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Training Manual

on

"Technological Interventions in Processing, Value addition and Packaging of Aquatic Resources"

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कृषिअनुसंधान ICAR

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ITEC Training Manual

'Technological Interventions in Processing, Value Addition and Packaging of Aquatic Resources'

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FOREWORD

ICAR - Central Institute of Fisheries Technology (ICAR-CIFT) is the only National institute in the Country where research in all disciplines relating to harvest and post harvest is undertaken. This premier institute has a dynamic post-harvest technology section which focus on complete utilization of fishery resources with proper and efficient handling, processing, value addition and waste management techniques. The Institute has developed technologies for preservation and processing of commercially important as well as unconventional varieties of fish and shell fishes. Creation and expansion of food processing/preservation capacities of the fish processing sector is addressed in this regard. Techniques/technology for a broad array of specialty products based on new ingredients, functional foods, alternate protein sources, bioactive formulations have been developed by the institute based on consumer demand. The post-harvest research outcomes of the Institute are disseminated through structured and demand driven training programmes on fish processing, value addition, packaging and waste management for the benefit of potential entrepreneurs in fisheries sector.

The international training on '*Technological Interventions in Processing, Value addition and Packaging of Aquatic Resources*' sponsored by Indian Technical & Economic Cooperation Programme (ITEC), Ministry of External Affairs, Government of India, assumes a greater importance as the technical expertise developed over many decades by the institute is shared with researchers and officials from different countries. Over a duration of three weeks, twenty-one participants from thirteen countries were exposed to various fish processing technologies. The topics for the programme were meticulously selected to impart comprehensive knowledge on processing and packaging of fish and shellfish, conversion of the waste generated to high value products as well as its safety and quality aspects. This training manual covers different aspects of fish processing, value addition, waste utilization and packaging as well as includes topics like microbial quality and food safety, ISO 22000/HACCP, and the role of extension in fisheries. I am sure that this manual will be beneficial for the ITEC trainees in adopting the lessons learned for effectively implementing in the post-harvest sector of their respective countries.

Dr. George Ninan
Director
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Importance of Fish in Human Nutrition

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1. Introduction

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

Many fish (especially fatty fish) are a source of long-chain omega-3 fatty acids, which contribute to visual and cognitive human development, especially during the first 1000 days of a child's life. The fat content and fatty acid profiles of farm raised fishes affected by the feed used in culture practice. Though the fish consumption has increased, people are obtaining smaller amounts of omega-3 fatty acids from aquatic foods, because these fats are more prevalent in marine fishes than in freshwater fish. Fish also provides essential minerals such as calcium, phosphorus, zinc, iron, selenium and iodine as well as vitamins A, D and B, thus helping to reduce the risks of both malnutrition and noncommunicable diseases which may co-occur when high energy intake is combined with a lack of balanced nutrition. Nutritional content is especially high in small fish species consumed whole and in fish parts that are not usually consumed (such as heads, bones and skin) which are having lower economic value. It

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

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

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

2. Nutritional Value of Fish and Shellfish

2.1 Fish Proteins

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

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

Table 1. Protein content of fish and shellfish

Fish group	Protein content (%)
White finfish	16–19
Fatty finfish	18–21
Crustaceans	18–22
Bivalves	10–12
Cephalopods	16–18

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

Table 2. Essential amino acids in fish and shellfish (g/100g)

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

2.2 Fish lipids

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

The triacylglycerol depot fat in edible fish muscle is subject to seasonal variation in all marine and freshwater fishes from all over the world. Fat levels tend to be higher during times of the year when fishes are feeding heavily (usually during the warmer months) and in older and healthier individual fishes. Fat levels tend to be lower during spawning or reproduction. When comparing fat contents between farmed and wild-caught food fish, it should be remembered that farmed species have a tendency to show a higher proportion of muscle fat than their wild counterparts. Also, the fattyacid composition of farmed fish depends on the type of dietary fat used in raising the fish.

Cholesterol in Fish

Cholesterol is independent of fat content and is similar in wild and cultivated fishes. The fish and shellfish contain well under 100 mg of cholesterol per 100 g, and many of the leaner types of fish typically have 40–60 mg of cholesterol in each 100 g of edible muscle. It is known that most shellfish also contain less than 100 mg of cholesterol per 100 g. Shrimp contain somewhat

higher amounts of cholesterol, over 150 mg per 100 g, and squid is the only fish product with a significantly elevated cholesterol content, which averages 300 mg per 100 g portion. Fish roe, caviar, internal organs of fishes (such as livers), the tomalley of lobsters, and the hepatopancreas of crabs can contain high amounts of cholesterol.

A note on Omega-3 PUFA in Fish and Shellfish

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

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

2.3 Vitamins in Fish

The vitamin content of fish and shellfish is rich and varied in composition, although somewhat variable in concentration. In fact, significant differences are neatly evident among groups, especially regarding fat-soluble vitamins. Furthermore, vitamin content shows large differences among species as a function of feeding regimes. Of the fat-soluble vitamins, vitamin E (tocopherol) is distributed most equally, showing relatively high concentrations in all fish groups, higher than those of meat. However, only a part of the vitamin E content is available as active tocopherol on consumption of fish, because it is oxidized in protecting fatty acids from oxidation. The presence of vitamins A (retinol) and D is closely related to the fat content, and so they are almost absent in most low-fat groups. Appreciable but low concentrations of vitamin A are found in fatty finfish and bivalve molluscs, whereas vitamin D is very abundant in fatty fish.

Water-soluble vitamins are well represented in all kinds of fish, with the sole exception of vitamin C (ascorbic acid), which is almost absent in all of them. The concentrations of the rest are highly variable; however, with few exceptions, they constitute a medium-to-good source of such vitamins, com-parable with, or even better than, meat. The contents of vita-mins B_2

(riboflavin), B_6 (pyridoxine), niacin, biotin, and B_{12} (cobalamin) are relatively high. Indeed, 100 g of fish can contribute up to 38, 60, 50, 33, and 100%, respectively, of the total daily requirements of those vitamins. Fatty fish also provides a higher supply of many of the water-soluble vitamins (namely pyridoxine, niacin, pantothenic acid, and cobalamin) than does white fish or shellfish. Crustaceans also possess a relatively higher content of pantothenic acid, whereas bivalve molluscs have much higher concentrations of folate and cobalamine.

2.4 Fish Minerals

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

Calorific value

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

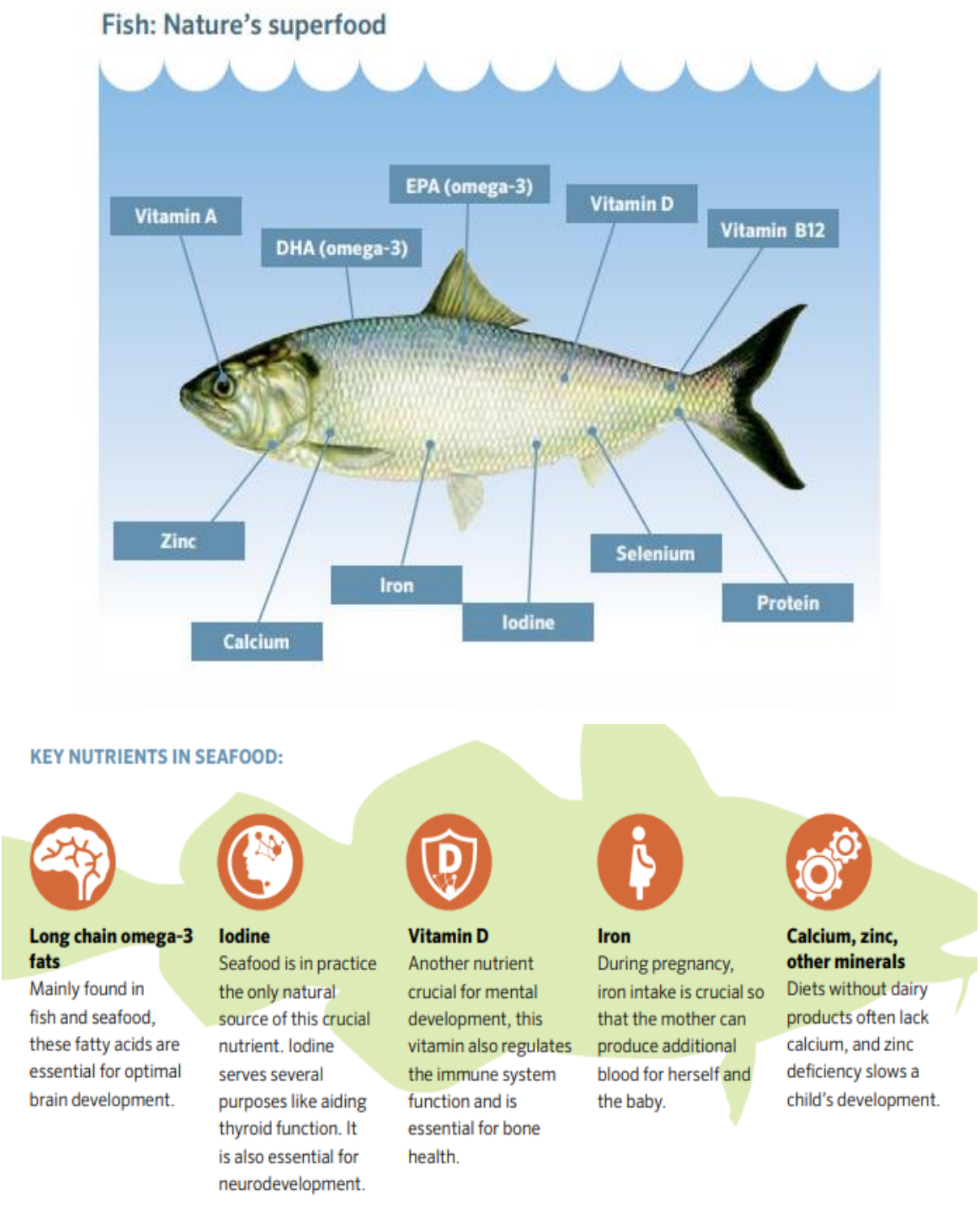
Table 3. Vitamin content of the different groups of fish and shellfish (mg or mg per 100 g), and relation to DRIs

