ICAR sponsored (SCSP) capacity building training programme

Marine Bioprospecting: Extraction and Quantification approaches

(12th December to 17th December 2022)

Course Director

Dr. Suseela Mathew

Course Coordinators

Dr. Renuka V

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BIOCHEMISTRY & NUTRITION DIVISION

ICAR- CENTRAL INSTITUTE OF FISHERIES TECHNOLOGY

CIFT ROAD MATSYAPURI, WILLINGDON ISLAND

KOCHI, KERALA 682029

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Foreword

ICAR-Central Institute of Fisheries Technology is the leading institute in the field of harvest and post-harvest sector including the nutrition quality of seafood. Fish and fishery products are not only nutritionally important but also important in global trade as foreign exchange earner. The marine environment is a relatively unexplored source of functional ingredients that can be used in food processing, storage, and fortification in a variety of ways. There is currently much interest in biologically active compounds derived from marine resources, especially compounds that can efficiently act on molecular targets, which are involved in various diseases. I am very happy to note that Biochemistry and Nutrition Division of ICAR-CIFT regularly impart training to the researchers /Academicians / college students from different parts of country who gets a hands on training experience in modern analytical techniques in the bioactive compounds. To cater to the needs of researchers, there is pressing need for manual that details about the extraction, characterization and analysis of bioactive compounds in a systematic way. I am sure that the techniques and lectures described in this book will be a very useful guide for students, researchers and teachers working in the field of life science and biochemistry and support to the candidates preluding to the laboratory works.

Dr. George Ninan
Director
ICAR-CIFT, Kochi

Contents

ICAR- CIFT INTERVENTION IN MARINE BIOPROSPECTING

Dr. George Ninan
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Introduction

Marine ecosystems have a high diversity of living organisms compared to terrestrial ecosystems providing numerous resources for human nutrition and health. Marine resources have received great attention recently; research on marine-derived molecules has discovered new bioactive compounds with important properties increasing their applicability as nutraceuticals in the food and supplement industries. During the past several years, an array of biologically active molecules has been extracted/isolated and purified from numerous sources of marine origin with the aid of distinct techniques and methodologies for newer applications. ICAR - Central Institute of Fisheries Technology (ICAR-CIFT) set up in 1957 is the only national center in the country where research in all disciplines relating to fishing and fish processing is undertaken. The institute started functioning at Cochin in 1957 with three Research centers function at Veraval (Gujarat), Visakhapatnam (Andhra Pradesh) and Mumbai (Maharashtra). Realising the paramount significance of marine nutraceuticals, ICAR-CIFT is working in the various research arenas staring from the harvest aspects of marine resources, designing and development of energy efficient fishing systems for responsible fishing and sustainable management, other basic and strategic research in fishing and processing, development of implements and machinery for fishing and fish processing etc. Some of the notable contributions from ICAR-CIFT in the marine nutraceutical sector is briefed below:

Chitosan

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). It has a number of commercial and possible biomedical uses. Chitosan is produced commercially by deacetylation of chitin. Chitin $(C_8H_{13}O_5N)_n$ is a long-chain polymer of a N-acetylglucosamine, a derivative of glucose, and it is found in many places throughout the natural world. It is the main component of the cell walls of fungi, the exoskeletons of arthropods, such as crustaceans (like the crab, lobster and shrimp) and the insects, including ants, beetles and butterflies, the radula of mollusks and the beaks of the cephalopods, including squid and octopuses. ICAR-CIFT has developed a method for the extraction of chitin from shrimp shell waste The wet prawn shell collected from the peeling

centers is initially converted in to chitin which is then converted to chitosan by a chemical process deacetylation. Then the alkali free dried and powdered chitosan can be bagged in polythene lined HDPE (high density polythene) woven sacks.

Technology Benefits:

- Chitosan find various industrial applications like, biotechnology, food processing, pharmacy and medicine.
- Boiler chicks diet with chitin was found to improve the feed efficiency, resulting in about 10-12% weight gain in the birds compared to a chitin free diet
- Use of chitin for the production of Glucosamine hydrochloride finds applications in antibiotics and baby food formulations.
- Chitosan can be used as sizing material for textiles
- It can be used as a water/ wine clarifying agent and also in the preparation of cosmetics and pharmaceuticals etc.
- Recent studies have shown the effectiveness of Chitosan (in the form of microfined powder) impregnated gauze and film for treatment of chronic wounds and external ulcers and to arrest/ minimize bleeding in brain surgery.

Glucosamine hydrochloride

ICAR-CIFT has developed a method for preparation of glucosamine from the shells of shrimp, lobster, or crab. Glucosamine is also fight joint inflammation and inhibit the production of enzymes that destroy cartilage. It plays a major role in lubricating joints, increasing their mobility and strengthening of cartilage. Glucosamine is commonly used in the treatment of osteoarthritis.

Collagen Chitosan Membrane for Plastic surgery and Dentistry

ICAR-CIFT has developed a Collagen chitosan membrane derived from collagen of fish air bladder and chitosan from prawn shell is intended to be used as an artificial space making barrier over periodontal bony defects- specifically in infrabony 2-3 walled defects and grade II furcation defects in dentistry and as covering membrane preventing fluid loss and blood loss in burns/wounds during healing. It finds applications in plastic surgery also. Collagen-Chitosan films are flexible, tough, transparent, clear and oxygen permeable with good tensile strength.

Development of a seaweed NutraDrink:

Based on a novel solvent free extraction of sulphated polysaccharide and phenolics from seaweed, a nutraceutical drink (NutraDrink) was developed. A macroporous adsorbent resin was used to selectively remove the compounds responsible for seaweed off-smell.

Iron-Calcium-Fortified-Fish Soup Powder (FSP)

FSP was developed with the objective of addressing micronutrient malnutrition with special reference to improving iron levels in vulnerable population. Intervention among adolescent girls of West Jaintia Hills District, Meghalaya using fortified FSP showed promising trends in blood hemoglobin levels.

Chitosan based vitamin microparticles

Microencapsulation of thiamine and pyridoxine Vitamin B1 and Vitamin B6 were encapsulated with vanillic acid grafted chitosan and subsequently spray dried. The wall material was synthesized following optimized protocol, starting with 20 g of chitosan and 20 g of vanillic acid to obtain microparticles of encapsulated thiamine and pyridoxine. Dietary supplementation of Thiamine - pyridoxine-vanillic acid-grafted chitosan is an effective means to prevent cardiovascular disease.

Fish oil Powder

A stable fish oil powder was developed using emulsion and encapsulation technology. Cardioprotective effect of encapsulated fish oil powder was stablished in cardiomyoblast cell lines.

Fish oil rich in PUFA supplementation effects the mRNA and protein expression of enzymes of lipid metabolism. Fish consumption has been associated with several health benefits as demonstrated by epidemiological studies worldwide. The positive effects are thought to be due to the predominance of n-3 PUFA in fish. To elucidate the possible mechanisms of action, we used RNA expression studies for determining the level of expression of four genes in liver of wistar strain rats namely acetyl co a carboxylase (ACC), fatty acid synthase (FAS) and steroyl Co A desaturase-1 (SCD-1) in response to feeding fish oil. Using western blotting technique the levels of ACC, FAS and SCD-1 enzymes expressed in the liver were determined.

Development of a co-delivery system of Betalain and PUFA

Multiple emulsification and microencapsulation followed by spray drying was adopted to prepare stable PUFA. Microencapsulation is one of the promising methods that can minimize oxidative deterioration of ω-3 oils by converting into a stable free-flowing powder.

Development of stable squalene powder

Microencapsulation of squalene with maltodextrin and whey protein isolate gave an encapsulation efficiency of 96% and oxidative stability of more than four months. Wall material optimization for squalene encapsulation led to the development of stable emulsion of chitosan (CS) and whey protein isolate as the emulsifier. Cakes fortified with squalene microencapsulated with chitosan-whey protein complex had superior oxidative stability and textural quality.

Fucoxanthin

Supercritical fluid extraction of Fucoxanthin and lipid from brown seaweed Sargassum sp. Fucoxanthin content in the extract was determined by HPLC and found to be 1.5 mg/g of the extract. The extract was also found to be rich in different saturated and unsaturated fatty acids. Since fucoxanthin and seaweed lipids are known to possess bioactivities, the extract could potentially be used as nutraceutical supplement.

Seaweed based dietary fibre

Dietary fibre from *G.edulis, S.wightii* and *U.lactuca* extracted under optimized conditions were analysed for their physicochemical and functional properties like FT- IR analysis, hydration properties (water holding capacity and swelling capacity), oil holding capacity and antioxidant properties (total phenolic content, DPPH free radical scavenging activity and reducing power assay).

Nutraceutical potential of seaweeds

Nine tropical potentially edible seaweeds from Phaeophyta and Chlorophyta were studied for their nutritional composition, mineral content and nutraceutical potential. The seaweeds considered for the studies were green (*Ulva reticulata, Valoniopsis sp., Boodlea composita, Caulerapa sertularioides*) and brown (*Sargassum johnstonii, Padina gymnospora, Padina tetrastromatica, Cystoseira indica, Dictyopteris australis*). Acetone and chloroform extracts of Ulva lactuca showed antibacterial activity towards *Salmonella typhimurium, Morganella morganii, E.coli, Listeria monocytogenes* and *Staphylococcus aureus.*

Proteoglycans

ICAR-CIFT has Extracted, purified and characterized proteoglycans from *E. brucus*. Cytotoxic effect of proteoglycans from *E. brucus* against He La (Breast cancer) cell lines was elucidated.

Myctophids

Myctophid biochemical database was developed. Biochemical and nutritional evaluation of mycotphid fishes was carried out. Fatty acid profiles of Myctophid fishes were profiled and

compared. Fatty acid profile of Myctophid fishes were compared with common food fishes. Myctophid fishes were found to be rich in omega-3 Polyunsaturated Fatty Acid such as DHA. Myctophid fishes were solar dried and dried fishes were subjected to Supercritical Fluid Extraction for fatty acid extraction.

Conclusion

Marine Biomolecules have the potential to be used in a broad spectrum of products such as: food, biofuels, chemicals, cosmetics, medicines, etc. ICAR-CIFT is working on the novel frontiers of marine biomolecules research starting from developing sustainable extraction protocols for obtaining marine bioactives, structural elucidation, their detailed in vivo and in-vitro bioactivities and finally commercialization of these technologies. However, promotion of marine Biomolecules as nutraceuticals is still needed by generating consumer awareness for the acceptance of products even on a regional basis. Presently, the Institute is working towards the Comprehensive utilization of Marine Biomolecules by applying biorefinery approach in line with the UNs Sustainable development goals.

MARINE BIOMOLECULES: PRESENT & FUTURE PROSPECTIVE IN HUMAN HEALTH CARE

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Introduction

Nutraceutical based research has shown continuous growth and the progressive approach owing to the increased awareness among the general public regarding its significance in consumption and overall well-being. Researchers across the globe are hence working on to explore the possibilities to extract and isolate bio-active compounds from both terrestrial and marine sources. Nutraceutical is a combination of two words, "nutrition" and "pharmaceutical," and the word nutraceutical was coined by Stephen L. DeFelice in 1989 (Wildman *et al*., 2006). Nutraceuticals are food products of natural origin from both terrestrial and marine sources having healthcare importance. The word nutraceuticals comprise of variety of products derived from terrestrial and marine sources (isolated nutrients, dietary supplements, and genetically engineered designer foods, herbal products, processed foods, and Beverages). Recent report says that nutraceuticals provides a positive healthcare approach with tremendous therapeutic impacts on human body (Das *et al*., 2012; Bagchi *et al*., 2015). The nutraceutical industry has identified a wide range of phytochemicals described as phytoestrogens, terpenoids, limonoids, glucosinolates phytosterols, polyphenols, carotenoids, flavonoids, isoflavonoids, and anthocyanidins having therapeutic effects on human health as antioxidants, anti-inflammatory, antibacterial, antiallergic, anti-fungal, chemopreventive, immunomodulatory etc., (Gupta and Prakash, 2014; Karwande and Borade, 2015).

Classification of Nutraceuticals

Based on the bio-functional properties of bioactive compounds from terrestrial and marine sources are classified into following –

1. Dietary Supplements
2. Functional foods
3. Medicinal food

Dietary Supplements

A dietary supplement, as defined by the Food and Drug Administration (FDA), is a product intended to supplement the diet by increasing the total daily intake, or an extract, metabolite, concentrate, constituent, or combination of at least one of the following dietary ingredients: vitamins, minerals, herbs or other botanicals, amino acids (FDA, 2022).The "Dietary constituents" can be bioactive components comprising of amino acids, vitamins, minerals, fibres, important metabolites, and certain enzymes. The dietary supplements also include extracts available in tablets, capsules, powders, liquids, and in any other dosage form (Radhika *et al*., 2011).

Functional Food

The term "Functional foods have become popular in the scientific world, and mostly adopted in twenty first century by the food and nutritional researchers who are currently working on how to solve various crises arising from the degenerative diseases, Functional foods are foods derived from natural origin enriched in nutrients and are being fortified with essential nutrients (Jones, 2002). As per the Health Canada, functional food defines a regular food with an ingredient having specific therapeutic effect along with nutritional value (Wildman *et al*., 2006). Whereas in Japan, functional foods are assessed on the basis of three important standards: (1) functional foods must be derived from natural sources and consumed in their native state instead of processed in different dosage forms like tablet, capsule, or powder; (2) consumed regularly as a part of daily diet; and (3) exert a dual role in prevention and management of disease and contribute in biological processes (Arai, 1996).

Medicinal food

Medical foods are foods that are specially formulated to be consumed internally under the supervision of a physician, which is intended for the dietary management of particular disease that has distinctive nutritional needs that cannot be met by normal diet alone. Dietary supplements and functional foods do not meet these criteria and are not classified as medical food. (Radhika *et al*., 2011).

Nutraceuticals from marine sources

Chitin and chitosan

Chitin, a cationic amino polysaccharide, is a natural biopolymer composed of *N*-acetyl-d-glucosamine with β (1 → 4) glycosidic linkages. The term chitosan is used when nitrogen content of chitin is more than 7% by weight or the degree of deacetylation is more than 60% (Peter *et al*.,

1986; Gagne and Simpson 1993). Chitosan, also known as deacetylated chitin, is a naturally occurring polycationic polysaccharide derived from partial deacetylation of chitin. Chitin and chitosan can be obtained from the bio-waste generated from both terrestrial and marine sources. Chitin is abundant in the marine organisms like lobster, crab, krill, cuttlefish, shrimp, and prawn. Chitosan finds extensive application in multidimensional sectors, such as in food and nutrition, biotechnology, material science, drugs and pharmaceuticals, agriculture and environmental protection, dental and surgical appliances, removal of toxic heavy metals, wine clarification, industrial effluent treatment, etc. (Se-Kwon, 2010).

Glucosamine Hydrochloride

Glucosamine is obtained from the crustacean waste (Xu and Wang, 2004; Tahami, 1994). Glucosamine is part of the structural polysaccharides such as chitosan and chitin, which is present in the exoskeletons of crustacean and other arthropods. Though, glucosamine was discovered long back, market for glucosamine has gained popular interest due to its health benefits. Dietary supplementation of glucosamine (glucosamine sulphate, glucosamine hydrochloride, or N-acetyl-glucosamine) is proven to promising biomolecule for the treatment of osteoarthritis, knee pain, and back pain (Houpt *et al*., 1999; Luo *et al*., 2005). It is also known for its unique properties like anti-cancer, anti-inflammatory and antibacterial effects (Nagaoka *et al*., 2011).

Chondroitin sulphate

Chondroitin sulphate is a key component of the cartilage extracellular matrix (ECM). It is considered a member of the glycosaminoglycans (GAGs) family and is an unbranched sulphated, highly water-soluble anionic polysaccharide. Shark cartilage is found to be a good source of chondroitin sulphate. It has been shown that the incorporation of CS in scaffolds for cartilage tissue engineering induces the chondrogenic differentiation of mesenchymal stem cells (MSCs). CS provides a microenvironment that enhances clustering of cells (pre-cartilage condensation of mesenchymal cells), upregulates cartilage-specific genes, and provides cell-mediated degradable sites for the cell clusters to grow further and produce ECM. Studies have also demonstrated that CS increases the compressive stiffness of collagen scaffold (Rashidi et al., 2022).

Hyaluronic acid (HA)

Hyaluronic acid (HA) is a polysaccharide composed of alternating d-glucuronic acid and N-acetylglucosamine, which are naturally present in cartilage and synovial fluid. Compared with other polysaccharides, HA affects the regulation of cartilage function and repair of cartilage damage in many ways. Previous studies have demonstrated that HA can improve the lubricity of

cartilage boundaries, regulate inflammation at cartilage lesions, promote cell adhesion and proliferation, and ameliorate cartilage ECM deposition and cartilage regeneration, all of which have excellent application prospects in cartilage tissue engineering (Wang et al., 2022). HA can be obtained from the bio-waste like fish eyeball and it is also present in the cartilage matrix of fishes. HA finds several biomedical applications such as in drug delivery, tissue engineering applications, gene delivery applications, targeted drug delivery, tumor treatment, environmental applications and sensors (Mathew *et al*., 2017).

