quality and safety

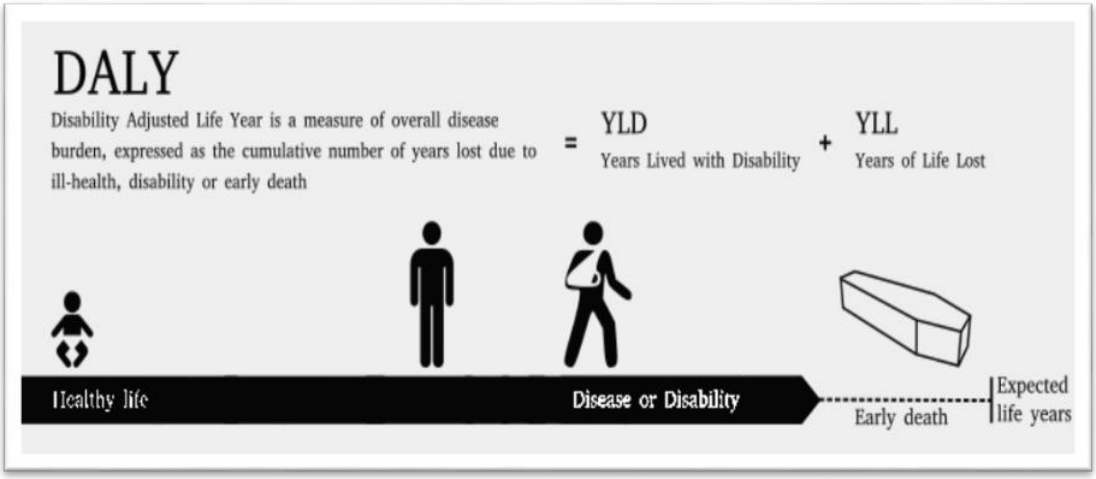
The occurrence of fish and fishery products illness is not evidence of the failure of our safety system. In fact, many of our prevention and control efforts have been and continue to be highly effective. Despite great strides in the area of microbiological safety of fish and fishery products, much remains to be done. In under developed and developing countries of Asia, Africa and Latin American Countries in the absence of good surveillance programs the task is much more complicated especially catering to needs of microbial safety of fish and fishery products of billions of populations.

Fish borne disease outbreaks are defined as the occurrence of 2 or more cases of a similar illness resulting from ingestion of a common fish and fishery products or observed number of cases of a particular disease exceeds the expected number. These can be confirmed (when at least one causal agent is identified) or suspected (based on clinical and epidemiological information). Although most cases are sporadic, these diseases draw attention to themselves due to outbreaks, thorough investigation of which can help in identifying control measures.

Annual burden of food borne diseases in the WHO South-East Asia Region includes more than: • 150 million illness • 175 000 deaths • 12 million DALYs Source: FERG Report 2010

The disability-adjusted life year (DALY) is a measure of overall disease burden, expressed as the number of years lost due to ill-health, disability or early death. It was developed in the 1990s as a way of comparing the overall health and life expectancy of different countries.

The DALY is becoming increasingly common in the field of public health and health impact assessment (HIA). It "extends the concept of potential years of life lost due to premature death...to include equivalent years of 'healthy' life lost by virtue of being in state of poor health or disability." In so doing, mortality and morbidity are combined into a single, common metric.



Despite significant success at improving the safety of the fish and fishery products, current science on which safety is based does not sufficiently protect consumers from emerging issues inherent to a complex to fish and fishery products. The evolving characteristics of food, technology, pathogens and consumers make it unlikely the marketplace will be entirely free of dangerous organisms at all times for all consumers. This is the conclusion made in the report, Emerging Microbiological food Safety Issues: Implications for Control in the 21st Century was released today at IFT's International Food Safety and Quality Conference and Expo in Atlanta one and half decades back.

The report, drew upon experts specializing in food borne pathogens and microbial evolution, fish and fishery products borne illness, food production and processing, testing methods and regulatory measures, reveals that diligent adherence to current methods that create and monitor the food supply cannot eliminate the risk of food borne illness. The report also offered the recommendations for providing the greatest possible reduction food safety risks.

Among its seven important issues addressed were:

1. Procedures from farm to table to significantly reduce illness due to mishandling,
2. Processes to recognize and respond to outbreaks and to reduce their scope.
3. Poor habits that make consumers more susceptible to foodborne illness,
4. Education and training recommendations necessary for reducing pathogenic influence at every step
5. From production to consumption (pond to plate/farm to fork
6. Recommendations to enhance monitoring, data generation, and risk assessment. &
7. The current state and future potential of rapidly evolving illness-causing pathogens and other key issues.

To gain the greatest measure of food safety, the report stressed on the necessity of implementing flexible food safety measures so as to utilize as quickly as possible the latest scientific information as it evolves. The report also urged manufacturers, regulatory and public health agencies and allied organizations to develop partnerships to improve risk assessment and food safety management.

Food safety goals must achieve more than end-product probes

The absence of pathogens in final-product testing does not ensure food free of virulent microorganisms, according to a new expert report on food safety issues, and as pathogen contamination decreases this form of testing becomes more deficient. So as today's food safety continues to improve, more emphasis should be placed on monitoring processing capabilities and conditions through the application of science-based food systems.

The microbiological testing of finished food products can be misleading for the following reasons

1. Due to statistical limitations based on the amount of product sampled.

2. The percentage of product contaminated.

3. The uniformity of the contamination distributed throughout the fish and fishery products.

The above-mentioned negative results imply an absence of pathogens in food, the report states, and can cause consumers to assume proper fish and fishery products and handling practices are unnecessary. Instead, the report urges everyone along the farm-to-fork sea food chain to be responsible for an important role in fish and fishery products safety management.

According to Douglas L. Archer of the University of Florida who contributed to IFT report "Current safety evaluations focus on microbes that may or may not be harmful to humans," he added, "For example, some subtypes of *Listeria monocytogenes* found in or on foods may not be associated with food borne illness. Yet their mere detection can be grounds for legal action against the manufacturer and force recalls of food that is unlikely to cause illness in the general population."

The need is for science-based approach called Safety Objectives that would place specific values on public health goals, with reassurances those values are reached at key points along the pond to plate process. Those values would be flexible as hazards and public health goals change, science progresses, and unfettered data sharing improves, allowing for the quickest implementation of new safety improvements as they evolve, and a safer fishery product.

The report urges intentional interaction of public health, regulatory, industrial and consumer agencies, calling the implementation of a flexible, science-based approach involving all these parties "as the best weapon against emerging microbiological food safety issues."

Steps in sea fish and fishery products Safety Management

Fish and fishery products borne illness in India is a major and complex problem that is likely to become a greater problem as we become a more global society where every 5th person walking on this planet is going to be Indian. Nearly 10 million fish and fishery products borne illnesses occur per year in India. To adequately address this complex problem, the need is to develop and implement a well-conceived strategic approach that quickly and accurately identifies hazards, ranks the hazards by level of importance, and identifies approaches for microbial control that have the greatest impact on reducing hazards, including strategies to address emerging hazards that were previously unrecognized.

Policy Development

Scientific research has resulted in significant success in improving sea foods safety, but the current science supporting the safety of our sea food is not sufficient to protect us from all the emerging issues associated with the complexity of the fish and fishery products. As new issues emerge, some will be best addressed through the application of control technologies during sea food production and processing, but others may be best addressed at the consumer level through modification of exposure or susceptibility.

Fish and fishery products safety policies should be developed as part of national initiatives, with input from all stakeholders. In addition, international coordination of fish and fishery products safety efforts should be encouraged. Globalization of the fish and fishery products has contributed to changing patterns of fish and fishery products

borne illness, and global fish and fishery products has the potential to introduce pathogens to new geographic areas.

To achieve the maximum benefits, our fish and fishery products safety efforts and policies must be carefully prioritized, both in terms of research and in application of controls. As scientific advances provide a better picture of pathogenicity, the need of the hour is whether to focus the efforts on those pathogens that cause many cases of minor illness or instead focus on those pathogens with the greatest severity, despite the relatively low number of cases. In the move toward making decisions based on risk, the fish and fishery products safety policies need to weigh these issues, and communicate information about risk to all stakeholders, especially the public.

The body of scientific knowledge must be further developed, with the research efforts carefully prioritized to yield the greatest benefit. Fish and fishery products safety and regulatory policies must be based on science and must be applied in a flexible manner to incorporate new information as it becomes available and to implement new technologies quickly. The seafood industry, regulatory agencies and allied professionals should develop partnerships to improve fish and fishery products safety management.

In essence:

Seafood Supply and exports: The amount of exported fish and fishery products has increased significantly, and this trend is likely to continue. Consistent, widespread application of fish and fishery products safety systems, including Hazard Analysis and Critical Control Points systems and good manufacturing (GMP), must be encouraged for international trade.

New Seafood Processing Technologies and Novel fish and fishery products. Scientists continue to be challenged to adequately address all the parameters associated with the introduction of a novel seafood or alternative processing technology. Once developed, new technologies must be appropriately used and regulated to ensure their proper application and the product's safety.

The use of manure as a fish pond fertilization is a significant concern. Methods are needed to reduce the presence of pathogens in manure and to effectively eliminate them before they contaminate the aquatic environment and fish.

Changes in Fish and fishery products Consumption. People's changing dietary patterns affect their risk of fish and fishery products from illness. The control and prevention methods will need to be adapted to these changing dynamics. For example, in India the number of high-end consumers who prefer ready to eat fish and fishery products are more than 300 million which is more or less equivalent to Europe.

At-Risk populations. It is likely that the number of persons at higher risk for fish and fishery products borne disease will continue to increase with time. In addition, there are an increasing number of transplant recipients, people undergoing treatment for cancer, people with AIDS, and others with compromised immune system function.

Pathogen Evolution. Microbial evolution has always happened and will continue to occur. Improved surveillance and new genomic technologies offer the potential to identify new potential fish and fishery products borne pathogens before they cause significant illness. Another hope for the future is a better understanding of how human actions affect fish and fishery products borne pathogens.

Consumer Understanding. Education and risk communication will be necessary to share with consumers our growing knowledge of fish and fishery products safety risks and to encourage behaviour modification, where needed.

Integrated Fish and fishery products Safety System. A farm to- fork or pond to plate table fish and fishery products safety system must involve many interested parties working together toward a common goal. The challenge is to build a system that applies science in a predictable, consistent, and transparent manner to enable harmonization within and between countries. The list of principal symptoms of Bacteria, potential fish and fishery products contamination are provided in table below.

List of bacterial fish and fishery products poisoning, symptoms and Fish and fishery products

Organism	**Common Name of Illness**	**Onset Time After Ingesting**	**Signs & Symptoms**	**Duration**	**Fish and fishery products**
Bacillus cereus	*B. cereus* fish and fishery products poisoning	10-16 h	Abdominal cramps, watery diarrhea, nausea	24-48 h	Meats, stews, gravies, vanilla sauce
Campylobacter jejuni	Campylobacteriosis	2-5 days	Diarrhea, cramps, fever, and vomiting; diarrhea may be bloody	2-10 days	Raw and undercooked poultry, unpasteurized milk, contaminated water
Clostridium botulinum	Botulism	12-72 hours	Vomiting, diarrhea, blurred vision, double vision, difficulty in swallowing, muscle weakness. Can result in respiratory failure and death	Variable	Improperly canned fish and fishery products, especially home-canned vegetables, fermented fish, baked potatoes in aluminum foil
Clostridium perfringens	Perfringens fish and fishery products poisoning	8–16 hours	Intense abdominal cramps, watery diarrhea	Usually 24 hours	Meats, poultry, gravy, dried or precooked fish and fishery products, time and/or temperature-abused fish and fishery products

Cryptosporidium	Intestinal cryptosporidiosis	2-10 days	Diarrhea (usually watery), stomach cramps, upset stomach, slight fever	May be remitting and relapsing over weeks to months	Uncooked fish and fishery products contaminated by an ill fish and fishery products handler after cooking, contaminated drinking water
Cyclospora cayetanensis	Cyclosporiasis	1-14 days, usually at least 1 week	Diarrhea (usually watery), loss of appetite, substantial loss of weight, stomach cramps, nausea, vomiting, fatigue	May be remitting and relapsing over weeks to months	Various types of fresh produce (imported berries, lettuce, basil)
E. coli (Escherichia coli) producing toxin	*E. coli* infection (common cause of "travelers' diarrhea")	1-3 days	Watery diarrhea, abdominal cramps, some vomiting	3-7 or more days	Water or fish and fishery products contaminated with human feces
E. coli O157:H7	Hemorrhagic colitis or *E. coli* O157:H7 infection	1-8 days	Severe (often bloody) diarrhea, abdominal pain and vomiting. Usually, little or no fever is present. More common in children 4 years or younger. Can lead to kidney failure.	5-10 days	Undercooked beef (especially hamburger), unpasteurized milk and juice, raw fruits and vegetables (e.g. sprouts), and contaminated water
Hepatitis A	Hepatitis	28 days average (15-50 days)	Diarrhea, dark urine, jaundice, and flu-like symptoms, i.e., fever, headache, nausea, and abdominal pain	Variable, 2 weeks-3 months	Raw produce, contaminated drinking water, uncooked fish and fishery products and

					cooked fish and fishery products that are not reheated after contact with an infected fish and fishery product handler; shellfish from contaminated waters
Listeria monocytogenes	Listeriosis	9-48 h for gastro-intestinal symptoms, 2-6 weeks for invasive disease	Fever, muscle aches, and nausea or diarrhea. Pregnant women may have mild flu-like illness, and infection can lead to premature delivery or stillbirth. The elderly or immunocompromised patients may develop bacteremia or meningitis.	Variable	Unpasteurized milk, soft cheeses made with unpasteurized milk, ready-to-eat deli meats
Noroviruses	Variously called viral gastroenteritis, winter diarrhea, acute non-bacterial gastroenteritis, fish and fishery products poisoning, and fish and fishery products infection	12-48 h	Nausea, vomiting, abdominal cramping, diarrhea, fever, headache. Diarrhea is more prevalent in adults, vomiting more common in children.	12-60 h	Raw produce, contaminated drinking water, uncooked fish and fishery products s and cooked fish and fishery products that are not reheated after contact with an infected fish and fishery products handler; shellfish from contaminated waters
Salmonella	Salmonellosis	6-48 hours	Diarrhea, fever, abdominal cramps, vomiting	4-7 days	Eggs, poultry, meat, unpasteurized milk or juice, cheese, contaminated raw fruits and

					vegetables
Shigella	Shigellosis or Bacillary dysentery	4-7 days	Abdominal cramps, fever, and diarrhea. Stools may contain blood and mucus.	24-48 h	Raw produce, contaminated drinking water, uncooked fish and fishery products s and cooked fish and fishery products that are not reheated after contact with an infected fish and fishery products handler
Staphylococcus aureus	Staphylococcal fish and fishery products poisoning	1-6 hours	Sudden onset of severe nausea and vomiting. Abdominal cramps. Diarrhea and fever may be present.	24-48 hours	Unrefrigerated or improperly refrigerated meats, potato and egg salads, cream pastries
Vibrio parahaemolyticus	*V. parahaemolyticus* infection	4-96 hours	Watery (occasionally bloody) diarrhea, abdominal cramps, nausea, vomiting, fever	2-5 days	Undercooked or raw fish and fishery products, such as shellfish
Vibrio vulnificus	*V. vulnificus* infection	1-7 days	Vomiting, diarrhea, abdominal pain, blood borne infection. Fever, bleeding within the skin, ulcers requiring surgical removal. Can be fatal to persons with liver disease or weakened immune systems.	2-8 days	Undercooked or raw fish and fishery products , such as shellfish (especially oysters)

Need for Quality Improvement in Fish

Costliest Tuna as case study

Kiyomura Co's sushi chefs react to a part of a 222 kg (489 lbs) Bluefin tuna after cutting its meat at the company's sushi restaurant outside Tsukiji fish market in Tokyo January 5, 2013. The tuna was sold nearly for 1.8 million USD and when it converted into local currency what could be cost of whole of 222kg, per/kg and also with 74% meat yield

amounting to 164.28kg what could be the cost price per kg in local currency of the participating countries is provided in the Table below.

Costliest Bluefin Tuna sold for 1.8 million USD and when it is converted into local currency what could be cost of whole of 222kg, per/kg and also with 74% meat amounting to 164.28kg and per kg						
S. No	Country (currency)	Local currency to USD	USD to local currency	A 222Kg Bluefin tuna cost	Per Kg out of 222kg	With 74% meat yield when the weight is 164.28 Kg and the cost per/kg
1	Afghanistan (Afghani)	75.97	0.013	136,746,000	615,972.97	**832,395.91**
2	Algeria (Dinar)	118.61	0.0084	213,498,000	961,702.70	1,299,598.25
3	Bangladesh (Bangladeshi Taka)	83.79	0.012	150,822,000	679,378.37	918,078.88
4	Ethiopia Ethiopia Birr	27.93	0.036	50,274,000	226,459.46	305060.68
5	Iraq Iraqi Dinar	1191.7	0.000884	2,145,060,000	9662432.43	13,057,341.12
6	Mynmar (Myanmar Kyat)	1598.1	0.00063	2,876,580,000	130,753,636.36	17,510,226.44
7	Sebia (Sebrbian Dinar)	104	0.0096	187,200,000	843,243.24	**1,139,517.89**
7	Sudan (Sudanese pound)	47.62	0.021	85,716,000	386,108.10	**521,767.71**
8	Tanzania (Tanzania Shilling)	2290.40	0.00044	4,122,720,000	18,570,810.81	**25,095,690.28**

Factors contributing to outbreaks of fish borne disease	
Contributing factors	Percentage
Factors relating to microbial growth	
Storage at ambient (room) temperature	43
Preparation too far in advance of serving	41
Improper warm holding	12
Use of leftovers	5
Extra large quantities prepared	22

Factors contributing to outbreaks of fish borne disease	
Contributing factors	Percentage [a]
Factors relating to microbial survival	
Improper reheating	17
Inadequate cooking	13
Factors relating to contamination	12
Fish and fishery products workers	7
Contaminated raw fish and fishery products s	11
Cross-contamination	7
Inadequate cleaning of equipment	5
Unsafe source	

Fish and fishery products hazards: Perception of the consumer verses epidemiological data		
Case	Perception	Relative importance
Microbial contamination	22	49.9
Nutritional imbalance		49.9
Environmental contaminants	31	0.05
Natural toxins	10	0.05
Fish and fishery products additives	30	0.0005
Others, e.g., packaging materials	7	

Chlorine use in different stages	
Purpose	**In PPM**
Washing for processing	5-10
For making ice	5-10
To disinfect after washing with detergents	100
Washing floors and gutters	500-800
Washing product	10
Washing of boat deck, fish holds and wooden boxes.	1000
Cleaning of fish containers, carrier vans, refrigerated wagons	100
Washing of utensils, processing tables etc	100
Washing of hands	20

Tools for quality improvement

- Empowerment
- Benchmarking
- Kaizen (Continuous improvement approaches)
- 6-Sigma applications
- 5-S A requirement for TQM
- Good manufacturing Practices (GMP)
- Hazard Analysis Critical Control Point (HACCP)

5s good housekeeping

- Sort: take out unnecessary items and dispose
- Systematize: Arrange necessary items in good order
- Sweep: Clean your work place
- Standardize: Standardize the process of sorting, arranging and cleaning
- Self-discipline: Do things spontaneously as a habit.