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

*T denotes Trace

Table 4. Selected mineral content of the different groups of fish and shellfish (mg per 100 g), and relation to DRIs

	Na	K	Ca	Mg	P	Fe	Zn	Mn	Cu	Se	Cr	Mo	I
White finfish	50–150	200–500	10–50	15–30	100–300	0.2–0.6	0.2–1.0	0.01–0.05	0.01–0.05	0.02–0.1	0.005–0.02	0.005–0.02	0.01–0.5
Fatty finfish	50–200	200–500	10–200	20–50	200–500	1.0–5.0	0.2–1.0	0.01–0.05	0.01–0.05	0.02–0.1	0.005–0.02	0.005–0.02	0.01–0.5
Crustaceans	100–500	100–500	20–200	20–200	100–700	0.2–2.0	1.0–5.0	0.02–0.2	0.1–2.0	0.05–0.1	0.005–0.02	0.01–0.05	0.01–0.2
Molluscs	50–300	100–500	50–200	20–200	100–300	0.5–10	2.0–10	0.02–0.2	0.02–10	0.05–0.1	0.005–0.02	0.01–0.2	0.05—0.5
Cephalopods	100–200	200–300	10–100	20–100	100–300	0.2–1.0	1.0—5.0	0.01–0.1	0.02–0.1	0.02–0.1	0.005–0.02	0.01–0.2	0.01–0.1
RDA	1500	4700	1000	320/420	700	18/8	8/11	1.8/2.3	0.9	0.025/0.055	0.035	0.045	0.15
% RDA per 100 g	3–33	2–10	1–20	4–50	15–100	2–50	1–90	0–10	1–100	25–100	15–60	10–100	8-100
% RDA/Md			6	5	30	18	2		2	100			100



Source: FAO - Fish and human nutrition

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Introduction to Fish Processing Techniques

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The demand for healthy and safe food products has driven significant interest and growth in the food processing industry. The focus on health, nutrition, and convenience is reshaping the global food industry. Fish products, in particular, have garnered attention due to their rich nutritional profile, including high-quality protein, essential vitamins, minerals, and healthful polyunsaturated fatty acids. The growth of fresh fish and seafood as a category is noteworthy, ranking third among the food categories with the fastest overall growth worldwide. This places it just behind drinkable yogurt and fresh soup, both of which have experienced an 18% growth rate. The increasing consumption of freshwater and seawater fish is expected to persist in the future. As fish is highly nutritious, it is also highly susceptible to spoilage, due to intrinsic and extrinsic factors. Proper processing and packaging help in maintaining the eating quality of fish for extended period. Worldwide, an array of processing and packaging methods are followed. This ranges from a simple chilled or ice storage,salted and drying to most recent and advanced high pressure and electromagnetic field applications, which attracts opportunities from both small scale and industrial level entrepreneurs.Fish products in live, fresh chilled, whole cleaned, fillets steaks, battered and breaded products, variety of dried products, smoked fish, fish sausage and traditional products are the range of low-cost processing methods which can be readily adopted by small-scale fishers. The processing methods like canning or heat processing, freezing, vacuum and modified atmosphere packaging, analogue products, high pressure processing, pulsed light processing, irradiation, electromagnetic field etc are the processing methods which requires higher investments can be adopted by large scale entrepreneurs, apart from the above-mentioned processing methods.

The advantages of food processing are numerous and play a crucial role in meeting the diverse needs of consumers, ensuring food safety, and contributing to economic development. Here are some key advantages:

1. Conversion of Raw Food: Processing transforms raw food into edible, usable, and palatable forms, making it more convenient for consumers to incorporate into their diets.
2. Preservation and Storage: Processing helps in the preservation and storage of

perishable and semi-perishable agricultural commodities. This is essential for preventing spoilage, extending shelf life, and reducing post-harvest losses.

3. Market Stabilization: Food processing helps avoid market gluts by enabling the storage and distribution of produce, ensuring a more stable supply throughout the year. This, in turn, reduces the impact of seasonal variations and helps manage market prices.
4. Employment Generation: The food processing industry generates employment opportunities across various stages of production, processing, packaging, and distribution, contributing to economic development.
5. Ready-to-Consume Products: The development of ready-to-consume convenient products saves time for consumers in meal preparation, aligning with the demands of modern, fast-paced lifestyles.
6. Improvement of Palatability: Processing enhances the palatability and organoleptic quality of food products through value addition, making them more appealing to consumers.
7. Inhibition of Anti-nutritional Factors: Certain processing methods can help inhibit anti-nutritional factors present in raw food, making the final products safer and more nutritious.
8. Facilitation of Marketing and Distribution: Processed foods are often easier to market and distribute, contributing to efficient supply chain management.
9. Long-Distance Transportation: Processing enables the transportation of delicate perishable foods across long distances, facilitating the availability of a wider variety of food products in different regions.
10. Microbial Safety: Processing helps make foods safe for consumption by controlling and eliminating pathogenic microorganisms through methods such as pasteurization, canning, and irradiation.
11. Specialized Diets: Modern food processing allows for the development of healthy foods tailored for individuals with specific dietary needs, such as those with allergies, diabetes, or other health conditions.
12. Nutritional and Food Security: Food processing can contribute to overall nutritional security by creating fortified products and enhancing the availability of nutrient-rich foods.

13. Export Potential: Processed food products can be exported, providing opportunities for earning foreign exchange and contributing to the growth of the national economy.

1. Chilled Fish Products

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

2. Frozen Fish Products

Freezing is an age-old practice to retain the quality and freshness of fishery products for a long time. This involves the conversion of water present in fishery products to ice i.e., a phase change from liquid to solid phase takes place in freezing. This retards the microbial and enzymatic action by reducing the water available for their action. This involves exposing fish products to very low temperature (<-35^{0}C) to enable freezing of free water and maintained at -18^{0}C till it is consumed. Plate freezing, air blast freezing, cryogenic freezing and individual quick freezing arethe methods adopted by the industry to preserve food products.