Collagen, gelatin and collagen peptides

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. Collagen derived from marine sources is finding wide applications in various sectors due to its biocompatibility, biodegradability, high cell adhesion properties and weak antigenicity (Yamada *et al*., 2014). Another major application of collagen is to act as a source for extraction of collagen hydrolysates, peptides, gelatin and gelatin peptides. Collagen peptides are reported to have bioactive properties like antioxidant, antimicrobial, antihypertensive, metal chelating, tyrosinase inhibitory, immunomodulatory, neuroprotective, antifreeze, wound healing, cell-proliferation, activities (Zhuang *et al*., 2009; Chi *et al*., 2014). Gelatin, the denatured form of collagen, by virtue of its surface active properties finds extensive applications in food, pharmaceutical and biomedical industries. Gelatin peptides are reported to have antihypertensive, antioxidant properties. The major difference between fish and mammalian gelatin lies in the iminoacid composition, viz, proline and hydroxyproline contents. (Mathew *et al*., 2017).

Fish lipids

Fish is considered as a good source of high quality, easily digestible protein rich in essential aminoacids (AHA 2012). Moreover, fats and oils from fish is an excellent dietary sources of long chain highly unsaturated fatty acids of omega-3 type such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) which are not contained in the fats of terrestrial animals or in vegetable oils. The major omega-3 PUFA, such as eicosapentaenoic acid (EPA C20:5) and docosahexaenoic acid (DHA C22:6) are very much essential for human beings, and hence are considered as essential fatty acids. The intake of long chain omega-3 PUFA is promoted by many health organizations owing to the health benefits associated with it. An average intake of 0.2 g and 0.65g of EPA and DHA a day is recommended by the European Academy of Nutritional Sciences (EANS) and International Society for the Study of Fatty Acids and Lipids (ISSFAL) respectively

(Dedeckere, *et al*., 1998). The American Heart Association recommends adults eat fish (in particular fatty fish) at least two times per week.

Squalene

Squalene, a bioactive isoprenoid, have evoked an unparalleled interest in pharmaceutical, drug delivery, cosmeceutical and clinical arenas by virtue of its wide range of bioactivities such as antioxidant, chemopreventive, anticancerous, antilipidemic, membrane stabilizing properties, immune system enhancer, antiaging, detoxification etc. It is widely present in nature, such as wheat germ, rice bran, shark liver and olive oils and among all the sources identified, shark liver oil is considered to be the richest source accounting for about 40% of its weight. Based on its diverse bio-active properties, squalene finds applications in field of biomedical, cosmetic, drug delivery systems and even in food industries.

Minerals

Marine organisms especially fish are considered as important source of minerals such as sodium, potassium, calcium, phosphorous and magnesium. Fish bone which is often discarded after the removal of protein is an excellent source of calcium and hydroxy apatite. Being rich in minerals, fish bone powder can be fortified into several food products. However, for fortification, the fish bone should be converted into an edible form by softening its structure by pre-treatment with hot water or hot acetic acid or superheated steam. Calcium powder processed from the backbone of tuna is a potential nutraceutical. It can be used to combat calcium deficiency in children. Fortification of calcium in foods helps consumers in meeting the calcium requirements and may reduce the risk of osteoporosis.

Nutraceutical industry in India: Current scenario and future trends

During the year 2015, global nutraceutical industry, valued at US$ 182.6 billion and is one of the fastest growing industries today and expected to grow at a Compound Annual Growth Rate (CAGR) of 7.3% from 2015 to 2021. As on today, the United States, Europe and Japan account for about 93% of the total global nutraceutical market and seems to have attained maturity in all three major regions. Hence, nutraceutical industries across the world are now showing their interest to emerging markets like India and China. Nutraceuticals industry in India is one of the rapid growing markets in the Asia-Pacific region. As per the record, the nutraceuticals industry in India is worth about US$ 2.2 billion and is expected to grow at 20% to US$ 6.1 billion by 2019-2020.

Innovative work done at Central Institute of Fisheries Technology, Cochin

By adopting grafting and micro-encapsulation technology, ICAR-Central Institute of Fisheries Technology, Cochin has developed some of the nutraceuticals products, such as thiamine and pyridoxine-loaded vanillic acid-grafted chitosan microspheres; sardine oil loaded vanillic acid grafted chitosan microparticles; microencapsulated squalene powder; vanillic acid and coumaric acid grafted chitosan derivatives; thiamine and pyridoxine loaded ferulic acid-grafted chitosan. These nutraceuticals products were shown to have health beneficial and immunomodulatory response in animal models.

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SEAWEED BASED NUTRACEUTICALS – PRESENT AND FUTURE PROSPECTS

Lekshmi R.G.Kumar, Tejpal C.S., Anas K.K., Renuka V & Suseela Mathew

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Seaweeds, commonly known as marine macroalgae are photosynthesizing plants that constitutes a major biomass in the intertidal zone. They are a varied group, with sizes ranging from a few centimeters to 100 m in length. In general, they are divided into three main classes based on pigmentation, green (chlorophytes), red (rhodophytes), and brown (phaeophytes). As seaweeds lack many of the distinct organs (roots, stems, leaves) found in terrestrial plants, whole parts can be used as a source of food, cosmetics, and other products. They have high nutritional value, in both fresh and dried forms, and act as ingredients in a wide variety of prepared foods.

Seaweeds were being used as traditional food and complementary medicine. Recently, it has gained popularity as a functional food too owing to the presence of potent biological molecules in it. It is being used directly or indirectly as a functional food ingredient. They are low caloric food but rich in vitamins, minerals and essential trace elements, polyunsaturated fatty acids, bioactive metabolites, proteins, polysaccharides and dietary fibres.

Bioactive compounds from seaweeds

Polysaccharides and Sulphated Polysaccharides

Polysaccharides are the most important macro-molecule in seaweed constituting around more than 80% of its weight. Seaweeds contain a high total dietary fibre content: 10–75% for brown seaweed, 10–59% for red seaweed, and 29–67% for green seaweed. Seaweeds are particularly rich in soluble dietary fibre, which accounts for 26–38%, 9–37%, and 17–24% in brown, red, and green seaweed, respectively. The major polysaccharides from phaeophyceae (brown algae) include alginates, laminarin, sargassan, fucoidans, sulphated galactofucans and ascophyllans. Polysaccharides derived from Rhodophyceae (red algae) include floridean starch, agars, carrageenans, xylans, galactans, sulphated galactans and sulphated rhamnans. Chlorophyceae contain sulphated galactans, xylans and ulvans as the major polysaccharides. Among the seaweed polysaacharides, the one which have spurred great deal of interest in the last decade is fucoidan. Fucoidans are a class of sulfated polysaccharides mainly composed fucose in the cell walls of brown algae, and they have demonstrated various activities, including anticancer, antioxidant, antiobesity, anti-

inflammatory, antimicrobial, antiangiogenic, immunomodulatory and neuroprotective activities (Holdt and Kraan, 2011). Fucoidans may play a role as dietary fiber uptake contributing to lower cancer incidence risk (Tiwari et al., 2015).

Proteins and aminoacids

The proportion of protein in seaweed ranges up to 45% DW and it differs from species, season and geographical area. Seaweed based proteins and peptides are proven to have antioxidant, antihypertensive, and anticoagulant activities. Seaweeds are widely recognized as cheaper protein alternative source due to its high-value proteins containing essential amino acids. The protein content in brown, green and red algae is 1–24%, 4–44% and 5–50% of the dry weight respectively. The major proteins in seaweeds include lectins and phycobiliproteins (Aneiros & Garateix, 2004). Phycobiliproteins are water-soluble and coloured components of the photosynthetic system in red macroalgae.

Lipids

Seaweed lipids mostly contain long-chain fatty acids, especially polyunsaturated fatty acids (PUFA) with 18- and 22- carbon atoms, depending on species. In general, lipid in seaweed ranges from 0.4% to 5% DW, with abundant saturated fatty acids (SFA) and palmitic acid in all species. Likewise, essential fatty acids (EFA) and PUFA were found abundant in brown seaweeds followed by green and red seaweeds which can regulate blood pressure and reduce the risk of cardiovascular diseases, osteoporosis, diabetes etc. (Maeda et al., 2008). Furthermore, green seaweeds like *Ulva pertusa* are reported to have sufficient amounts of hexadecatetraenoic, oleic, and palmitic acids (Ortiz et al., 2006).

Minerals

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

Vitamins

Algae are the richest source of vitamins almost contain all essential and non-essential vitamins in it. Numerous seaweeds like Porphyra umbilicalis, Himanthalia elongata and Gracilaria changii contains a high level of vitamin C compared to land vegetables. It has been reported that vitamin C is present in very high amounts of 2000 mg/kg dry matter in red seaweed *Eucheuma denticulatum* and 3000 mg/kg dry matter in green seaweed *Enteromorpha flexuosa* (McDermid and Stuercke, 2003). Furthermore, B group vitamins, especially thiamine and riboflavin are found in substantial amounts in most red and brown seaweeds whereas vitamin E content is higher in brown seaweed. Hence, it can be said that seaweeds are good source of vitamins also.

Fucoxanthin

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

Bioactive properties of seaweeds

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

Anti-coagulants are therapeutics which have ability to prevent blood coagulation or stop the formation of blood clots. Heparin, a sulfated polysaccharide is one of the most common anti-coagulant drugs used in the world against thromboembolic disorders (Fan et al., 2011). However, because of the several side effects associated with it, scientists are looking for suitable alternatives

for heparin. Sulfated polysaccharides of anti-thrombotic and anti-coagulant properties have been isolated from different marine algae. Fucoidan, a sulphated polysaccharide from seaweeds are reported to display strong anticoagulant properties. The degree of its anticoagulant property is related to its sulphate and polysaccharide content. It was reported that C-2 sulfate and C-2, 3 disulfate in fucoidans is mainly associated with anti-coagulant activity. The outcomes of many studies have proposed that fucoidans can be used as suitable alternatives to heparin and even certain fractions of fucoidan can be qualified as heparinoids.

Sulfated polysaccharides from marine algae are reported to have effects on innate immunity by modulating the ability of immune cells to produce nitric oxide and thereby reducing inflammation (Leiro, Castro, Arranz, & Lamas, 2007). The two important biomolecules from marine algae, fucoidan and arabinogalactan are reported to have immunomodulating effects. As fucoidan can influence the activation and maturation of human monocyte-derived dendritic cells, it can be used for cancer immunotherapy. Fucoxanthin, another marine bioactive compound, has shown anti-inflammatory activities both in vitro and in vivo assays. Because of its strong anti-inflammatory properties, it can be comparable with predinisolone, a commercially available steroidal anti-inflammatory drug.

Marine algae are reported to possess an extensive range of bioactive compounds which can be used to cure various types of cancers. Many of these compounds have been found to destroy tumor cells by initiating apoptosis or activating signalling enzymes that affect cell metabolism and eventually lead to cell death, however the clinical trials are limited due to the risk factors.

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

Future prospects

Seaweeds are being used as food in many Asian countries. However, the increasing scientific evidences suggests its wider application in various sectors such as biomedical, nutraceutical and cosmetic arenas. However, Apart from all the scientific aspects, another important lacuna associated with the seaweed research is the considerably low consumer awareness about health

benefits of seaweeds. Hence, these all issues need to be properly addressed before this resource can be trapped for its benefits at a large scale.

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MARINE BIOACTIVE COMPOUNDS AND THEIR ROLE IN HUMAN HEALTH CARE IMPORTANCE

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

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

Protein based bioactive compounds

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

Marine Proteins

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

Fish muscle proteins and microalgae contains all the essential amino acids in close to the right proportions for humans. Spirulina, for example, has high protein content (60% to 70%), with great balance of the essential amino acids and bioavailability. Spirulina appears to be one of the most important microalgae used by humans. A daily supplement of Spirulina is believed to reduce allergy symptoms in human being.

Collagen

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

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

Gelatin

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

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

Marine Peptides

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

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

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

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

Amino Acids

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

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

Minerals

Fish frames and bones would be a great potential source of high quantity minerals. In the total mass of fish bone nearly 60-70% is made up of minerals such as calcium, phosphorous and hydroxyapatite. Consumption of small fish along with bones in the regular diet will prevent the calcium deficiency. Since fish bones are the good source of hydroxyapatite, it can be extracted

from fish processing waste. It is mainly used in medical and dental field as a bone graft material for produce artificial bone.

Enzymes

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

Natural Pigments

The photosynthetic pigments are bioactive compounds that are able to capture solar energy. They are used by autotrophs for photosynthesis. For macroalgae, the major pigments are carotenoids and chlorophylls. These pigments are formed by algae, plants, fungi, and other microorganisms; however, humans and animals require ingesting them in their diets. Dietary carotenoids have nutritional and therapeutic importance since they act as provitamin A, which is converted into vitamin A. Carotenoids are known to be active agents for the protection against cancer, Cardio vascular disease, and macular degeneration. Microalgal formation of carotenoids, including β-carotene and astaxanthin, is an active area of research as they can be present at relatively high concentrations. β-Carotene is one of the major natural colorants and it has been employed to a vast spectrum of food and drinks in order to enhance their aspect. Moreover, β-carotene with intense antioxidant properties helps to reduce the harmful effects of free radicals, which have been related to various life-threatening conditions, such as different kinds of cancer, CHD, premature aging, and arthritis.

Lipids and fatty acids

The long-chain omega-3 fatty acids such as eicosapentaenoic acid (EPA, C20:5) or docosahexaenoic acid (DHA, C22:6) are the most common omega-3 fatty acids generated from marine sources which have been well documented as essential for human health. Humans are incapable of synthesizing PUFAs with more than 18 carbons thus, they should get them from food. Seafood are the major sources of long-chain PUFAs, although the synthesis actually occurs in the algae eaten by the fish. The amount and composition of these oils depend on the species, season and location of catching sites. The long chain fatty acids help to regulate the blood clotting and

blood pressure, and develop function of the brain and nervous systems. They also decrease the risk of many chronic diseases such as arthritis, diabetes and obesity. Moreover, PUFAs regulate inflammatory responses by producing inflammation mediators called eicosanoids (Lordan and others 2011). The rate of omega-3 to omega-6 of macroalgae is close to ideal, therefore they are used as dietary complement as part of a balanced diet.

Sterols

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

Marine polysaccharides

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

Chitin

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

Chitosan

Chitosan is the most important derivative of chitin. The term chitosan usually refers to a family of polymers obtained after chitin deacetylation to varying degrees. In fact, the acetylation degree, which reflects the balance between the N-acetyl glucosamine and D-glucosamine residues, differentiates chitin from chitosan. When the Degree of acetylation is lower than 50%, the product

is named chitosan and becomes soluble in acidic aqueous solutions. Chitin can be converted to chitosan by enzymatic preparations or chemical process. Chemical methods are used extensively for commercial purpose of chitosan preparation because of their low cost and suitability to mass production.

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

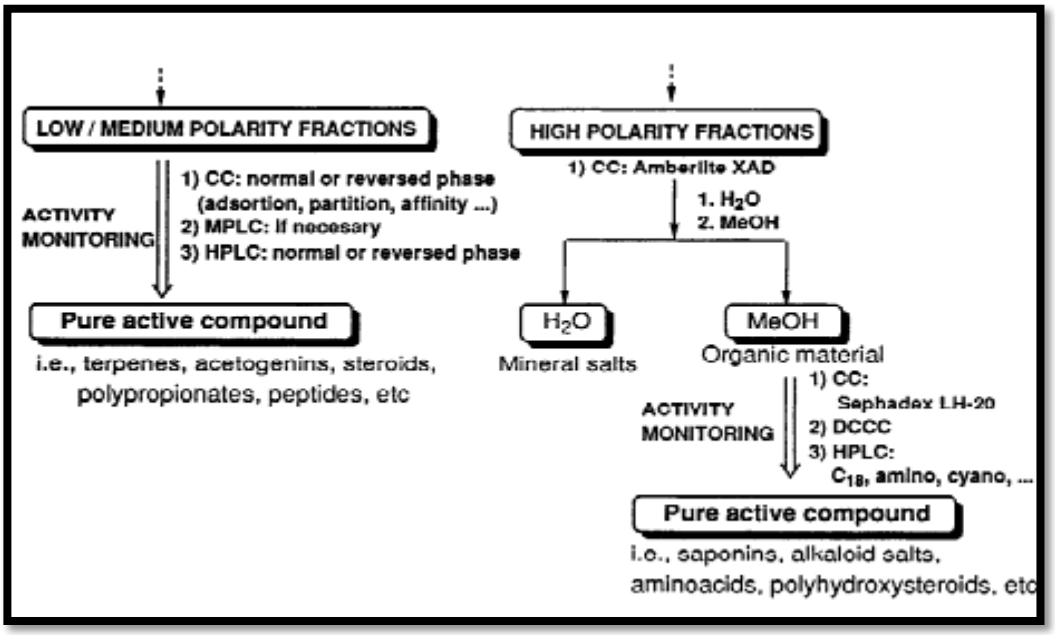

Figure 1: Procedure for isolating bioactive compounds from low/medium and high polarity fractions (adopted from Ricardo Riguera, 1997)

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

Table 1: Potential bioactive compounds obtained from different marine sources

Conclusion

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

FISH OIL: NUTRACEUTICAL AND BIOMEDICAL APPLICATIONS

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Fish oil is the primary natural long chain omega-3 fatty acid source containing two human health beneficial fatty acids. They are rich in polyunsaturated fatty acids (PUFA) belong to the class of simple lipids and have two or more double bonds. The location of the first double bond, counted from the methyl end of the fatty acids, is designated by the omega or n- number. Two broad categories of PUFAs that are of concern with respect to cardiovascular homeostasis are E-PUFAs (essential PUFAs) and NE-PUFAs (nonessential PUFAs). The essential PUFAs must be provided in the diet as they can't be synthesized from simple carbon precursors in mammalian organisms. The presence of a high proportion of highly polyunsaturated fatty acids-those having more than four double bonds- makes the fish oil unique in nature. While fish oils contain primarily ω-3 series of fatty acids, vegetable oils contain mainly ω-6 series of fatty acids. The most important PUFA present in fish are eicosapentaenoic acid (EPA, C20:5n-3) (**Fig.1)** and docosahexaenoic acid (DHA, C22:6n-3) (**Fig 2**). These belong to ω-3 series of fatty acids. They cannot be synthesized by the body but are needed, particularly, for the formation of the retina and of the brain. α-Linolenic acid can be converted to EPA and DHA in the human body. However, the extent of this conversion is not precisely known and, at best, very limited.