Evolution of the quality profession

- ’50s---Inspection & Conformance to specification
- ’60s---Customer requirements or fitness for use
- ’70s---Human dimensions of quality (Quality people do quality work)
- ’80s---Relationships at the work place (Quality work depends on quality of work life)
- ’90s--- partnerships between employees, customers and stakeholders.
- 2010: management of Data, Information and Knowledge

5M’s of Quality

- Manpower
- Materials
- Methods
- Machines
- Measurement

5r’s of Unquality

- Reject
- Rework
- Return
- Recall
- Regrets

PPM of quality responsibility

- Planning
- Prevention
- Monitoring

DIFFERENT LEVELS OF QUALITY PRACTICE

- LEVEL 1- QUALITY AWARENESS (QAW)
- LEVEL II- QUALITY CONTROL (QC)
- LEVEL III- TOTAL QUALITY CONTROL (TQC)
- LEVEL IV- TOTAL QUALITY MANAGEMENT (TQM)
- LEVEL V-PARTNERSHIPS FOR QUALITY, PRODUCTIVITY AND PROFITABILITY (PQP2)

Principles of Total Quality Management

- A Aim for customer satisfaction
- C Communicate and coordinate all activities
- C Commit and cooperate towards improvement
- E Empower the employees
- P Promote use of problems solving tools
- T Training for quality is forever

Stages in TQM Development

- G Get management commitment
- R Review recorded procedures
- A Assess quality practices
- C Compare records and practice
- E Evaluate results
- O Overview total situation
- F Find areas requiring improvement
- G Get fully involved
- O Out do your own performance
- D Document changes in procedures
- A Assessment, identification and preparation
- M Management, understanding and commitment
- E Energizing for improvement
- N New initiatives, new targets and critical monitoring

Requisites for Total Quality Commitment

- C Customer orientation whether inside or outside the set up
- H Human resource striving for excellence
- A Acquisition of products and process leadership
- M Management leadership for quality
- P Practice quality as a way of life inside and outside work place
- S Sustained quality culture in the company

CARES

- C Communicate management plans for quality
- A Accessibility to one another in the organization
- R Revitalization of problem solving capabilities
- E Embarrassments are avoided if all agree that inspection is not the way to achieve quality
- S Sustain the desire to personally commit to quality

Code of Conduct In Team Meeting

- Co-operate with each other
- Listen to other's ideas
- Keep an open mind
- No personal attacks
- Stick to the facts
- Every one participates
- Be tactful, be honest
- No hidden agendas

Importance of Delivering Both Quality Products And Service

- 68% customers stop purchases due to poor service
- Customers are five times more likely to leave for poor service than poor product quality or high cost
- The average unhappy customer tells nine other people about experience
- When 50 to 75% of the complaints attended to 95% unhappy customers can be saved
- Average happy customer tells five others.

Conclusion

The disposal of wastes generated by fishery processing industries represents an increasing environmental and health problem. However, these by-products have attracted considerable attention as an alternative feedstock and energy source, since they are abundantly available. Various microbes are capable of using these substances

as carbon and energy sources beneficial in enzyme production process. A number of such substrates have been tested for the cultivation of microorganisms to produce several enzymes (protease, lipase, chitinases, peroxidases, laccases, oxidases, etc.). This may have numerous advantages for enzyme production process, such as superior productivity, simpler techniques, reduced energy requirements and reduced production costs. Generally, fish waste pre-treatments may be necessary to maximize microbial growth and enzyme production. However, each microbial strain has its own special conditions for maximum enzyme production. Therefore, it is of great significance to optimize the medium composition, taking into consideration the variability of fish waste composition, the nutrient requirements of microbial strain and fermentation parameters (pH, temperature, aeration, agitation, etc.). Nevertheless, the improvements in fish waste technology (pre-treatments, characterization, formulation, etc.) are still necessary before large-scale application of this new strategy can be realized.

****

AMR in Fisheries Sector, Detection & Control

Murugadas. V. and M. M. Prasad

Microbiology, Fermentation and Biotechnology Division

ICAR-Central Institute of Fisheries Technology, Cochin

E-mail: murugadascift81@gmail.com

Introduction

Living organisms that multiply frequently and spread rapidly are very tiny in nature and cannot be seen in naked eye are microorganisms. Majority of the organisms are existing as beneficial flora in each and every niche and contributing to the basic biogeochemical cycle of the life. However, some of the microbes do exists as pathogenic to either human or animals including the fish/shellfish. Examples are Bacteria (e.g., *Staphylococcus aureus*, *Streptococcus pneumoniae*), viruses (e.g., Measles, Mumps), fungi (e.g., *Candida albicans*,), parasites (Coccidia etc).

Any chemical or drug that normally kill or limit their growth are called antimicrobials. It may be produced from other microbes as natural or synthetics by chemical process. Due to the exposure of several chemicals or to environments, the microbes are continuously evolving and enabling them to efficiently adapt to new environments and it makes them harder to be eliminated from the particular environment. Examples of antibiotic penicillin and ciprofloxacin, whereas antimicrobial refers to all microbes *viz*., bacteria, viruses, fungi, and parasites. Hence, antibiotic or antimicrobial resistance (AMR) denotes the ability of microbes to resist the effects of drugs, so that either their growth is not stopped or they are not killed or both.

Antimicrobial resistances is the ability of microbes to grow in the presence of antimicrobial substances. Major factors which potentiates the spread of antibiotic resistances are misuse or overuse of antibiotics with or without professional oversight, growth promoting substances in food producing animals, inadequate or inexistent programmes for infection prevention and control (IPC), poor-quality medicines, weak laboratory capacity, inadequate surveillance, insufficient regulation of the use of antimicrobial medicines. WHO has predicted AMR as a major threat to the public health and estimated that by 2050 many human beings succumb to death due to treatment failure in AMR Fig.1.

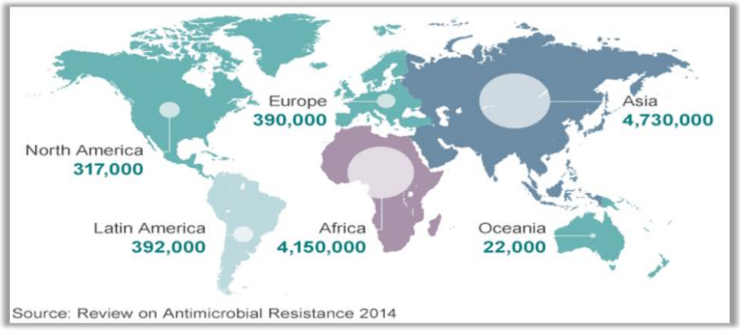


Fig.1. WHO report on death due to AMR by 2050.

Antibiotics are very critical compounds for preventing, controlling, and treating disease in human as well as farm animals. They act by preventing the essential metabolic process of bacteria which includes cell wall synthesis, DNA proliferation, and protein synthesis, antibiotics. However, the development of antibiotic resistance ie ineffectiveness of the antibiotics against bacterial pathogens makes situation critical than before. Multiple drug resistance is the resultant of the inadvertent or indiscriminate usage of antibiotics not only in the therapeutic both for human and animals but also for the prophylactic usage in the food producing animals including aquaculture. Steep increase in the antibiotic resistances have been reported in human as well as animals &and plants.

The relevance of detection and quantification of antimicrobial resistance or the genes responsible for the resistances are now shifted to bacteria from food producing animals or environment from clinically important pathogens. A decade back, clinically important and relevant organisms were *Enterobacteriaceae, Haemophilus* spp., *P. aeruginosa*a, *Neisseria gonorrhoeae, Staphylococcus* spp, *Streptococcus pneumoniae, Enterococcus* spp., *Streptococcus* spp., *Vibrio cholera, Helicobacter pylori and* potential agents of bioterrorism like *Bacillus anthracis, Yersinia pestis, Burkholderia mallei, and Burkholderia pseudomallei.* However, the current situation is more alarming due to the rapid emergence of antimicrobial resistances to newer pathogens and or evolution of more antibiotic resistances to already existing AMR pathogens. The condition is more alarming in bacteria from environment including aquatic environment. At this conjuncture, it is very important to understand the prevalence of antibiotic resistant pathogens in their defined geographical region inorder to control their rapid spread. Detection or determination of antimicrobial resistances in bacteria can be achieved by phenotypic or genotypic method either qualitatively or quantitatively. In this context, the chapter will introduce you to the antimicrobial resistances, relevance in fisheries sector, methods of detecion of antimicrobial resistance, and its control measures.

Routes of transmission of Antimicrobial resistance

AMR is not a single sector problem. AMR is complex in nature and spreads across sectors such as human, animals, food and environment (Fig.2.). Finally, all the microbial populations with or without AMR enters the environment either soil or water. The picture nicely depicts transmission routes of the AMR pathogens including transboundary or intercontinental. It includes the run-off from the agriculture as well as animal agriculture.

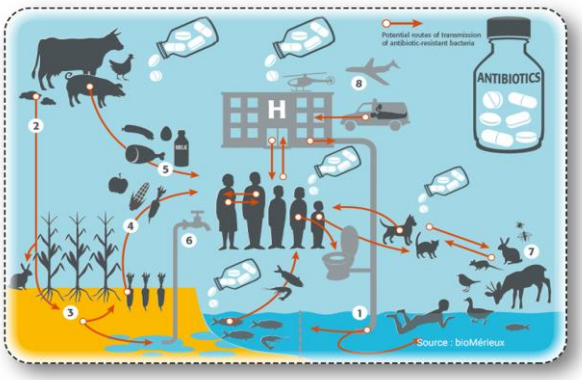


Fig. 2. Biomerieux's recent publication on probable routes of transmission of AMR pathogens across the sectors.

WHO has listed the pathogens of AMR in several categories as prioritized list of pathogens. The pathogens which are economically very important in hospital infections, community as well as food producing animals is depicted in Fig.3. The pathogens which are very relevant to fisheries sector are *Vibrios* sp, *Aeromonas* sp, and *Edwardsiella* sp.

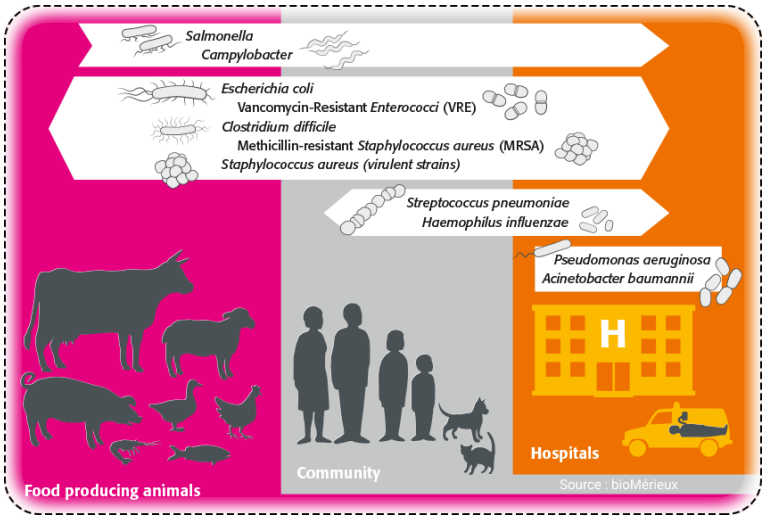


Fig.3. Pathogens of economic relevance in each sectors.

Trends in Fisheries sector

Fisheries sector plays an important role in the food security and fish is now traded internationally. Shift in the trading policies (import and export) of seafood/aquatic products are happening at a rapid pace and consumption of seafood increased globally in substantial quantum. It is estimated that aquatic products export from Asian countries outcompetes earlier contributions to their importing partner's year after year. Aquatic products include chordates, molluscs and arthropods from freshwater, brackish and marine system. They are nutrient rich diet and perishable too in nature and this prompted the industry to process the seafood in to different forms such as frozen, canned, cured and dried to extend its shelf life and recently value addition step being followed to improve the customer satisfaction. Nevertheless, the risk associated with the transboundary exchange of pathogens of seafood importance and its antibiotic resistance are generally cannot be disregarded. Majority of the pathogens are not a native flora of fish. Each step in the aquatic products production chain either in the captured or cultured fisheries involves the contact of the seafood to the environment where they are grown, various implements used, contact surfaces, handlers, water etc. This post harvest handling makes the seafood contaminated with the pathogens of seafood importance's such as *Escherichia coli*, *Salmonella* spp, *Clostridium botulinum*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Vibrio cholerae*, *Vibrio parahemolyticus*, *Shigella* sp, *Aeromonas hydrophila*, *Plesiomonas shigelloides* and viral pathogens such as hepatitis A virus etc. Among these pathogens, *Escherichia coli*, *Salmonella* spp, *Staphylococcus aureus* and *Shigella* spp are non-indigenous to the aquatic environment and others are indigenous to the aquatic environment. Depending on the nature of the environment (contaminated water source), feeding habits (filter feeders), season of harvest (summer season) are very crucial factors which cause seafood inherently contaminated in nature.

AMR is an increasing global public threat in various facets of healthcare system because of their rapid emergence of newer resistances and spread across the various countries. Its impact is felt across the globe. This results in prolonged illness, complications in surgical conditions due to infection with resistant organisms, severe fatal forms are also encountered. Antibiotic resistance development is a natural process occuring during due to change in genetic makeup of microbes in a longer time, however the current situation is happening at an elevated speed. In the present scenario, the risk is potentiated not only by the presence of these pathogens but also on the antibiotic resistances they possess. Worldwide research deviation is noticed on antibiotic resistant pathogens both from clinical sector and in the food producing animals. Antibiotic resistant pathogens of seafood importance are Methicillin-resistant *Staphylococcus aureus*, Extended spectrum Beta-lactamase producing Enterobacteriaceae *viz*., ESBL *E. coli*, ESBL Salmonella; carbapenamase resistant Enterobacteriaceae viz., Klebsiella, *E. coli*; Vancomycin resistant Enterococci and so on. The link between the use of antimicrobial substances in food production and the presence of antibiotic resistant foodborne pathogens *Salmonella,* pathogenic *E. coli, Campylobacter, Staphylococcus* spp., *Enterococcus* spp. and extended-spectrum beta-lactamase (ESBL) has been already proved by various researchers. This perhaps shows the importances of studies on AMR pathogens in the food producing animals with special reference to the seafood or aquatic products development.

In general to exception of commercially sterile and other pro,pre and synbiotics food products, food have the proximity of getting contaminated to various microbes during entire production and processing chain. The raw food in general have the highest culturable bacterial concentrations, followed by minimally and fully processed foods. Minimally or fully processed food including ready-to-eat food contamination depends on the level of sanitary hygiene followed during the processing and preservation steps (Fig.4.).

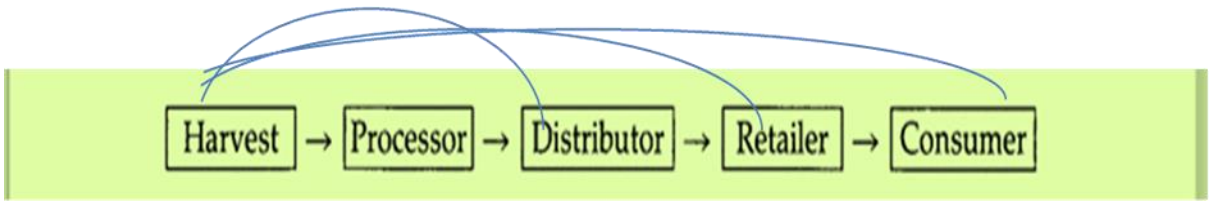


Fig.4. Steps contributes to the entry of microbes in the food chain

The food with acceptable microbiological quality range may also serve as the sink for the development of antibiotic resistances through bacteria, bacteriophages, bacterial DNA and mobile genetic elements, some of which may include AMR genes. Hence, the food chain ecosystem may be conducive niches for gene transfer, selection and persistence of AMR bacteria and this route cannot be generally disregarded.

V. parahaemolyticus, V. vulnificus, V. alginolyticus, and V. cholerae are autochthonous Gram-negative bacilli to estuarine and marine environments and found associated with disease through wound infection or through consumption of contaminated seafood especially shellfish. Antimicrobial resistant Pathogenic bacteria released into aquatic environments through wastewater acts as potential spread of antibiotic resistant genes spread. In general Vibrios sp showed higher resistances towards Ampicillin and low

tetracycline resistances. The frequency of resistance reported in aquatic products ranged from 16.6 to 50% level and 10 to 69% of the vibrio strains showing resistance to more than 4 molecules. Common antibiotics showed resistances are teicoplanin, pencillin, oxacillin, vancomycin and low level resistance for cephalosporin groups.

Highly resistant to penicillin, ampicillin, tetracycline, and vancomycin was observed in *L. monocytogenes* isolated from seafood and low level less than 10% for Tetracycline, enrofloxacin, and ciprofloxacin. The antibiotic resistance pattern and number changes between the serotypes of *L. monocytogenes* isolated from seafood, serotype 1/2a was found to be more resistant than other serotypes.

S. aureus isolated from fishery products were resistant to penicillin, chloramphenicol and ciprofloxacin and most of them were also resistant to tetracycline. In general, to the β-lactams, Macrolides, aminoglycosides, ciprofloxacin, co-trimoxazole (4.7%) and tetracycline resistances were observed in most of the studies with varied percentage. Pencillin, Macrolides are above 50% and others were less than 50% level. Multidrug resistant strains were also reported in many studies.

Salmonella isolated from seafood were in general resistant to the pencillin, erythromycin, tetracycline and other antibiotics were less than 15% level. In a study conducted on imported seafood in to US from 20 countries, S. enterica strains of 36 serovar were isolated and twenty isolates showed resistance to at least one antibiotics. Five strains (serovars Bareily, Oslo, Hadar, Weltevreden and Rissen) were resistant to two or more antibiotics. Two *S. enterica* strains (serovars Bareily and Oslo) from seafood from Vietnam and India were resistant to trimethoprim/sulfamethoxazole, sulfisoxazole, ampicillin, tetracycline and chloramphenicol. Multidrug resistant strains were also observed in Salmonella isolated from seafood.

In addition to this, Fish are reservoirs for zoonotic pathogens not only infecting the host animal but also humans in contact during aquaculture activity.The infections includes *Aeromonas hydrophilia, Mycobacterium marinum, Streptococcus iniae, Vibrio vulnificus,* and *Photobacterium damselae* etc are noted few.

All the study demonstrated that there is a change in the trend of antibiotic resistances which depends on the country of origin of the seafood, antibiotic usage in particular country for aquaculture practices etc.

Work done at ICAR-CIFT

ICAR-CIFT has been working in four states of India viz., Kerala, Andhra Pradesh, Maharastra and Gujarat for the past five years on AMR. *Staphylococcus aureus, Escherichia coli, Vibrio cholerae, Vibrio parahaemolyticus, Listeria monocytogenes and others.* Some of the important findings in AMR in public health significance bacteria are

The prevalence of MRSA (Methicillin resistant *Staphylococcus aureus*) was 13.8%, 9.3%, 12%, 15.3% in fish, crustaceans, molluscs and environment samples collected from Kerala, respectively. *spa* (Staphylococcal protein A) typing of the MRSA isolates revealed that MRSA from the fish landing centre (t311 and t15669) was carried to the retail fish market. T15669 Novel clone identified in the landing centre. Coagulase positive Staphylococci isolated from seafood samples from Veraval, Gujarat demonstrated resistance to at least three groups of antibiotics (multidrug resistance – MDR). 97.67% of the *S. aureus* isolates were resistant to azithromycin, ciprofloxacin and gatiflaxacin while 93.33% were resistant to lomefloxacin. Antimicrobial resistance to

Ceftriaxone, a third-generation cephalosporin antibiotic, was found in *L. monocytogenes* isolate from fish.