3. Dried and Salted Fishery Products

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

4. Smoked Fishery Products

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

5. Retort Pouch Processing

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

6. Extrusion Technology

In order to improve the utilization of underutilized fisheries resources, there is a need to minimizethe post-harvest losses, develop innovative processing technologies and utilize processing waste for industrial and human use. One such technology, which will be suitable for utilization of low value fish or by catch, is extrusion technology. Use of fish mince with cereals for extrusion processwill enable production of shelf-stable products at ambient temperature. Extrusion cooking is usedin the manufacture of food products such as ready-to-eat breakfast cereals, expanded snacks, pasta,fat-bread, soup and drink bases. The raw material in the form of powder at ambient temperature isfed into extruder at a known feeding rate. The material first gets compacted and then softens and gelatinizes and/or melts to form a plasticized material, which flows downstream into extruder channel. Basically, an extruder is a pump, heat exchanger and bio-reactor that simultaneously

transfer, mixes, heats, shears, stretches, shapes and transforms chemically and physically at elevated pressure and temperature in a short time. At times, the extrusion cooking process is also referred as High Temperature Short Time process. In extrusion process gelatinization of starch anddenaturation of protein ingredient is achieved by combined effect of temperature and mechanical shear. The conversion of raw starch to cook and digestible materials by the application of heat andmoisture is called gelatinization. During extrusion the conditions that prevail are high temperature,high shear rate and low moisture available for starch may lead to breakdown of starch molecules to dextrins.

7. Irradiation

Irradiation is a physical treatment that consists of exposing foods to the direct action of electronic,electromagnetic rays to assure the innocuity of foods and to prolong the shelflife. Irradiation of food can control insect infestation, reduce the numbers of pathogenic or spoilage microorganisms, and delay or eliminate natural biological processes such as ripening, germination, or sprouting in fresh food. Like all preservation methods, irradiation should supplement rather than replace good food hygiene, handling, and preparation practices.

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

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

Different forms of irradiation treatment are raduarization (for shelf life extension), radicidation (for elimination of target pathogens) and radappertization (for sterilization). Radiation processingis widely used for medical product sterilization and food irradiation. Moreover, the use of irradiation has become a standard treatment to sterilize packages in aseptic processing of foods and pharmaceuticals.

Irradiation produces some chemical changes, which, although lethal to foodborne bacteria, do notaffect the nutritional quality of the food but lead to the production of small amounts of radiolytic products. Gamma irradiation has been considered as an interesting method of preservation to extend the shelf life of fish and also to reduce qualitatively and quantitatively the microbial population in fish and fish products. Irradiation doses of 2–7 kGy can reduce important food

pathogens such as Salmonella, Listeria, and Vibrio spp., as well as many fish-specific spoilers such as *Pseudomonaceae* and *Enterobacteriaceae* that can be significantly decreased in number.

8. Microwave Processing

The applications of microwave heating on fish processing include drying, pasteurization, sterilization, thawing, tempering, baking etc. Microwaves are electromagnetic waves whose frequency varies within 300 MHz to 300 GHz. Microwave heating is caused by the ability of the materials to absorb microwave energy and convert it into heat. Microwave heating of food materials mainly occurs due to dipolar and ionic mechanisms. Microwave heating also occur due to the oscillatory migration of ions in the food which generates heat in the presence of a high frequency oscillating electric field. Studies showedthat chemical changes involved during different microwave cooking practices of skipjack tuna andwill retain omega-3 fatty acids compared to frying/canning. Microwave blanching can be carried out for color retention and enzyme inactivation which is carried out by immersing food materials in hot water, steam or boiling solutions containing acids or salts. Microwave drying is used to remove moisture from fish and fishery products. Microwave drying has advantage of fast drying rates and improving the quality of product. In microwave drying, due to volumetric heating, the vapors are generated inside and an internal pressure gradient is developed which forces the wateroutside. Thus, shrinkage of food materials is prevented in microwave drying. Microwave combined with other drying methods such as air drying or infrared or vacuum dryingor freeze drying gave better drying characteristics compared to their respective drying methods ormicrowave drying alone.

9. Ohmic heating

Ohmic heating is an emerging technology with large number of actual and future applications. Ohmic heating technology is considered a major advance in the continuous processing of particulate food products. Ohmic heating is direct resistance heating by the

flow of an electrical current through foods, so that heating is by internal heat generation. Ohmic heating is defined as aprocess wherein electric current is passed through materials with the primary purpose of heating the object. During ohmic heating, heating occurs in the form of internal energy transformation (from electric to thermal) within the material. Therefore, it can be explained as an internal thermalenergy generation technology and it enables the material to heat at extremely rapid rates from a few seconds to a few minutes. Ohmic heating have a large number of actual and potential future applications, including its use in blanching, evaporation, dehydration, fermentation, extraction, sterilization, pasteurization and heating of foods. The microbial inactivation due to ohmic heatingcan be explained by the presence of electric field. The additional effect of ohmic treatment may be its low frequency (usually 50e60 Hz), which allows cell walls to build up charges and form pores. As a main consequence of this effect, the D value observed for the microbial inactivation under ohmic heating is reduced when compared to traditional heating methods. More research is needed to completely understand all effects produced by ohmic heating to food products, effects of applied electric field, the applied electric frequency during ohmic heating over different microorganisms and foods, cold spot determination etc.

10. Infrared and Radiofrequency Processing Technologies

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

The main advantage of electromagnetic heating over conventional electric and gas oven-based heating is its high thermal efficiency in converting the electrical energy to heat in the food. In ordinary ovens, a major portion of the energy is lost in heating the air that surrounds the food, fairly a good amount escapes through the vent, besides being lost

through the conduction to the outside air. In contrast, almost all the heat generated by electromagnetic radiations, which reachesthe interior of the oven, is produced inside the food material itself. According to the reports the energy efficiency of EMR based systems is 40-70%, as compared to approximately 7-14% in caseof conventional electric and gas ovens.

11. High Pressure Processing

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

12. Ultrasound Processing

Ultrasound refers to sound that is just above the range of human hearing, i.e. above frequency of 20 MHz. Ultrasound when propagated through a biological structure induces compressions and depressions of the medium particles imparting a high amount of energy to the material. The soundranges for food applications employed can be divided into two, namely, low energy, highfrequency diagnostic ultrasound and high energy low frequency power ultrasound. Low energy applications involve the use of ultrasound in the frequency range of 5-10 MHz at intensities below1 W/cm^2. Ultrasonic waves at this range are capable of causing physical, mechanical, or chemicalchanges in the material leading to disrupting the physical integrity, acceleration of certain chemical reactions through generation of immense pressure, shear, and temperature gradient in the medium.Ultrasonics has been successfully used to inactivate *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes*, *Staphylcoccus aureus* and other pathogens.

13. Bio Preservation

Bacteriocins are a heterogeneous group of antibacterial proteins that vary in spectrum of activity, mode of action, molecular weight, genetic origin and biochemical properties.

Various spices and essential oils have preservative properties and have been used to extend the storage life of fish andfishery products. Natural compounds such as essential oils, chitosan, nisin and lysozyme, bacteriocins have been investigated to replace chemical preservatives and to obtain green label products.

14. Application of enzymes

Enzymes have been used for the production of various cured and fermented fish products from centuries. Because of their appreciable activity at moderate temperature, products and process haveemerged that utilizes enzymes in a deliberate and controlled fashion in the field of food processing. Cold active enzymes including elastase, collagenase, chymotrypsin extracted from Atlantic cod were used in various food processing applications. The other applications of cold active enzymes include caviar production, extraction of carotenoprotein etc. Treatment with protease under mild treatment conditions extending for a few hours can result in the recovery of the proteins from fishframe or shrimp shell waste. The role of transglutaminase in surimi production is well established. The gel strength of surimi can be improved by the application of extracellular microbial transglutaminase. Lipase extracted from Pseudomonas spp can be used to produce PUFA enrichedcod liver oil. Enzymatic de-skinning of fish fillets was done by partial denaturation of skin collagenusing a gentle heat treatment followed by immersion in enzyme solution for several hours at low temperature (0-10^{0}C). De-skinning of tuna, Herrin, Squid were also carried out by using differentenzyme technology.

Hygienic Handling of Fish and Fishery Products

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Fish is nutritionally significant due to its balanced profile of nutrients, including easily digestible proteins with a balanced amino acid profile, healthy long-chain polyunsaturated fatty acids, minerals, and vitamins. This nutritional composition makes fish a valuable food source for maintaining health and well-being. Among animal proteins, fish protein is noted as one of the cheapest options. The fast growth rate of fish, combined with its affordability and nutritional richness, positions fish as a tool for achieving nutritional and social security. This suggests that incorporating fish into diets can address nutritional needs and contribute to overall food security. The consumption of fish and other seafood is growing rapidly each year, driven in part by the recognition of their nutritional benefits. More than one billion people worldwide rely on fish as a crucial source of animal proteins, obtaining at least 20% of their protein from fish. The consumption of fish has been linked to various health benefits, including cardiovascular health, due to the presence of omega-3 fatty acids. While the contribution of agriculture to Gross Domestic Product (GDP) is decreasing globally, the fisheries sector is experiencing growth in most countries. Fishery products play a substantial role in international trade, providing valuable foreign exchange to many developing countries.