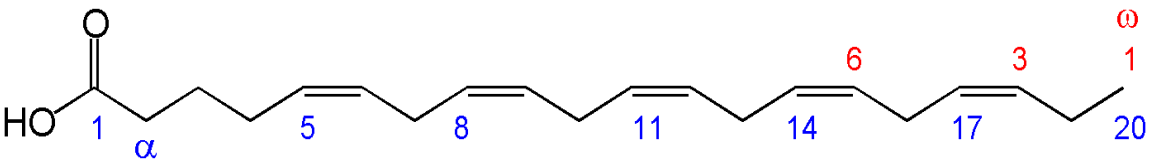

Fig 1 Chemical structure of eicosapentaenoic acid (EPA)

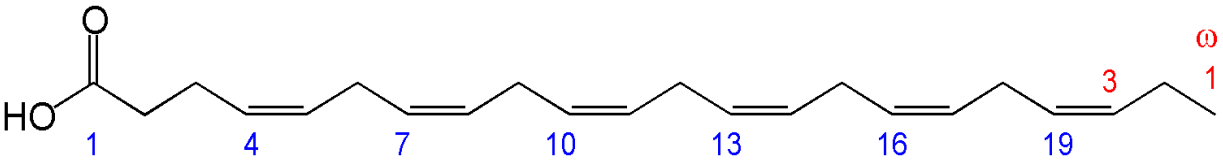

Fig 2.3.1.2 Chemical structure of docosahexaenoic acid (DHA)

Metabolism

Depending on nutritional intake, ω-3 fatty acids are incorporated in the phospholipid pool of cellular membranes and replace the ω-6 fatty acids, thereby increasing membrane fluidity and influencing lipid mediator and cytokine production. ω-3 fatty acids affect biophysical characteristics of cellular membranes by alteration of the membrane phospholipid composition and the content of cholesterol, which improves membrane fluidity. The associated increase in the deformability of blood cells might account for improvement of blood rheology after fish oil intake. Furthermore, ω-3 fatty acids modify the function of membrane-linked enzyme systems, signal transduction and receptor functions. LDLs bind many fatty acid molecules and nearly half of them are PUFA. Linoleic acid accounts for 86% of PUFA and is mainly (65%) contained in the cholesteryl esters, whereas arachidonic acid accounts for 12% and is mostly (68%) found in the phospholipids. Docosahexaenoic acid is present in trace amounts, mainly in phospholipids (Esterbauer *et al.,* 1989).

Essential linoleic acid (C18:2 ω-6), which is present in the food, is converted either into arachidonic acid of class ω -6 (whose first double bond is located at the 6^{th} carbon atom of the CH_3-end of the hydrocarbon chain) or, by a shunt via γ-linoleic acid (C18: 2n-3), into n-3 PUFA - eicosapentaenoic (C20:5, EPA) and docosahexaenoic (C22:6, DHA) acids. ω-3 PUFA can directly inhibit the metabolism of n-6 PUFA, especially at the desaturation stage. Despite certain structural distinctions, arachidonic acid, on the one hand, and EPA and DHA, on the other, compete naturally with one another for the same enzymes and are synthesized from the same precursors - linoleic and linolenic acids. These FAs are metabolized via the arachidonic acid pathways. Besides its ability to incorporate into membrane phospholipids, arachidonic acid serves as a substrate for two important enzymes - lipoxygenase, which gives rise to leukotrienes, and cyclooxygenase, which produces endoperoxides, the substrates for platelet synthetases forming thromboxanes, and

endothelial synthetases which make prostacyclins. EPA and DHA effectively compete with n-6 PUFA for cyclooxygenases initiating the synthesis of prostaglandins with changed properties. The formation of metabolites via lipoxygenase and cyclooxygenase routes (which minimizes the risk of clot formation) is one of the most beneficial effects of n-3 PUFA-enriched diets for the cardiovascular system.

Nutritional and health benefits of Fish oil and PUFA consumption

Fish and fish oils contain very-long chain and highly unsaturated n-3 PUFA such as eicosapentaenoic acid and docosahexaenoic acid. Fish oils reduce the synthesis of chylomicrons by the intestine and/or increase their removal from circulation, thus decreasing postprandial lipemia (Harris *et al.,* 1988). Chylomicron remnants are selectively cleared after the ingestion of n-3 PUFA. Labeled [1-^{14}C] oleate and [1,2-^{3}H] cholesterol in chylomicrons remnants derived from fish oil are incorporated into phospholipids more efficiently than those derived from olive, corn or palm oil remnants and that fish oil remnants are metabolized more rapidly than palm oil remnants. The hypolipidemic effect of fish oil is stronger on hyperlipidemic patients than on normal subjects. Cholesterol concentration in plasma is decreased by fish oil and by n-3 PUFA in patients with type V hyperlipidemia who do not tolerate any other type of dietary fat.

PUFA and lipid profile

Monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid-rich diets decrease the levels of total plasma cholesterol and LDL-cholesterol and increase HDL-cholesterol in healthy normolipidemic subjects and in mouse models of atherosclerosis (George *et al.,* 2000). Although the ingestion of n-3 PUFA has a lower effect than MUFA on plasma cholesterol and on LDL and HDL cholesterol levels. The slight effect of fish oil on plasma LDL and HDL, as against the decrease in very low-density lipoproteins and triacylglycerol concentrations, is the result of factors such as the smaller very low-density lipoprotein particle produced, which is more likely to be converted to LDL by the direct effect on the synthesis of LDL by the liver and by lowering the saturated fat intake. These effects depend largely on the dose and type of n-3 PUFA content of fish oil used. Altogether PUFA diet decreases triacylglycerol level, increases the HDL/LDL-cholesterol ratio and decreases the total cholesterol/HDL-cholesterol ratio, thus reducing the risk of atherosclerosis and coronary artery disease.

PUFA and coronary heart disease

Fish oils contain a large proportion of long chain *n*–3 fatty acids and supplementation with these materials has been recommended for patients with ischaemic heart disease and in disorders such as psoriasis. Now concern has been raised over the fact that the *n*–3 fatty acids present in fish oils are more susceptible to degradation by free radical species (Wills, 1985). Studies have shown that consumption of fish oils exacerbates the susceptibility of the tissues to free radical-mediated lipid peroxidation in vitro. PUFA are particularly susceptible to lipid peroxidation and modification of the polyunsaturated content of the myocardial membranes would be expected to influence their susceptibility to lipid peroxidation. n–3 PUFA supplementation in humans did not have a deleterious effect on skin damage caused by exposure to ultraviolet light, a process associated with epidermal lipid peroxidation. Van den Berg *et al.,* (1991) reported an increased n–3 fatty acid content of red blood cells from fish oil-fed rabbits caused an increase in in vitro lipid peroxidation, but decreased the rate of haemolysis. They proposed that the increased *n*–3 fatty acid content following fish oil supplementation in the erythrocytes, acted as an oxidizable buffer, competing for a limited supply of free radicals, which were generated under times of oxidative stress and so preventing the peroxidation of other molecules.

Observational studies indicate that intake of fish is associated with less fatal coronary heart disease in several populations. The low occurrence of fatal coronary heart disease in Eskimos could be related to their high intake of marine n−3 PUFA (10-14g/day). This ecological study was the basis for the hypothesis that consumption of marine n−3 PUFA could protect against coronary heart disease. Fish consumption was inversely related with fatal coronary heart disease and sudden cardiac death -but not with non-fatal myocardial infarction- in a dose-dependant way, where each 20 g/day increase in fish intake was associated to a 7% lower risk of fatal coronary heart disease. Recently, in a large prospective randomized clinical trial of 11,324 patients with recent myocardial infarction, administration of 850 mg EPA plus DHA daily, in addition to pharmacological treatment, led to a 45% reduction in mortality at 42 months (Marchioli *et al.,* 2002).

The mortality following myocardial infarction was reduced by 29% after 2 years in a group of men who were advised to eat oily fish at least twice weekly as compared to others who had not received any recommendation. Interestingly, this decrease in mortality was not associated with reduced ischemic heart disease or total cholesterol levels, and thus may be related to protection of

the heart muscle itself. Major beneficial effects of marine n-3 PUFAs in coronary heart disease are relating to their ability to decrease level of triglycerides, platelet reactivity, leukocyte reactivity and blood pressure and their antiarrhythmic properties (Schmidt *et al.*, 2006). Dietary n-3 PUFA rapidly incorporate into cardiac phospholipids (predominantly, phosphatidylethanolamines and phosphatidylcholines) which constitute up to 90% of the overall phospholipids pool of the myocardial membranes. It would be natural to expect that the cardioprotective effect of n-3 PUFA is not confined to just reducing the risk of clot formation but is manifested also at the cardiomyocyte level as the alteration of the fatty acid composition of the membrane structures.

PUFA and 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. As removal of free EPA with added bovine serum albumin quickly reversed this protective effect, 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. 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. Interestingly, exogenous DHA supplementation has also been demonstrated to correct calcium homeostasis and mitochondrial dysfunction in diabetic cardiomyocytes. As inhibition of protein kinase C is associated with a reduction in reactive oxygen species generation, DHA has been shown to inhibit generation of superoxide from neutrophils. It is possible that DHA, through its prevention of PKC activation and oxidative stress, could limit premature apoptosis of diabetic cardiomyocytes. In vitro, long-chain n-3 PUFAs decrease myocyte excitability and reduce cytosolic calcium fluctuations via inhibition of Na^+ and L-type Ca^{2+} channels, supporting a potential antiarrhythmic effect of these fatty acids

PUFA and cancer

Many trials using fish oil or PUFAs from fish oil as 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. In humans, dietary (n-3) fatty acid treatment offers possibilities in malignant diseases. In contrast, low α-linolenic acid (precursor of EPA and DHA) levels in mammary adipose tissue are associated with an increased risk of breast cancer in women. In patients with prostate cancer, fish intake was inversely related to cancer. In the great majority of colon adenocarcinomas taken from humans, COX-2 levels are 2- to 50-fold higher than levels in adjacent normal intestinal mucosa, while COX-1 levels are unchanged. Although the mechanism of action of PUFA is still unclear, the identification of an enzyme COX-2 catalyzing fatty acid oxidation as a rate limiting step in the progress from normal cell growth through hyperplasia on to neoplasia has opened up a new field of research. This enzyme is a participant in the pathway of colon carcinogenesis, especially when mutation of the Adenomatous Polyposis Coli tumour suppressor gene is the initiating event. It seems that there is a correlation between COX-2 expression and the size of the tumours and their propensity to invade underlying tissues. DHA down-regulates the expression of COX-2 and induces apoptosis. Inhibition of COX activity, decreases eicosanoid production and prevents lung cancer in animal models. There is a report that feeding menhaden oil in place of corn oil or EPA in place of linoleic acid decreases the development of 1,2- dimethyl hydrazine- or it's metabolite azoxymethane- induced colon tumors, but adding menhaden oil to a low fat diet does not affect colon carcinogenesis. Thus, consumption of diet enriched in n-3 PUFA, specifically EPA and DHA provide a significant mechanism for the prevention of human cancers. Some conflicting results are also there regarding the ability of PUFA in preventing cancers.

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. 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 nonsignificantly) inhibited carcinogenesis. It is possible that oxidation products of polyunsaturated fatty acids could act on signal transduction pathways leading to altered cell proliferation or apoptosis. In addition, lipid peroxidation products can form DNA adducts such as 8-hydroxyguanosine (Beckman & Ame which have the potential to exert genotoxicity and therefore could bring about tumor initiation.

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. Despite never being measured, the activity of liver desaturases is probably decreased in human cirrhosis, mainly because of liver insufficiency which explains PUFA deficiency in cirrhosis. 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 or hepatocytes of patients with cirrhosis as compared with healthy controls. Arachidonate deficiency may lead to impaired platelet aggregation often occurring in advanced cirrhosis. It has been reported that changes in membrane lipid composition hamper the insulin receptor function in the erythrocytes of cirrhotic patients and that the infusion of polyunsaturated lecithin improves such a derangement. Eicosapentaenoic acid (EPA; 20:5n-3) up-regulates the metabolic action of insulin and inhibits cell proliferation. It has been found that fish-oil rich in EPA inhibit DEN-induced hepatocarcinogenesis in rats. 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.

An explicative hypothesis proposed is that PUFA increase lipid peroxidation. It is suggested that while giving PUFA supplements to cirrhotic patients balance between n-6 and n-3 long chain PUFAs should be ensured as administering only latter (as fish oil) might further impair the already deranged platelet aggregation of these patients. As the cirrhotic patients are deficient in antioxidant vitamins providing the same along with PUFA may create some beneficial effects. Polyunsaturated fatty acids deficiency is common in patients with alcoholic liver disease. They found that PUFA deficiency reverses alcohol-related mitochondrial dysfunction via an increase in phospholipid arachidonic over linoleic ratio, which raises cytochrome oxidase activity.

PUFA and aging

PUFA deficiency is related to a number of diseases like Alzheimer's disease, Liver cirrhosis, Parkinson's disease, hypertension, cancer, diabetes, inflammatory and auto-immune disorders,

depression, schizophrenia, multiple sclerosis etc. Indeed, deficits in the peripheral amounts of PUFA have been described in subjects suffering from neurological and psychiatric disorders. n-3 PUFA deficiency is found to elevate and n-3 PUFA enrichment is found to reduce the brain 2-2-Arachidonoylglycerol level in mice. 2-Arachidonoylglycerol is a putative endogenous ligand for cannabinoid receptors and was suggested to play an important role in both physiological and pathological events in the central nervous system (CNS) as well as in peripheral organs. Studies conducted in pregnant rat dams by Armitage *et al.* (2003) showed that inadequate levels of DHA in the perinatal period are associated with altered blood pressure control in later life.

A distinct decrease in the ratio of mitochondrial membrane ω-3 to ω-6 polyunsaturated fatty acids (PUFA) and a decrease in the mitochondrial phospholipid cardiolipin in aged rat hearts. It has been found that rat cardiomyocytes are devoid of the ability to convert PUFA C20 into C22, although the reverse reaction precedes rather effectively. Moreover, EPA and DHA biosynthesis in animal and human organisms is rather a slow process which is further decelerated with ageing.

From birth to aging the heart undergoes functional changes which reflect biochemical and ultrastructural modifications. Indeed, while in the fetus the right ventricle is the dominant pumping chamber, after birth a functional left ventricular dominance develops, following a postnatal increase in systemic vascular resistance and a decrease in pulmonary vascular resistance. Yet cardiac output at rest and during exercise is similar in both young and old healthy subjects. Diastolic function decreases with age, since left ventricular filling is fast in young subjects and slow in the old ones and is due, respectively, to rapid and slow ventricular relaxation and atrial contraction. These functional changes are paralleled by modifications in the number of myocytes (smaller and more abundant in neonatal than in adult and aged heart), in the number of specialized conduction cells, in the development of cardiac fibrosis due to changes in the amount and composition of connective tissue, in the reduction in calcium transport across membranes, in the lower capillary density, and in the changes in mitochondrial function. In both humans and animals, the aging process in the heart has been associated with a decrease in the total number of myocytes, mainly confined to left ventricle and reactive hypertrophy of the remaining cells. This cell loss occurs with aging even in the absence of pathologies known to cause heart damage, such as atherosclerosis, diabetes, hypertension, and ischemic heart disease.