The antibiotic resistance profile of 382 *V. cholerae* isolates from seafood samples from Kerala revealed that 26, 40, 62 and 84% of non 01 and non 0139 strains were resistant to Cefpodoxime, Ticarcillin, Augmentin and Colistin, respectively. *V. cholerae* isolates from Andhra Pradesh revealed that 37.5% isolates were resistant to cefotaxime, and 12.5% of the isolates were resistant to ceftriaxone and nitrofurantoin. However, all the *V. cholerae* isolates were sensitive to 21 other antibiotics. Multidrug resistant *Aeromonas hydrophila* was recovered from fish farms.

Methods of detection of Antimicrobial resistance

In general, the methods involved in the determination of antimicrobial resistances are categorized into phenotypic and genotypic and phenotypic further classified into qualitative as well as quantitative (Fig. 5)

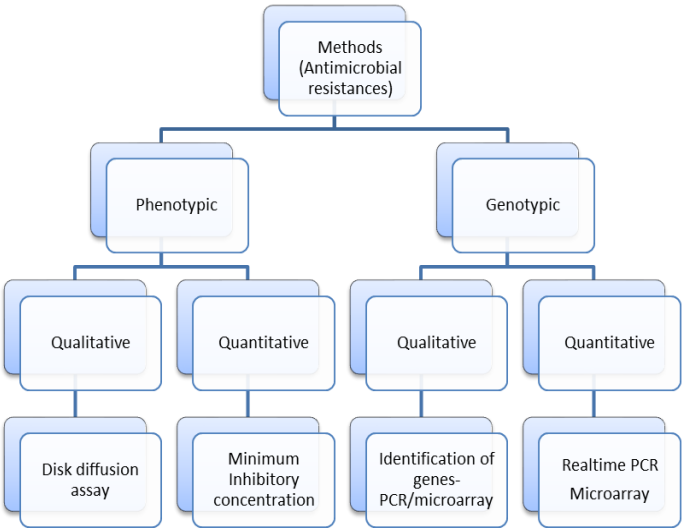


Fig.5. Methods of determination of antimicrobial resistances

Qualitative methods

Phenotypic methods

Disk diffusion assay

Bauer, Kirby, Sherris, and Turck harmonized the procedure of disk diffusion by controlling the variables such as the media, temperature, and depth of agar and published the detailed paper in 1966.

Detailed steps involved in the disk diffusion assay

Selecting the colonies: Preparing the inoculum involves pick or selecting 3–5 well-isolated colonies using an inoculating loop or a cotton swab from the primary plate, suspending them in broth or standardizing the suspension. Avoid testing mixed cultures or subculture the organism to a fresh plate for purity checking.

Prepare inoculum suspension: The turbidity of the test suspension from Direct colony (not more than 18–24 hours) or log phase growth (Broth culture approximately 8

hours) in saline or Mueller hinton or Tryptic soy broth standardized to match that of a 0.5 McFarland standard (approximately 1.5 X 10^8 CFU/ml) and use inocula within 15 minutes. Compare the turbidity of the suspensions by placing the tubes in front of a white paper or file card with black line. Direct colony suspension is commonly recommended for Staphylococci and Streptococci. Log phase culture for most of the non-fastidious microorganism. In general avoid over grown cultures or old cultures.

In general Mueller Hinton agar is prepared as pre-set with depth of 4 mm at least, dried, kept at incubator for 10-15 min to absorb excess moisture in the plate. Bring the antimicrobial disk to the room temperature 1-2 hour before impregnating to reduce the condensation. Vortex the suspension to mix well and dip a fresh, sterile cotton-tipped swab into the suspension. Remove the excess liquid from the swab by pressing it against the side of the tube. Inoculate the surface of the MHA plate to cover the entire plate back and forth and from edge to edge, then rotate the plate approximately 60° angle, repeat the procedure two times and finally encircle it, so as to ensure the even distribution of the inoculum.

Apply antimicrobial disk within 15 min and incubate the plate within 15 min. 12 and 5 disks can be applied to a 150 mm and 100 mm diameter plate respectively. Alternatively the disk can be applied with the dispenser. Invert and incubate plates with agar side up, for nonfastidious bacteria in general incubate in ambient air at 35°C for 16–18 hours and for fastidious organism and other environmental bacteria incubate as appropriate to the desired organism.

After incubation, examine plates for the lawn of growth is even and confluen and clear unobstructed zones. Measure zones from the back of the plate using reflected light by holding the plates a few inches above a black non-reflecting surface using vernier caliper or zone measurement ruler. Reflected light for all the organism and transmitted light for vancomycin or oxacillin resistant Staphylococci or Enterococci.

Plates with fuzzy or double zone lines or feathered edges or swarming and colonies inside zones should be taken adequate care in reporting. This occurs in mixed culture or resistant subpopulation within the culture. Measure the inner most zone for double zone. Pick the inner colony and check for the purity and repeat the antimicrobial susceptibility testing once again. In general colony free zone is taken. For feathered edge the region demarcating growth and no growth is taken as zone region. Ignore generally swarming and measure the zone region only.

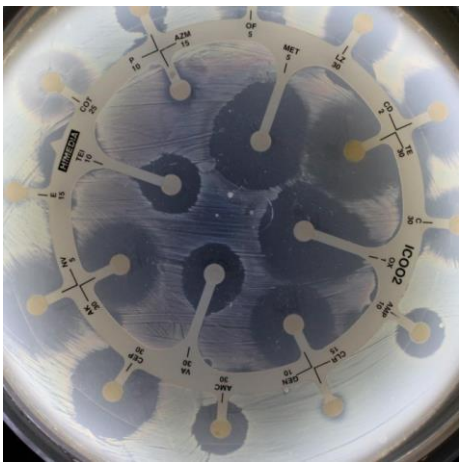


Fig.6. Results of Disk diffusion assay

Quantitative methods

MIC

The minimal inhibitory concentration (MIC) of an antimicrobial agent is the lowest (i.e. minimal) concentration of the antimicrobial agent that inhibits a given bacterial isolate from multiplying and producing visible growth in the test system. MIC tests can be performed in broth or agar media dilution or broth microdilution in microtitre plates.

Broth microdilution MIC is performed in a polystyrene panel containing approximately 96 wells which may be sufficient enough for a panel of 12 antibiotics upto 7–8 dilutions with one well each for a positive growth control (broth plus inoculum) and negative control (broth only). In general volume of 0.1 or 0.2 mL in each well the MIC is performed. Use separate panel for gram-positive bacteria and gram-negative bacteria and special media for fastidious bacteria. In general, Mueller-Hinton broth with appropriate divalent cation (Ca++ and Mg++) is recommended for susceptibility for rapidly growing aerobic, or facultative organisms non-fastidious organism. The pH of the medium adjusted to 7.2 and 7.4 at room temperature (25˚C). For fastidious organisms Mueller-Hinton broth may be supplemented with 2–5% lysed horse blood or according to the organism.

In the agar dilution method, the antimicrobial agent is incorporated at different concentration into the each agar medium Mueller-Hinton agar. The pH of the agar must be between 7.2 and 7.4 at room temperature. The inoculum is applied on surfaces as replicators transfer 32–36 inocula to each plate.

Follow the same procedure of inoculum suspension preparation as mentioned in disk diffusion assay. Within 15 minutes of adjusting the inoculum to the 0.5 McFarland turbidity standard, dilute it further so that the final concentration in each well is 5×10^5 CFU/mL. Deliver 2.0 mL of the original suspension into 38 mL of water (1:20 dilution). Inoculate 0.01 mL (1:10 dilution) into each well and finally inoculate MIC panel carefully to avoid splashing from one well to another. Cover the plate to avoid dehydration during incubation. Incubate non-fastidious bacteria in ambient air at 35˚C from 16–20 hours. Staphylococci incubate oxacillin and vancomycin for 24 hours. For vancomycin, high level gentamicin resistance (synergy test) and high level streptomycin resistance (synergy test) for 24 hours.

At the end of the testing, check the plate for purity. Check the positive control well for growth of turbidity or a button of growth >2 mm should be present. Check the negative control well for no growth or clear. Read the MIC endpoint as the lowest concentration of antimicrobial agent that completely inhibits growth of the organism as detected by the unaided eye. Always include the MIC breakpoint for the particular organism for that particular antibiotic.

The performance of each batch of broth or antimicrobial are evaluated with standard set of quality control organisms.

Other commercial manual and automated systems used for determination and quantitation of phenotypic antimicrobial resistances are E-test,l vitek system, microscan etc

Genotypic methods

Looking for genes involved in the conferring resistance to antibiotics are now currently more followed in well established laboratories. The detection of mechanism of

antibiotic resistance can be better elucidated from the genes present in the organism. This is achieved by polymerase chain reaction targetting against genes involved in each class or generic or group of antibiotics. Over 30 class and subclass of antibiotics are available. Resistance mechanism developed against these antibiotics were mediated by over 100 different genes and their mutants or variants. In general, the PCR is targeted against these genes to look for the possible molecular mechanism involved in the acquiring of antibiotic resistances. Even the researchers moved to the quantitation of antibiotic resistance for particular antibiotics by a pathogen using realtime PCR or microarray analysis.

Control

AMR is a complex and indisciplinary issue, coherent efforts are required to bring down the burden of AMR among public. WHO, FAO and OIE have taken collective tripartite one health approach to control AMR spread which are considered as national action plans to each countries. The proposed the action plan against AMR control is depticted in Fig.7 (WHO).

Fig.7. The four pillars of the FAO plan of action to support the food and agriculture sector in addressing AMR

Key action plans proposed to control AMR are

1. Strengthen the surveillance system in healthcare, food producing animals on antimicrobial usage and antimicrobial drug resistant bugs
2. Emphasis need to be given to the food and environmental sectors also
3. Strengthening the laboratory capacity for surveillance system
4. Guideline for the optimised use of antibiotics in human and animal health
5. Reduce the infection loss due to AMR pathogens by providing assured quality medicines
6. Awareness and understanding among the general public
7. Effective infection prevention and control programmes
8. Development of alternate to antibiotics protocols

9. Controlling the resistances development in bacteria for medically important antibiotics

Whole world is looking for alternatives to the antibiotic in combating the bacterial infections which may consequently reduce the amount of antibiotic used in therapies and lower doses in other animal agriculture practices. Many alternatives are looked in and few promising among them are lytic phages, probiotics, antimicrobial peptides, quorum quenching molecules etc.

Suggested reference

- CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
- CLSI. *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition.* CLSI document M02-A11. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
- CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That grow Grow Aerobically; Approved Standard—Tenth Edition.* CLSI document M07-A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
- https://www.cdc.gov/drugresistance/about.html
- http://www.who.int/antimicrobial-resistance/publications/ situationanalysis/ en/
- Cahill, S. M., Desmarchelier, P., Fattori, V., Brunoand, A., Cannavan, A. (2017) Global Perspectives on Antimicrobial Resistance in the Food Chain. Food Protection Trends, Vol 37, No. 5, p. 353–360

****

HACCP (Hazard Analysis and Critical Control Point)

Pankaj Kishore

Quality Assurance and Management Division

ICAR- Central Institute of Fisheries Technology, Cochin

Email: pkishore2007@gmail.com

Introduction:

Food safety is growing concern day by day as it has direct impact on human health. Various hazards viz. physical, chemical, biological,or allergens resulting food-borne illness acrossthe world lead to provide a thought in all processes through which food goes to the consumers. Each year, millions of illnesses can be attributed to contaminated food.Hence a food safety action aimed at ensuring that all food is as safe as possible is must. Food safety policies and actions need to cover the entire food chain, from production to consumption.Food safety in the beginning of twenty-first century is an international challenge requiring close cooperation between countries in agreeing standards and in setting up transnational surveillance systems.The behaviour of consumers has been gradually changing. They currently require not only much higher dietary quality, hygiene and health standards in the products they purchase, but they also look for certification and reassurance of products' origins (national or geographical) and production methods. HACCP or Hazard Analysis Critical Control Points is a scientific and systematic approach to identify, assess and control of 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.

Introduction of HACCP:

Hazard Analysis and Critical Control Point (HACCP) was developed in the 1960s in the United States to ensure food safety for the first manned National Aeronautics and Space Administration space missions (NASA). NASA required a 'zero defect' program to guarantee safety in the foods astronauts consumed in space.

HACCP is endorsed by the:

- FAO (Food and Agriculture Organization)
- Codex Alimentarius (a commission of the United Nations)
- USFDA (US Food and Drug Administration)
- European Union
- WHO (World Health Organization)

Time Line Development of HACCP:

- **1959** - The Pillsbury Company developed concept for NASA.
- **1971** - HACCP, as we presently know it, took form at the US National Conference on Food Protection, where risk assessment was combined with the critical point concept (1st mention of HACCP).

- **1972** - The Pillsbury Company in the United States began the application of its HACCP concept to the manufacture of its consumer food products. Pillsbury published the first comprehensive treatise on HACCP in 1973.
- **1973**- An HACCP system was adopted for the Low-Acid Canned Food Regulations following the Bon Vivant Vichyssoise Soup botulism incident, in which several people died after eating the soup, due to botulism poisoning.
- **1980** - WHO/ICMSF report on HACCP.
- **1983**- WHO Europe recommends HACCP.
- **1997** - Codex Document on HACCP principles and application
- **1997 December**- FDA's Seafood HAACP program becomes mandatory.
- **1998**- FAO/WHO provide guidance for regulatory assessment of HACCP
- **1998 January** - HACCP becomes mandatory for large meat and poultry manufacturers.
- **2003** - FAO/WHO developed HACCP guidelines.
- **2004** - EC 852/2004 requirement for all food businesses to adopt HACCP principles in EU.
- **2006** - Legal requirements to apply HACCP in food businesses (other than primary production) across EU
- **After 2006** - Increased worldwide use of HACCP in food safety legislation

HACCP- Aglobal requirement for food safety assurance:

An effective food safety assurance methodis required due to emergence of food-borne pathogens and food-borne diseases which has widespread public health problem. Increased knowledge and awareness of the serious and chronic health effects associated with unsafe food products had made HACCP indispensible in all exporting food processing industries. An effective food safety assurance method such as HACCP is important due to the followings:

- New food technologies and processing methods are introduced now and then
- Increased awareness of the economic consequences of foodborne disease
- Increase in the number of vulnerable people
- Industrialization and mass production
- Urbanization
- Changes in lifestyle
- Increase intourism and international trade for foodstuffs
- Increase in consumer awareness about food safety

HACCP Concept:

It is important to always remember that the establishment of effective HACCP programs involves primarily the application of good common sense and preventive considerations to address situations before they become problems. The emphasis is on prediction

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.

The objectives of application of the HACCP system:

- Prevention of food-borne illness
- Reduction of losses due toproduct recall
- Protection of reputation
- Reduction of costsof food analysis
- More efficient quality assurance system
- Focuses on identifying and preventing hazards from contaminating food, based on sound science.
- Permits more efficient and effective government oversight, primarily because record keeping allows investigators to see how well a firm is complying with food safety laws over a period, rather than how well it is doing on any given day.
- Helps food companies to compete more effectively in the world market.
- Reduces barriers to international trade.

Guidelines in the application of HACCP system:

1. Assemble the HACCP team
2. Describe product
3. Identify intended use
4. Construct flow diagram
5. On-site verification of flow diagram
6. List all potential hazards, conduct a hazard analysis and determine control measures
7. Determine CCPs
8. Establish critical limits for each CCP
9. Establish a monitoring system for each CCP
10. Establish corrective actions
11. Establish verification procedures
12. Establish record keeping and documentation

1. <u>Assemble the HACCP Team</u>:

   A multi-disciplinary HACCP team needs to include knowledge of the following aspects:Raw materials, specialist (quality assurance/technical), operation activities, engineering/equipment technical knowledge of HACCP, process, finished product, hazard expertise, environment (premises, property, surroundings)

2. <u>Describe the product</u>:

   Describe the product giving detail of its composition, physical/chemical structure, and packaging, safety information, processing treatments, storage and

method of distribution. Product name, composition, end product characteristics, method of preservation, primary packaging, shipping, storage conditions, distribution method, shelf life, special labeling, customer preparation

3. Identify the intended use:

   Identify the intended use of the product, its target consumer with reference to sensitive population.Five sensitive groups in the population are categorized such aselderly, infants, pregnant, sick andimmuno-compromised.

4. Construct a process flow diagram:

   Details of all process activities including inspections, transportation, storage and delays in the process are to be given. Inputs into the process in terms of raw materials, packaging, water and chemicals and output from the process e.g. waste – packaging, raw materials, product-in-progress, rework and rejected products also need to be mention

5. On site verification of the process flow diagram:

   It should be done by all members of the HACCP team during all stages and hours of operation. Validate process flow diagram by HACCP team, observe process flow, sampling activities, interview and outline / non routine operations.

Prerequisite Programs (PRP):

PRP focus on employees, facilities, and equipment. Examples of prerequisite programs includes illness policy, cleaning and sanitizing procedures, garbage removal, pest control, equipment selection, employee hygiene.

Principles of HACCP (CODEX):

1. Conduct a hazard analysis
2. Determine the CCPs
3. Establish critical limit(s)
4. Establish a monitoring system
5. Establish corrective actions
6. Establish verification procedures
7. Establish documentation

1) Conduct a hazard analysis:

Identify hazards associated with a specific menu item by preparing a flow diagram that outlines all handling/preparation steps from receiving to service.Listing of likely hazards associated with each step and identification of how to prevent the hazards at each step.Hazards can be biological, chemical, physical or allergens. Also a listof hazards need to be projected that are likely to occur andthat will cause severe consequences if not controlled.Hazards that are low risk and are not likely may not need to be considered.

2) Determine CCPs:

A control point is any point, step, or procedure where biological, physical, or chemical factors can be controlled.A critical control point(CCP) is a point, step, or

procedure where an identified hazard can be prevented, eliminated, or reduced to acceptable levels.Critical control points are monitored much more frequently than are control points.

3) Establish critical limits:

This step involves establishing criteria that must be met to prevent, eliminate, or reduce the identified hazard at the CCP so that the food is safe to eat.Examples of critical limits are temperature, time, physical dimensions, water activity, pH, and available chlorine. Critical limits can come from regulatory standards and guidelines, scientific literature, experimental studies, and consultation with experts.

4) Establish monitoring procedures:

Monitoring is a planned observation or measurement to determine if a CCP is under control. Examples of monitoring include visual observations, temperature measurements, time assessment, pH measurements, water activity measurements, etc.

5) Establish corrective actions:

Corrective actions focus on what to do when a food does not meet the critical limit.Example of a corrective action is temperature of a cooker, throwing out food might be a corrective action.Maintains records of all corrective actions taken.

6) Establish verification procedures:

Four phases of verification needed for a HACCP plan:

1. Determine that the critical limits at all CCPS are sound.
2. Make sure that the establishment's HACCP plan is being properly implemented.
3. Have regulatory personnel review the plan to make sure that it is being properly implemented.
4. Check the accuracy of all monitoring equipment.