However, this nutritious nature of fish along with the unpredictable fish catch makes it necessary to preserve it under hygienic conditions to ensure its quality till it reaches the consumers. Food hygiene relates to "all conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain". These hygienic measures cover aspects related to the hygienic design of facilities during harvesting, transportation, processing and distribution, to personnel hygiene, cleaning, sanitation and pest control.

The major objective of effective handling and transportation is to land the fish inthe same condition as it is at the time of catch within the limits of practicability under good commercial practice. For this, the general rules to be followed include:

- Maintain the fish at low temperature throughout the post-harvest chain by proper icing
- Avoid mishandling of the fish
- Sorting of fish - catch wise (species-wise, size-wise)
- Use of clean containers/surface for the holding/transportation of fish
- Use of good quality water and ice
- Personnel hygiene at every handling stage

1. Hygienic Fish Handling practices during harvesting

Careful and hygienic handling of fish during harvesting can ensure enhanced longevity of fish. These mainly include proper harvesting and handling practices, cleanliness of premises, workers hygiene and maintenance of cold chain. For this:

- Harvesting of fish/shell fish in the early morning or night hours isrecommended as the ambient temperature is lower to avoid drastic temperature swings.
- Line fishing or trapping are the best capture techniques that can be adoptedfor harvesting fin fish as these are the least damaging techniques, thus less stressful to the species.
- Nets with knotless mesh is always recommended for harvesting fishes as they minimize the damage caused to skin and scales on account of smooth surface.
- The time period for which they are left in gear should be minimized to minimize spoilage.
- Fish should be removed carefully from the gear to reduce the damage or stress caused. Physical damage to skin, scales etc may be unappealing and further can affecting the value of fish.
- On harvesting, air exposure should be kept to absolute minimum as it may lead to drying out. Practices like dropping or throwing of fish should be avoided.
- Equipment, materials, surfaces and surface coatings that come into contact with fish and fishery products must be corrosion-proof, durable, non-toxic as well as easy to clean and disinfect.
- Farm should have provision to maintain cold chain like availability of ice and chilling facilities to preserve fresh fish and fishery products throughout the fishing period.

- Workers must maintain a reasonable standard of hygiene to prevent spoilage of fish or fishery products.

Live Fish Harvesting in aquaculture farm

2. Hygienic Fish Handling in Domestic Market

Domestic markets play a very crucial role in the development of fisheries sector in the country as about 85% of the total fish landing is distributed through domestic markets. They play a major role in strengthening the nutritional and food security. Ensuring hygienic handling practices in domestic market helps to minimize post-harvest losses and leads to food safety. Following minimum basic requirements can ensure good hygiene in domestic market:

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

Domestic fish market

3. Hygienic Fish Handling in Processing Units

Processing units aims towards value addition of the fish thus improving the market value of the products. Following hygienic practices in these units will ensure improved fish quality which in turn is critical to increase marketing opportunities.

- Appropriate design and layout comprising sufficient working space under adequate hygienic conditions, an area for machinery, equipment and storage, separation of operations preventing cross-contamination, adequate natural or artificial lighting, ventilation and protection against pests.
- All food contact surfaces shall be smooth, durable, non-absorbent type, easyto maintain and clean and non-toxic.
- Availability of uninterrupted supply of portable water throughout for all processing operations.
- Availability of suitable facilities for temperature, humidity and other controls.
- All pre-processing and processing activities should be scheduled under HACCP system with proper documentation.
- Regular monitoring of processing unit for plant sanitation with an in-house laboratory and an in-process product quality check.
- Effective maintenance and sanitation systems including cleaning and sanitation procedures, pest control systems, waste management and monitoring effectiveness.
- All fish handlers should follow the recommended hygienic handling practices such as periodic medical examinations, regular cleaning and disinfection procedures prior and post to processing activities.

Fish Processing Unit

4. Proper Transportation of fish

Proper transportation should be ensured throughout the supply chain right fromharvest till it reaches the customer for prime quality fish and fishery products. During transportation, measures should be taken to protect food from potential sources of contamination and damage likely to render the food unsuitable for consumption. Proper transportation maintaining low temperatures provide an environment which effectively controls the growth of pathogenic or spoilage microorganisms. Care during transportation includes:

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

Interior of a refrigerated truck Fish layered with ice in insulated box

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

Refrigeration enabled Mobile Fish Vending Kiosk developed by ICAR-CIFT forhygienic seafood handling and storage in domestic/retail markets

- No odour, flies or contamination
- Fish display in chilled condition: 25-30 Kg (2-4 ^{0}C)
- Shelf-life extension: 3-4 days compared to conventional icing methods
- Attached with Fish Descaling machine
- Provisions for Cutting, Cleaning and Packing
- Separate provisions for collecting liquid and solid wastes
- Storage facility in Ice-Box (70-75 Kg)
- Mobile unit with wheels
- Plug-in unit with provision for inverter battery/DG set
- Designed considering the maximum weight that a man pulls on a rickshaw
- Can be integrated with Solar power system
- Detachable roof and seat for roadside vending

Value Addition in Fishery Sector

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Seafood is a highly valued and versatile food source, appreciated worldwide for its nutritional benefits, quality, and safety. These valuable commodities are simultaneously convenient due to its potentiality for high product diversification. It is rich in essential nutrients, including high-quality protein, omega-3 fatty acids, vitamins, and minerals (such as iodine, selenium, and zinc). These nutrients are vital for overall health, making seafood an important component of a balanced diet. Though an excellent source of nutrients, it is highly perishable too, which demands its effective processing and preservation. Effective approaches in this regard is crucial to maintain its inherent quality characteristics and extended stability for better returns, to abate post-harvest losses as well as for consumer safety and satisfaction. Food preservation methods are designed to maintain the quality and safety of food products by controlling factors that lead to spoilage. This can be achieved by controlling the storage temperature, maintaining proper water activity, proper pH, use of preservatives, alone or in combination. By carefully selecting and combining preservation techniques, the shelf life of various foods can be extended, reducing waste and ensuring a safe and stable food supply. Among the various preservation methods available, low temperature preservation viz., chilling as well as freezing has attracted interest of many researchers on account of its minimal changes in the texture and other characteristics of fish if properly processed and stored. Traditional preservation techniques, collectively known as curing techniques viz., drying, salting, smoking, pickling etc. are commonly adopted methods for seafood preservation. The demand for minimally processed fishery products has led to the exploration of innovative processing and preservation technologies like retort processing, high pressure processing, irradiation, pulse light technology, cold plasma processing, ohmic heating etc. to preserve and extend the quality and stability of these valuable commodities These innovative technologies are helping meet the growing demand for minimally processed seafood products that offer convenience, safety, and quality without compromising the nutritional and sensory characteristics. As consumers seek healthier and more convenient food options, these advancements play a critical role in the seafood industry's efforts to meet their needs.

Options for Value Addition

India's status as one of the fastest-growing economies and the second-largest consumer market globally provides a strong foundation for the processed seafood industry. The fish processing sector is poised for significant growth, and value addition represents a key strategy to enhance profitability in this competitive and cost-intensive industry. In foods, value is a combination of functional attributes as well as emotional benefits arising on account of nutritional as well as sensory facets at superior quality as well as affordable price. In addition, it promises utilization of the under-exploited nutrient rich resources in the most effective manner.

Value addition in fish and fishery products is a flexible strategy that responds to the diverse needs of different markets and consumer segments. It allows the industry to create convenient, flavorful, and nutritious products while meeting various dietary, cultural, and sustainability preferences. This approach enhances the appeal and competitiveness of fish products in the global marketplace. Some approaches for value addition include:

1. **Variations in Form:** Value-added fish products can be presented in different forms, including dressed or trimmed, minced, filleted, or deboned. These forms make it more convenient for consumers to use the products in their cooking, reducing the preparation time and effort.