Oxidative stress is one of the major factors that induce apoptosis (Phaneuf & Leeuwenburgh, 2002)**.** The low degree of tissue fatty acid unsaturation of longevous homeothermic animals could have been selected during evolution to protect the tissues against oxidative damage. The heart is one of the organs in which the effects of oxidative damage would be more readily detectable due to its high dependence on oxidative phosphorylation to derive energy. It is generally agreed that isolated mitochondrial preparations from old compared to young hearts produce more reactive oxygen species (ROS), reflecting an age-related decline in coupling of electron transport to ATP production. Despite an increase in manganese superoxide dismutase and selenium-dependent glutathione peroxidase activities, there was an increase in lipid peroxidation in the cytosol in myocytes of the old animals compared with the young and middle-age groups (Phaneuf & Leeuwenburgh, 2002)**. 7.**

PUFA and Neuroprotection

In the fetal brain, DHA, a structural constituent of membranes accumulates mainly during the last trimester of pregnancy and remains at very high proportions up to the end of the second year of life. Consumption of DHA may contribute to optimal conditions for brain development as the endogenous formation of DHA appears to be pretty low. Researches reveal that DHA is essential for brain function mainly for neuronal cell growth, differentiation as well as in neuronal signaling. (Lauritzen et al., 2016). Triglyceride form of DHA facilitates neuroprotection in experimental Parkinson's disease, a neurodegenerative disorder and many studies suggest that omega-3 polyunsaturated fatty acids provides protection against brain damage. (Gómez-Soler et al., 2018).

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MARINE FUNCTIONAL PROTEINS

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Introduction

In today's scenario, a large portion of the global population is very much aware of health benefits one can achieve through consumption of aquatic food products. Particularly, fish and shellfish are highly nutritious and delicious. The demand for fish is ever increasing. On the other hand, aquatic animals like fish and shellfish are highly perishable compared to meat from land animals due to near neutral post mortem pH, low glycogen reservoir, low connective tissue content and high moisture content. Immediately after harvesting of fish (immediately after death), it undergoes various bio-chemical and microbiological changes which lead to spoilage. Functional proteins can be obtained from edible portion of meat from aquatic animals as well as waste from the aquatic food waste. Hence, fish is essentially processed and preserved to make the fish available in edible condition. As a result of processing, a greater portion of raw material is discarded as waste which is biochemically equivalent to edible portion. This chapter doesn't include the content or process details for obtaining proteins from aquatic plants, planktons and microalgae.

Fish muscle proteins

The proteins in fish muscle can be classified into three groups as given by Huss (1995).

1. *Sarcoplasmic proteins* (myoalbumin, globulin and enzymes) which are soluble in neutral salt solutions of low ionic strength (< 0.15 M). This fraction constitutes 25-30 percent of the total proteins. About 100 different proteins are known to be present in the sarcoplasmic fraction.
2. *Structural proteins* (actin, myosin, tropomyosin and actomyosin) which constitute 60-70 % of the total protein content. These proteins are soluble in neutral salt solutions of fairly high ionic strength (0.5 M and above)

3. *Stroma proteins*, which constitute approximately 3 % of the protein in teleostei and about 10 % in elasmobranchii (compared with 17 % in mammals). They include connective tissue, collagen and elastin.

Sarcoplasmic proteins

The sarcoplasmic proteins consist mostly enzymes and include proteinases, peptidases and cathepsins. The cathepsins are a group of muscle proteases and can cause softening of the fish tissue (Ladrat *et al*., 2003). Apart from enzymes, pigments such as myoglobin and a low molecular weight protein parvalbumin are present in sarcoplasm which can significantly influence the quality of fish meat and FPH. Myoglobin is a conjugated protein which has high binding co-efficient with oxygen and acts as a storage means of oxygen (Baron and Andersen, 2002). Depending on the oxidation state of iron atom, the colour of the product including FPH will vary. Parvalbumin is a small protein with a molecular weight of 12 kDa and is involved in calcium signale either for binding and release (Taylor *et al*., 2004). There are reports that the allergic reactions are caused in some populations of human by consumption of parvalbumin.

Myofibrillar proteins

The myofibrillar protein comprises of more than 15 different fractions and myosin is the abundant molecule (Sikorski, 1994). The different myofibrillar protein fractions with their molecular mass and number of subunits are given in Table 1. Myosin constitutes nearly 55 % of the total myofibrillar proteins (Murray *et al*., 1993). The myosin molecule is relatively large in size with a molecular weight of 4.8 - 5.0 x 10^5 Da (Rayment *et al*., 1993). The number of subunits in myosin molecule vary from 4 - 6. The two polypeptides in the myosin molecule are highly coiled and terminate into the globular head. These two are referred as myosin heavy chains (MHC). The other subunits are small in size and referred as light chains (Wang *et al*., 2009). The average number of residues in myosin molecule is 2300 - 2700. The primary structure of different myosins from the muscle system has been determined by protein sequencing and cDNA or genomic DNA cloning. The sequence showed considerably high homology between carp, chicken and rabbit myosin. The relative instability of fish myosin has been attributed to the nature of residue in S2 fraction. The amino acid at position 1078 in pollock and carp myosin is serine which has hydrophilic side chain while that in rabbit and chicken is leucin which has hydrophobic side chain (Ojima *et al*., 1998). It has been established that native myosin molecule do not have disulfide bridge despite 40 free sulfhydryl residues found in the molecule. The globular head possess

binding site for nucleotide and for actin molecule. In myosin head three different domains have been identified together constitute the molecular mass of 95 kDa. It consists of 25 kDa N-terminal domains, a 50 kDa central domain and a 20 kDa C-terminal domain. The 25 kDa and 50 kDa domains form the ATP binding site while 50 kDa and 20 kDa domains form actin binding site. The two light chains binding sites are located at 20 kDa domain and they wrap around 20 kDa domain.

Actin is ubiquitous in muscle tissue and is the major constituent of thin filaments. It makes up to 25 % of myofibrillar protein by weight (Murray *et al*., 1993). Actin exists in two forms - one is globular (G-actin) and another one is fibrous (F-actin). F-actin is formed by polymerization of G-actin. G-actin consists of a single polypeptide chain, a nucleotide (ATP) and a divalent cation bound to a specific site. The average molecular weight of actin is 42 kDa and consists of 375 amino acid residues among which 5 are free sulfhydryl residues (Dominguez and Holmes, 2011).

Tropomyosin is a regulatory protein in the myofibril (Xiong, 1997). It is a filamentous molecule composed of a coiled coil of two α-helices. The two α-helices are dissimilar, designated as α- and β-tropomyosin with molecular weight of 34 and 36 kDa respectively. Each subunit α-helix usually consists of 284 amino acid residues (Inoue *et al*., 1998).

Troponin is a complex of three polypeptide chains (Tn C-18 kDa; Tn I-24 kDa; Tn T-37 kDa). The number of amino acid residues in TnC, TnI and TnT is 159, 179 and 259 respectively (Stryer, 1995, Inoue *et al*., 1998; Nishita *et al*., 1994).

The other myofibrillar proteins include α actinin which constitutes the Z-disk, the C-protein associated with myosin, M-line proteins (Myomesin, M-protein and creatine kinase) found in the M-line region and H-and X-proteins associated with myosin at discrete sites on the surface of the thick filaments (Xiong, 1997). The FPH prepared from fish meat / processing waste has to take into consideration the properties and nature of residues in major protein fractions which will have a bearing on the end product.

Stroma proteins

The stroma protein mainly comprises of connective tissue and collagen. The collagen content of fish is lower as compared to mammalian counterpart (Bremner, 1992). Apart from providing the structural integrity to the muscle fiber, collagen is a major component of skin and bones. Collagen is a multimeric protein with 3 subunits. The 3 polypeptide chains are highly coiled and lack α-helical structure. The proportion of proline and hydroxyl proline is about 10 % of total amino acid residues together referred as imino acids. The special feature of collagen is occurrence

of glycine at every 3rd residue. The technological significance of collagen during processing is related to the texture. During chilled storage of blue grenadier (*Macruronus novaezelandiae*), it was observed that the attachment between muscle fibers and myocommata and the whole sarcolemma was degraded leading to detachment of muscle fiber (Bremner and Hallett, 1985; Hallett and Bremner, 1988). A similar degradation was observed with king salmon (*Oncorhynchus tshawytscha*) (Fletcher *et al*., 1997), Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*) (Ofstad *et al.*, 1996). The intermolecular cross-links in collagen are thought to be responsible for the stability, physical strength and mechanical properties of the connective tissue and other components of the extracellular matrix (Bracho and Haard, 1995). Breakdown of the connective tissue by endogenous collagenases may lead to undesirable textural changes in the fish.

Secondary raw material

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

Protein content in secondary raw material

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

Table 4. Protein content in major fish waste parts

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

(Source: Rustard, 2007**)**

Proteins from secondary raw material and the possible industrial products

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

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

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

Fish protein concentrate

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

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

Fish Collagen

Collagen is a structural protein found mainly in the skin and bones of all animals. Collagen is the most abundant protein originating from the animal source, comprising approximately 30% of total animal protein. It is composed of three α-chains which are intertwined to form a triple-helix. It is present in the connective tissue matrix that makes the framework of skin, bones and joints, cornea, blood ducts, and the placenta. There are many types of collagen, but 90% of our body's collagen protein is Type-I collagen. It is found to be rich in amino acids such as glycine, valine, alanine, proline and hydroxyproline (Burghagen, 1999). Glycine constitutes one third of the total amino acid content of collagen followed by hydroxyproline and proline, which account for another one-third. Owing to this structural uniqueness of collagen molecule, there is increasing interest for the direct consumption of collagen in the form of their easily digestible derivatives. Worldwide, this interest has been taken-up by the nutraceutical industry, especially in developing countries.

Currently, collagen is used in many pharmaceutical and cosmetic products, due to its structural role and better compatibility with human body. It is commonly used in the cosmetic

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

Fish Gelatin

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

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

Fish enzymes

Fish visceral waste can serve as a source of large amount of enzymes which have potential applications in different sector starting from laundry application to pharmaceutical applications (Simpson and Haard, 1987). The nature of fish visceral enzymes is different from the enzymes found in the digestive system of terrestrial animals. Hence, they can be exploited for certain distinct applications. Fish pepsins can act even at low temperature and higher pH optimum than

the pepsins from terrestrial source. Moreover, fish pepsins do not undergo autolysis at low pH (Raa, 1990). The differences in the properties of pepsins from fish and other sources could be attributed to the difference in the sequence and composition of aminoacids (Gildberg and 0verbj, 1990). Fish enzymes can be used as processing aids in the following applications

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

Hemoproteins

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

Carotenoproteins

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

Protein derivatives from secondary raw material

Fish protein hydrolysates (Bioactive peptides)

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

Application of fish protein hydrolysates

Nutritional application

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

Table 6. Proximate composition of fish protein hydrolysate

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

(Source; Chalamaiah et al., 2010)

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

Nutraceutical applications

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

Table 7. Commercially marketed fish protein 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
Nutripeptin®	Manufactured by enzymatic hydrolysis of Cod fish fillet/muscle protein	It helps in the blood glucose stabilization<comma> and weight management.	UK and USA

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

(Source: Chalamaiah et al., 2010)

Fish protein hydrolysate as a functional ingredient

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

Collagen peptide/gelatin hydrolysate

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

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

Enzymatically hydrolyzed collagen have shown better biological activities compared to the peptides derived from fish muscle protein with antioxidants and antihypertensive agents.

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

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

Conclusion

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

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PREPARATION CHARACTERIZATION AND APPLICATION OF PROTEIN ISOLATES

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Consumers' demand for healthy food products is increasing worldwide. Development of functional foods involves incorporation of specific compounds (or ingredients) with demonstrated health benefits (Hamer, Owen, & Kloek, 2005). In addition to the importance of the health effects; sensory attributes such as taste, texture, and flavor as well as convenience remain crucial factors for consumers. Fish and fishery products play a major role in human health due to presence of essential amino acids, fatty acids, vitamins and minerals. In the fish industry, processing of raw fish into food products generates large quantities of by-products (heads, skin, bones, frames, scales etc.) that contain proteins and lipids. If these proteins and lipids are recovered, it could be a source of nutrients for humans; and therefore, could be used in the development of food products destined for human consumption. These problems can be overcome by using Isoelectric solubilization/precipitation (ISP) method or pH shift technology which allows the separation of proteins and lipids. Isoelectric solubilization/precipitation (ISP) is a pH-shift process that induces water solubility of meat proteins. While proteins are dissolved, they are separated from lipids and other insoluble materials such as skin, bones, scales, etc.

Recovery of proteins from fish and fish processing by-products using isoelectric solubilization/precipitation

In fish muscle homogenates, myofibrillar proteins are present as aggregates that are held together by weak protein–protein hydrophobic interactions (Undeland, Kelleher, Hultin, McClements, & Thongraung, 2003). The solubility of fish muscle proteins can be "turned" on or off by providing conditions that either favor or disfavor protein solubility, respectively. When acid is added to a solution, it dissociates yielding hydronium ions (H_3O+). Protonation of negatively charged side chains on glutamyl or aspartyl residues results in an increased net positive surface charge. Similarly, when base (− OH) is added to a solution, deprotonation of side chains on tyrosyl, tryptophanyl, cysteinyl, lysyl, argininyl or histidinyl residues contributes to an increased net negative surface charge. When the charge equilibrium is reached

and a protein solution attains homeostasis, the final status of a protein surface electrostatic charge at a given pH is referred to as the net charge. The accumulation of a net positive or negative charge induces protein–protein electrostatic repulsion and an increased hydrodynamic volume due to expansion and swelling (Kristinsson, Theodore, Demir, & Ingadottir, 2005; Undeland et al., 2003). As proteins assume more positive or negative net charge, they gradually start electrostatic interactions with water (i.e., protein–water interactions). Due to increased protein– water interactions, the protein–protein hydrophobic interactions decrease. Therefore, as the protein molecules become more polar (charged), more water associates on and around the protein surface and proteins become water soluble. However, it is possible to adjust the pH of a protein solution so that the number of negative charges on the protein's surface is equal to the number of positive charges, and therefore, the protein molecule assumes a zero net electrostatic charge. The pH at which the net electrostatic charge of a protein is equal to zero is called the isoelectric point (pI). The pI is very specific for different proteins, and isoelectric focusing is often used to pinpoint the pI. The iso electric solubilization and precipitation (ISP) can be universally applied to recover muscle proteins from animal processing by-products, including fish. Fish protein isolates (FPIs) have thus far been recovered using ISP in a batch mode at the laboratory scale (Choi & Park, 2002; Kim, Park, & Choi, 2003; Kristinsson & Hultin, 2003; Undeland Kelleher, & Hultin, 2002) and pilot scale (Mireles DeWitt, Nabors, & Kleinholz, 2007). ISP processing has been applied to beef, chicken and fish processing by-products (Chen & Jaczynski, 2007a; Mireles DeWitt, Gomez, & James, 2002; Tahergorabi, Beamer, Matak, & Jaczynski, 2011).

Protein isolate from fish meat

Fish protein isolate is a concentrated protein mainly consists of myofibrillar proteins (eg. actin and myosin). Fish protein isolate (FPI) is prepared from fish meat and discards different kinds of raw materials by the pH-shift technology. The process of protein recovery by pH-shift was proposed by Hultin and Kelleher (1999). In the pH-shift process, proteins of the muscle tissue are first solubilized at either acid (pH below 1.5-3.0) or alkali pH (near 10.5-13.0) and centrifuged. Then, the top lipid layer and sedimentation (insoluble impurities such as bones and skin) at the bottom are discarded. The middle layer of protein solution is collected and precipitated by adjusting the pH to a value near the isoelectric point (5.2-5.5) Then it was centrifuged and precipitate is collected which is called protein isolate (Kristinsson et al. 2005).

Protein isolate from fish / shrimp head waste

The shrimp head homogenate is mixed with distilled water at different ratio (1:2 to 1:4 w/v). Then, pH (9.43) is adjusted with 1M NaOH. Then it is centrifuged and supernatant is collected. After that pH is adjusted to 4.5 using 1M HCl to precipitate the protein in the supernatant. The precipitated proteins are collected by centrifuging at 10,000 rpm for 15 min at 4° C followed by decantation of supernatant. The precipitants are resuspended in distilled water and the pH is adjusted to 7 using 1M NaOH. Then the precipitate is collected by centrifugation at 10,000 rpm for 15 min at 4° C. The precipitate can be washed thrice to remove the salts using distilled water for further application.