7) Establish record keeping:

The following make up the records of a HACCP Plan

- List of HACCP team and their assigned responsibilities
- Description of each menu item
- Flow diagram for each menu item indicating CCPs
- Hazards associated with each CCP and preventive measures
- Critical limits
- Monitoring procedures
- Corrective actions plans
- Record keeping procedures
- Procedures for verification of the HACCP plan

Advantages of HACCP:

Most important advantages related to implementation of HACCP in food sector comprises:

- identifying and preventing hazards resulting food unsafe
- Scientific approach
- permits more efficient and effective government oversight, primarily because the recordkeeping allows investigators to see how well a firm is complying with food safety laws and following practices that reduce the risk of unsafe food over a period rather than how well it is doing on any given day
- rendering responsibility for ensuring food safety appropriately on the food business operators
- reduces barriers to international trade which make food companies to compete more effectively in the world market

Conclusion:

HACCP forms the foundation of European and international legislation for the food industry and is a key component of international trade in food products.HACCP program to be successful need proper implementation and management. This depends largely on regularly scheduled verification activities. The HACCP plan should be updated and revised as requirement. An important aspect of maintaining the HACCP system is to assure that all individuals involved are properly trained so they understand their role and can effectively fulfill their responsibilities. Today food industry standards play a major role in assisting food businesses to achieve compliance with legislation and in many cases exceed legislative requirements. Many of these integrate business operations such as good manufacturing practices (GMP), GHP and HACCP; thereby, providing food businesses with a means to develop an integrated food safety management system.This is a scientific and cost effective system for controlling product safety and quality. This may enable food business operators to ensure consistency in terms of product safety and quality with fair trade across globe.

References:

- Codex Alimentarius Commission. (1997). Hazard analysis and critical control point (HACCP) system and guidelines for its application. *Annex to CAC/RCP*, *1*(1969), Rev-3.
- Ehiri, J. E., Morris, G. P., & McEwen, J. (1995). Implementation of HACCP in food businesses: the way ahead. *Food Control*, *6*(6), 341-345.
- HACCP in microbiological safety and quality. International Commission on Microbiological Specifications for Foods (ICMSF), 1989. Boston, Massachusetts, USA, Blackwell Scientific Publications.
- Pierson, M. D. (2012). *HACCP: principles and applications*. Springer Science & Business Media.
- Reilly, A., &Käferstein, F. (1997). Food safety hazards and the application of the principles of the hazard analysis and critical control point (HACCP) system for their control in aquaculture production. *Aquaculture research*, *28*(10), 735-752.

****

Statistical Methods for Research and Product/Process Development

Joshy C G
Fish Processing Division
ICAR-Central Institute of Fisheries Technology, Cochin
Email: cgjoshy@gmail.com

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 and can be presented in the form of descriptive statistics.

Descriptive Statistics

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

Measures of Central Tendency

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

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

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

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

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

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

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

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

Measures of Dispersion

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

a. Range
b. Variance
c. Standard Deviation

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

Range= H-L

Variance measures the variability of observations from its mean. It computes the sum of squared 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 arewidely 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.

Tests of Significance

Once sample data has been gathered, statistical inference allows assessing evidence in favor or some claim about the population from which the sample has been drawn. The method of inference used to support or reject claims based on sample data is known as testing of hypothesis. Statistical test is a procedure governed by certain rules, which

leads to take a decision about the hypothesis for its acceptance or rejection on the basis of the sample values. These tests have wide applications in agriculture, medicine, industry, social sciences, etc.

Definitions:

Statistic: It is a function of units in the sample, like sample mean, sample variance

Parameter: It is a function of units in the population, like population mean, population variance

Statistical Hypothesis: A definite/tentative statement about the population parameters

Simple Hypothesis: If all the parameters are completely specified, the hypothesis is called a simple hypothesis

Composite hypothesis: If all the parameters are not completely specified by a hypothesis is called as composite hypothesis

Null Hypothesis (H_0): The hypothesis under test for a sample study

Alternative Hypothesis (H_1): The hypothesis tested against the null hypothesis

$H_0: \mu = \mu_0$

$H_1: \mu \neq \mu_0$ *(Two-Tailed Test)*

$\mu < \mu_0$*(Left-Tailed Test)*

$\mu > \mu_0$ *(Right-Tailed Test)*

Level of Significance (α): The maximum size of the error (probability of rejecting H_0 when it is true) which we are prepared to risk. The higher the value of α, less precise is the result

Test Statistic: It is a quantity calculated from sample of data. Its value is used to decide whether or not the null hypothesis should be rejected in the hypothesis test

Critical value(s): The critical value(s) for a hypothesis test is a value to which the value of the test statistic in a sample is compared to determine whether or not the null hypothesis is rejected. The critical value for any hypothesis test depends on the significance level at which the test is carried out, and whether the test is one-sided or two-sided.

Procedure of Testing Hypothesis

Step 1: Setting up the hypothesis and level of significance

Null hypothesis (H_0) and Alternative hypothesis (H_1)

Level of significance formulation (α)

Step 2: Data Collection and selection of appropriate test procedure

Compute the Test Statistic

Step 3: Test Criteria

i) reject the null hypothesis, or

ii) not reject the null hypothesis

Step 4: Draw the Inference

The major statistic's used for tests of significance are

1. **Normal Test**
2. **t - Test**
3. **Chi - Square Test**
4. **F - Test**

Normal test

Test for the Mean of a Normal Population

When Population Variance is known

If x_i(i =1,…,n) is a r.s of size n from N(μ, σ^2), then

H_0 : $\mu = \mu_0$ or

H_0 : the sample has been drawn from the population with mean μ_0

H_1 : $\mu \neq \mu_0$ (two-tailed) or $\mu > \mu_0$ (right-tailed) or $\mu < \mu_0$ (left-tailed)

$$\text{Test Statistic} \quad Z = \frac{\bar{x} - \mu}{\sigma/\sqrt{n}} \sim N(0, 1)$$

with n-1 degree of freedom

Depending on the alternative hypothesis selected, the test criteria is as follows:

H_1	Test	Reject H_0 at level of significance α if
$\mu \neq \mu_0$	Two-tailed test	$\lvert Z \rvert > Z_{\alpha/2}$
$\mu < \mu_0$	Left-tailed test	$Z < -Z_\alpha$
$\mu > \mu_0$	Right-tailed test	$Z > Z_\alpha$

Z_α is the table value of Z at level of significance α.

Test for Difference of Means

Normal PopulationI: Sample size n1

Normal PopulationII: Sample size n2

H_0 : $\mu_1 = \mu_2$

Test Statistic: Normal test

$$\text{Test statistic } Z = \frac{\bar{x}_1 - \bar{x}_2 - (\mu_1 - \mu_2)}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}}$$

$$\text{Under H0}\quad Z=\frac{\bar{x}_1-\bar{x}_2}{\sqrt{\frac{\sigma_1^2}{n_1}+\frac{\sigma_2^2}{n_2}}}=\frac{\bar{x}_1-\bar{x}_2}{\sigma\sqrt{\frac{1}{n_1}+\frac{1}{n_2}}}\quad \text{if } \sigma_1^2=\sigma_2^2=\sigma^2$$

Population Variances are unknown but equal $\sigma^2=\frac{n_1s_1^2+n_2s_2^2}{n_1+n_2}$

t - tests

1. Test for the Mean of a Normal Population when Small Sample (n < 30) and Population Variance is Unknown

A r.s $x_1,\ldots,x_n \rightarrow N(\mu, \sigma^2)$

$H_0 : \mu = \mu_0$

$H_1 : \mu \neq \mu_0$ or $\mu > \mu_0$ or $\mu < \mu_0$

Test Statistic: $\text{t - test } t=\frac{\bar{x}-\mu_0}{s/\sqrt{n}} \sim t_{n-1}$ with n-1 degree of freedom,

where $\bar{x}=\frac{1}{n}\sum_{i=1}^{n}x_i$ and $s^2=\frac{1}{n-1}\sum_{i=1}^{n}(x_i-\bar{x})^2$

The null hypothesis is accepted or rejected accordingly.

2. Test for the Difference of Two Population Means: when the population variances are unknown but assumed to be equal

Let $\bar{x}_1$ be the sample mean of a sample of size n_1 of first population with mean μ_1 and $\bar{x}_2$ be the sample mean of a sample of size n_2 from a population with mean μ_2.

$H_0 : \mu_1 - \mu_2 = \delta_0$ ie $\bar{x}_1 - \bar{x}_2 = 0$

Test Statistic: Under H_0 is $t=\frac{\bar{x}_1-\bar{x}_2-\delta_0}{\sqrt{s^2(\frac{1}{n_1}+\frac{1}{n_2})}} \sim t_{n_1+n_2-2}$

S^2 is estimated from the sample $s^2=\frac{(n_1-1)s_1^2+(n_2-1)s_2^2}{n_1+n_2-2}$

$s_1^2=\frac{1}{n-1}\sum_{i=1}^{n}(x_{1i}-\bar{x}_1)^2$ and $s_2^2=\frac{1}{n-1}\sum_{i=1}^{n}(x_{2i}-\bar{x}_2)^2$

3. **Paired t-test for Difference of Means**

Paired t-test is used when

- Sample size n_1 = sample size n_2 = n
- Two samples are not independent (paired)
- Let (x_i, y_i), i=1,..,n be a random sample from a bivariate normal population
- Let $d_i = x_i - y_i$

The null hypothesis is $H_0 : \mu_1 - \mu_2 = 0$ i.e $\bar{d} = 0$

The Test Statistic under H_0 is $t = \frac{\bar{d} - \mu_0}{s/\sqrt{n}} \sim t_{n-1}$

Where $\bar{d} = \frac{1}{n}\sum_{i=1}^{n} d_i$ and $s^2 = \frac{1}{n-1}\sum_{i=1}^{n}(d_i - \bar{d})^2$

Chi - Square Test

1. Test for the variance of a normal population

Let $x_1, x_2, \ldots, x_n$ $(n \geq 2)$ be a r.s from $N(\mu, \sigma^2)$.

$H0 : \sigma^2 = \sigma_0^2$

Test Statistic under H_0 is $\chi^2 = \sum_{i=1}^{n} \left(\frac{x_i - \mu}{\sigma_0} \right)^2 \sim \chi_n^2$ when μ is known and

$\chi^2 = \sum_{i=1}^{n} \left(\frac{x_i - \bar{x}}{\sigma_0} \right)^2 \sim \chi_{n-1}^2$ when μ is unknown

2.Test of Goodness of Fit

To test the discrepancy between the observed and the expected frequency

H_0 : the fitted distribution is a good fit

H_1 : not a good fit

Test Statistic:

$O_i \rightarrow$ Observed frequency of i^{th}class

$E_i \rightarrow$ Expected frequency of i^{th}class, i =1,…,n.

The test statistic is $\chi^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i} \sim \chi_{n-r-1}^2$

3.Test of Independence

H_0: The attributes are independent

H_1: They are not independent

Test Statistic: $\chi^2 = \sum_{j=1}^{c} \sum_{i=1}^{r} \frac{(O_{ij} - E_{ij})^2}{E_{ij}} \sim \chi_{(r-1)(c-1)}^2$

$O_{ij} \rightarrow$ Observed frequency ,$E_{ij} \rightarrow$ Expected frequency i =1,…,r; j =1,…,s

H_0 is rejected at level α if $\chi^2 > \chi_{(r-1)(c-1)}^2$

F- tests

1. To Test for the comparison of two population variances

Consider a sample of size n1 from a normal population N(μ_1, σ_1^2) and another sample of size n2 from second normal population **N(μ_2, σ_2^2). The null hypothesis to test the significance of two population variances is** $H0: \sigma_1^2 = \sigma_2^2$ ie $s_1^2 = s_2^2$

The test statistic is $F = \frac{s_1^2}{s_2^2} \sim F_{n_1-1, n_2-1}$

The computed value of F is compared with the tabulated value and the inference is drawn accordingly.

2.Equality of Several PopulationMeans

The null hypothesis to test the equality of several means is **$H_0 : \mu_1 = \mu_2 = ... = \mu_k$**

The total variability in the data is being partitioned into different known variability components using a statistical technique called analysis of variance (ANOVA). But the statistic used to the test the significance of equality several means is F-test/F-statistic

The statistic is $F = \frac{\text{Variation among the sample means}}{\text{Variation within the samples}}$

Fundamentals of Design of Experiments

Introduction

Any scientific investigation involves formulation of certain assertions (or hypotheses) whose validity is examined through the data generated from an experiment conducted for the purpose. Thus experimentation becomes an indispensable part of every scientific endeavour and designing an experiment is an integrated component of every research programme. Three basic techniques fundamental to designing an experiment are *replication*, *local control* (*blocking*), and *randomization.* Whereas the first two help to increase precision in the experiment, the last one is used to decrease bias. These techniques are discussed briefly below.

Replication is the repetition of the treatments under investigation to different experimental units. Replication is essential for obtaining a valid estimate of the experimental error and to some extent increasing the precision of estimating the pairwise differences among the treatment effects. It isdifferent from *repeated measurements*. Suppose that the four animals are each assigned to a feed and a measurement is taken on each animal. The result is four independent observations on the feed. This is *replication*. On the other hand, if one animal is assigned to a feed and then measurements are taken four times on that animal, the measurements are not independent. We call them *repeated measurements*. The variation recorded in repeated measurements taken at the same time reflects the variation in the measurement process, while variation recorded in repeated measurements taken over a time interval reflects the variation in the single animal's responses to the feed over time. Neither reflects the variation in independent animal's responses to feed. We need to know about the latter variation in order to generalize any conclusion about the feed so that it is relevant to all similar animals.

For inferences to be broad in scope, it is essential that the experimental conditions should be rather varied and should be representative of those to which the conclusions of the experiment are to be applied. However, an unfortunate consequence of increasing the scope of the experiment is an increase in the variability of response. Local control is a technique that can often be used to help deal with this problem.

Blocking is the simplest technique to take care of the variability in response because of the variability in the experimental material. To block an experiment is to divide, or partition, the observations into groups called blocks in such a way that the observations in each block are collected under relatively similar experimental conditions. If blocking is done well, the comparisons of two or more treatments are made more precisely than similar comparisons from an unblocked design.

The purpose of randomization is to prevent systematic and personal biases from being introduced into the experiment by the experimenter. A random assignment of subjects or experimental material to treatments prior to the start of the experiment ensures that observations that are favoured or adversely affected by unknown sources of variation are observations "selected in the luck of the draw" and not systematically selected.Lack of a random assignment of experimental material or subjects leaves the experimental procedure open to experimenter bias.

Contrasts and Analysis of Variance

The main technique adopted for the analysis and interpretation of the data collected from an experiment is the analysis of variance technique that essentially consists of partitioning the total variation in an experiment into components explainable to different sources of variation due to the controlled factors and error. Analysis of variance clearly indicates a difference among the treatment means. The objective of an experiment is often much more specific than merely determining whether or not all of the treatments give rise to similar responses.

Contrasts

Let $y_1, y_2, \ldots, y_n$ denote n observations or any other quantities. The linear function $C = \sum_{i=1}^{n} l_i y_i$, where l_i's are given number such that $\sum_{i=1}^{n} l_i = 0$, is called a *contrast* of $y_i's$.

Let $y_1, y_2, \ldots, y_n$ be independent random variables with a common mean μ and variance σ^2. The expected value of the random variable C is zero and its variance is $\sigma^2 \sum_{i-1}^{n} l_i^2$. In what follows we shall not distinguish between a contrast and its corresponding random variable.

Sum of squares (s.s.) of contrasts. The sum of squares due to the contrast C is defined as $C^2 / \sigma^{-2} Var(C) = C^2 / \left(\sum_{i=1}^{n} l_i^2 \right)$. Here σ^2 is unknown and is replaced by its unbiased estimate, *i.e.mean square error*. It is known that this square has a $\sigma^2 \chi^2$ distribution with one degree of freedom when the $y_i's$ are normally distributed. Thus the sum of squares due to two or more contrasts has also a $\sigma^2 \chi^2$ distribution if the contrasts are

independent. Multiplication of any contrast by a constant does not change the contrast. The sum of squares due to a contrast as defined above is not evidently changed by such multiplication.

Orthogonal contrasts. Two contrasts, $C_1 = \sum_{i=1}^{n} l_i y_i$ and $C_2 = \sum_{i=1}^{n} l_i y_i$ are said to be orthogonal if and only if $\sum_{i=1}^{n} l_i m_i = 0$. This condition ensures that the covariance between C_1 and C_2 is zero.

When there are more than two contrasts, they are said to be mutually orthogonal if they are orthogonal pair wise. For example, with four observations y_1, y_2, y_3, y_4, we may write the following three mutually orthogonal contrasts:

(i) $y_1 + y_2 - y_3 - y_4$

(ii) $y_1 - y_2 - y_3 + y_4$

(iii) $y_1 - y_2 + y_3 - y_4$

The sum of squares due to a set of mutually orthogonal contrasts has a $\sigma^2 \chi^2$ distribution with as many degrees of freedom as the number of contrasts in the set.

Response Surface Methodology for Product/Process Optimization

Response surface methodology (RSM) is such a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes. It also has important applications in the design, development and formulation of new products, as well as in the improvement of existing product designs. The most extensive applications of RSM are in the industrial world, particularly in situations where several input variables potentially influence some performance measure or quality characteristic of the product or process.

In general, suppose that the scientist or engineer is concerned with a product, process or system involving a response Y that depends on controllable input factors x_1, x_2,------ x_p.The relationship between Y and the x's is defined as

$$Y = f(x_1, x_2, \cdots\cdots\cdots, x_p) + \varepsilon$$

Where the form of the true response function f is unknown and perhaps very complicated, and ε is a term that represents other sources of variability not explained or accounted by *f.* Thus ε includes effects such as measurement error on the response, other sources of variation that are inherent in the process or system, the effect of other variables, and so on. We treat ε as a statistical error term with mean zero and constant variance i.e. $\varepsilon \sim N(0, \sigma^2)$, then

$$E(Y) = E[f(x_1, x_2, \cdots\cdots\cdots, x_p)] + E(\varepsilon)$$

$$= f(x_1, x_2, \cdots\cdots\cdots, x_p)$$

Because the form of the true response function f is unknown, we must approximate it. In fact, successful use of RSM is critically dependent upon the experimenter's ability to develop a suitable approximation for *f*. Usually, a low order polynomial in some relatively small region of the independent variable space is appropriate. In many cases a first order or a second order model is used. The major objectives and applications of RSM are

1. To determine and quantify the relationship between response variables and settings of a group of experimental factors (independent variables) i.e. Mapping a response surface over a particular region of interest
2. To find the settings of experimental factors that produces the best value or the best set of values of the response variables i.e. Optimization of the responses

The major steps involved in RSM to improve an existing process/product or formulation of new product are

1. Formulation of experimental design in terms of independent variables
2. Formulation of hypothesis
3. Execution of experiments and generation of experimental data
4. Development of empirical model to predict the response variables in terms of independent variables
5. Model adequacy checking and testing of hypothesis
6. Optimization of response variables in terms of independent variables

Formulation of Experimental Design

Factorial designs are widely used in experiments involving several factors (independent variables) to investigate the main and interaction effects of the factors on response variables. The factorial designs can be classified into two groups viz: symmetrical and asymmetrical factorial experiments. A good response surface design should possess the properties *viz.*, detectability of lack of fit, the ability to sequentially build up designs of increasing order and the use of a relatively modest, if not minimum, number of design points. Examples on some experimental situations, where response surface methodology can be usefully employed are

Example 1: To Optimize the high pressure process parameters viz: pressure, ramp rate and holding time and to see its effect on high pressure treated Indian white prawn. The levels of various factors are

	Factors	**Levels**
1.	Pressure (MPa)	150, 250, 350
2.	Ramp Rate	300, 400, 500
3.	Holding Time(Min)	5, 10, 15

Example 2: For value addition to the agriculture produce, food-processing experiments are being conducted. In these experiments, the major objective of the experimenter is to obtain the optimum combination of levels of several factors that are required for the product. To be specific, suppose that an experiment related to osmotic dehydration of

the banana slices is to be conducted to obtain the optimum combination of levels of concentration of sugar solution, solution to sample ratio and temperature of osmosis. The levels of the various factors are the following

	Factors	Levels
1.	Concentration of sugar solution	40%, 50%, 60%, 70% and 80%
2.	Solution to sample ratio	1:1, 3:1, 5:1, 7:1and 9:1
3.	Temperature of osmosis	25^0C, 35^0C, 45^0C, 55^0C and 65^0C

In this situation, response surface designs for 3 factors each at five equispaced levels can be used.