2. **Added Ingredients and flavours:** Value-added products can include additional ingredients to enhance flavor, nutrition, or convenience. Adapting products to cater to diverse global tastes and culinary traditions allows the industry to tap into a wider consumer base. For example, adding a coating to fish can provide a crispy texture, while incorporating bioactive or functional constituents can boost the nutritional value of the product. Marinating fish products with flavor-enhancing ingredients or pre-packaging them with sauces can improve taste and convenience, making them more appealing to consumers seeking easy and flavorful meal options.

3. **Convenience Products:** Ready-to-cook or ready-to-eat fish products, such as fish fillets with seasoned coatings or microwaveable seafood meals, cater to consumers looking for quick and easy meal solutions.

4. **Health and Dietary Preferences:** Value addition can cater to specific dietary preferences or health needs.

5. **Innovations in Packaging:** Innovative packaging techniques such as vacuum packaging, modified atmospheric packaging etc. can extend the shelf life of fish products and preserve their quality and safety.

6. **Sustainable and Eco-Friendly Packaging:** Responding to the growing interest in sustainability, the use of eco-friendly packaging materials can be a value addition, aligning with the environmental concerns of consumers.

7. **Traceability and Labeling:** Providing clear information about the source and quality of fish products through traceability and transparent labeling can enhance consumer trust and perception of value.

8. **Customization:** Some manufacturers offer customizable fish products, allowing consumers to choose ingredients, flavors, or preparation methods to suit their preferences.

A number of such diverse products have already invaded the industry, globally ranging from live fish and shellfish to ready to serve convenience products. Value added fishery products primarily fall under the categories viz., mince/mince-based products, surimi/surimi-based products, enrobed or coated products, ready to serve retorted products, cold/hot extruded products, speciality products, ethnic products like marinated, dried products etc.

Fish mince is a valuable and cost-effective seafood product, which can be defined as deboned and unwashed fish flesh from fillets or frames. It is obtained from the initial steps of surimi manufacturing or through direct processing of raw material. It offers a versatile ingredient for culinary applications and provides economic and nutritional benefits to the seafood industry and consumers alike. When compared to surimi, fish mince can be obtained at a significantly higher yield with much less capital investment. Fish mince also offers nutritional advantages, economic benefits as well as functional advantages compared to the other intermediate materials. Fish mince can also be successfully used directly in various food systems and in a physically or chemically altered form to produce an array of nutritional and functional products. It finds application in processing several convenience foods like fish finger, cutlet, burger, fish momos and also in some

low cost salted and dried products. For preparation of fish finger, stick, etc., the mince stripped from the bone frame is incorporated to increase the yield.

Surimi, a Japanese term refers to mechanically deboned fish flesh that has been water washed and mixed with cryoprotectants for good frozen shelf life. Washing removes fat and undesirable matters such as blood, pigments and odoriferous substances. Further it intensifies the concentration of myofibrillar protein, the content which improves the gel strength and elasticity of the product. This property facilitates in developing a variety of products like fish sausage, balls, burgers as well as fabricated products like shellfish analogues which draws good demand in both domestic and export markets. Low value fishes can also be conveniently used for the preparation of surimi. Surimi and derived products are popular and of high demand, especially in South east Asian countries.

Enrobed/Coated/battered and breaded commodities are highly appreciated form of value-added products on account of their convenience, sensory appealness and nutritional attributes. In view of the increasing consumer demand, the technology has made several advancements. The most important advantage of coating is value addition as it increases the bulk of the product. This technology also paves way for better utilization of underutilized seafood resources. A wide array of seafood products can be categorized in it, with the first commercially launched coated product being fish finger/fish stick followed by commodities in similar line viz., coated fish fillet, fish portions, fish cakes, fish medallions, fish nuggets, breaded oysters and scallops, crab balls, fish balls, coated shrimp products, coated squid rings etc. The most popular battered and battered products in India include fish nuggets, cutlet, balls, finger, patties etc. In value-added markets, where consumers are willing to pay a premium for high-quality, convenient, and flavorful products, ready-to-eat battered and breaded snacks can find a strong foothold. Their ability to cater to diverse tastes and preferences makes them an attractive option for manufacturers and consumers alike.

Ready-to-serve retorted fish products cater to the demands of modern consumers for convenience, quality, and variety. Their extended shelf life and versatility make them a convenient option for domestic and international markets, and they contribute to the growing popularity of seafood-based convenience foods. A wide array of products is categorized under this including retorted fish curries, rice-fish combos, seafood biriyanis etc. These products have a shelf life of more than

one year at room temperature. The most common retortable pouch consists of a 3-ply laminated material consisting of polyester/aluminium/cast polypropylene. As there is increasing demand in domestic and International market for ready to serve products, proper exploration of this technology can provide a lively market for these commodities. The technology for retort pouch processing of several varieties of ready to serve fish and fish products including curries from mackerel, rohu, sardine, tuna, pomfret, prawn, seer fish molly, pearl spot molly, fried mussel, fish sausage, prawn kurma, prawn manchurian, fried mussel masala etc. has been standardized at ICAR-CIFT and this technology has been transferred successfully to entrepreneurs.

Food extrusion is a versatile food processing technology that offers numerous benefits in the development of a wide range of food products, including cereal-based snacks and convenience foods. Extruded products are gaining importance nowadays on account of their unique flavour, texture and convenience. Extruded products, including various snack foods, breakfast cereals, and convenience foods, are often made from starchy ingredients like grains, cereals, or legumes. These ingredients can indeed be lower in protein content compared to other food sources. Hence it is beneficial to fortify extruded products with protein-rich ingredients to enhance their nutritional value. One of the possible ways for alleviating this problem is to utilize fish and fish proteins to enrich cereal-based extruded products. Formulation of appropriate types of products using fish meat and fish portions will add value to the low-cost and underutilized fish and shellfish, thus promoting their utilization. Attractive packaging for the products and market studies are needed for the popularization of such products. These products can command very high market potential particularly among the urban elites.

Product diversification is indeed a key strategy for effective marketing, and the trend toward specialty and convenient products is gaining significant consumer acceptance. The most popular products under the speciality product category include those like stretched shrimp (Nobashi), sushi (Cooked butterfly shrimp), skewered shrimp, shrimp head-on cooked (centre peeled), fish wafers, fish crackers, fish soup powder etc.

Ethnic seafood products are food items that are deeply rooted in the culinary traditions of specific regions or cultures and have been prepared and consumed by people in those areas for generations. These products are often associated with the unique flavors, ingredients, cooking techniques, and cultural significance of a particular ethnic group or geographical area. Some of these EFP are

preserved or processed using centuries-old indigenous knowledge of fermentation/drying/smoking etc. Globalization has resulted in high demand for these ethnic food products and hence approaches towards its popularization by adopting various processing techniques can bring a huge market potential for these commodities.

Fermentation is a traditional preservation technique adopted mainly in the north eastern part of the India. During the fermentation process, beneficial microorganisms in the food produce enzymes that break down proteins and improve the digestibility of nutrients. It leads to improved taste and texture of fish products. The development of characteristic flavors, such as umami, can make fermented fish a delicacy in many cultures. Fermentation is particularly useful in tropical climates, where the hot and humid conditions can lead to rapid spoilage of fresh fish. The process helps extend the shelf life of fish products.

Smoking fish imparts a unique and desirable smoky flavor and aroma to the product, making it a popular choice among consumers who appreciate this distinct taste. The use of typical flavor extracts is an innovative approach in smoking. By incorporating flavored extracts, it's possible to achieve desired taste and aroma profiles more quickly, reducing the smoking time required. Further by experimenting with different flavor profiles and ingredients, it's possible to create a variety of flavored fish products that cater to different tastes and preferences. Minimal processing protocols can involve the use of modern technologies, such as smoking equipment, to expedite the production process and maintain quality and safety standards. These innovations can make these preserved fish products more accessible and appealing to a broader range of consumers.

Curing and drying, even though an age-old practice, opens up new dimensions and possibilities towards value addition in domestic as well as overseas markets. According to estimates in India, a substantial portion of the total catch, around 17-20%, is processed into dried products and dry fish export contributes to about 7.86% of total fish exports. This practice not only reduces post-harvest losses but also creates value-added products. The major importing countries are Sri Lanka, Malaysia, Indonesia, Singapore and United Arab Emirates. However, there are several factors hindering the addition of dried fishery products to the product profile. The major one being, drying is still considered a traditional method of processing, and hence standard operating procedures are seldom followed. Moreover, there is a general conception that drying is a secondary method for preserving low value varieties and quality compromised materials. Attempts towards improving

the handling practices right from the point of raw material harvesting till marketing, popularisation of improved packaging practices, use of hygienic energy efficient mechanical driers, and adequate extension services can facilitate better adoption of drying practice in seafood sector.