Characterization of protein isolate:

Moisture

The AOAC method is used to measure the moisture of the samples. Calculation of the moisture was done according to the following formula;

$$Moisture\ (\%) = \frac{Pre - dry\ weight\ (g) - After\ dry\ weight}{Pre - dry\ weight\ (g)} x100$$

Pressure Induced Drip

Pressure Induced Drip is an important factor to estimate the water content of FPI and the quality of the protein in the product. 50 g of the test sample is transferred to a circular cylinder with an inner diameter of 35 mm and 120-150 mm in length, made of stainless steel and a perforated plate with holes 1 mm in diameter in the bottom. Pressure is applied with a 1 kg cylindrical rod 34 mm in diameter and left for 20 minutes. The weight of the dripped liquid is measured and the percentage of the weight of the test sample is calculated.

Objectionable matter

The term "objectionable matter" is used here means skin, small bones and any objectionable matter other than fish muscle. In this method 10 g of the test sample is spread to the thickness of 1 mm or less, and the number of visible objectionable matters more than 2 mm in diameter is noted.

Whiteness

The colour and whiteness of protein isolate gel is another important factor and it can measured by using colour [L*(lightness), a* (red-green colours) and b* (yellow-blue colours)] analyzer. Whiteness, as an index for the general appearance of surimi gel, can be calculated as:

Whiteness = L* - 3b*.

Determination of water holding capacity by measuring expressible moisture

Water holding capacity of protein isolate is easily determined by measuring expressible moisture of cooked isolate gel. A small amount of test sample (around 2 g) is placed between filter papers and pressed using texture analyzer under a fixed pressure (10 kg/cm²). The expressible water is calculated according to the following formula to the first decimal place:

$$Expressible\ moisture\ \ (\%) = \frac{Pre-pressed\ weight\ (g) - After-pressed\ weight}{Pre-pressed\ weight\ (g)} x100$$

Determination of functional properties

Solubility

Protein sample (10 mg ml^{-1}) in distilled water at neutral pH is vortexed for 30 min at room temperature and centrifuged at 7500 g for 15 min. Protein contents in the supernatant is determined by Kjeldahl method and protein solubility is calculated as follows (Morr et al., 1985).

$$Protein\ solubility\ \ (\%) = \frac{Total\ protein\ content\ in\ supernant}{Total\ protein\ content\ in\ sample} x100$$

Foaming properties

Foaming capacity and stability of fish protein isolate is determined by the method described by Sathe and Salunkhe (1981). Protein solution (1.0 %) is homogenized (230 VAC T-25 digital Ultra-turrax, IKA, India) at a speed of 16,000 rpm for 2 min to entrap air and foaming capacity is determined instantly whereas foam stability after a time period of 3 minutes as:

Foaming capacity/stability (%) = [(A-B) / B] x100

Where A is the volume immediately after whipping (foam capacity) and after 3 min standing (foam stability); B is the volume before whipping.

Emulsifying properties

Emulsifying properties is determined according to the method of Pearce and Kinsella (1978). A pre-mix containing 1% protein solution and vegetable oil (3:1 (v/v)) is homogenized (230 VAC T-25 digital Ultra-turrax, IKA, India) for a period of 1 min at 20,000 rpm and an aliquot of the emulsion (50 µl) is carefully taken from the bottom of the container at 0 and 10 min after homogenization. Further it is mixed with 5 ml of 0.1% sodium dodecyl sulphate (SDS) solution and the absorbance measured at 500 nm (Lambda 25 UV/Vis, Perkin Elmer Life and Analytical Sciences, Singapore) immediately (A0) and 10 min (A10) after emulsion formation to evaluate the emulsifying activity index (EAI) and the emulsion stability index (ESI) as:

$$\text{EAI (m2 /g)} = \frac{2 \times 2.303 \times A_0}{0.25 \times \text{wt of protein}}$$

$$\text{ESI (min)} = A_{10} \times \Delta t / \Delta A$$

Where $\Delta A = A_0 - A_{10}$ and $\Delta t = 10$ min

Oil absorption capacity

OAC of sample is determined as the volume of edible oil held by a known quantity of the material as per the method of Shahidi et al., (1995). A 0.5 g sample with oil added (10 ml) is vortexed (Expo Hitech, India) for 30 sec following centrifugation (K-24A, Remi Instruments, Mumbai) at 2800 g for 25 min. The free oil is decanted and the OAC was determined by weight difference (g of oil per gram of sample).

Quality characteristics of protein Isolate

In general, protein isolate prepared by pH shift technology offer high quality protein consists of all essential amino acids. Protein isolate will have a protein content of 87–95%. Lipid and ash content ranged from 1–5%, 2–6%, respectively. Proximate composition of protein isolates varied depends on the raw material and process applied (acid or alkali solubilization). The nutritional quality of a protein source is determined based on the presence of all nine essential amino acids (EAAs) in adequate quantities to support human or animal health. ISP at basic (i.e., alkaline) pH allows recovery of FPIs with higher nutritional quality as assessed by a greater content of EAAs when compared to ISP at acidic pH (Chen, Nguyen, Semmens,

Beamer, & Jaczynski, 2007; Chen, Tou, & Jaczynski, 2009). The biological value (BV) of FPIs is higher than soybean protein concentrate and similar to milk protein (Bridges, Gigliotti, Altman, Jaczynski, & Tou, 2010; Gigliotti, Jaczynski, & Tou, 2008). Egg protein is commonly the reference protein due to its high nutritional quality, while lysine is often considered a limiting EAA. It needs to be emphasized that FPIs from fish processing byproducts have a similar concentration of lysine as the whole egg, and the concentration of lysine is even greater in the FPI from whole carp (gutted, but bone-in, skin-on) and whole krill (Chen et al., 2009; Taskaya, Chen, & Jaczynski, 2009). The protein isolate could be considered a source of high-quality complete protein.

Factors affecting quality of fish protein isolate

i) **Source of raw material:** Properties of fish protein isolate varies by types of fish or processing discard used and freshness of raw material

ii) **Process of homogenization:** The first step in preparation of fish protein isolate is homogenization. This process enhances the solubility of protein and recovery. Moreover, time given for homogenization also influence the yield and solubility of protein. Solubility of proteins also depends on pH, and/or temperature.

iii) **Ratio of fish to water:** FPI production involves homogenization of fish meat by using 10-20 parts of water (1:10 or 1:20). The ratio of fish to water influences the viscosity of protein solution. It has been reported that low viscosity is preferable to isolate insoluble material during centrifugation process.

iv) **Process Time and temperature:** Quality of protein isolate depends on time and temperature used for its production.

v) **Presence of hem proteins:** In case of dark muscle protein, presence of heme protein will affect the quality of fish protein isolate . Hence it should be removed from soluble fraction to reduce the fat oxidation.

vi) **Protein denaturation:** Denaturation of protein results loss of protein functionality. It can be overcome by control of temperature during processing

Fish protein isolate Vs Surimi

Fish protein isolate (FPI)	**Surimi**
Processing discards, under-utilized fish can be used for fish protein isolate production.	Fresh fish mince prepared from fillet or by using deboner is used for surimi production
pH meter, homogenizer and centrifuge is required	It requires high-cost instrument like filleting machine/ deboner, washing tank, strainer etc.
Water usage is less for fish protein isolate production (5-10lit/kg of isolate)	Water usage is high (25-30lit/ kg of Surimi production)
Less quantity effluent generated during process results less environmental pollution	Huge water uses during process generate large quantity of effluent for disposal
Production process involves acid and alkali usage. Hence its needs stronger safety requirement.	Production process does not involve chemical usage except sodium bicarbonate is used for gel strength improvement in dark meat process. Skilled person required for machineries operation
Yield of protein ranged from 70-90%	Yield of protein ranged from 55-70%
Fat is removed effectively during processing	Fat removal from fish mince depends on fish and number of washing cycle used
Cryoprotectant is required to improve the stability of fish protein isolate during frozen storage	Cryoprotectant is required to improve the stability of surimi during frozen storage
Fish protein isolate process is well developed. But fish protein isolate based products are under developmental stage.	Surimi process and surimi-based products are well established

Applications

- ✓ Fish protein isolate used as functional ingredients for preparation of ready to eat/ ready to cook value-added products
- ✓ Dried fish protein isolate powder can be used as protein supplements in food products.
- ✓ Fish protein isolate has an excellent functional property which results in wider applications in developing restructured products
- ✓ Unlike Surimi, FPI also used for production of seafood analogue products
- ✓ Fish protein isolate also used to develop nutraceutical products (eg. Fortification of FPI with omega -3 fatty acids)

Raw fish protein

Freeze dried fish protein

Restructured product from isolate

Fish protein isolate incorporated

Conclusion

The utilization of processing discards and fishes not suitable for human consumption is one of the biggest challenges in the seafood sector. These can be overcome by using Isoelectric solubilization/precipitation (ISP) method or pH shift technology to convert muscle tissue to edible protein and it can also serve as a substitute to surimi. However, production process of fish protein isolate is under developmental stage. If the production process and isolate based product is well established, fish protein isolate will serve as excellent protein source for human consumption. Moreover, it offers wider applications in functional food development.

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CHITOSAN AND ITS FUNCTIONAL DERIVATIVES

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Chitosan (CS) is a copolymer of glucosamine and N-acetyl glucosamine branched by β-(1-4) linkages. It is derived from chitin, which is among the most abundant biopolymers on Earth. The word "chitin" is derived from the Greek language, meaning "envelope" or "tunic". Chitin was the first polysaccharide identified by the French scientist Braconnot in 1811 and was fully described in 1884 as a natural poly-β-(1-4)-N-acetyl-d-glucosamine.

Chitosan is a natural-based biopolymer derived from chitin, the second most abundant polymer in nature next to cellulose. Chitin can be found from many sources in nature, including exoskeletons of crustaceans, insects, as well as mollusks and fungi. Chitosan becomes water-soluble in acidic pH (lower than its pKa~6.2), in which condition the protonation of $–NH_2$ groups occurs resulting in solubilization. After protonation, chitosan carries positive surface charges on its D-glucosamine repeat unit and therefore becomes the only pseudo natural cationic biopolymer.

Chitosan is also well-known for its biocompatibility, biodegradability and low toxicity. Due to these unique properties, chitosan is considered a versatile biopolymer that can be developed into different forms, such as gels, films, nano/micro-particles, beads, etc., and find numerous applications in various fields, including food, pharmaceutical, and cosmetic sciences. Due to the hydroxyl and amino groups on its backbones, chitosan is an amenable molecule that can be easily modified by various methods. Modifications of chitosan are aimed to improve the physicochemical properties of chitosan and thus expand its applications in different situations. Several modification methods have been well-documented for chitosan, including chemical, physical, and enzymatic approaches.

Chitosan Modification and Functionalization

Due to their exceptional properties and biological activities, CS and its derivatives are having growing success regarding the number of publications concerning their description and application in foods, environmental, material, cosmetic, pharmaceutical, and biomedical sectors. However, their applications are strongly limited by their solubility in many polar solvents and water. Overcoming this issue is possible by modifying CS through

chemical/enzymatic methods to generate depolymerized and/or new derivatives. Due to the presence of reactive amino (-NH_2) and hydroxyl (-OH) groups, chitosan is very easily modifiable. These modifications aim to enhance their biological and chemical properties and modify their solubility as a function of the final applications. The different types of chitosan derivatives and its application are mentioned below.

Quaternized Chitosan Derivatives

Quaternization is an example of a procedure to enhance the solubility of chitosan in water. CS is positively charged at pH under 6.5, whereas quaternized CS is still permanently positively charged at pH above 6.5. A quaternization reaction occurs between alkyl iodide and CS under basic conditions media. *N*,*N*,*N*-trimethyl chitosan chloride (TMC) is the best known quaternized chitosan.

N-acyl Chitosan Derivatives

N-acylation gives hydrophobic properties to chitosan by grafting different fatty acids. The reaction is a specific amidation between -COOH groups from fatty acids and -NH_2 groups from chitosan.

Cross-Linked Chitosan Derivatives

Making cross-linked chitosan requires the use of specific chemical agents for linking the chains together and thus creating a three-dimensional macromolecular network. Chitosan is most often crosslinked by covalent bonds in the presence of aldehyde derivatives, such as glyoxal, formalin, or glutaraldehyde, in acid or basic medium to generate chitosan-based hydrogel.

Chitooligosaccharides

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

Carboxy methyl Chitosan

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

Hydroxy propyl Chitosan

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

Phosphorylated Chitosan

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

Glucosamine hydrochloride

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

Glucosaminoglycans

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

Application of chitosan derivatives

Specific properties	**Main applications**
Bioactivity	Antimicrobial additive to fibers and textile products, food packaging, wound healing and anticholesterolemic agent
biodegradability	Controlled release of drugs, agro-chemical, food packaging and toiletries production
Chelation ability	Reduction of surface water and waste water pollutions by chelating of heavy metal ions and radionuclide
Absorption capacity	Efficient electrostatic painting, clarification of juices and beverages.
Film forming ability	Production of dialysis membranes and dental fluids, separation membranes for medicine and food processing.

Conclusion

Chitosan is insoluble in water and most organic solvents, which seriously limits both its application scope and applicable fields. However, chitosan contains active functional groups that are liable to chemical reactions; thus, chitosan derivatives can be obtained through the chemical modification of chitosan. The modification of chitosan has been an important aspect of chitosan research, showing a better solubility, pH-sensitive targeting, an increased number of delivery systems, etc.

ROLE OF LC-MS/MS IN MARINE BIOACTIVE COMPOUNDS

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Introduction

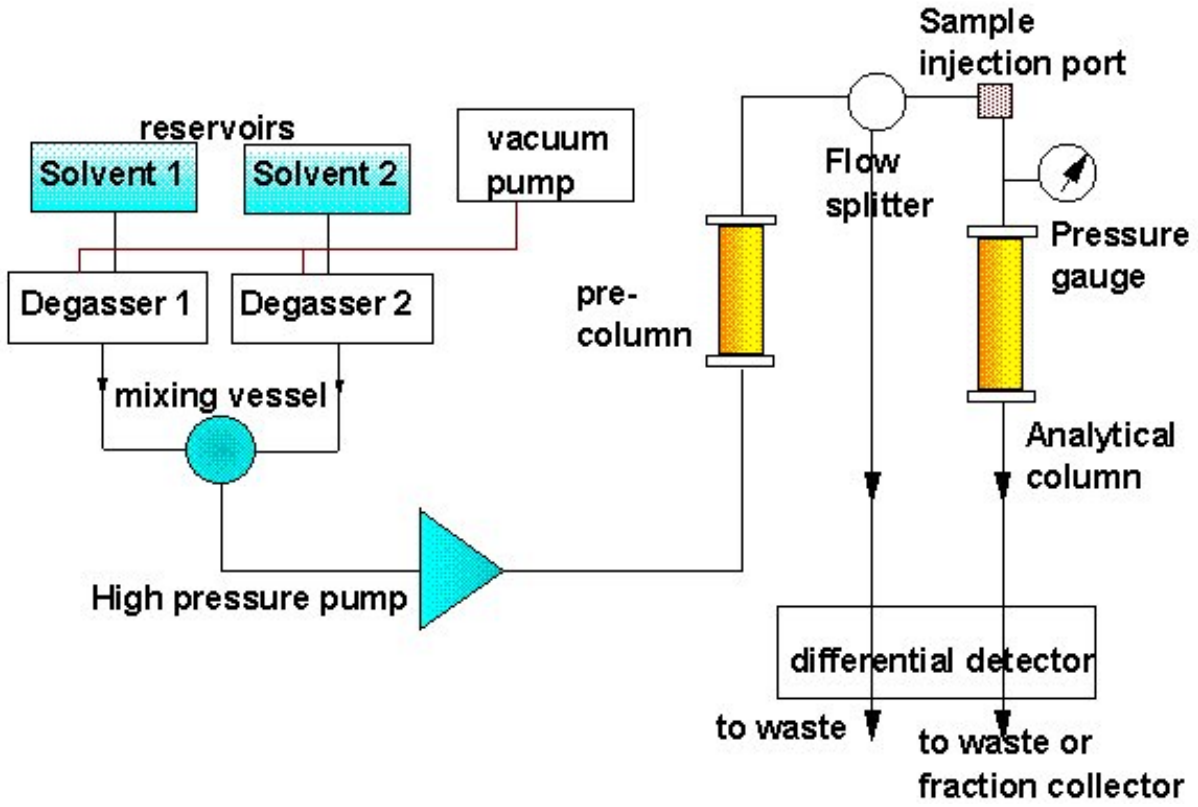
A Mass Spectrometer is an analytical instrument that measures the masses of individual molecules which have been converted into gas-phase ions. Molecules in a liquid-phase need to be converted into a gas-phase for the mass spectrometer to be able to measure them. Ions are separated, detected and measured by their mass-to-charge ratios (m/z). Mass spectrometers hyphenated with liquid chromatograph (LC-MS/MS) are widely used in chemical and biological research now a days, to identify and elucidate structures of unknown metabolites, protein etc. in biological tissue, plant material, microbial broth etc. It is also used for targeted analysis of known compounds such as pesticides, antibiotics, vitamins, amino acids, phospholipids etc. In the field of marine bioactive compounds, LC-MS/MS are widely used to determine metabolite profile of marine plants, fish, crustaceans, micro algae, and microbes. Structure elucidation of marine bioactive peptides is another possible application of LC-MS/MS. It is also used for targeted analysis of phenolic acids, flavonoids, carotenoids, vitamins, phospholipids etc. in the marine plants and animals.