In general response surface methodology is useful for all the factorial experiments in agricultural experimental programme that are under taken so as to determine the level at which each of these factors must be set in order to optimize the response in some sense and factors are quantitative in nature.

Examples of experimental design setup for RSM

1. All the factorial experiments where the factors are quantitatively measured
2. Central Composite Design
3. Box-Behnken Design
4. Simplex lattice mixture design
5. Simplex centroid mixture design
6. D-optimal design

Development of Empirical Models

In practice the mathematical form of '*f*' discussed in the introduction is not known; we, therefore, often approximate it, within the experimental region, by a polynomial of suitable degree in variables x_{iu} (independent variables). The adequacy of the fitted polynomial is tested through the usual analysis of variance. Polynomials which adequately represent the true input-response relationship are called **Response Surfaces** and the designs that allow the fitting of response surfaces and provide a measure for testing their adequacy are called **response surface designs**. If the function '*f*' is of degree one in x_{iu}'s *i.e.* the response can be represented as

$$y_u = \beta_0 + \beta_1 x_{1u} + \beta_2 x_{2u} + \ldots + \beta_v x_{vu} + e_u$$

And we call it a first-order response surface in $x_1, x_2, \ldots, x_v$.

The second-order (quadratic) response surface can be represented as

$$y_u = \beta_0 + \sum_{i=1}^{v} \beta_i x_{iu} + \sum_{i=1}^{v} \beta_{ii} x_{iu}^2 + \sum_{i=1}^{v-1} \sum_{i'=i+1}^{v} \beta_{ii'} x_{iu} x_{i'u} + e_u$$

This functional form has many applications in most of the agricultural experiments

The analysis of variance table for a second order response surface design is given below.

Analysis of variance for second order response surface

Source	d.f.	S.S.
Due to regression coefficients	$2v+\binom{v}{2}$	$\hat{b}_0\sum_{u=1}^{N}y_u+\sum_i\hat{b}_i\left(\sum_{u=1}^{N}x_{iu}y_u\right)+\sum_i\hat{b}_{ii}\left(\sum_{u=1}^{N}x_{iu}^2y_u\right)$ $+\sum_{i\neq i'}\sum\hat{b}_{ii'}\left(\sum_{u=1}^{N}x_{iu}x_{i'u}y_u\right)-CF$
Error	$N-2v-\binom{v}{2}-1$	*By subtraction = SSE*
Total	$N-1$	$\sum_{u=1}^{N}y_u^2-CF$

In the above table CF = correction factor = $\frac{(Grand\ Total)^2}{N}$. For testing the lack of fit the sum of squares is obtained using (2.16) and then sum of squares is obtained by subtracting the sum of squares due to pure error from sum of squares due to error. The sum of squares due to lack of fit and sum of squares due to pure error are based on $N'-2v-\binom{v}{2}-1$ and $N-N'$ degrees of freedom respectively.

The lack of fit is tested using the statistic $F=\frac{SS_{LOF}/(N'-p)}{SS_{PE}/(N-N')}$

where N is the total number of observations, N' is the number of distinct treatments and p is the number of terms included in the model. SS_{PE}(sum of squares due to pure error) has been calculated in the following manner: denote the l^{th} observation at the u^{th} design point by y_{lu}, where $l=1,\ldots,r_u(\geq 1)$, $u=1,\ldots,N'$. Define $\bar{y}_u$ to be average of r_u observations at the u^{th}design point. Then, the sum of squares for pure error is

$$SS_{PE}=\sum_{u=1}^{N'}\sum_{l=1}^{r_u}(y_{lu}-\bar{y}_u)^2 \qquad (2.16)$$

Then sum of squares due to lack of fit (SS_{LOF}) = sum of squares due to error - SS_{PE}

It is suggested that in the experiments conducted to find an optimum combination of levels of several quantitative input factors, at least one level of each of the factors should be higher than the expected optimum. It is also suggested that the optimum combination should be determined from response surface fitting rather than response curve fitting, if the experiment involves two or more than two factors.

Optimization of Response

The result of model-building procedure is an equation. Once the model is developed, the next stage is to optimize the process. Different type of optimization methods are

1. Method of steepest ascent/descent
2. Method of graphical evaluation of response surface plot

3. Method of desirability function analysis
4. Method of genetic algorithm

References

- Snecdecor.G.W. and Cochran.W.G. 1989.Statistical Methods.John Wiley & Sons. New York. P 503
- Das.M.N and Giri. N.C. 1986. Design and analysis of experiments. New age international P. Ltd. New Delhi. P 488.
- Khuri, A.I. and Cornell, J.A. (1987).*Response Surfaces: Designs and Analysis*. New York: Marcel Dekker.
- Myers, R.H. and Montgomery, D.C. (1995).*Response Surfaces Methodology: Process and product optimization using designated experiments.* John Wiley and Sons.

****

Innovative Extension Approaches for Fish Waste Management

Rejula K. and A. K. Mohanty

Extension, Information and Statistics Division

ICAR-Central Institute of Fisheries Technology, Cochin

Email:rejula.iari09@gmail.com

Introduction

Fish is one of the most perishable food commoditiesand it becomes unfit for human consumption within a short span of capture unless it is subjected to some form of processing. Presently, post-harvest fish losses (PHFL) have become a major concern, which occur mostly in the fish distribution chains starting fromharvesting, transporting, marketing, processing, packaging till consumingwhich result into an estimated global loss/wastage of 27 percentof landed fish in the process. It has been has estimated that postharvest losses in fishes in developing countries alone shares 50% of their domestic fish production(FAO, 2016).In Africa only, estimates indicate an alarming rate of post-harvest losses with a wide range of variation i.e.20-50 percent. Post-harvest losses in small-scale fisheries are considered as highest of all the commodities in the entire food production system. It is known that substantial losses of fish occur at all stages in the supply chain starting from harvesting to consumption. These losses have a direct impact on fishers, processors, traders and other stakeholders involved in ancillary operations in the sector in reducing theirpotential income and creating health hazards due to microbiological spoilage. Thus it has a negative implicationon food security.Post-harvest fish losses (PHFL) are often caused by enzymatic, oxidative and microbiological spoilage that leads to the quality deterioration of fishes, then it becomes waste that require proper management to convert it to wealth.

Fisheries wasteis a major source of surface pollution in coastal areas. Aquatic pollution has become a global concern, but even so, most developing nations are still producing huge pollution loads and the trends are expected to increase (Islam & Tanaka, 2004).Fish waste is costly to dispose due to its high organic content (Knuckey*et al.,* 2004).At present, India registers an unparalleled average annual growth rate of 4.8% in fishery that establishes its position as second largest in global fish production (11.41 MT) next to China. A minimum of 4MT of fishery waste is being generated every year, even though it is scattered in the domestic and industrial sector (Zynuddeen, 2017).The unregulated disposal of seafood solid and liquid wastescreates environmental and social ill effects in the nearby area. One of the major challenges faced by the coastal communities especially in developing countries like India is the negative externalities exerted by the industries on the environment, affecting their livelihood (Abhilash, 2013). Fish and shellfish processors are facing a rise in the cost and difficulty of waste disposal. This is of particular concern in remote areas where alternative uses (e.g.fishmeal) are neither accessible nor economically viable and therefore, cost effective and environmentally-sound solutions for the disposal of thesefish wastes need to be explored (Mazik, *et al.,* 2005).A comprehensiveand fine-tuned participatory extension system is required to face this challenging task in a more strategic way.

Management of waste at Seafood industries

The utilization of by-products is an important production opportunity for the seafood industries, as it can potentially generate additional revenue as well as reduce disposal

costs for the waste materials.In the past, fish by-products, including waste, were considered to be low value commodity and used as feed for farmed animals or thrown away as waste materials. In the last two decades, utilization of fish by-products has been gaining momentum because they can represent a significant additional source of nutrition. Increasingly, the utilization of fish by-products is encouraging business opportunities for the industries, with a growing focus on their handling in a controlled, safe and hygienic way, thereby also reducing waste (FAO, 2016). There are different methods to manage the waste generated from seafood industries for sustainable seafood waste management which areexplained below: -

- Segregation of waste materials into solid and liquid waste
- Shell waste for chitin, chitosan production
- Liquid waste management by treating through ETP
  Channelizing solid seafood waste for the production of seafood silage-which could be further utilized for organic liquid fertilizer production-livestock feed thus leading to employment generation as a small scale business unit (Abhilash, 2013).

Innovative Work Done at Central Institute of Fisheries Technology, Cochin

The fish wasteutilization technology evolved by ICAR-CIFT helps to eliminate harmful environmental effects and improve quality in fish processing. About 30% of the total fish weight remains as waste in the form of skins and bones during preparation of fish fillets. This waste is considered as an excellent raw material for the preparation of high value products including protein foods.Research carried out at the Central Institute of Fisheries Technology, Cochin, paved the way for production of valuable food and industrial products namely protein extract, chitin and its derivatives chitosan and glucosamine hydrochloride from the head and shell waste of prawns, crab and squilla. Fish skin and scales which constitutes about 30% and 5% of the total seafood processing discards, respectively are considered as the richest source for collagen and gelatin, which have wide applications in nutraceutical product development due to its biocompatibility, biodegradability, and bioactive properties like antioxidant, antimicrobial, antihypertensive (Mathew, S. 2017).

Role of extension for fish waste management

Commercialization and income generation from fishery by-products require sincere efforts from researchers, extension scientists and also economists who can develop a professional business model for each product developed by the institute from fish waste. CIFT has technical collaborations with 13organizations comprising industries and also public sector firms for chitin production. There is need to develop a successful business model for entrepreneurship development in fisheries. Sensitization, ideation, incubation, acceleration and seed funding need to be done at the various growth stages of the entrepreneurship development.

Waste minimization and recycling

Waste minimization and recycling is one of the management strategies which can be applied in industries. It was found that the water consumption was substantially reduced & total water saving was upto 45% in ananchovy thawing and gutting industry in Adana Turkey which adopted waste minimization and recycling applications. They could save 48,175$ annually due to water and energy saving. This shows that tangible economic gains can be achieved if waste minimization and recycling applications are successfully realized in seafood processing industry (Alkayaa&Demirerb, 2016).

Fish composting

Composting is one of the low-investment fish waste management option as per a study conducted by Australian Seafood Co-products (ASCo) Fertilizers. (Knuckey*et al.,* 2004). Hydrolyzed fish offal as a stable liquid fish concentratewhich blended with rock phosphate and inoculated with bacteria and fungi using advanced composting technology was developed. (Burdon &M.Elliott, 2005).

In an effort to help the Michigan fish processing industry forbetter solutions to handle fish processing waste materials, it was recommended that fish waste compost can be used as a component of a growing mix that meets a more demanding specification and for which the consumer is accustomed to pay a higher price. Market led extension need to be adopted while going for technology development which integrates agriculture and allied sectors.

- Conducting demonstration for popularizing the fish waste compostingas a source of manure among sea food processors, fish farmers, agricultural farmers and farm women is very much essential.
- Encourage fish processors to plan for fish waste management in terms of a sustainable production system.
- Putting fish waste in a form that processors either use or that will bring an additional revenue stream into the business.

Developing Cooperation Between Industries

- To commercialize value addition in fish waste working relationships need to be formed between the seafood industry and fertilizer manufacturers.
- It was acknowledged that if the utilization of fish waste were to be successful on a broad scale, it would require a considerable level of coordination and cooperation, both within the seafood industry supply chain and across a range of different stakeholders.
- Involvement with a range of seafood companies that may have an interest in adding value to the seafood supply chain through productionand utilizationof waste fish products.
- Develop an agreed structure for the fish waste utilization company that meets the needs of entire supply chain.
- Develop a business plan for a fish waste utilization company that includes a feasibility/economic analysis and a marketing plan (Knuckey*et al.*, 2004)

Following Collaborative Approach

- Fish waste management requires a concerted assessment and further discussion between all parties.
- There is a need for a collaborative approach between the industry and the regulators with input from scientific, technical and economic expertise(Mazik, *et al.,* 2005).

Increasing Awareness On Marine Pollution

- Since scientific knowledge on marine pollution is patchy, knowledge gaps have been identified as one of the major problems in introducing effective management strategies for its control.

Strategies for Waste Management in Aquaculture

- Sustainable growth of the aquaculture industry requires profitability, economic development, and waste management.
- Waste management decisions must be made on an individual basis due to site characteristics on the farm and within the watershed.
- Although the costs incurred with waste management seems to be high, they are considered as minor compared to the costs of controlling the environmentpollution.
- Policy options to address this issue include, cost-sharing, incentives, feed related taxes, education, and water quality testing that would be used to establish total maximum daily loads (TMDL) as cited by Dan Miller and Ken Semmens (2002).

Innovative Extension Approaches for Technology Dissemination

Earlier in developing countries, the extension personnel were involved in diffusion of farm technologies generated by public research organizations, mostly disseminated through appropriate mechanism, *viz.,* On Farm Trials (OFT), Front Line Demonstrations (FLD), field visits, farmers' meetings, media use, etc. These process had the conceptual backup from the 'diffusion of innovation' model. But in the last two decades, the paradigm shifts in development pivots to the enhanced concern for future generations to meet their basic needs, accordingly the nature of agricultural technology design and integration is drawing attention of the extension professionals and practitioners across the globe. In India, different models for transfer of farm technology have been tested and also robust extension management approaches have been validated for successful diffusion of technologies. Furthermore, the frontline extension system of the country has been sharpened through more farmer-centric approaches for technology adaptation and dissemination. The extension system in India has been redesigned to move beyond technology and beyond commodity through ensured reciprocal farmer-research-extension linkages. Farm producers located at far-off and those unreached still suffer from lack of access to appropriate services like credit, inputs, market, extension, technologies etc.

Keeping eye upon this, the World Development Report had focused on need to restructure and revamp agricultural extension system as a pivot for realizing the growth potential of farm sector against the widening demand–supply pressures for ensuring sustainable growth and pro-poor agricultural and economic development. Therefore, farmer's participatory technology development and client's participatory extension approaches emerged as a part of integration of the '*interdependence model*' and the '*innovation systems framework*' that offered more inclusive ways of involving the institution in technology generation, diffusion and use of new knowledge. Extension approaches have been redefined depending upon the components involved for sustainable growth and livelihood security of the farmers for which a conceptual framework has to be developed in response to recognizing and considering different livelihood assets viz., *social, physical, natural, human and financial resources*. Some of the following innovative extension approaches originating from multiple sources must be adopted on trial basis to make technologies more accessible to provide food, nutrition and livelihood security to farmers, which can be replicated in the fishery sector interwoven with numerous issues including increased production with sustained natural resources, growing market demand for processed products having entrepreneurial opportunities, protection and conservation of environment, and even international trade.

An analysis of national extension systems in the Asia and Pacific region by Qatar (2006) observes that agricultural extension is undergoing a major transformation as a result of failure of public extension systems perceived to be outdated in the context of globalization, decentralization, and ICT revolution. Extension systems in many developing countries have undergone a paradigm shift to more farmer-oriented approaches to rural innovation that emphasize the importance of interactive, integrated and multidisciplinary oriented mutual learning between formal and informal knowledge systems (Friederichsen, 2009).

Asset Based Community Development (ABCD) approach

As per the traditional approach to development, poor people see themselves as people with special needs that can only be met by outside supporting agencies. But Asset Based Community Development (ABCD) approach intends for the development of community based on the principle of identifying and mobilizing individual and community 'assets', rather than focusing on problems and needs. It is an extension approach in which a community's micro-assets are linked with its macro environment. It believes that communities can initiate and sustain the process of growth and development themselves by recognizing and harnessing the existing, but often unrecognized assets, and thereby promoting local economic potential to drive its development process (Rans & Green, 2005). The approach is optimistic in nature, because the focus is on what is possessed by the community, rather than the problems of the community.

The focal point in this approach is asset and not the need of the community. Assets of individuals, associations and institutions are identified after an extensive survey and assets are then matched with the need of the people to empower communities to control their futures and create tangible resources such as services, funds and infrastructures etc. *(Foot and Hopkins, 2010).* In agriculture, ABCD approach gives greater emphasis on reducing the use of external inputs and on a high degree of social mobilization in which the assets of the poor (social, physical, financial as well as human) can be utilized to bring sustainable livelihoods through variety of different agricultural and non-agricultural activities.

Five Key Assets inAbcd

As per ABCD approach there are 5 categories of asset inventories such as individuals, associations, institutions, physical assets and connections

1. **Individuals**: Every individual has got certain assets, gifts and qualities; such individual is at the center of ABCD approach.
2. **Associations**: Groups of people working with a common interest are critical to community mobilization.
3. **Institutions**: The assets of institutions help the community capture valuable resources and establish a sense of civic responsibility.
4. **Physical Assets**: Physical assets such as land, buildings, space, and funds are other assets that can be used.
5. **Connections:** These are the exchange between people sharing their assets by various methods.

Rural Advisory Services

Rural Advisory Services (RAS) provide the information and services needed and demanded by fishers and other actors in rural settings thereby assist in providing their livelihoods by developing their technical, organizational and management skills and practices (GFRAS, 2011; FAO, 2010). The large farms are mostly self-reliant and need only regulatory support, while medium-sized farms need mobilization and facilitation support in addition to regulatory support. Small aquaculture farms need more education and input provision alongside facilitation (Kumaran, 2014).

Role of RAS

- Provide management and business development support appropriate to the scale, resources and capacities of each fisherman.
- Help fishermen to better understand markets (prices, seasonality, standards, value addition etc.)
- Link fishermen to other stakeholders involved in provision of varied support and services
- Create platforms to facilitate interaction and sharing among the various stakeholders
- Promote institutional and policy change to enable and support family fish farms.

Market Led Extension Approach

In order to make agriculture more enterprising, extension professionals need to be pro-active beyond the regular objective of maximizing the productivity of the farmers/producers by transferring improved technologies rather farmers should be sensitized on various aspects of produce like quality, consumer's preference, market intelligence, processing and value addition and other marketing information. This will help the farming community to realize high returns for the produce, minimize the production costs, and improve the product value and marketability that may lead to realize the concept of doubling farmers' income. With the globalization of agriculture, emphasis on productivity and profitability to the farm enterprises increased and, therefore the demand driven agriculture (and allied sectors) has led to the paradigm shift from production-led extension to market led extension. There are many challenges in the agricultural marketing system which can be resolved through the efforts of market led extension models.

In this approach farmer/producer is viewed as an 'Agripreneur' who expects high returns 'Rupee to Rupee' from his produce by adopting a diverse baskets of package of practices suitable to local situations/ farming systems with optimum cost -benefit ratio (C:Bratio) ensuring maximum share of profit by exploring the market demand. Market led extension focuses on harnessing the ICT tools to access market intelligence including likely price trends, demand position, current prices, market practices, communication network, etc. besides production technologies.