Live seafood is often perceived as a premium product, attracting customers who value the highest level of freshness. Live seafood often commands premium prices, making it a financially attractive option for farmers and suppliers. Several internal and external factors must be carefully considered to ensure the success of this approach. Internal factors include the species-specific requirements and their tolerance limits. External factors encompass the logistics of transportation, packaging, and handling. Gentle handling of live seafood is crucial to minimize stress and injury, which can affect the quality and survival of the animals. Different seafood species have varying requirements and tolerances and it's essential for farmers, suppliers, and retailers to have a thorough understanding of the specific needs of the species they are dealing with. This knowledge helps in setting up the right conditions for transportation and storage. Regulations and standards related to the transportation and sale of live seafood must be adhered to. Compliance with food safety and animal welfare regulations is crucial to ensure that live seafood meets the required quality and safety standards. With the right approach, live seafood can be a highly attractive product for both producers and consumers.

Market Scenario

Seafood processing and marketing has become highly competitive that the exporters are shifting towards value addition for increased margins. Understanding what drives consumers to make purchasing decisions is critical for businesses to tailor their offerings and marketing strategies effectively. By understanding the factors that drive individuals to consider purchasing a product or service, helps the sellers to design marketing campaigns and product features that align with these intentions. Preferences can vary by demographic, location, and other factors. Further, product quality also plays a significant role in meeting consumer expectations and creating value.

Summary

Value addition involves transforming the raw seafood materials into more valuable and marketable products. This includes processes like filleting, marinating, freezing, preparing ready-to-eat seafood dishes etc. On account of value addition, seafood products can cater to a wider

range of consumer preferences and demands. Value-added seafood products are often better positioned in the food and nutraceutical markets. These products can be marketed as healthier alternatives, rich in essential nutrients. This appeals to consumers seeking nutritious and convenient food options.

Further, one of the significant advantages of value addition is the reduction of post-harvest losses. Proper processing, preservation, and packaging can extend the shelf life of seafood products, reducing waste and economic losses within the seafood supply chain. By adding value to seafood products, the seafood industry can generate more revenue from the same quantity of raw materials contributing to economic growth by increasing the value of the seafood sector and creating employment opportunities in processing, packaging, and distribution. This approach also plays a vital role in addressing nutritional security by providing readily available, nutritious food options. These products can help meet the dietary requirements of populations and combat malnutrition, particularly in regions where seafood is a dietary staple. Technology plays a crucial role in the value addition process. Advanced processing, packaging, and monitoring technologies can enhance the quality, safety, and efficiency of seafood production. Innovations in seafood processing methods, such as minimal processing, can help maintain the nutritional quality of the products. Further smart packaging concepts and intelligent quality monitoring systems can ensure that seafood products remain fresh, safe, and of high quality throughout the supply chain. This builds consumer confidence and reduces the risk of spoilage or contamination.

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Traditional Methods of Fish Preservation

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

1. Drying

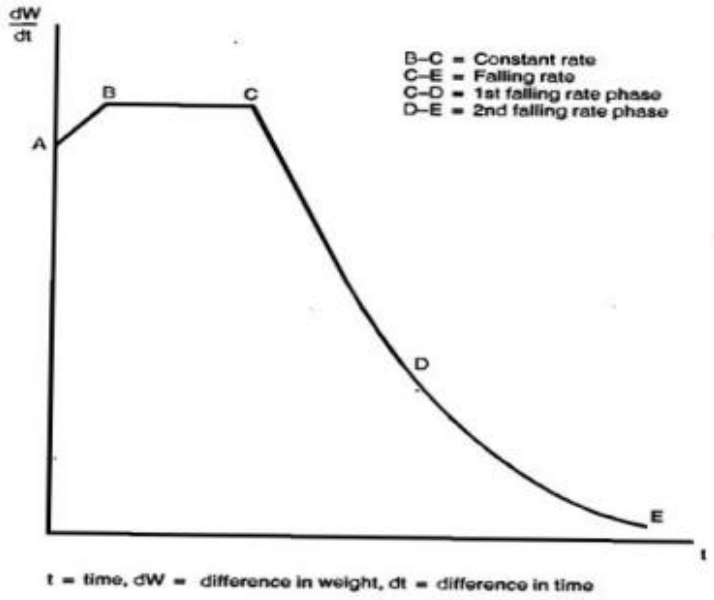
The term 'drying' implies the removal of moisture by means of evaporation. Water being the essential component for all living organisms, its removal facilitates microbial retardation, arrest of autolytic activity as well as oxidative changes and hence can be used as a method of preservation. In any process of drying, the removal of water requires an input of thermal energy. The thermal energy required to drive off moisture can be obtained from a variety of sources, e.g., the sun or the controlled burning of oil, gas or wood, electrical heating etc.

1.1 Drying phases

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

During air drying, water is removed from the surface of the fish and water moves from the deeper layers to the surface. Drying takes place in two distinct phases. In the first phase, whilst the surface of the fish is wet, the rate of drying depends on the condition (velocity, relative humidity etc.) of

the air around the fish. If the surrounding air conditions remain constant, the rate of drying will remain constant; this phase is called the 'constant rate period'. Once all the surface moisture has been carried away, the second phase of drying begins and this depends on the rate at which moisture can be brought to the surface of the fish. As the concentration of moisture in the fish falls, the rate of movement of moisture to the surface is reduced and the drying rate becomes slower; this phase is called the 'falling rate period'.



Drying rate curve.

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

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

Constant rate drying phase

- Air temperature
- Relative humidity of the air
- Air velocity
- Surface area of the fish

Falling rate drying phase

- Nature of the fish
- Thickness of the fish
- Temperature of the fish
- Water content

1.2 Methods of Drying

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

Natural or sun drying

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

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

Artificial / Mechanical Dryers

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

2. Salting

Salting, one of the traditional methods of preservation is usually done alone or in combination with drying or as a pretreatment to smoking. The presence of sufficient quantities of common salt (sodium chloride) in fish can prevent or drastically reduce bacterial action. Salting amounts to a process of salt penetration into the fish flesh when fish is placed in a strong solution of salt (brine) which is stronger than the solution of salt in the fish tissue. Penetration ends when the salt concentration of the fish equals that of the surrounding medium. This phenomenon is known as osmosis. It is based on different factors like diffusion and biochemical changes in various

constituents of the fish. This process facilitates preservation of fish by reducing the water activity. A concentration of between 6–10 % salt in the tissue together with the removal of some water from the tissue during the salting process will prevent the activity of most spoilage bacteria. If fish are salted before drying, less water needs to be removed to achieve preservation. A water content of 35–45%, depending on the amount of salt present, will often prevent, or drastically reduce, the action of bacteria.

2.1 Salt

Source

Based on the source as well as method of manufacture, common salt (sodium chloride, NaCl). can be grouped as:

- **Solar salt:** prepared by the evaporation of sea or salt lake waters
- **Brine evaporated salts:** produced from underground salt deposits which are brought to the surface in solution form and evaporated.
- **Rock salt:** obtained as natural deposits from interior rock mines

2.2 Types of Salting

2.2.1 Dry salting: This is the most widely used method of fish curing. Dry salting is advisable for fishes of any size, except fatty fishes. The fish is gutted, beheaded or ventrally split open and the viscera removed followed by washing. Scoring is also practiced if the flesh portion is thick for facilitating better salt penetration. Salt is then applied in the ratio 1:3 to 1: 10 (salt to fish) depending upon the size of the fish. The fish is then stacked in clean cement tanks or other good containers layered with salt and weight is applied from top for better salt penetration. The fish is kept in this condition for 24-48 hours. After salting period, the fish is taken out, washed in brine to remove adhering salt and drained. It is then hygienically dried to a moisture content of about 25%. Yield of the product by this method is about 35-40% with a storage stability of up to three months under ambient conditions.