Liquid Chromatography

A high-performance liquid chromatograph (HPLC) or ultra-high performance liquid chromatograph is a common front end of a LC-MS/MS system. HPLC/UHPLC separates mixture of compounds based on the principle of adsorption chromatography where the mobile phase is liquid solvent and the stationary phase is solid sorbent particles tightly packed inside a metal column. When the stationary phase is polar in nature, the type of chromatography is called normal phase chromatography; while in case of reverse phase chromatography the stationary phase is non polar. Reverse phase chromatography is most commonly used with mass spectrometry because of its repeatability, relatively lower maintenance, and chromatographic resolution for wide range of mid-polar to non-polar compounds. Most common type reverse phase stationary phase material is C18, where the silica particle surface is modified with 18 carbon chain length hydrocarbons. Similarly, C30 and C8 columns are used for separation of highly nonpolar and relatively polar compounds respectively. Normal phase

chromatography commonly uses unmodified silica as stationary phase and used for chromatographic separation of polar compound mixture such as fatty acids and tocopherol isomers. In reverse phase chromatography water in combination with acetonitrile or methanol is most common type of mobile phase, where the solvent elution programme starts with high aqueous content and gradually ramped to high organic content. In case of normal phase chromatography, water can not be used as mobile phase because of its interaction with silica particles. A combination of nonpolar and relatively polar solvents is used as mobile phase, where the elution programme starts with high content of nonpolar solvent and the content of polar solvent is gradually increased. The following figure presents different parts of the HPLC/UHPLC.

Figure. Different parts of a liquid chromatograph



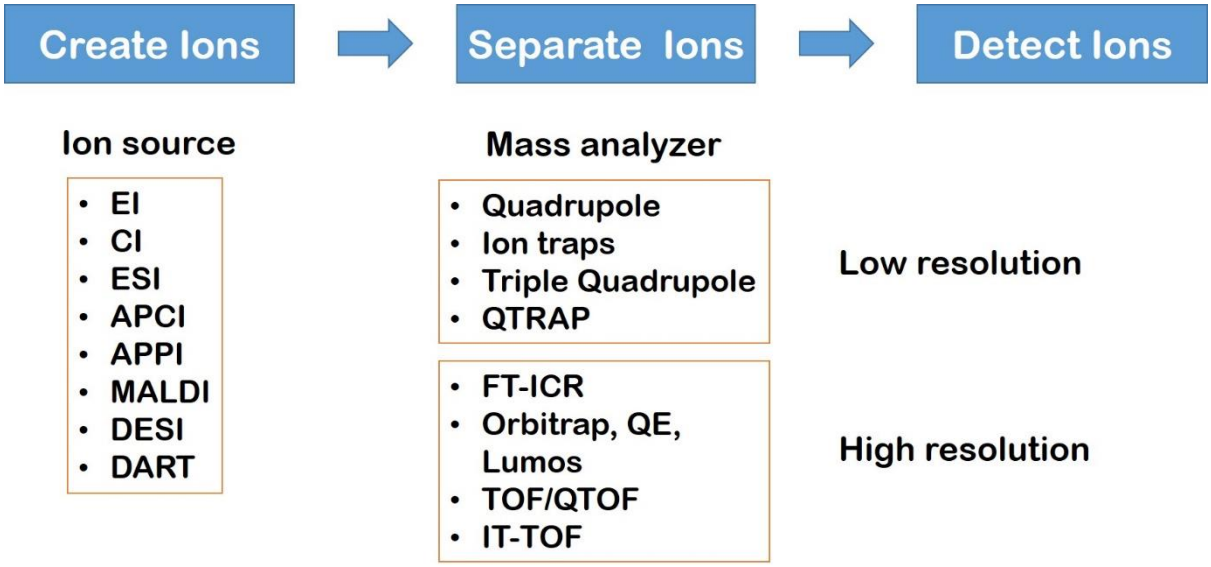
Chromatographic resolution is directly proportional to the length of the chromatographic column, and inversely proportional with the particle size, inner diameter, and pore size. Hence, a short length column with finer particle size, shorter inner diameter, and smaller pore size can achieve the same chromatographic resolution in less time which will take longer in a column of higher length, with bigger particle size, longer inner dia and bigger pore size. However, the solvent back pressure is extremely high in such short columns and can be used only with

UHPLC where the pump is equipped to handle back pressure up to 18000 psi. UHPLC is a popular front end of mass spectrometer due to short analysis time, sharp peak shape, and less consumption of organic solvents.

Mass spectrometer

In a mass spectrometer the compounds introduced in liquid phase form gas phase ions in the ion source. Next the ions are separated in a mass analyzer and finally they reach the detector. The detector shows the output in the data system as a mass spectrum, total ion chromatogram (TIC), base peak ion (BPI) chromatogram, or extracted ion chromatogram (XIC). There are different possible ion sources and mass analyzer combinations in different mass spectrometers which are used for different application needs. The following figure shows a schematic of major parts of a mass spectrometer and lists different possible ion sources and mass analysers.

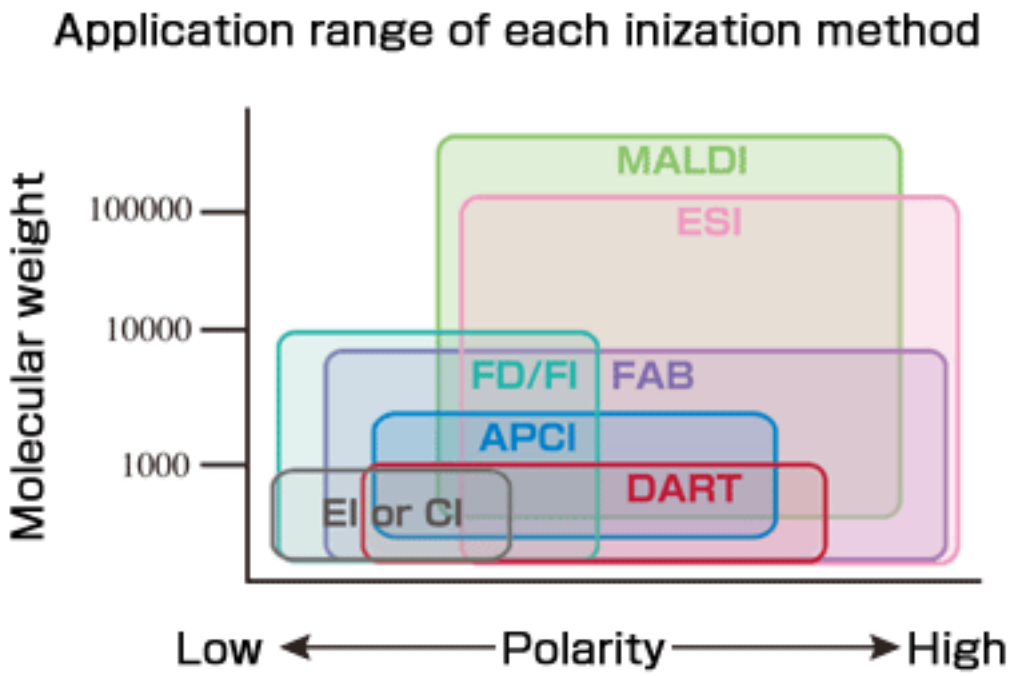
Figure. Schematics of different parts of mass spectrometer



The electron impact (EI) and chemical ionization (CI) ion sources are found in gas chromatograph hyphenated mass spectrometer (GC-MS) and the ionization happens under complete vacuum. EI is a hard ionisation technique, where the molecular weight ion is almost completely broken down into fragments. Hence, for molecular weight determination, CI ion source is preferred in GC-MS; where a pseudo molecular ion with reagent gas (most commonly methane or ammonia) is formed through a soft ionisation technique. Electron spray ionisation (ESI), atmospheric pressure chemical ionisation (APCI), atmospheric pressure photo ionisation (APPI), fast atom bombardment (FAB), matrix assisted laser desorption ionisation (MALDI), desorption electron spray ionisation (DESI), direct analysis in real time (DART) are prominent

ion sources in different LC-MS. These ion sources are used based on the polarity and molecular weight range of the target analytes or analyte classes, as shown in the following schematics.

Figure. Application range of different ion sources



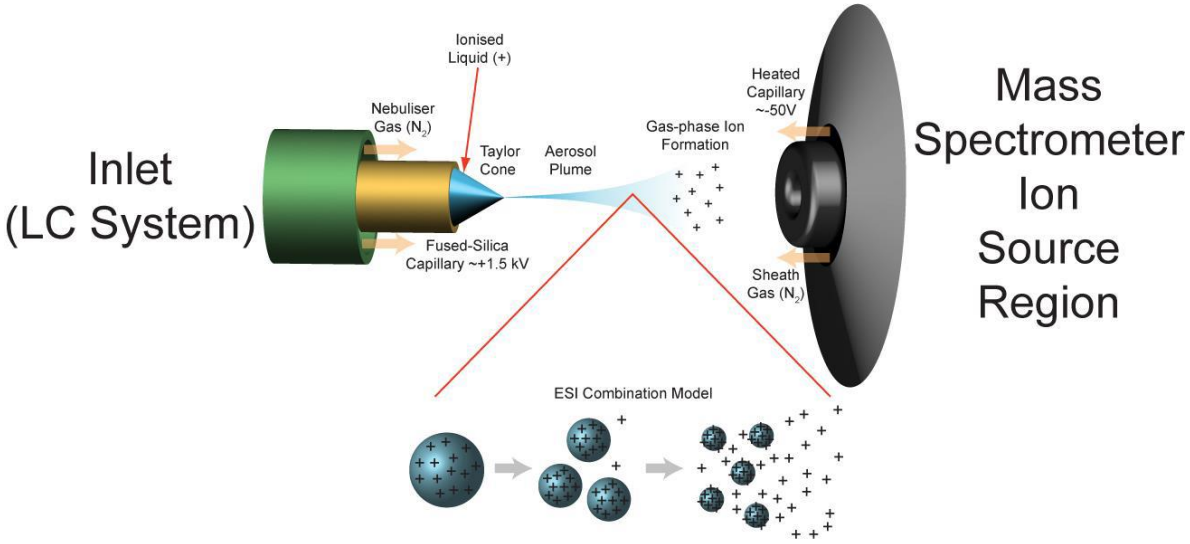
ESI is most commonly used ion source with LC-MS as a wide range of compounds with medium polarity to high polarity, and low to high molecular weight can be analysed. APCI is suitable for compounds with low polarity which do not ionise sufficiently in ESI. APPI is suitable for highly non polar compounds such as persistent organic pollutants. MALDI is prominently used for intact mass determination of proteins.

In ESI ion source, the compound in liquid phase is nebulised through a charged capillary. In positive ionisation mode a positive charge is applied, where in negative ionisation mode a negative charge is applied. The solvent around the droplets containing charged ions is rapidly evaporated by the heater gas and ion source temperature. Hence, the droplets become smaller and smaller, finally releasing only gas phase ions. The ESI is a soft ionisation technique, where the most commonly formed ions are $[M + H]^+$, and $[M - H]^-$, depending on the ionisation operating mode. These ions of a compound are called adduct/pseudo molecular weight ion/parent ion/precursor ion. Some other common adducts found in ESI ion source are listed below.

Positive polarity adduct	Mass difference*	Negative polarity adduct	Mass difference*
$[M + H]^+$	+1.0078	$[M - H]^-$	-1.0078
$[M + NH_4]^+$	+18.0344	$[2M - H]^-$	-
$[M + Na]^+$	+22.9898	$[M - H + H_2O]^-$	+18.0106
$[M + K]^+$	+38.9637	$[M - H + CH_3OH]^-$	+32.0262
$[M + H_2O + H]^+$	+18.0106	$[M - H + CH_3CN]^-$	+41.0265
$[M - H_2O + H]^+$	-17.0027	$[M + Cl]^-$	+36.4609
$[M - 2H_2O + H]^+$	-35.0133	$[M + Br]^-$	+79.9040

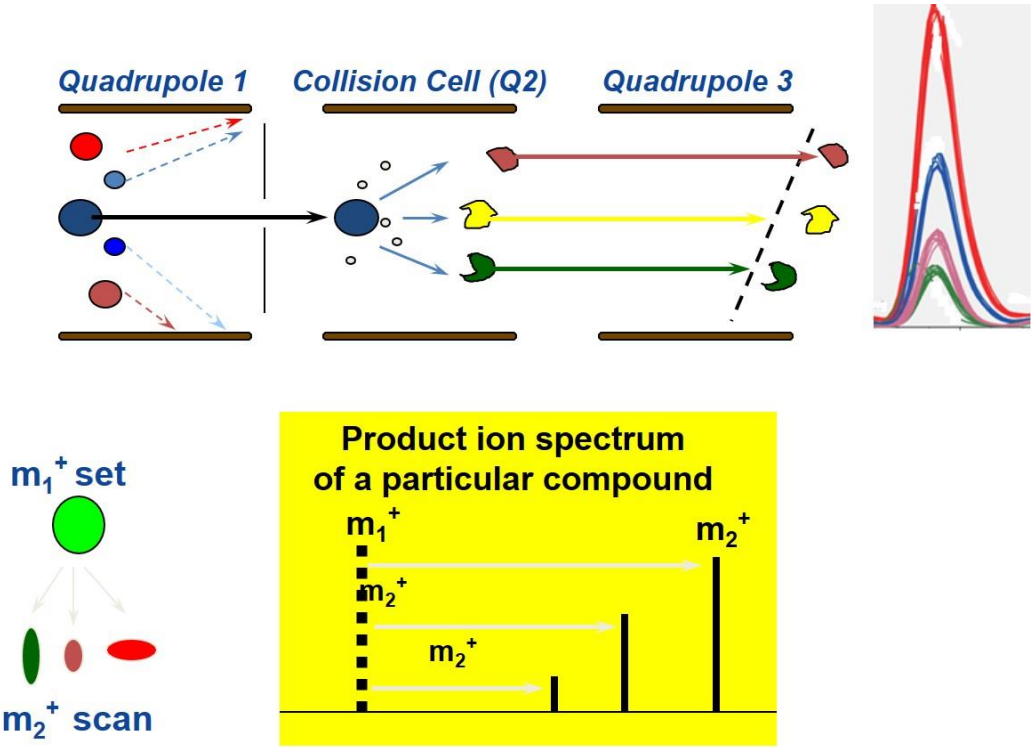
A cone voltage or declustering potential is applied on the ion source cone to further push the generated gas phase ions towards the mass analyser. Hence, the flow rate of nebuliser gas, heater gas, ion source temperature, cone voltage/declustering potential are important parameters that need to be optimised in analyses using ESI ion source. Following is a schematic of the operation of ESI ion source.

Figure. Schematic of ESI ion source.



A quadrupole system uses four cylindrical magnets that are set parallel to each other and function to filter ions based on their mass-to-charge ratio (m/z). The analyzer consists of two pairs of like charged magnets that oppose each other and keep the ions within the ion path of the quadrupole under vacuum. Ions are filtered based on their masses as they traverse the linear ion path. When a linear series of three quadrupoles is used, the resulting triple stage quadrupole analyzer is able to both filter and fragment the ion stream. In most cases, the first (Q1) and third (Q3) quadrupoles act as mass filters, while the second (Q2) quadrupole dissociates ions by having them collide with argon, helium or nitrogen gas. Quadrupole-based mass analysers excel at tracking single ions or reactions for extended periods of time. This is why they are preferentially used in the targeted analysis of compounds, especially known compounds such as drugs and pollutants. This is also why quadrupole mass analysers are often used in the fields of food safety, environmental analysis, clinical and forensic toxicology studies. The triple quadrupole (QQQ) mass spectrometer (MS) consists of a series of three quadrupoles and selects ions of specific mass-to-charge ratios (m/z) when a specific DC/RF voltage combination is applied. The first and third quadrupoles (Q1) act as mass filters, while the Q2 acts as a collision cell. Triple quadrupole MS systems can be operated in a tandem MS/MS assay called Selected

Reaction Monitoring (SRM) (sometimes also called Multiple Reaction Monitoring (MRM)) mode. SRM is a highly selective mode whereby a fixed set of DC and RF voltages is applied to the quadrupole, permitting only one precursor ion, which is measured by its m/z, to pass. After the Q1 filters that specific precursor ion, the Q2 produces product ions via collision of the precursor ion with a neutral gas (e.g., nitrogen) in a process called collision-induced dissociation (CID). Product ions progress to the Q3, where only a specific m/z is permitted to pass. By breaking the ion apart into its component fragments, a given molecular species can be identified not only by its mass but by product identity. In this way, SRM reduces noise and increases selectivity. Following schematic presents the working of a triple quadrupole mass analyser in MRM mode.