For farmers, as the extension system is more credible source of farm technologies, the extension personnel ought to be knowledge and skill-oriented in relation to production and marketing of agricultural goods. Thus, revamping the extension system will have a catalytic role for ushering in farmer-led and market-led extension; which can subsequently alleviate poverty and ensure livelihood security. In this context, the

challenge is to motivate the extension personnel to learn the new knowledge and skills of marketing before assigning them marketing extension jobs to establish their credibility and facilitate significant profits for the farming community. SWOT analysis of the market, Organization of Farmers' Interest Groups (FIGs), capacity development, establishing linkage and synergy, harnessing ICTs, digital marketing etc. are the competencies required by the extension personnel in order to effectively implement market led extension.

Digital extension approach

Extension reforms brought a transformation in agricultural extension system through introduction of Information and Communication Technologies (ICTs). The ICT-enabled extension systemreferred to as Digital Extension has the potential for enabling the empowerment of farming communitiesby improving their access to information and sharing knowledgewith innovative e-agriculture initiatives (Saravanan, 2010a).

With the phenomenal growth in information and communication technology, use of IT application in agriculture will bring remarkable change in the attitude and knowledge level of user. Basic requirement is to provide most appropriate information in such a capsule that can be easily understood and used by them. This approach will strengthen the extension system for better dissemination of technology. As a case study the contribution of Digital Green, a NGO that uses an innovative digital platform for community engagement to improve lives of rural communities across South Asia and Sub-Saharan Africa is remarkable. Digital Green associate with local public, private and civil society organizations to share knowledge on improved agricultural practices, livelihoods, health, and nutrition, using locally produced videos and human mediated dissemination. *As per the study, the Digital Green project (participatory digital video for agricultural extension) increased the adoption of certain agriculture practices seven times higher compared to traditional extension services and the approach was found to be 10 times more cost-effective per dollar spent.*Hence, along with ICT-based advisory services, input supply and technology assessment need to be integrated for greater impact and content aggregation from different sources require to be sorted in granular format and customized in local language for rapid adoption of technologies (Balaji et al., 2007&Glendenning and Ficarelli, 2011).

The use of internet, mobile and video- conferencing assists the IT enabled farmers to utilize the facilities for their favors for which the most suitable permanent infrastructure is the basic requirement. Strong linkages need to be established between direct ICT interventions and it should be part of the national level program on agricultural development.

Conclusion

Vital role of extension in fisheries waste management mostly emphasizes on commercialization of technologies through public private partnership, commercialization through farmer producer organizations, implementing through sustainable extension approach, popularizing through awareness campaign and grassroots level problem analysis so that policy level changes can be achieved.

****

Engineering Tools and Technologies for Fish Processing: A Profitable Venture in Agri-Business

Manoj P.Samuel, S. Murali, Anies Rani Delfiya and P.V. Alfiya

Engineering Division,

ICAR-Central Institute of Fisheries Technology, Cochin

Email:manojpsamuel@gmail.com

Fisheries comprise a major economic activity within complex interactions between human beings and water - 'the first among equals' of the natural resources (Ahmed, 1992). Fisheries data assembled by the Food and Agriculture Organization (FAO) suggest that global marine fisheries catches increased to 86 million tonnes in 1996, then slightly declined. In the past three decades, employment in fisheries and aquaculture has grown at a higher rate than the growth of *world* population. The fishery engineering is evolving as an important domain in view of depleting stocks on both pre and post-harvest scenarios. It will also aid in fish processing technologies, optimizing energy and water use in seafood industries, mitigating climate change related issues and reducing carbon foot print. It is important to explore novel ways to obtain, quantify, and integrate industry responses to declining fishing stocks and increasing management regulations into fishery- and ecosystem-based management advice. The technological interventions help to reduce the wastage of fishes, which is otherwise a highly perishable commodity by preservation technologies and converting it into value added products with higher shelf life. Use of appropriate technologies along the fish value chain will help in producing better quality products and fetch more markets and higher price.

Major areas of technological interventions in the field of fishery engineering cover design and development of fish processing equipment and machineries, energy efficient and eco-friendly solar fish dryers, fuel efficient fishing vessels and fiberglass canoes, indigenous electronic instruments for application in harvest and post-harvest technology of fish, quality improvement of Indian fishing fleet and energy and water optimization techniques for fish processing industries. Focused areas include development of cost effective solar dryers with LPG, biomass, Infra-Red or electrical back-up heating systems, fish de-scaling machines, Fish freshness sensor etc.

1. Technologies For Fish Processing and Value Addition

Post-harvesting processing of fishes are important to reduce the wastage, increase shelf-life, add more value to the products and ensure higher returns. The major engineering interventions for fish post-harvest operations, processing and value addition are given below:

1.1 Solar dryers:

Out of total catch 30-40 % of fish is dried or processed for export and local consumption. Sun drying (open air drying) is the traditional method employed in most parts of the state to dry fishery products. It denotes the exposure of a commodity to direct solar radiation and the convective power of the natural wind. This form of energy

is free, renewable and abundant in any part of the world especially in tropical countries. Also it offers a cheap method of drying but often results in inferior quality of product due to its dependence of weather conditions and vulnerability to the attack of dust, dirts, rains, insects, pests, and microorganisms. Solar drying is an alternative which offers numerous advantages over the traditional method and environmentally friendly and economically viable in the developing countries. In solar drying, a structure, often of very simple construction, is used to enhance the effect of the solar radiation. Compared to the sun drying, solar dryers can generate higher air temperatures and consequential lower relative humidity, which are conducive to improved drying rates and lower final moisture content of the final products. However, there exist some problems associated with solar drying i.e. reliability of solar radiation during rainy period or cloudy days and its unavailability during night time. To overcome this limitation, an auxiliary heat source and forced convection system are recommended for assuring reliability and better control, respectively.

In a hybrid solar drying system, drying can be continued during off-sunshine hours by utilizing back up heat source and stored heat energy of daytime sunshine. In this way, drying becomes continuous process and the product is saved from possible deterioration by microbial infestation. These types of Hybrid solar dryers find useful applications in developing countries where the conventional energy sources are either scarce or expensive and the heat generating capacity of the solar system alone is not sufficient. Further, to assist the drying process (forced convection) in a hybrid dryer, a small blower is attached in between solar collector and drying chamber or inside the drying chamber which is powered by solar PV panels installed on drying chamber. Moreover, power from PV panels can be used for street lighting purpose. In addition, if the proposed setup is not used for drying purpose (kept idle), then the same can be used to draw hot water for domestic use. Therefore, in a single set up it is envisaged to have multiple utilities i.e. drying of fish, hot water and electricity generation.

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

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

ICAR-CIFT design includes small capacity dryers like solar tent dryers, natural convection dryers *etc*. which will be useful to dry fish hygienically during sunny days. Solar tunnel dryers, solar fish dryers with alternate electrical back up (SDE-10, SDE-20 and SDE-50) and solar fish dryers with fire wood or biomass alternate back up heating system (SDF-20, SDF-50) *etc*. can be efficiently used to dry fish using renewable solar

energy which is abundantly and freely available. The details of solar dryers with different backup systems are given below:

1.2 Solar Dryer with LPG back-up:

ICAR-CIFT designed and developed a novel system for drying of fish using solar energy supported by environment friendly LPG back up (Fig.1). In this dryer during sunny days fish will be dried using solar energy and when solar radiation is not sufficient during cloudy/ rainy days, LPG back up heating system will be automatically actuated to supplement the heat requirement. In the solar fish drier with LPG back up heating system, water is heated with the help of solar vacuum tube collectors installed on the roof of the dryer and circulated through heat exchangers provided in the PUF insulated stainless steel drying chamber loaded with fish. Thus continuous drying is possible in this system without spoilage of the highly perishable commodity to obtain a good quality dried product.

This dryer is ideal for drying of fish, fruits, vegetables, spices and agro products without changing its colour and flavour. It helps to dry the products faster than open drying in the sun, by keeping the physico-chemical qualities like colour, taste and aroma of the dried food intact and with higher conservation of nutritional value. Programmable logical Controller (PLC) system can be incorporated for automatic control of temperature, humidity and drying time. Solar drying reduces fuel consumption and can have a significant impact in energy conservation.

Fig.1. CIFT Solar-LPG Dryer

a. **Solar Dryer With Electrical Back-Up:** Effective solar drying can be achieved by harnessing solar energy by specially designed solar air heating panels and proper circulation of the hot air across the SS trays loaded with fish (Fig.2). Food grade stainless steel is used for the fabrication of chamber and perforated trays which enable drying of fish in a hygienic manner. Since the drying chamber is closed, there is less chance of material spoilage by external factors. An alternate electrical back-up heating system under controlled temperature conditions enables the drying to continue even under unfavourable weather conditions like rain, cloud, non-sunny days and in night hours, so that the bacterial spoilage due to partial drying will not occur. Improved shelf life and value addition of the

product fetches higher income for the fisher folk. The eco-friendly solar drying system reduces fuel consumption and can have a significant impact in energy conservation.

a. Fig.2 CIFT Solar-Electric Dryer

b. **Solar-Biomass Hybrid dryer**: A dryer working completely on renewable energy was designed and developed for eco- friendly operation. Solar Biomass Hybrid Dryer consists of well insulated and efficient solar air-heating panels, drying chamber, SS mesh trays, photo-voltaic cells, fans and biomass heating system (Fig.3). Hot air is generated by virtue of solar energy inside the heating panels and passed into the drying chamber. Continuous flow of hot air is maintained with the help of Photo Voltaic cells and fans to enable drying process. During cloudy days when sufficient solar energy is not available to maintain required temperature within the dryer, an alternate biomass heating system is manually actuated. Thus a fully green technology for fish drying is achieved by this.

Fig.3 CIFT Solar-Biomass Dryer

c. **Solar Tunnel dryer:** Solar tunnel dryer utilizes solar energy as the only source of heat for drying of the products. Heat absorbing area of 8 m^2 is made of polycarbonate sheet (Fig.4) . Products to be dried are placed on nylon trays of dimension 0.8X0.4 m. The dimensions of the whole drying unit is 2.21X2.10X0.60 m. The capacity of the dryer is 5 kg. Drying takes place by convection of hot air within the drying chamber. Apart from fishes, this dryer is also suitable for other agricultural products like fruits, vegetables and spices.

Fig.4 CIFT Solar-Tunnel Dryer

d. **Solar Cabinet dryer with electrical back-up:** This offers a green technology supplemented by electrical back up in case of lacunae in solar radiation. The dryer consists of four drying chambers with nine trays in each chamber (Fig.5). The trays made of food grade stainless steel are stacked one over the other with spacing of 10 cm. The perforated trays accomplish a through flow drying pattern within the dryer which enhances drying rates. Solar flat plate collectors with an area of 7 m^2 transmit solar energy to the air flowing through the collector which is then directed to the drying chamber. The capacity of the dryer is 40 kg.

Electrical back up comes into role once the desired temperature is not attained for the drying process, particularly during rainy or cloudy days.

Fig.5. CIFT Solar-Cabinet Dryer with Electrical back-up

e. **Infrared drying** – CIFT has recently developed an Infra Red (IR) dryer heat transfer is happening by radiation between a hot element(infrared lamps) and a material (to be dried). Thermal radiation is considered to be infrared in the electromagnetic spectrum between the wavelength of 0.78 μm and 1000 μm.Infrared emitters offer efficient heat and much more advantages compared to other conventional heat technologies:
   - No direct contact with the product
   - High drying/heating rate
   - Infrared radiation can be focused where it is needed in a defined time,
   - Cost savings thanks to high overall efficiency and optimal infrared heaters lifetime.

1.3 Fish Descaling Machines

(a) Fish descaling machine with variable drum speed: Fish de-scaling machine is designed and fabricated for removing the scales of fishes easily. This equipment can remove scales from almost all types/sizes/ species of fishes ranging from marine to freshwater species like Sardine, Tilapia to Rohu. The machine is made of SS 304 and has 10 kg capacity. It contains a 1.5 HP induction motor and a Variable Frequency Drive (VFD) to vary the speed of the drum depending on the variety of the fish loaded. The drum is made of perforated SS 304 sheet fitted in a strong SS Frame. Water inlet facility is provided in the drum for easy removal of the scales from the drum so that area of contact to the surface will be more for removal of scales. The water outlet is also provided to remove scales and water from the machine. An Electronic RPM meter was attached with the de-scaling machine which directly displays the RPM of the drum. Speed of the drum is a factor influencing the efficiency. The machine takes only 3-5 minutes to clean 10 kg fish depending on the size.

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

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

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

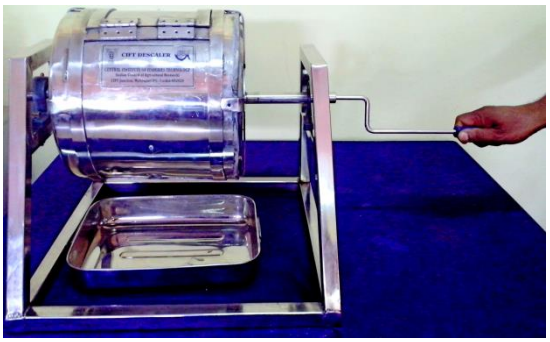


Fig.7 Fish de-scaling machine hand operated

1.4 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 customised for specimen during 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 utilising specimen other than for which the yield has been originally customised. The meat yield of this machine was about 60% against 35% in imported models. Capacity of the machine is 100kg/hour.

1.5 Modern Hygienic Mobile fish vending kiosk: Most of the fisher folk across India sell fish in an open basket without any hygienic practices. The fish is kept in an open bag or container, it loses its freshness.They use ice purchased at high cost for temporary preservation and at the end of the day, if the fish is not sold, they give it at a low rate to customers with little or no profit. More over fish gets contaminated under unhygienic handling practices. The fish vending persons, especially women folk find it difficult to carry the fishes as head load and subsequently sell it in the local markets or consumer doorsteps. In this context, the ICAR-CIFT have designed and developed a mobile fish vending kiosk for selling fish in the closed chilled chamber under hygienic conditions at consumer doorstep.

The major advantages of the new Kiosk are as follows:

- The mobile kiosk was designed considering the maximum weight that a man pulls on rickshaw.
- The mobile unit is mounted on frame with wheels at the bottom. The kiosk can carry 100kg fish with 20kg under chilled storage display in glass chamber and remaining in insulated ice box (developed by CIFT).
- The main components of the kiosk are fish storage & display chilled glass chamber, hand operated descaling machine and fish dressing deck with wash basin, water tank, cutting tool, waste collection chamber and working space.
- The vending unit has been fabricated mainly using stainless steel (SS 304 Food Grade) and frame and supports are made with MS and GI sheets.
- The kiosk main part *i.e* chilling unit & display for fish storage which was envisaged to power by solar energy through solar PV cells,however presently powered by AC current.
- The stored fish is covered with transparent glass cover through which consumer can see the fishes and select according to their choice of purchase.
- Kiosk is attached with hand operated descaling machine for removal of scales. The fishes coming out of descaler is free of scales, dirt or slime.
- It also reduces human drudgery and avoids cross contamination, consumes lesser time. Fish dressing deck with wash basin also designed conveniently to prepare fresh clean fish under hygienic conditions.

Chilling of fish using electricity/PV cells or by adding large quantity of iceadds to cost to the selling price. Since this technology has well insulated storage space for fish with

provisions for refrigeration, it reduces the ice melting rate and its cost, thereby reducing the selling price. The unit also extends the keeping quality of fish for 4- 5 days and increases marginal benefit to fish vendors. It also helps change the practice of unhygienic handling and marketing of fish.

1.6 Electronics and Instrumentation:

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

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

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

2. Commercialization and Agri-Business Incubation

Agri-Business Incubators (ABI) open new entry points in the agricultural value chains, which in turn can use to access new markets. They afford leverage through these entry points to accelerate agricultural development and offer the unique potential to develop small and medium-sized enterprises (SME's) which can add value along these chains in ways which other development tools do not offer. There is no single "right way" to perform agribusiness incubation. Rather the work of agribusiness incubation depends on the state of development of the agribusiness ecosystem and changes over time as that ecosystem matures and develops. In its earliest phases, incubators demonstrate the viability of new business models and look to create and capture additional value from primary agricultural products. In underdeveloped agricultural economies, incubators help by strengthening and facilitating linkages between enterprises and new commercial opportunities. They open new windows on technologies appropriate to agribusiness enterprises and help agricultural enterprises discover new, potentially more competitive ways of doing business. In subsequent phases of development, incubators operate as network facilitators: they link specialized service providers to

agribusinesses and link separate agribusinesses to one another. Finally, in a more advanced state of business development, incubators operate as conduits for the exchange of technology, products, inputs and management methods across national borders.

A more pragmatic system for business incubation and promoting start-up companies with respect to agricultural technologies have been evolved in recent times within the ICAR-CIFT.The Agri-Business Incubation (ABI) center along with Institute Technology Management Unit (ITMU) seeks to provide business consulting services to agriculture-related businesses and helps to develop a strategic business plan. ABIs facilities for incubation of new business ideas based on new agricultural technologies by providing cheap space, facilities and required information and research inputs. The Agribusiness Incubator Program also seeks to provide business consulting services to agriculture-related businesses and helps to develop a strategic business plan.

The Engineering Division of ICAR-CIFT has commercialized its technologies like solar fish dryers, fish descaling machines, refrigeration enabled fish vending machines etc through the ABI. In the financial year 207-18 itself, two entrepreneurs have taken up Solar fish drying technology and three start-ups came up by establishing CIFT designed fish vending kiosks. Three firms fish descaling machines were also successfully handed over to sea-food industries located both in Andhra Pradesh and Kerala. Apart from these, 10 numbers of fish dryers of 10 kg capacity were distributed among women SHG groups located in Kerala, Manipur and Assam for demonstration purposes. Furthermore, 3 incubatees(one physical and two virtual) have already registered under ABI in the current year for using engineering technologies.

Bibliography:

- Ahmed, M., D. Capistrano and M. Hossain. 1992. Redirecting benefits to genuine fishers: Bangladesh's new fisheries management policy. Naga: The ICLARM Quarterly, October 1992, p. 31-34.
- FAO.2014.The State of World Fisheries and Aquaculture- Opportunities and challenges.Food and Agriculture Organization of the United Nations Rome, 2014

****

Scope of Entrepreneurship Development in Fisheries

George Ninan

Fish Processing Division

ICAR- Central Institute of Fisheries Technology, Cochin

Email: george66jiji@gmail.com

Introduction

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

According to the recent statistics, the annual capture and culture based fish production in India is around 90, 00000 MT. Seafood export sector is one of major foreign exchange earner in India. In 2015-16, India has exported 945892 MT of Seafood worth Rs.30, 420 crores. USA and South East Asia are continued to be the major importers of Indian seafood. Frozen Shrimp continued to be the major export item followed by frozen fish. Marketing of value added products is completely different from the traditional seafood trade. It is dynamic, sensitive, complex and very expensive. Market surveys, packaging and advertising are a few of the very important areas, which ultimately determine the successful marketing of a new product. Most of the market channels currently used is not suitable to trade value added products. A new appropriate channel would be the super market chains which procure directly from the source of supply of the products and control most of the components of production and supply chain like packaging, advertising and retail marketing. Appearance, packaging and display are all important factors leading to successful marketing of any new value added product. The retail pack must be clean, crisp and clear and make the contents appear attractive to the consumer. The consumer must be given confidence to experiment with a new product launched in the market. Packaging requirements change with product form, target group, market area, species used and so on. The packaging technology needs to be evolved which should be attractive, convenient and adding to the shelf life of the processed products.