2.2.2 Wet salting: The initial stages of processing and salting are the same as for dry curing. However, the fish kept in tank is allowed to remain in self-brine till marketing without further drying. For marketing, as per the demand the wet salted fish is drained and packed in palmyrah leaf baskets or coconut leaf baskets. This method is particularly suitable for fatty fishes like oil sardine, mackerel etc. Wet salted fishes have short shelf stability with a moisture content of 50-55% and a salt content of around 25%.

2.2.3 Pickle salting: Pickle curing is a type of wet salting where the fish is layered by granular salt which, dissolves in the surface moisture of the fish forming solution which penetrates into the fish removing moisture from the fish. The fish is allowed to remain in this self-brine. If the self-brine is not sufficient, saturated brine is added to immerse the fish.

2.2.4 Kench salting: In this method, salt is rubbed on to the surface of the fish and stacked in layers of salt and fish. The self-brine formed is allowed to drain away. This method cannot be recommended for general use in the tropics as the fish are not covered by the brine or pickle and are therefore more susceptible to spoilage and insect attack. Exposure to the air and the presence of salt also encourages the rate of fat oxidation which gives rise to discoloration and the characteristic rancid flavours.

2.2.5 Mona curing: Mona curing is mainly adopted for medium to small size fishes. Before salting, the intestine and entrails are removed by pulling out through the gill region without split opening the fish. The flesh is not exposed during salting thereby causing less contamination and the product has a shelf stability of about two months. The yield obtained by this method is about 70%.

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

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

2.3 Quality issues in dried and salted fish

2.3.1 Pink/Red: Salt content prevents the growth of microflora in the fish, but halophiles can survive at 12-15% of salt concentration. Halophilic bacteria are present in most of the commercial salt, which will cause Red / Pink patches on wet or partially dried salted fish by decomposing protein. Usage of good quality salt is recommended to avoid this condition. This spoilage is mostly found in heavily salted fish.

2.3.2 Dun: In salted fish, brownish black or yellow brown spots are seen on the fleshy parts, referred to as "dun". This is mainly caused by growth of halophilic mould called *Sporendonema epizoum*, which grows at relative humidity above 75% and optimum temperature of 30-35 ºC. To avoid the mould growth, it is necessary that the fish to be dried, packed and stored properly to avoid uptake of moisture. Chemical method of prevention includes dipping the fish in a 5% solution of calcium propionate in saturated brine for 3-5 minutes depending upon the size of the fish.

2.3.3 Salt burn: A mixture of large and small grain sizes is recommended for dry salting of fish. If fine grain is used directly on the fish, salt burn may occur due to the rapid removal of water from the surface with no penetration of salt to the interior of the fish.

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

2.3.5 Rancidity: This is caused by the oxidation of fat, which is more pronounced in oil rich fishes like mackerel, sardine etc. At this stage the colour of the fish changes from yellowish to brown referred to as rust and cause unpleasant flavour and odour, leads to consumer rejection of the product.

2.3.6 Insect infestation: Spoilage due to insect infestation occurs during initial drying stages as well as during storage of the dried samples. The flies which attack the fish during the initial drying stage are mainly blowflies belonging to the family Calliphoridae and Sarcophagidae. The most commonly found pests during storage are beetles belonging to the family Dermestidae. Mites are also an important pest, which are found infesting dried and smoked products. Infestation can be reduced by proper hygiene and sanitation, disposal of wastes and decaying matter, use of physical barriers like screens, covers for curing tanks etc, and use of heat to physically drive away the insects and kill them at 45 ºC.

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

3. Smoking

Smoking is an ancient method of food preservation, which is also known as smoke curing. Smoking is a process of treating fish by exposing it to smoke from smouldering wood or plant materials to introduce flavour, taste, and preservative ingredients into the fish. This process is usually characterised by an integrated combination of salting, drying, heating and smoking steps in a smoking chamber. Smoked seafood includes different varieties like, smoked finfish and smoked bivalves. Many of the smoked products are in the form of ready-to-eat.

Developments of modern food preservation technology, have eclipsed the preserving functions of many traditional methods including smoking. Nowadays, the main purpose of smoking has been shifted for sensory quality rather than for its preservative effect. Depending upon how the smoke is delivered into the food and smoking temperature, four basic types of smoking can be defined: hot smoking, cold smoking, liquid smoking, and electrostatic smoking.

3.1 Hot smoking

Hot smoking is the traditional smoking method using both heat and smoke, which usually occurs at temperatures above 70 °C. Therefore, after hot smoking, products are fully cooked and ready for consumption. Torry smoking kiln was introduced in the early 1960s by United Kingdom's Torry Research Station. The Torry smoking kiln is considered as a model for the modern smokers/smokehouses by enabling the precise controls of the heating temperature, air ventilation, and smoke density. Some recently designed smokehouse may also be equipped with more precise time and temperature controls, humidity control, and product internal temperature monitor probes. Thus, the products produced by the modern smokehouses are much more uniform than those produced with traditional smokers. Hot smoking is typically not a single process. Several other steps such as brining, drying and smoking are also involved to produce a product of good quality.

3.2 Cold smoking

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

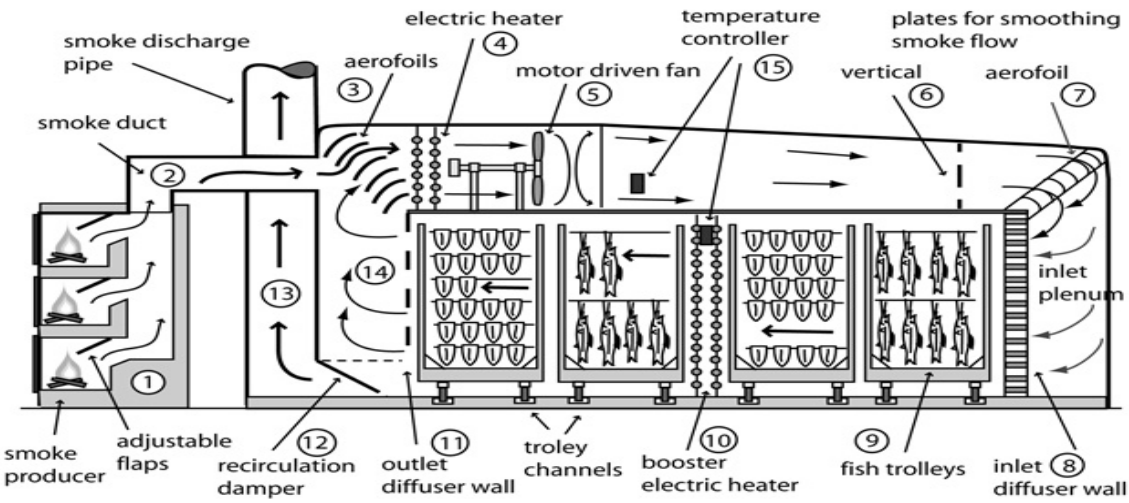


Fig. Illustration of the hot smoke airflow in the Torry smoking kiln

3.3 Liquid smoking

Liquid smoke is smoke condensate that is dissolved in a solvent, such as water or oil. Liquid smoke can be used directly on products by dipping or spraying. It is rapid and much easier to achieve a uniform smoke flavour than traditional cold and hot smoking processes, although the flavour and colour from the traditional smoking cannot be exactly duplicated. Some potential harmful ingredients (e.g. polycyclic aromatic hydrocarbons, PAHs) in the nature smoke can be separated out and excluded from the liquid smoke. Other advantages of liquid smoke include easy modification, application to food items that traditionally are not smoked, lower operation cost, and less environmental pollution. However, the application of liquid smoking may be expensive compared to other methods.

3.4 Electrostatic smoking

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

4. Conclusion

Traditional methods of preservation are age-old practices followed on account of its simplicity and effectiveness. However, a major drawback with this traditional processing is the lack of standard operating procedures being followed which affects the quality of the products. Moreover, there is a general a conception that the methods are secondary method for preservation, which is applicable for low value as well as inferior quality varieties. Efforts towards effective and hygienic handling practices in the process chain, popularization of improved methods and packaging practices, and adequate extension services can facilitate better adoption of traditional fishery products in the fishery sector.

Suggested Readings:

- Daniela Borda, Anca I. Nicolau and Peter Raspor, (2017). Trends in Fish Processing Technologies, CRC Press, Taylor & Francis Group, Boca Raton, FL
- G. M. HALL, (1997). Fish Processing Technology (Second edition), Published by Blackie Academic and Professional, an imprint of Chapman & Hall, 2-6 Boundary Row, London, UK
- J. Clucas, ‘’Fish Handling, Preservation and Processing in the Tropics’’. Part 2. Report of The Tropical Development and Research Institute (TDRI). 45-50. 1982
- FAO, ‘’Handling of Fish and Fish Products. Fisheries and Aquaculture Department’’. Rome. Updated 31st October, 2001. Retrieved 9th, January, 2012. 2005.