LC-MS/MS is a versatile technology with wide range of application in marine bioactive compound analysis. LC-MS/MS can be used for free amino acid analysis in serum, tissue or plant material extracts. The instrument with ESI and MALDI ion source has prominent application in the field of proteomics and peptide sequencing of bioactive peptides. The technique is also used for structural elucidation of bioactive compounds through molecular weight determination, and tandem mass spectra fragmentation pattern. High resolution mass spectrometer can be used for high throughput metabolomic profile of biological materials and can derive important insights in biological experiments.

FT-NIR AND ITS APPLICATION IN FISHERIES SECTOR

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Introduction

Although in 1800, Herschel discovered light in the near-infrared region (NIR), the acceptance of the NIR region of the electromagnetic spectrum as a valuable tool can be credited to two investigators. First, Karl Norris in the 1960s who worked on instruments that could record NIR spectra and applied multivariate treatment of the spectra to determine major plant components (Norris, 1996). Second, Phil Williams in the 1970s, who recognized the potential of the technology to segregate wheat grain according to protein content (Williams, 1995). Since then, the development of equipment featuring improved electronic and optical components, as well as the advent of computers capable of effectively processing the information contained in NIR spectra, has facilitated the expansion of this technique in an increasing number of fields. In recent past, the use of near-infrared spectroscopy (NIRS) for the assessment of mineral composition and trace elements in plant and animal tissues, has opened new horizons to NIR spectroscopists dealing with the application of this technique to the agricultural, medical, food safety and environmental fields (Font et al., 2007). Infrared energy is the electromagnetic energy of molecular vibration. The energy band is defined for convenience as the near infrared (0.78 to 2.50 microns); the infrared (or mid-infrared) 2.50 to 40.0 microns; and the far infrared (40.0 to 1000 microns). In the recent past, the FT-Near-infrared (NIR) spectroscopy has gained much importance as a non-destructive analytical technique and it has become the tool of choice in several fields of application (Bec et al., 2021). Similar to the IR and Raman techniques, NIR spectroscopy extracts information from the sample through molecular vibrational. Spectral region for NIR is 12 500–4000 cm^{-1} or 800–2500 nm, and it measures the absorption of light from the sample in the NIR region at different wavelengths. The recorded NIR spectrum consists of overtones and combination vibrations of molecules that contain CH, NH or OH groups. This makes NIR spectroscopy the first choice for the analysis of organic materials in the chemical and pharmaceutical industry, as well as in the food, feed and agricultural industries. Near-infrared spectroscopy is primarily a more of quantitative technique (Whetsel, 1968).

Advantages of FT-NIR

- The low absorptivities of absorption bands are compatible with moderately concentrated samples and longer path lengths than those used in the mid-infrared region.
- It is a non-destructive technique because sample preparation is avoided.
- As the sample preparation step has been avoided in NIRS analysis being very simple to perform so that there are few operator-induced sources of error.
- Measurement and result delivery is fast.
- It is an environmentally friendly analytical technique, as no chemicals are used during the process.
- Many components of the material being analyzed can be determined simultaneously from a unique spectrum, and not only chemical but also physical parameters can be determined on the sample.
- The accuracy of an NIR analysis is comparable to that of the chemistry reference method, and its precision is usually high because the avoidance of sample treatment.

Disadvantages of FT-NIR

- The complexity of the NIR spectrum requires chemometric techniques to extract the relevant information to the component being measured.
- In constructing the calibration models the whole physical and chemical variability predicted to be present in the population must be added to the calibration set of samples. This implies continuous addition of new samples to the original set to encompass all variations.
- No specific methodology for transferring calibrations between instruments has gained widespread acceptance in recent years.

Relevance of IR and NIR in Agriculture

Infrared radiation is absorbed, transmitted, and emitted by compounds, depending on the vibrations of the chemical bonding between component atoms or molecules. The vibrations' frequencies determine the parts of the infrared spectrum that they absorb, transmit, or reflect. Thus, each molecule or compound has its unique fingerprint or spectral signature. Based on the spectral signature, it is possible to know the size, shape of atoms and molecules, and the bonds holding them together. The information on the structure of the compounds helps to identify the

makeup of compounds or plants. Depending on the interaction with light, it is also possible to determine the amount of these compounds. Therefore, infrared light is valuable in studying the different compounds and their concentrations.

Applications of NIRS in fish and fishery products

In the recent years, NIR spectroscopy has given wide array of applications many fields. But this chapter we are restricting to applications in fishery sector. NIR is generally used in fish quality control, fish authenticity and fish safety aspects. Fish quality control refers specially to the determination of the proximate composition of the fish samples and the evaluation of the freshness of the products (Liu. et al., 2013). The proximate composition determines the organoleptic quality of a specific fish product. At the same time, the determination of the freshness of samples is another indicator of the quality of the product. Nowadays, NIRS is becoming an alternative as a quality control method, due to its advantages over traditional analysis (Karlsdottir et al., 2014). The globalization and expansion of the fish and aquaculture sector, in addition to the increasing public concern about food quality, have caused a growing interest in several issues related to fish authenticity. According to the European Regulation (EU) n. 1379/2013 (European Regulation (EU) n. 1379/2013), fishery and aquaculture products must be labelled with the commercial designation, proper scientific name of the species, production method (e.g. caught, farmed), fishing gear (e.g. hook, trap, trawl), catch or production area and storage method (unfrozen or frozen-thawed). Both the geographical origin and the production method, among others, can strongly affect the characteristics of the two types of products, whose discriminating properties are usually difficult to determine. Several analytical techniques have been traditionally used to assess fish authenticity. However, even though they are well established, there is still a necessity for faster, easier and more affordable methods. The substitution of valuable species with cheaper ones is most commonly happing food fraud in fish and fishery products. However, the differences in the economic value, the exploitation of endangered species, the replacement with poisonous fish and their difficult identification, make the substitution of fish species an extended problem, especially severe after processing, at the retailers and supermarkets (Blanco-Fernandez et al., 2021). The mentioned frauds particularly affect fish fillets and ready-to-eat products, such as fish products, which cannot be recognized through the traditional morphological analysis. The use of NIRS with the objective of discriminating between species has been explored during the recent years (Ghidini et al., 2019).

NIR Applications

NIR is a technology seemingly tailor-made for agriculture, not only for research but also for growers, packers, and distributors. Today's devices are sophisticated and give rapid measurements that are easy to understand and use in real-time. They are precise enough for use in research and complement existing equipment, taking the guesswork out of farm management, while becoming ever-more user friendly. NIR scanners are more widespread and developed for spot measurements, with imaging applications gradually gaining popularity. Smaller and portable tools are more affordable than ever and will continue to be an essential step in bringing quantifiable scientific value to the food supply chain, saving stakeholders time, money, and resources.

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BIOACTIVE COMPOUNDS FROM FAMILY VIBRIONACEAE

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The need of new bioactive compounds always makes the marine environment as a fascinating source. The immense biodiversity helps to quench the thirst of new molecules from the secondary metabolites by bacteria, sponges, marine plants and invertebrates. Most of the secondary metabolites from the marine environment are used as antibacterial, fungal, inflammatory, tumour and viral medicines

The Vibrionaceae family is the most diversified family under the bacterial classification of aqua bacteria. The vibrios are the most successful group of aquatic bacteria that can be found as symbionts in the aquatic animals, corals, shrimps, plants and free living as well. Some species are found as symbionts in specialized luminous organs of marine fish and invertebrates, whereas a number of other species are well-known pathogens of humans or animals. Vibrios account for the 80% of heterotrophic bacteria in marine environment which makes them important in biogeochemical cycling. As a Bacteria occurring in aquatic ecosystems vibrio are reported to have the ability to inhibit the growth of other microorganisms by producing antimicrobial substances such as antibiotics and bacteriocins. The analysis of coral associated vibrios have revealed they produce secondary metabolites of bioactive importance

Vibrindole and other compounds

Vibrindole is a bis-indole derivative isolated from *Vibrio parahaemolyticus* An3 strain. It is an antagonistic compound for many microbes and thus can be used as antimicrobial. Phenyl acetic acid pyrrolidine carboximidamide, pyrrolopyrazines, tetramethyl pyrazine and phenolic compounds also isolated from *Vibrio parahaemolyticus* and has the activity against different bacteria, fungi and other microbes

Anticorrosion substance

The symbiotic bacteria in corals belonging to the vibrio group produce many bioactive compounds for the protection of corals. The bacteria *V.neocaledonicus* produce anticorrosive paint like substance to form an inhibitory layer on the surface of corals

Acetylcholinesterase inhibitors (AChEIs) from vibrios

This can reduce the activity of the enzyme AChE that degrades the neurotransmitter acetylcholine (ACh). ACh is essential for processing memory and learning. Both the concentration and function of ACh are found to be decreased in patients with neuro degenerative diseases like Alzheimer‘s disease. AChEIs may alter the cholinergic synapse, which is involved in the etiology of Alzheimer’s disease

Snake bite venom inhibitors

Snakebite envenoming kills more than 100,000 people and maims more than 400,000 people every year . The pathophysiological effects induced by snakebite envenomations are induced by the biological activities of several enzymes, mainly phospholipases A_2 (PLA_2), zinc dependent metalloproteinases (SVMPs) and serine proteinases (SVSPs). Snake venom induce hemorrhage by the proteolytic degradation of endothelial cell surface proteins and extracellular matrix components of capillaries and venules. PLA_2 hydrolyze the sn2 ester bond of cell membrane glycerophospholipids, inducing systemic and local myotoxicity, mionecrosis and edema. Indole and indole-3-formaldehyde from *V. neocaledonicus are reported to have the potential of inhibiting the snake venom.*

Vibrio as a source of polymers

Vibrios are the known species to produce biopolymers as a virulence mechanism biofilm. The biopolymers can be utilized for various polysaccharide usages. The Vibrio cholerae biofilms are extensively studied for bioactive compounds. The biofilms are found to have bioactive lipid called sebestenoic acid. This is helpful in controlling the species distribution, signalling to production in biofilm. This can be used for beneficial biofilms.

Vibrio as the source of enzymes

Vibrio have the successful history of producing enzyme for almost all sugars present on earth. Their sugar utilizing diversity helps them for ubiquitous distribution. They produce enzyme with different metabolic and physiological utilities. Vibrios produce chitinase enzyme which can utilize the shrimp shell waste for production of other bioactive compounds. The

presence of chitinases and chitinase encoding genes has been confirmed for several members of the family. Vibrios are able to degrade other complex carbohydrates such as fucoidan and laminarin found in algal species.

Vibrio as a source of antibiotics

The red pigment and antibiotic prodigiosin has been isolated from *V. psychroerythreus,V. gazogenes* and *V. ruber*. Prodigiosin have a broad range of biological activities, including antimicrobial, antimalarial, immunosuppressive, and. The clinical potential as antibiotics is, however, limited due to a low therapeutic window and considerable toxic effects. The production of prodiginines in a *Vibrio* sp. isolated from estuaries conferred competitiveness against a *Bacillus* sp. from the same sample, suggesting that prodigiosin might act as an antibiotic in the natural environment.

BIODEGRADABLE FILMS FROM MARINE BIOPOLYMERS

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Production and properties of biodegradable film

Worldwide annual plastics manufacturing is appraised to exceed 330 million tons by 2020, growing at a CAGR (Compound Annual Growth Rate) of 5.3% from 2014 to 2020 (Plastics market to reach $654.38 billion by 2020: grand view research, Inc.), revealing trillions of dollar in terms of world economic returns. More than 40% of the plastics are used for packaging and almost half of them are used for food packaging in the form of films, sheets, bottles, cups, tubs, and trays, etc (Rhim et al., 2013).

Petroleum resources are widely employed in manufacturing these polymers, which generates issues regarding both economic and environmental protection. After their beneficial life, it is preferable for the packing materials to biodegrade in a reasonable time without generating environmental problems (Rhim et al., 2013). Though, synthetic plastic materials have been extensively utilized for the packaging of several kinds of food, they caused serious environmental issues because they are not easily degraded in the environment after use.

So, there is an increasing trend to exert environmentally friendly polymers with the aim of substituting non-degradable materials, thus lowering the environmental pollution as a result of waste accumulation. To address environmental issues, and simultaneously increase the shelf-life and quality of food, decreasing packaging waste has catalyzed the exploration of new bio-based packaging materials like edible and biodegradable films. Structuring biopolymers, such as polysaccharides, lipids, and proteins have been applied for the formulation of edible films.

Biodegradable films

The most common keywords in the recent literature of food packaging are “biopolymer” “edible”, “biodegradable”, “biocompatible”, “Environmental friendly” and renewable resources. This implies the consumer’s awareness about the environmental safety. The main objective of food packaging is to maintain the integrity of food products till it reaches the consumer without affecting the environment. It should also protect the food products from external factors like light, oxygen, moisture, permeable volatile compounds and unavoidable circumstances. In order to achieve, these packaging materials should have excellent physical, chemical barrier and biological properties to ensure safety and improve the quality of packed food products. “Socially responsible products” is the recent trend in food processing and

packaging industries for sustainability of food and food-related issues (FAO, 2015). Among the agricultural industries, seafood processing generates large amount of by-products (50-70%) which contains protein, carbohydrates and minerals. Biodegradable material used for biodegradable film can be divided into 4 categories: hydrocolloids (protein and carbohydrates), lipids, resins and composites (Krochta et al., 1997).

Biodegradable film formation

The biodegradable film can be formed either using wet method or dry method. In wet method of edible film production uses water and solvent medium whereas, dry method includes molden casting, extrusion and heat pressing. Dry method mostly used for thermoplastic polymers and the thermoplastic properties of polymer, suitable additives, and plasticizer should be taken into consideration in film formation process (Krochta et al., 2002 & Guilbert et al., 1997). In dry process, heat is applied to above the melting point of the film forming polymer, cause them to flow.

	Dry method	**Wet method**
Process	heat	liquid
Film properties depend	Pressure, temperature and time	pH, temperature and time
Film forming medium	solid	liquid
Drying technique	Solvent evaporation	Compression, moulding and extrusion

Ref: Guerrero et al., 2010

Benefits of and possible uses for natural biopolymer-based packaging materials

- Edible
- Biodegradable
- Supplement the nutritional value of foods
- Enhanced organoleptic characteristics of food, such as appearance, odor, and flavor
- Reduced packaging volume, weight and waste
- Incorporated antimicrobial agents and antioxidants
- Extended shelf-life and improved quality of usually non-packaged items
- Control over intercomponent migration of moisture, gases, lipids, and solutes
- Individual packaging of small particulate foods, such as nuts and raisins

- Function as carriers for antimicrobial and antioxidant agents
- Microencapsulation and controlled release of active ingredients
- Possible use in multilayer food packaging materials together with non-edible films
- Low cost and abundant
- Annually renewable resources

Protein based biodegradable films

Protein-based films from animal sources (gelatin, collagen, casein, whey protein, etc.) have been studied for the development of biodegradable films due to their high abundance, acceptable mechanical properties, excellent gas barrier properties to non-condensable gases (oxygen, carbon dioxide, and nitrogen) and aromas.

Fish gelatin based biodegradable film

Gelatin has attracted attention for the development of edible film due to its biodegradability, availability and its fimogenic property. Gelatin films used as a delivery system for medical and pharmaceutical industry. In food industry, gelatin used as a casting component or coating material. As a rule, the physical properties of gelatin films depend chiefly on the properties of the raw materials extracted from the different animal species and on the processing conditions of gelatin manufacturing. They also depend on the physical parameters used in film processing, such as temperature and drying time, and on formulation ingredients, such as the inclusion of plasticizers or cross-linker. Sorbitol and glycerol are the plasticizers most commonly used in producing gelatin-based films.

Although gelatins have good film forming ability, in order to accommodate drawbacks (high solubility and hydrophilicity) and designated applications (high strength and seal ability) various modifications have been reported. To improve the strength and functionality of the fish gelatin films, mixing with some other polymers of similar nature is desirable. Polysaccharides addition improved the tensile strength and water vapour permeability properties, although the transparency of films were reduced. Gelatin-based edible films and coatings have already been proposed to protect, maintain or extend the shelf-life of food products. Factors that should be considered when designing this type of system include the chemical nature of food, controlled release mechanisms, food organoleptic characteristics and additive toxicity, storage and distribution, physical and mechanical properties of packaging materials and regulations to be applied.

Polysaccharide based films

Polysaccharides, such as chitosan, carrageenan and alginate have been used as biopolymer materials to create coatings and edible films to reduce traditional plastic packages. Polysaccharides are one of the materials that have been recently used as a sustainable material in coatings and edible films formulation. Polysaccharides are not toxic and widely available materials in nature, and have selective permeability to carbon dioxide and oxygen. These characteristics allow polysaccharide-based coatings and edible films to extend the shelf life fruits, vegetables and meat products.