Technology developments in fish processing sector offer scope for innovation, increase in productivity, increase in shelf life, improve food safety and reduce waste during processing operations. A large number of value added and diversified products both for export and internal market based on fish, shrimp, lobster, squid, cuttlefish, bivalves etc. have been identified. However, the commercialisation of fish products still pose lot of challenges to the entrepreneur and researcher in terms of optimization of technologies

and ultimately developing the technologies into a commercially viable business plan. In this regard, the Indian Council of Agricultural Research (ICAR) has started a Business Incubation Unit at the Central Institute of Fisheries Technology (CIFT) exclusively for the Fisheries sector through the World Bank funded National Agricultural Innovation Project (NAIP). It is designed to accelerate the growth and success of entrepreneurial start-up efforts through the mobilization of an array of business resources and services. Later in 2016 an Agri-Business Incubation Centre (ABI) was established in CIFT under the XII plan scheme of National Agriculture Innovation Fund (NAIF) of ICAR. The role of the ABI Centre is to facilitate the innovator and the researcher to turn their ideas into commercial ventures with focus on incubation and business development programme, including entrepreneurship, skill development and Grassroot innovators activities.

Health benefits of fish

As a rich source of nutrients, fish provide a good balance of protein, vitamins and minerals, and a relatively low caloric content. In addition fish are excellent sources of Omega-3 polyunsaturated fatty acids which appear to have beneficial effects in reducing the risk of cardio- vascular diseases and are linked with positive benefits in many other pathological conditions particularly, certain types of cancer and arthritis.

Fish represents an excellent option as a major source of nutrients. On a unit caloric basis fish can provide a broad range of nutrients. A high intake of fish is compatible with a reduction of both calorie and saturated fatty acid intakes. Coronary heart disease, hypertension, cancer, obesity, iron deficiency, protein deficiency, osteoporosis and arthritis are contemporary health problems for which fish provide a number of nutritional advantages and some therapeutic benefits. Nutritional factors of importance are calories, proteins, lipids, cholesterol, minerals and vitamins.

Conventional finfish and fishes potentially provide from 100 to 200 kcal/100g, which is mainly attributed to the protein and fat contents of fish. The amount of carbohydrates in fish is very small. Finfish usually contains less than 1% carbohydrate whereas shellfish have very low fat content. Compared to other muscle food, they contribute very low fat calories to the average diet. For example, each gram of fish muscle provides only 0.05 – 0.2g of fat compared to 0.25 – 0.5 fat per gram of red meat. The most important constituent of fish muscle is protein. The protein content in fish varies from 17 to 25%, though values as low as 9% are sometimes encountered as in the case of Bombay Duck. Fish protein is highly digestible because of very low stroma protein and has an excellent spectrum of essential amino acids. Like milk, egg and mammalian meat proteins, fish protein has a high biological value. Cereal grains are usually low in lysine and/ or the sulfur containing amino acids, whereas fish protein is an excellent source of these amino acids. In diets based mainly on cereals, fish as a supplement can, therefore, raise the biological value significantly.

Fish oil contains primarily the Omega -3 series of fatty acids. The polyunsaturated components of fish lipids can be effective in reducing plasma lipids. Epidemiological data from Japan and the Netherlands indicate that frequent consumption of fish even in quantities as low as 30g/ day may have beneficial effects in reducing heart disease. Consumption of medium (100g) to large amounts especially triglycerides, prevent thrombosis and ameliorate ischemic heart disease. These effects are mediated by the Omega -3 PUFA of fish lipids which alter the production of certain biologically important components called eicosanoid. The efficiency of the Omega -3 PUFA

components is influenced by the amount ingested and the concentration of other unsaturated fatty acids in the diet, especially Omega -6 PUFA. Squalene, an isoprenoid molecule present in shark liver oil in higher quantities, has been reported to possess antilipidemic, antioxidant and membrane stabilizing properties. Fish and shellfish, particularly anchovies, clams, oysters and sardines are rich sources of vitamin B_{12}.

Fish consumption is compatible with optimum dietary practices / recommendations and that substitution of fish for other foods can help to maintain a balanced nutrient intake compatible with a low fat consumption. In addition, the consumption of fish- or more precisely, fish lipids – may provide significant health benefits.

Entrepreneurship Initiatives in Fisheries Sector

Fisheries sector with its important role played in the socio-economic development of the country has become a powerful income and employment generator, and stimulates the growth of a number of subsidiary small, medium and large scale industries. In order to translate the research results arising from the field of fisheries and other agricultural sectors, ICAR have set up an innovation based Business Incubation Centre (BIC) at the ICAR-Central Institute of Fisheries Technology (CIFT), Cochin. BIC is managed by Zonal Technology Management – Business Planning and Development (ZTM-BPD) Unit and aims at establishment of food business enterprises through IPR enabled ICAR technologies.

BIC supports operations on business projects as a measure of enhancing the foundation for new technology based industries and establishing a knowledge-based economy. It focuses on finding new ways of doing business in fisheries and allied agricultural fields by finding doors to unexplored markets. The Centre helps prospective entrepreneurs, by providing pro-active and value-added business support in terms of technical consultancy, infrastructure facility, experts' guidance and training to develop technology based business ideas and establish sustainable enterprises. It acts as a platform for the speedy commercialization of the ICAR technologies, through an interfacing and networking mechanism between research institutions, industries and financial institutions. The Incubator at ICAR-CIFT differs from traditional Business Incubators as it is tailored specifically for technology based industries and is operational at an area with a high concentration of fish production. This industry-specific incubator also allows new firms to tap into local knowledge and business networks that are already in place. BIC offers their services to industries not only in Cochin, but also all over India through virtual incubation. Beyond promoting business growth, the Centre is also trying to bring its benefits to all the fisheries communities in India.

This unique Business Incubator is now known as a "One Stop Shop", where entrepreneurs can receive pro-active, value-added support in terms of technical consultancy, and access to critical tools such as entrepreneur ready technologies, vast infrastructure and other resources that may otherwise be unaffordable, inaccessible or unknown. With the aim of transforming the incubator into a symbol of entrepreneurship and innovation, the ZTM-BPD Unit has created an environment for accessing timely scientific and technical assistance and support required for establishment of technology based business ventures. The activities of the ZTM-BPD Unit focuses on finding creative and innovative ways for linking public sector resources and private sector initiatives within and across regional and national boundaries for promoting economic growth. The Centre uses the right expertise in relevant fields to

identify and analyze the constraints and barriers hindering the growth of a business, and devise appropriate strategies. It explores the various structures and strategies to help small enterprises to grow and ensure a promising future in the global market. It fosters corporate and community collaborative efforts, while nurturing positive government-research-business relationships.

Process of Incubation

The Business Incubation Centre targets entrepreneurs, from fledgling start-ups 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 nine 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. BIC apart from being a multi-tenant facility with on-site management that delivers an array of entrepreneurial services to clients operating with the facility, it also serves clients that are not located in the facility through virtual incubation or incubation without walls.

The Centre regularly conducts industry interface and technology promotional programmes for sensitization of entrepreneurs and to identify interested potential candidates for physical and virtual incubation. The Clients at BIC gets the privilege of meeting Scientists, Business Manager and Business Associates directly, to discuss and finalise the strategies to be adopted to take the business forward. It is also the peer-to-peer relationships that develop within the incubator, that ensures the delivery of basic services such as how to actually incorporate a business; what are the legal issues; how to take intellectual property protection; how to do basic accounting and cash flow; how to do business presentations etc. Those kinds of skills are what are transmitted as part of the incubation process.

The residency period for direct incubatees is normally for two years, 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. Each incubatee of the Unit will have to pay to the Institute a charge for utilization of space, at a rate concessional to the benchmark rate which is the prevailing market rent realizable. Incubatee mentoring will continue in virtual mode after graduation, on need basis.

Services and facilities offered by ICAR-CIFT Business Incubator

The Centre through its business support services provides links to supporting industries; upgrade technical / managerial skills; provide scientific / technical know-how; assist in market analysis, brand creation and initial test marketing; protect IP assets; and find potential investors and strategic partners.

Incubation facilities under one roof are:

- Furnished office suites within the premises of ICAR-CIFT, with shared facilities like secretarial assistance, computing, copying, conferencing, video conferencing, broad band internet and communication services.
- Pilot level production lines

- Culinary facility
- Access to modern laboratory facilities for product testing and quality control
- Access to well-equipped physical and digital libraries

Pilot Level Production Lines

A state-of-the-art generic semi-commercial production facility is made available to incubating entrepreneurs for developing value added products from fish. BIC provides access to these facilities along with support of manpower, and assists the entrepreneurs in production and testing of new product formulations. For the tenants, the pilot plant is an ideal testing arena to determine the commercial viability of new products. The plant also serves as a process lab, a place to see how processing equipment impacts food products under varying conditions. There are production lines for pre-processing, cooking, retort pouch processing, canning, sausage production, extruded products, chitin & chitosan, smoking, curing & drying, breading & battering and product packaging. By providing access to these resources, the Centre greatly reduces one of the major barriers to the commercialization of institute technologies by smaller firms - the high capital cost of intermediate or large scale process equipment.

Business Services

The business oriented services offered by BIC include assistance in complying with business regulations and licensing procedures, financing, information services, marketing, and tailor-made services designed for the various tenant enterprises. Incubator clients can also gain special advantage in terms of tax savings through special regulations for Business Incubators. BIC also offers a wide variety of services, with the help of strong associations throughout the Business Incubation Network

Conclusion

Fish processing and value addition has evolved over the years as the sunrise sector in Agriculture domain. Globally many new species are being introduced in the Aquaculture sector. A comprehensive study on the suitability of these species for value addition has to be carried out to propose optimized utilization protocols. Functional fish products will be in much demand in future; the challenge will be to retain the functional benefits of fish & shellfish meat by way of adopting product specific processing protocols or alternate delivery systems for sensitive components. These issues offer ample scope for Innovation coupled with entrepreneurial skills for the creation of wealth and employment in fisheries sector.

****

Labour Challenges in Fisheries Processing Industries

Nikita Gopal

Extension, Information and Statistics Division

ICAR-Central Institute of Fisheries Technology, Cochin

Email: nikiajith@gmail.com

Introduction

FAO observes that fish was one of the most traded food items in the world and an estimated 35% of all fish produced in the world, about 60 million tonnes worth about USD 143 billion, entered international trade in 2016 (FAO, 2018). The average per capita annual fish consumption was to the tune of 24.9 kg in developed countries, 20.5 kg in other developing countries, 12.6 in LDCs and 7.7 kg in low-income food-deficit countries (Table). This shows the key role fish plays in nutritional security of the world.

Total and per capita apparent fish consumption by region and economic grouping

Region/ economic Grouping	Total food fish consumption (million tonnes live weight equivalent)	Per capita food fish consumption (kg/year)
World	148.8	20.2
World (excluding China)	92.9	15.5
Africa	11.7	9.9
North America	7.7	21.6
Latin America and the Caribbean	6.2	9.8
Asia	105.6	24.0
Europe	16.6	22.5
Oceania	1.0	25.0
Developed countries	31.4	24.9
Least-developed countries	12.0	12.6
Other developing countries	105.4	20.5
Low-income food-deficit countries	20.8	7.7

Source: FAO, 2018

More than 50% of of trade in seafood is from the developing to the developed world and the net trade income was valued at US$ 37 billion in 2016 (UNCTAD, 2018). Trade is essential for meeting demand from consumers along with economic expectations of

countries. The increase in trade has been driven by globalization and liberalization of trade barriers and facilitation of freer trade between countries. Seafood is a major foreign exchange earner for several developing countries, and provides employment and income to millions of people. Fish is traded in different forms from live to value added and even or non-food uses; and is supported by a processing sector, which can be vary in scale from small to very large. The processing industry itself has undergone tremendous changes from traditional methods of processing to more advanced and sophisticated processing technologies, inclduing increasing machine based processing in developed countries. The harvest, procesing and codnsumption of fish can be in different countries. Almost 45% of fish is consumed in fresh, live or chilled form followed by forzen (31%), preserved (12%), cured – salted, smoked or dried (12%) forms (FAO, 2018). Also almost 56 % of consumption in developing countries is catered to mostly in frozen or prepared forms, which is the major market for processed seafood from the developing countries.

World fisheries and aquaculture production and utilization

	2012	**2013**	**2014**	**2015**	**2016**	**2026**
Production (million tons)						
Capture						
Inland	11.2	11.2	11.2	11.4	11.6	
Marine	78.4	79.4	79.9	81.2	79.3	
Total Capure	**89.5**	**90.6**	**91.2**	**92.7**	**90.9**	**91.7**
Aquaculture						
Inland	42.0	44.8	46.9	48.6	51.4	
Marine	24.4	25.4	26.8	27.5	28.7	
Total aquaculture	**66.5**	**70.3**	**73.8**	**76.6**	**79.5**	**102.1**
Total world fisheries and aquaculture	**157.8**	**162.9**	**167.2**	**169.2**	**170.3**	**193.9**
Utilization (in million tons)						
Human consumption	136.9	141.5	146.3	148.8	150.9	177.4
Non-food uses	20.9	21.4	20.9	20.3	19.4	16.3
Population (billions)	7.1	7.2	7.3	7.3	7.4	8.1

Per capita food fish supply (Kg)	**19.3**	**19.7**	**20.1**	**20.3**	**20.4**	**21.6**

Sourced from UNCTAD, 2018

Original Source: OECD-FAO (2017), FAO, (2018). Source of population figures: United Nations, 2015.

Labour in the Sector

Fisheries and aquaculture have important roles in providing employment to millions of people in the world. FAO estimates that about 10-12% of the world's population may be employed in these sectors and 60 million people are directly and about 200 million people otherwise employed along the fisheries value chain (FAO, 2016), in activities as diverse as land based work in harbours, in processing facilities; and in other services.

Seafood exports from India

With a production of 35,99,693 tonnes in 2016 from marine and 14,62,063 tonnes from inland capture and 57,00,000 tonnes fronm aquaculture, India is one of the largest producers of fish in the world (FAO, 2018). It is also one of the top exporters of saefood with 5546 million USD and a share of 3.9% during the same year, with an annual average grwoth rate of 12.1% during 2006-16.

This seafood export is supported by a strong processing sector that has established itself ovr the past few decades. There are 551 processing plants with an insatlled capacity of 27813.81 MT, of which 313 plants are EU approved plants (http://mpeda.gov.in). Besides, there is a total storage capcity of 366315 MT , which included cold storage, chilled storage, dry fish storage and other storage.

Fish processing is a major post harvest activity and will involve multiple activities:

- Gutting and cleaning (for domestic markets)
- Pre-processing (for further processing)
- Processing (in factories for export)
- Drying, salting curing, smoking (largely traditional, catering to domestic markets)

Employment in the organisedfish processing sector at various levels depending on the activity profiles of the industry, includes, the shop floor workers where the actual processing work takes place; the middle level and top level management; the loading/unloading workers at the shop floor; the supervisors (at the shop floor); the quality control professionals etc. In unorganized and traditional processing there is more informality in employment.Thetotal workforce in seafood processing is not readily available, but several region or country based assessments are available (https://www.tsic.org.au; http://www.fpsc-ctac.com http://www.iuf.org).

Labour related policies in fisheries

While there are no specific policies related to fish processing workers, several international and national policies cover the workforce in the industry. Some of the international covenants available are given below:

Access to decent forms and conditions of employment are enshrined in the Sustainable Development Goals. SDG8 is on 'Decent work and economic growth'. This was, among other things, necessitated because of '…..widening inequalities, and not enough jobs to keep up with a growing labour force.' The targets specifically mentions 'decent job creation' and '…. achieve full and productive employment and decent work for all women and men, including for young people and persons with disabilities, and equal pay for work of equal value' (http://www.undp.org/content/undp/en/home/sustainable-development-goals/goal-8-decent-work-and-economic-growth.html).

The ILO has set out the core labour standards (https://www.ilo.org) that are applicable in all employment situations. They are as follows:

- Freedom of association and the effective recognition of the right to collective bargaining (Convention No. 87 & No. 98)
- The elimination of all forms of forced and compulsory labour (Convention No. 29 & No. 105)
- The effective abolition of child labour (Convention No. 138 & No. 182)
- The elimination of discrimination in respect of employment and occupation (Convention No. 100 & No. 111)

With special reference to fisheries, concerned specifically with work on board fishing vessels is the Work in Fishing Convention, 2007 (No. 188). The Committee on Fisheries (COFI) of the FAO has also in its various Sessions decided on '…..legally mandated rights to decent working conditions…..' and '…..give priority to ensure decent working and living conditions in small scale fisheries……..' (http://www.fao.org/3/a-i5980e.pdf).

The four pillars of decent work are:

Employment & Enterprise Development Availability of an adequate number of productive, quality jobs, which provide income to cover atleast basic needs	**Social protection** Protection from work-related injury and from lack of income due to unemployment, illness or age	**Standards & Rights at Work** Fundamental rights: Freedom of choice & equality of treatment, freedom of association & opportunity at work	**Governance & Social dialogue** Participation to decision-making about work conditions and representation of interests in negotiations

Source:http://www.fao.org/3/a-i5980e.pdf

While the international covenants give the broad framework for developing specific policies, countries have their own policies to regulate work in industries, including fish processing industries. The Ministry of Labour& Employment of the Government of India looks into policy making on labour and employment. An important national policy relates to ***Safety, Health and Environment at Work Place***. Policy making is guided by

provisions under the Constitution as well in line with international instruments (https://labour.gov.in/policies/safety-health-and-environment-work-place). In the goals of the policy mention is made of 'providing a statutory framework on Occupational Safety and Health in respect of all sectors of industrial activities….' and in objectives '…..continuous reduction in the incidence of work related injuries, fatalities, diseases, disasters and loss of national assets'. Several acts support policy and some of them that are general in nature are applicable to the fish processing sector as well, like 'those related to compensation, wages, insurance and provident fund, maternity, contract labour (regulation), inter-state migrant workers, unorganized workers etc. which are enacted under various sections like Industrial Relations, Industrial Safety & Health, Child & Women Labour, Social Security, Wages, Labour Welfare, Employment, Labour Reforms etc.

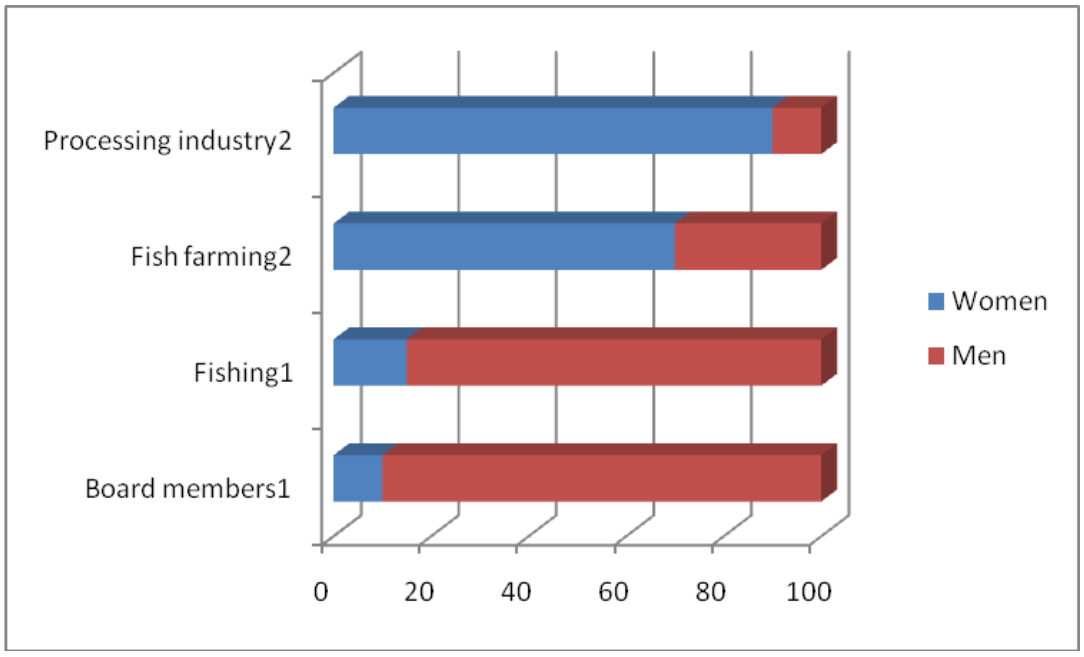
The EU has a Common Fisheries Policy (https://eige.europa.eu/..) that looks at employment in the sector, including Fisheries, Aquaculture and Processing. Individual states have their own bills and regulations for the sector. Several other international guidelines are also formulated like the Environmental, Health, and Safety Guidelines for Fish Processing by the World BankGroup (https://www.ifc.org/wps/wcm/.....).

Work in the seafood processing sector

Work in the processing sector can be in organised processing plants or in the unorganized sector where traditional processing activities are carried out.

Traditional processing activities are curing –salting, smoking or drying. These activities are generally small scale and are community or homestead based. They are also mainly carried out by women. The major issues that arise in these activities are in relation to the repetitive nature as well as the conditions of work. Smoking results in release of gases that may be harmful to the persons involved. Sun drying exposes the women and men to harsh weather conditions and possibilities of sun burns. Excessive handling of salt and water also results in injuries to the palms.

Organized processing work involves primary processing like grading, peeling, cutting, gutting, washing of fish and shell fish or may involve other processing steps like brining, cooking, freezing, canning etc. Other jobs like handling, loading and unloading are also carried out. More than 80% of the workforce in seafood processing iswomen . (Monfort, 2015; World Bank 2010; Siason et. al. 2002; Jeebhay et al. 2004, Gopal et. al., 2009; Gopal et. al., 2007, De Silva, 2011; FAO, 2012; Ancy, 2016).. The processed product is then set into marketable forms or sizes before being frozen. Frozen products are once again packed by the women workers. The characteristics feature of this job is the need for dexterity and skill, but the work is repetitive and involves drudgery. The skill and dexterity that the women possess as well as the patience and ability to bear drudgery are the precise reasons for the domination of women in the sector. Montford (2015), in her summary of various studies observes that the women are preferred because they 'are perceived to be trustworthy, dedicated, meticulous, flexible, compliant, quality minded and cheaper than men.' However, sadly, they are still categorized as semi or unskilled in many countries.



1 WSI Article 2018 https://wsi-asso.org/media/

2 FAO. World Bank 0%

1The International Organisation for Women in the Seafood Industry (WSI)conducted a 'Gender on the Agenda' online survey from September to December 2017. Complete results are available at : https://wsi-asso.org/wsireports/

The work environment is generally cold as a very perishable commodity is being processed. This leads to exposure problems for most of the women involved in this job.Also slippery floors may cause slips and falls and injuries thereof. Constant standing or squatting leads to musculoskeletal disorders and repetitive strain injuries ((Gopal et. al., 2007; Jeyanthi et. al., 2015; Gopal et. al., 2016; Garcia and de Castro, 2017). Use of sharp tools may also lead to injuries. Constant exposure of the hands to ice, cold water and the raw material which could harbour pathogens also leads to infections of the palms.

India

Most factorieshave a health check-up done before the start of the season. In all the processing plantssurveyed, visiting doctor provision has been made usually on a monthly interval forcheck-ups. A few factories also reimburse the employees' medical bills. However, itis observed that they do not undergo any regular or periodic medical check-ups. The only source of information and entertainment is television and newspapers. However, all the workers possess mobile phones.

Jobs can be repetitive and this can lead to fatigue and drudgery. Infrastructure may not always be adequate to meet personal hygiene requirements and women develop bladder related issues. Since work is related to availability of raw material and wages piece rate, there is practically no break time for the women workers. Work is generally done individually and spaces are confined with little interaction among workers. Shifts during the night may also lead to sleep disorders. There is also a risk of exposure to chemicals. The industry is increasingly catered to by migrant labour and this brings in its wake issues of cultural differences.

To minimize occupational health and safety issues proper guidelines are available, implementation of which can reduce the risks associated (https://www.ifc.org/wps/wcm/.....) like following sector-specificrecommendations for accident prevention, including providing workers with training in the proper use andmaintenance of cutting equipment and personalprotective equipment. Plants should be so designed that process flows smoothly. Several guidelines like provision of hand rails, separate transport corridors, enclosed conveyer belts, etc. will ensure safe work environments. Use of gloves to prevent cold bites and infections from pathogens is also recommended. Proper ventilation, protective clothing, lighting, temperature control, workspace design to minimize ergonomic distress can be ensured.

Conclusion

The issues associated with labour working in the different nodes of the fisheries value chain have been existent since the time fishing has been an avocation. While there are areseveral international and national laws addressing labour issues, the on the sector level policy formulation and program implementation need to be strengthened to ensure safe and decent work and working conditions for fishers and fish workers.

References

- Ancy, V.P. and K.V. Raju. 2016. Seafood Export Processing Sector in Kerala – Issues and Challenges. International Journal of Development Research, Issue 04, pp. 7575-7582.

- De Silva, D. 2011. Faces of Women in Global Fishery Value Chains: Female Involvement, Impact and Importance in the Fisheries of Developed and Developing Countries.
- FAO. 2016. Scoping study on decent work and employment in fisheries and aquaculture: Issues and actions for discussion and programming
- FAO. 2012. The State of World Fisheries and Aquaculture 2012. FAO, Rome (also available at www.fao.org/docrep/016/i2727e/i2727e00.htm).
- FAO. 2018. The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.
- Garcia, Gabriel Macasiray and de Castro, Butch. 2017. Working Conditions, Occupational Injuries, and Health Among Filipino Fish Processing Workers in Dutch Harbor, Alaska, Workplace Health Saf. 2017 May; 65(5): 219–226. doi:10.1177/2165079916665396
- Gopal, N, Geethalakshmi V, Unnithan G R, Murthy L N and Jeyanthi P. 2007. Women in the Seafood Processing Sector in the Post Globalization Scenario - An Analysis. Paper presented at 2nd Global Symposium on Gender and Fisheries held during the 8th Asian Fisheries Forum, 21 November 2007, Kochi_available at http://wif.icsf.net/icsf2006/jspFiles/wif/bibliography/biblioHome.jsphttp)
- Gopal, N., Jeyanthi, P., Geethalakshmi, V., &Unnithan, G. R. 2009. Indian finfish exports–an analysis of export performance and revealed comparative advantage.
- Gopal, N., Sruthi. P., Chacko Babu and LiyaJayalal. 2016. Women Workers in the Seafood Processing Sector Of Kerala, India - Structural Changes Due To Migration, The NIEW Journal The Voice of the NAM Woman, Gender, Women empowerment and Sustainability, Volume (2016), p.32-41
- Gopal, N., Geethalakshmi V, Unnithan G R, Murthy L N and Jeyanthi P. 2007. Women in the Seafood Processing Sector in the Post Globalization Scenario - An Analysis. Paper presented at 2nd Global Symposium on Gender and Fisheries held during the 8th Asian Fisheries Forum, 21 November 2007, Kochi_availableat (http://wif.icsf.net/icsf2006/jspFiles/wif/bibliography/biblioHome.jsphttp)
- Gopal, N., Geethalakshmi, V., Unnithan, G. R., Murthy, L.N., and Jeyanthi, P. 2009. Women in Seafood Processing, Yemaya, Issue 30
- http://mpeda.gov.in
- http://www.fao.org/3/a-i5980e.pdf
- http://www.fpsc-ctac.com
- http://www.iuf.org
- http://www.undp.org/content/undp/en/home/sustainable-development-goals/goal-8-decent-work-and-economic-growth.html
- https://eige.europa.eu/..
- https://labour.gov.in/policies/safety-health-and-environment-work-place
- UNCTAD (2018) Achieving the targets of Sustainable Development Goal 14: Sustainable fish and seafood value chains and trade BACKGROUND NOTE for the

Second Oceans Forum 16–17 July, 2018 Geneva, Switzerland _Available at https://unctad.org/meetings/en/SessionalDocuments/Background-Note-Second-Oceans-Forum-July2018-v4.pdf

- https://wsi-asso.org/media/
- https://www.ifc.org/wps/wcm/
- https://www.ilo.org
- https://www.tsic.org.au
- Jeebhay, M. F, T. G Robins, and A. L Lopata. 2004. World at work: Fish processing workers. Occupational and Environmental Medicine 61 (5): 471–474.
- Jeyanthi,P., Nikita Gopal, L. N. Murty, V. Geethalakshmi, 2015. Employment Status of Women in the Seafood Processing Sector of Gujarat, Fishery Technology, Vol 52, No 2, 135 – 139
- Monfort, M. C. 2015. The Role of Women in the Seafood Industry. GLOBEFISH Research Programme, Vol. 119, Rome, FAO 2015. 67 pp.
- OECD-FAO, 2017. Fish and seafood, in OECD-FAO Agricultural Outlook 2017-2026, OECD Publishing, Paris. DOI: http://dx.doi.org/10.1787/agr_outlook-2017-12-en
- Siason, I. M., Tech, E., Matics, K. I., Choo, P. S., Shariff, M., Heruwati, E. S., Susilowati, T., Miki, N., Shelly, A. B., Rajabharshi, K. G., Ranjit, R., Siriwardena, P. P. G. N., Nandeesha, M. C. and Sunderarajan, M. 2002. Women in fisheries in Asia. In: Global Symposium on Women in Fisheries: Sixth Asian Fisheries Forum. 29 November 2001, Kaoshiung, Taiwan. Williams, M. J., Chao, N. H., Choo, P. S., Matics, K., Nandeesha, M. C., Shariff, M., de, I., Tech, E. and Wong, J. M. C. (eds.). ICLARM-The World Fish Center. 209pp.
- World Bank. 2010. Field Research Data for the West African Regional Fisheries Programme in Sierra Leone. Freetown: Statistics

****

Biodata of Trainees

Name	Zainabu Hamisi Kilusungu
Country	Tanzania
Designation	Fisheries Officer
Address	P.O.BOX 9152 Dar-Es-Salaam Tanzania
Mobile	+255 655 944 141
E-mail	h.zainabu@yahoo.com
Date of Birth	01/04/1987

Educational Qualification

Masters	Life Science And Engineering
Bachelors	B.Sc. Aquaculture

Professional experience

Designation	Institution	Year	
Tutor	Fisheries Education and Training Agency (FETA)	2012	2015
Fisheries Officer	Ministry of Livestock and Fisheries	2015	Till date

Areas of Research/Interest:

- Fish feeds regimes and growth performance of fishes fed different locally available fish feeds
- Antimicrobial resistance profile genes in microbial isolates from Fish and Marine Environment

Name	Ihsan K. Sulaiman
Country	Iraq
Designation	Veterinarian
Address	Veterinary Hospital, Iraq Slah al deen, Tekreet Iraq
Mobile	009647711114629
E-mail	Ihsan.bazony@gmail.com
Date of Birth	22/07/1989

Educational Qualification

Bachelors	Veterinary Medicine and Surgery

Professional experience

Designation	**Institution**	**Year**	
Veterinarian	Veterinary Hospital	May 2013	Till date

Areas of Research/Interest:

- Fish diseases and Management of Aquaculture

Name	Tariq Mosawee
Country	Afghanistan
Designation	Fishery officer
Address	Bazarak District, Panjshir Province, Afghanistan
Mobile	0093783571020
E-mail	mosaweetariq@gmail.com
Date of Birth	22/04/1988

Educational Qualification

Bachelors	Faculty of Agriculture , Department of Agronomy

Professional experience

Designation	Institution		Year
Extension Worker	Panjshir DAIL	2012	2017
Fishery Officer	Panjshir DAIL	2017	Till date

Areas of Research/Interest:

- Fresh water farming

Name	Moe Theingi Hlaing
Country	Myanmar
Designation	Lecturer
Address	Department of Industrial Chemistry East Yangon University Myanmar
Mobile	8137048639 +959799684238, +959402245962
E-mail	moetheingi@gmail.com moetheingihlaing.dr@gmail.com
Date of Birth	26.5.1975

Educational Qualification

Others	LCCI Level 1 and 2
Ph.D	Ph.D. (2015) Thesis: An investigation into the quality improvement of some marine based food products
Masters	M.Sc. (Industrial Chemistry)
Bachelors	B.Sc. (Industrial Chemistry)

Professional experience

Designation	**Institution**	**Year**	
Demonstrator	Yangon University	2005	2012
Assistant Lecturer	Dagon University	2012	2016
Lecturer	Yadanabon University	2016	2018

Areas of Research/Interest:

- Related to extraction, preparation, production and standirization process, chitosan, chitin and gelatin is very interesting. It is carried out accompany with RSM.

Name	Khansaa Bdraldein Osman Ahmed
Country	Sudan
Designation	Assistant Researcher
Address	Animal Resources Research Corporation Fisheries Research Centre- Khartoum- Sudan Telephone No. 00249—912345891/ +249912808696 Email sfsaf-i@hotmail.com
Mobile	+249119429316/ +249919654099
E-mail	Khansabdr90@gmail.com
Date of Birth	20/9/1988

Educational Qualification

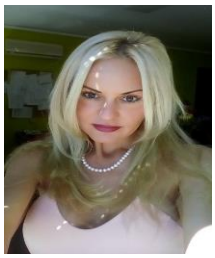
Masters	Master of Fish Science and Technology (2017) - Sudan University of Science and Technology - College of Animal Production Science and Technology
Bachelors	Bachelor of Science (Honours) in Fisheries and Wildlife Science (2012) - Sudan University of Science and Technology - College of Veterinary Medicine and Animal Production

Professional experience

Designation	Institution	Year	
Assistant Researcher	Fisheries Research Centre- Khartoum- Sudan	30/08/2018	Till date

Areas of Research/Interest:

- Fish Technology - Disease and Microbiology - Biotechnology



Name	Jaroslava Svarc-Gajic
Country	Serbia
Designation	University Professor
Address	Faculty of Technology Bulevar cara Lazara 1 21 000 Novi Sad Serbia
Mobile	+38163549664
E-mail	jsgajic@gmail.com
Date of Birth	2 March 1976

Educational Qualification

Ph.D.	Faculty of Technology, University of Novi Sad, Doctorate on Technical Sciences, Applied and Engineering Chemistry, 2006. Title of PhD thesis: *Chronopotentiometric stripping analysis of mercury at glassy carbon.*
M.Sc.	Faculty of Technology, University of Novi Sad, Applied and Engineering Chemistry, 2002. Title of master degree thesis: *Chronopotentiometric stripping analysis of selenium using mercury film electrode.*
B.Sc.	Faculty of Technology, University of Novi Sad, Pharmaceutical Engineering, 1993-1998. Title of bachelor degree thesis: *Extraction and isolation of flavonoids from Ginkgo biloba*(*Ginkgo biloba* L.)

Professional experience

Designation	Institution	Year	
Research assistant	Faculty of Technology, University of Novi Sad	1999	2006
Assistant professor	Faculty of Technology, University of Novi Sad	2006	2011
Associate professor	Faculty of Technology, University of Novi Sad	2011	2016
Professor	Faculty of Technology, University of Novi Sad	2016	-

Areas of Research/Interest:

- Subcritical water extraction/decomposition; modern extraction techniques; sample preparation; isolation and characterisation of natural bioactive compounds. Bioactivity of phytochemicals.Valorisation of biowaste. Development of functional food, pharmaceutical and cosmetic products. Instrumental analysis. Development and application of electroanalytical, chromatographic and spectroscopic techniques in trace analysis. Quality control of food, environmental and pharmaceutical samples.

Name	Inayaturahman Aazem
Country	Afghanistan
Designation	Planning & Program Manger
Address	Wardak Province, Agriculture Directorate, Afghanistan
Mobile	00930771069696
E-mail	inayat.aazem@gmail.com
Date of Birth	6 March 1991

Educational Qualification

Bachelors	In Agriculture(Animal Sciences)

Professional experience

Designation	**Institution**	**Year**	
Planning& program Manger	Wardak Province Agriculture Directorate	7/5/2015	TILL DATE
Regional Officer	National Horticulture and livestock Project(NHLP)	1/1/2014	30/4/2015

Areas of Research/Interest:

- Fresh water fish farming and Management, Planning Donor based projects

Name	Frank Festo Kabitina
Country	Tanzania
Designation	Fish Technologist
	Ministry Of Live Stock and Fisheries
	P.o Box 2847
Address	DODOMA
	Phone +255 2861910
	Email; ps@uvuvi.go.tz
Mobile	+255 712 037 056
E-mail	kabitinafrank@gmail.com
Date of Birth	23/01/1984

Educational Qualification

Others	Diploma in Fish Processing, Quality Assurance and Marketing, Fisheries Education and Training Agency
Bachelors	B.Sc. in Economics of Development, The Mwalimu Nyerere Memorial Academy

Professional experience

Designation	Institution	Year	
Fish Technologist	Ministry of Livestock and Fisheries	2014	Till date
Assistant Researcher	Tanzania Fisheries Research Institute.	2012	2014
Inspection Technician	Tanzania Bureau of Standards	2011	2012

Areas of Research/Interest:

- Fisheries Economics, Natural resources and Environmental Economics.

Name	Balogun Bukola Bimbo
Country	Nigeria
Designation	Business Development Officer
Address	Abuja Chamber of Commerce And Industry, Km, 8, Airport Road, Abuja, Nigeria
Mobile	07035573135
E-mail	florabimbo35@gmail.com
Date of Birth	1 December 1992

Educational Qualification

Others	Science Laboratory Technology (Biochemistry option)

Professional experience

Designation	Institution	Year	
Business Development Officer	Abuja Chamber Of Commerce And Industry	January 2018	Till date

Areas of Research/Interest:

- Small Scale Business Development

Name	Benfares Redhouane
Country	Algeria
Designation	Director of Division Research and Researcher
Address	National Center for Research and Development of Fisheries and Aquaculture (CNRDPA) 11, Bd Amirouche, PO Box 67, BouIsmaïl 42415 Tipaza, Algeria
Mobile	+213670211642
E-mail	benfaresredhouane@yahoo.fr
Date of Birth	12/02/1982

Educational Qualification

Ph.D	2010-2017 Biology (Vegetal Biotechnology)
Masters	2005-2009 Biology (Vegetal Biotechnology)
Engineer	2000-2005 Biology (Quality Control and Analysis)
Bachelors	2000 Natural science

Professional experience

Designation	**Institution**		**Year**
Senior Quality Inspector	Wilaya Trade Authority	2008	2010
Researcher	National Center for Research and Development of Fisheries and Aquaculture (CNRDPA)	2010	2017
Director of Division Research	National Center for Research and Development of Fisheries and Aquaculture (CNRDPA)	2017	2018

Areas of Research/Interest:

- Biotechnology, Biochemistry and Nutrition of Fisheries and Seaweed