Chitosan based biodegradable film

Chitosan is the hetropolysaccharide derivate from chitin which would be considered as most abundant polymer next to cellulose. Among polysaccharides, chitosan has received considerable attention from academics and industry for food packaging applications in the form of edible films and coatings due to its particular physicochemical features, biodegradability, non-toxicity, biocompatibility, good film-forming properties, chemical stability, and high reactivity. In food industry, chitosan was used as a food protecting as a coating material or bioplastic film. Sufficient data showed that the use of chitosan as an edible coating enhanced the food safety and appearance and increased the market share of the product (Renuka et al., 2016 & Mohan et al., 2012). However, Inherent drawbacks of chitosan films such as high sensitivity to water, low mechanical and thermal stability lead to a shorter food shelf life compared to the conventional food packaging material and consequently limited its applications in food packaging. Therefore, different strategies have been proposed to tackle these issues and to improve the properties of chitosan-based materials, such as cross-linking, high-energy irradiation and blending with other biopolymers.

Alginate based biodegradable film

Alginates are hydrophilic polysaccharide and can be found in brown seaweed, made up of 40% dry mater consisting another ionic polymer. Sodium alginate films has been widely used in biomedical applications such as wound dressing, tissue engineering and drug delivery system due to its biocompatibility, biodegradability as well as its properties which similar to the human tissues. Due to their composition, alginates can form strong films with fibrous structure (in solid state), and are considered good filmogenic materials with characteristics comparable to those of conventional materials. In combination with glycerol, sodium alginate has been used to create fruit and vegetable coatings, thus contributing to the delay of

degenerative processes and microbiological damage by maintaining color, preserving the content of polyphenols and anthocyanins, and completely improving the quality of fruits after harvest. Along with other hydrocolloids, it has been used to form coatings used in numerous subsectors of the food industry.

Lipid-based biodegradable films

Proteins and polysaccharides have acceptable mechanical and gas barrier properties, but they show high moisture sensitivity (Rhim & Ng, 2007). On the contrary, lipid films exhibit acceptable water vapor barrier property and high oxygen permeability, but they have poor mechanical properties.

Conclusions and Future Perspectives

In conclusion, regular plastic packaging materials used in the food industry, often for single uses, are polluting. Viable solutions to this problem include the use of alternative packaging materials—natural, obtained from bio-based polymers, biodegradable, and even edible. Studies and research in this regard highlight the use of films and coatings successfully applied to products across the entire food chain. The biopolymers from marine sources have biodegradability, non-toxicity, biocompatibility, good film-forming properties, chemical stability, and high reactivity. Developing awareness campaigns for producers and consumers can have a positive effect in amplifying the use of new packaging materials.

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SEAFOOD CONSUMPTION IN INDIA: PATTERNS AND DETERMINANTS

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

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

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

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

Drivers and barriers to fish consumption: important factors

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

Fish consumption: feedback from consumer behaviour studies

A study on knowledge and perception of fish consumers with respect to health benefits of fish consumption, safety and quality of fish and major drivers and barriers to consumption was done among consumers in Kerala State, India. The state was identified for the study due to its

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

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

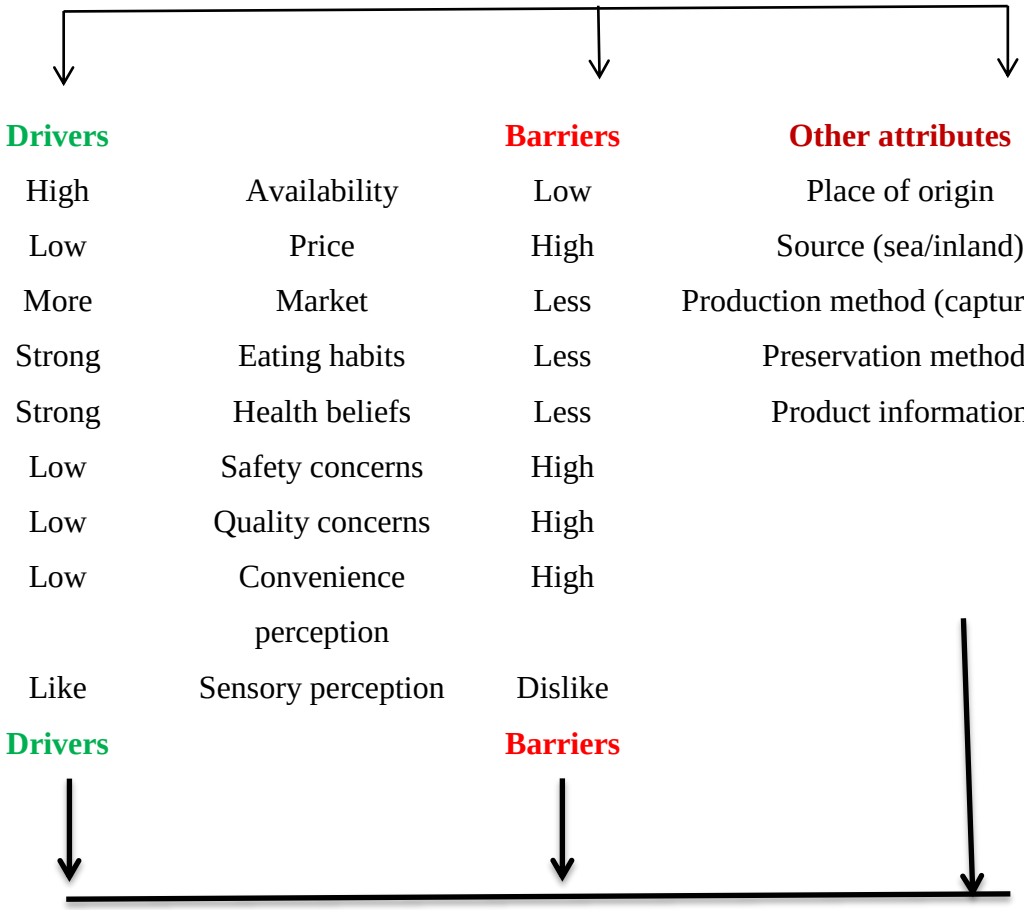
In another study conducted among urban consumers of Kerala, Conjoint analysis revealed that the factors like 'place of origin of fish', '24x7 accessibility' and 'sensory perception' were the most contributing drivers while 'price of fish' and 'availability of favourite fish' were the most important barriers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

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

Drivers and barriers to fish consumption

Personal factors (values, beliefs, attitudes, demographics),

Situational factors and Environmental factors



Quantity, frequency and characteristic of fish consumed

Sajeev *et al.*, 2018

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

Hence the most important drivers and barriers to fish purchase identified among the above studies has to be considered by existing and upcoming entrepreneurs.

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ICAR-CIFT ENGINEERING INTERVENTIONS IN POST HARVEST FISHERIES SECTOR

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

1. Fish Descaling Machines

1.1.Fish descaling machine with variable drum speed

The fish de-scaling machine is designed and fabricated for removing the scales of fishes easily. This equipment can remove scales from almost all types/sizes/ species of fishes ranging from marine to freshwater species like Sardine, Tilapia to Rohu. The machine is made of SS 304 and has a 10 kg capacity (Fig. 5). It contains a 1.5 HP induction motor and a Variable Frequency Drive (VFD) to vary the speed of the drum depending on the variety of the fish load. The drum is made of a perforated SS 304 sheet fitted in a strong SS Frame. A water inlet facility is provided in the drum for easy removal of the scales from the drum so that area of contact to the surface will be more for removal of scales. The water outlet is also provided to remove scales and water from the machine. An Electronic RPM meter was attached with the de-scaling machine which directly displays the RPM of the drum. The speed of the drum is a factor

influencing the efficiency. The machine takes only 3-5 minutes to clean 10 kg fish depending on the size.

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

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

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

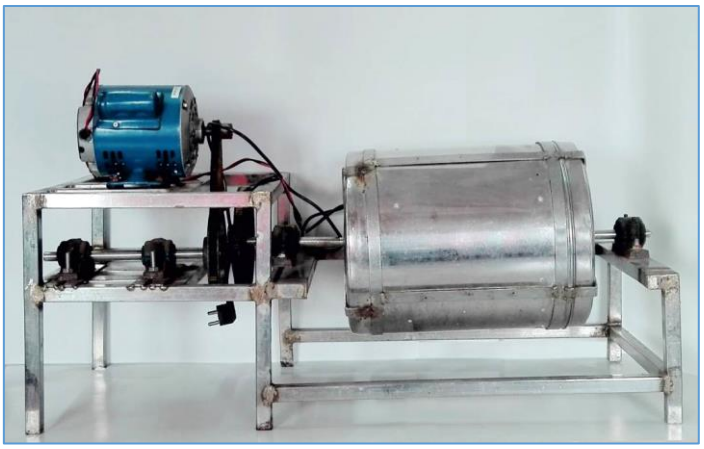

Fig. 6. Fish de-scaling machine with fixed drum speed

1.3. Hand operated Fish descaling machine

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

Fig. 7. Hand operated fish de-scaling machine

2. **Fish meat bone separator**

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

Fig. 8. Fish meat bone separator

3. Refrigerated mobile fish vending kiosk

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

Fig. 8. Refrigerated mobile fish vending kiosk

4. Electronics and Instrumentation

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

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

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

5. **Energy and Water Use Optimization in Seafood Processing Industry**

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

6. Development of portable fish quality and freshness assessment sensor

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

7. Commercialization of engineering technologies

A more pragmatic system for business incubation and promoting start-up companies concerning agricultural technologies have been evolved in recent times within the ICAR-CIFT.The Agri-Business Incubation (ABI) center along with Institute Technology Management Unit (ITMU) seeks to provide business consulting services to agriculture-related businesses and helps to develop a strategic business plan. ABIs facilities for incubation of new business ideas based on new agricultural technologies by providing cheap space, facilities, and required information and research inputs. The Agribusiness Incubator Program also seeks to provide business consulting services to agriculture-related businesses and helps to develop a strategic business plan.

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

ROLE OF ICAR-CIFT IN THE ENTREPRENEURSHIP DEVELOPMENT IN THE SEAFOOD SECTOR

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Introduction

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

Business Incubation at ICAR-CIFT

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

- Commercializing technologies developed by ICAR-ICAR-CIFT
- Helping entrepreneurs to commercialize business ideas utilizing the R&D back up of the institutes
- Providing pilot level production facilities in fisheries to entrepreneurs
- Imparting training for creating prospective entrepreneurs and value added manpower

Process of Incubation

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

Incubation facilities under one roof

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

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

Incubation Office facility for Clients

Pilot level production facility in fisheries

A state-of-the-art generic semi-commercial production facility is made available to incubating entrepreneurs for developing value added products from fish. The ABI Centre provides facilities and staff support on a fee for use basis to assist companies and individuals, with production and testing of product formulations provided by the client. The user can experiment with new equipment and processing steps on old products or to link old equipment in new ways. For incubatees, the pilot plant is an ideal testing arena to determine commercial run

viability of new products. The pilot plant also serves as a process lab, a place to see how processing equipment impacts food products under varying conditions. The pilot plant facility at ICAR-CIFT includes production facilities for:

1. Pre-processing and cooking
2. Chilled & Frozen fish
3. Coated fish products
4. RTE fish products
5. Extruded fish products
6. Fish sausage
7. Smoked fish products
8. Fish based Cookies
9. Fish pickle
10. Fish wafers
11. Chitin & Chitosan
12. Protein hydrolysate
13. Collagen peptide
14. Advanced packaging

Plant Plant Facility

Categories of entrepreneurs approaching the agri-business incubator

The fishing industry includes any industry or activity concerned with culturing, harvesting, processing, preserving, storing, transporting, marketing or selling fish or fish products. The commercial activity is aimed at the delivery of fish and other seafood products for human consumption or as input factors in other industrial processes. Directly or indirectly, the

livelihood of over 500 million people in developing countries depends on fisheries and aquaculture. The commercial sector of the fishing industry comprises the following chain: (i) Commercial fishing and fish farming which produce the fish (ii) Fish processing which produce the fish products and (iii) Marketing of the fish products. The clients approached the centre during the period 2010-19, for various services are classified based on the area of their expertise and represented as Fig: 1.

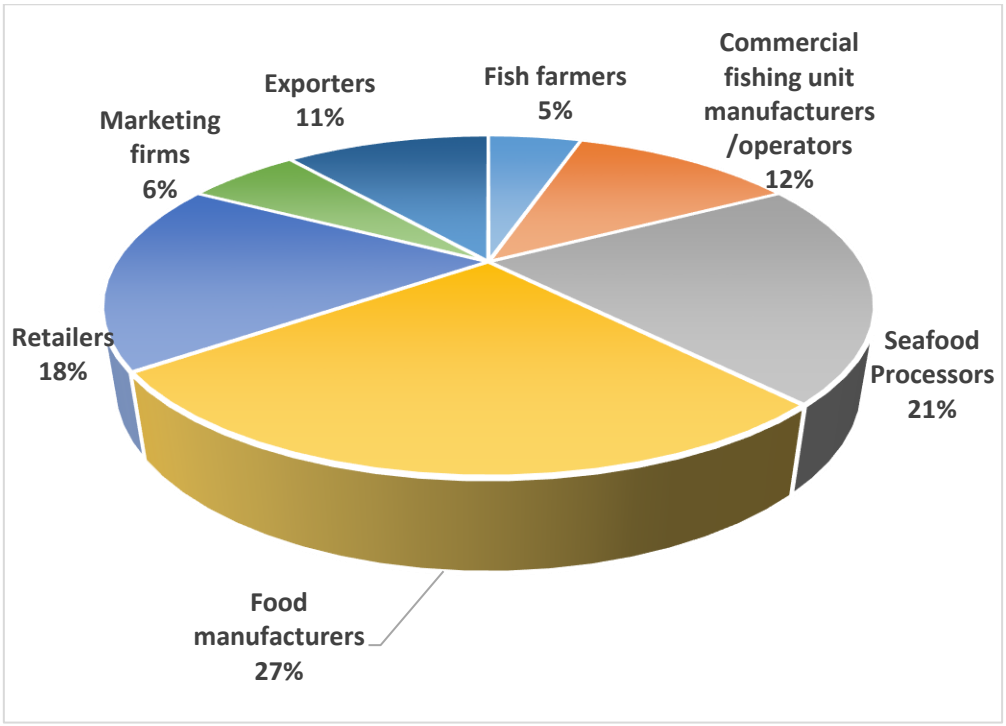

Fig 1: Categories of entrepreneurs incubated at ABI Centre

Activities of ICAR-CIFT Incubation Centre

- 200+ registered incubatees
- More than 150 successful graduates
- 16 companies had taken the office facility
- More than 175 clients have used the pilot plant facility for product development and trial production
- Around 850 people trained in various technology concepts
- 49 B2B Meets / Industry Meets / Workshops / Sensitization Programs conducted
- Around 62 new product brands in the market

Conclusion

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

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BIOCHEMISTRY & NUTRITION DIVISION OF ICAR-CENTRAL INSTITUTE OF FISHERIES TECHNOLOGY

About the Division

The Biochemistry & Nutrition Division of ICAR-CIFT focuses its research activities with a broad vision on Marine Bioprospecting. Starting from developing a strong database on nutritional and contaminant profiling of marine resources, the division has extended its research on different arenas such as development and validation of green extraction techniques for obtaining high value marine bioactives and their detailed structural elucidation by employing high end analytical techniques. The Division is also having well equipped facilities for carrying out in-vitro and in-vivo bioactivity studies of the commercially significant marine bioactives. Further, the division has already been into development of dietary supplements for the aquaculture sectors to improve the nutritional security. Research activities carried out at the division was instrumental in development and commercialization of several value added marine products of nutraceutical and pharmaceutical significance. Several consultancies and Training programmes are being organized from the division to cater the needs of a wider domain.

Major Areas of Research

- Nutrient and Contaminant profiling of fish and fishery products.
- Extraction, characterization and establishing bioactivity of Marine biomolecules
- Biomedical applications of commercially important biomolecules viz collagen, collagen peptide, squid peptide, proteoglycans, n-3 PUFA, fish oil, liver oil, fish proteins and peptides, chitosan derivatives, vitamins from marine sources.
- Bioprospecting of Seaweeds – Novel Frontiers
- Development of nutraceutical and functional foods of marine origin.
- Designing innovative dietary aquafeed supplements
- Antinutritional factors of aquafeed ingredients – Non-Destructive Method Development

General Facilities Available

Animal House Facility, Wet lab facility for fish culture

Instruments

- Gas Chromatography: FID, GCMS, LC -HR MS
- High Performance Liquid Chromatography (HPLC): PDA, Preparative HPLC
- Particle size & Zeta Analyzer (Malvern)
- Atomic Absorption Spectroscopy (AAS) & Flame photometer
- ELISA Reader and Western Blotting Unit
- Supercritical Fluid Extraction Unit
- Iatroscan
- Electrophoresis apparatus and gel imaging system

Major Nutraceuticals developed from the Division

Seaweed Nutridrink

Encapsulated β-carotene

Oyster peptide

Chitosan based sanitizer

Fish soup powder distributed to adolecent girls in a Jowai, Meghalaya

Collagen peptide

Sodium alginate - seaweed

Fucoidan - seaweed