Thermal Processing and RTE (Ready to Eat) Products

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Processing and preservation of food is an important activates to ensure safe food supply apart from reducing food loss. Fish being highly perishable food commodity, processing and preservation assumes great importance. Thermal sterilization of foods is the most significant part of food processing industry and is one of the most effective means of preserving food supply. Thermal processing, which iscommonly referred as heat processing or canning is a means of achieving long-term microbiological stability for non-dried foods without the use of refrigeration, by prolonged heatingin hermetically sealed containers, such as cans or retortable pouches, to render the contents of thecontainer sterile. The concept of thermal processing has come a long way since the invention of the process by French confectioner, Nicholas Appert. Later on Bigelow and Ball developed the scientific basis for calculating the sterilization process for producing safe foods. Today, thermal processing forms one of the most widely used method of preserving and extending shelf life of treatment for sufficient time to destroy all the microorganisms of public health and spoilage concerns. Normally, thermal processing is not designed to destroy all microorganisms in a packaged product, which may result in low quality product which destroys important nutrients. Instead of this, the pathogenic microorganisms in a hermetically sealed container are destroyed byheating and a suitable environment is created inside the container which does not support the growth of spoilage type microorganisms. Several factors must be considered for deciding the extent of heat processing which include,

- type and heat resistance of the target microorganism, spore, or enzyme present in thefood
- pH of the food
- heating conditions
- thermo-physical properties of the food and the container shape and size
- storage conditions

Thermal processing is designed to destroy different microorganisms and enzymes present in the food. Normally in thermal processing, exhausting step is carried out to before sealing the containers. In some cases, food is vacuum packed in hermetically sealed containers. In such casesvery low levels of oxygen is intentionally achieved. Hence, the prevailing conditions are not favorable for the growth of microorganisms that require oxygen (obligate aerobes) to create food spoilage or public-health problems. Further, the spores of obligate aerobes are less heat resistant than the microbial spores that grow under anaerobic conditions (facultative or obligate anaerobes). The growth and activity of these anaerobic microorganisms are largely pH dependent. From a thermal-processing standpoint, foods are divided into three distinct pH groups which are given below. Changes in the intrinsic properties of food, mainly salt, water activity and pH are known toaffect the ability of microorganisms to survive thermal processes in addition to their genotype. Due to health-related concerns on the use of salt, there is increased demand to reduce salt levels infoods. The United States Food and Drug Administration (FDA) have classified foods in the federalregister (21 CFR Part 114) as follows (Table 2):

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

Table 1. Approximate pH range of different food

Food	pH	Food	pH
Lemon juice	2.0 - 2.6	Sweet potato	5.3 – 5.6
Apples	3.1 - 4.0	Onion	5.3 – 5.8
Blueberries	3.1 – 3.3	Spinach	5.5 – 6.8
Sauerkraut	3.3 – 3.6	Beans	5.6 – 6.5
Orange juice	3.3 – 4.2	Soybeans	6.0 – 6.6

Apricot	3.3 – 4.0	Mushroom	6.0 – 6.7
Bananas	4.5 – 5.2	Clams	6.0 – 7.1
Beef	5.1 – 7.0	Salmon	6.1 – 6.3
Carrot	4.9 – 5.2	Coconut milk	6.1 – 7.0
Green pepper	5.2 – 5.9	Milk	6.4 – 6.8
Papaya	5.2 – 6.0	Chicken	6.5 – 6.7
Tuna	5.2 – 6.1	Whole egg	7.1 – 7.9

The acidity of the substrate or medium in which micro-organisms are present is an important factor in determining the extent of heat treatment required. With reference to thermal processing of food products, special attention should be devoted to *Clostridium botulinum* which is a highly heat resistant mesophilic gram positive, rod shaped spore-forming anaerobic pathogen that produces the toxin *botulin*. It has been generally accepted that *C. botulinum* and other spore forming, human pathogens does not grow and produce toxins below a pH of 4.6. The organisms that can grow in such acid conditions are destroyed by relatively mild heat treatments. For food with pH values greater than 4.5, which are known as low-acid products which includes fishery products, it is necessary to apply a time–temperature regime sufficient to inactivate spores of *C. botulinum* which is commonly referred to as a *botulinum cook* in the industry. Thermal processesare calibrated in terms of the equivalent time the thermal centre of the product, i.e. the point of theproduct in the container most distant from the heat source or cold spot, spends at 121.1°C, and thisthermal process lethality time is termed the F_0 value. Although there are other microorganisms, for example *Bacillus stearothermophilus*, *B. thermoacidurans*,and *C. thermosaccolyaticum*, which are *thermophilic* in nature (optimal growth temperature ~ 50–55°C) and are more heat resistant than *C. botulinum a* compromise on the practical impossibility of achieving full sterility in the contents of a hermetically sealed container during commercial heat processing, whereby the initialbacterial load is destroyed through sufficient decimal reductions to reduce the possibility of a single organism surviving to an acceptably low level. This level depends on the organism, usually*Clostridium botulinum*, which the process is designed to destroy. The time required to reduce the number of spores of this organism (or any other micro-organism) by a factor of 10 at a specific reference temperature (121.1°C) is the decimal reduction time, or *D* value, denoted D_0. The D_0 value for

Clostridium botulinum spores can be taken as 0.25 minutes. To achieve a reduction by afactor of 10^{12}, regarded as an acceptably low level, requires 3 minutes at 121.1°C, and is known as the process value, or *F* value, designated F_0 so, in this case, $F_0 = 3$, which is known as a botulinum cook which is the basis of commercial sterility.

1.0 Thermal resistance of microorganisms

For establishing a safe thermal processing, knowledge on the target microorganism or enzyme, its thermal resistance, microbiological history of the product, composition of the productand storage conditions are essential. After identifying the target microorganism, thermal resistance of the microorganism must be determined under conditions similar to the container. Thermal destruction of microorganism generally follow a first-order reaction indicating a logarithmic orderof death i.e., the logarithm of the number of microorganisms surviving a given heat treatment at aparticular temperature plotted against heating time (survivor curve) will give a straight line (Figure1). The microbial destruction rate is generally defined in terms of a decimal reduction time (D value) which represents a heating time that results in 90% destruction of the existing microbial population or one decimal reduction in the surviving microbial population. Graphically, this represents the time between which the survival curve passes through one logarithmic cycle (Fig. 1). Mathematically,

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

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

From the survivor curve, as the graph is known, it can be seen that the time interval required to bring about one decimal reduction, i.e. 90% reduction in the number of survivors is constant. This means that the time to reduce the spore population from 10,000 to 1000 is the same as the time required to reduce the spore population from 1000 to 100. This time interval is known as the numbers, but it is affected by the temperature of the heating medium. The higher the temperature,faster the rate of thermal destruction and lower the D value. Different micro-organisms and their spores have different D values as shown in Table 2.

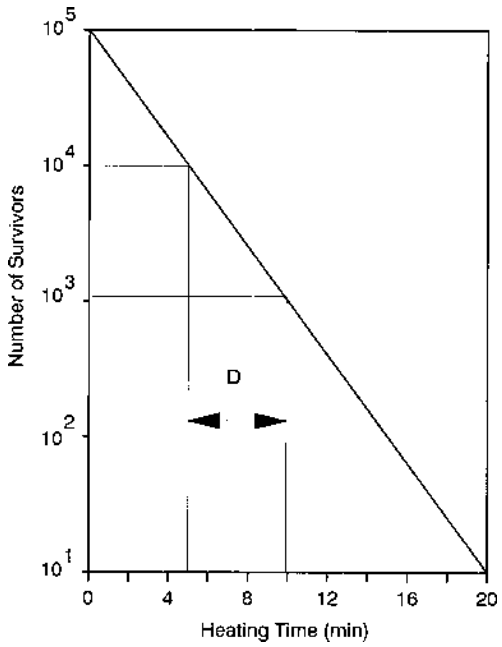


Fig 1. Survivor curve

Table 2. D value (at 121.1°C) of some bacterial spores

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

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

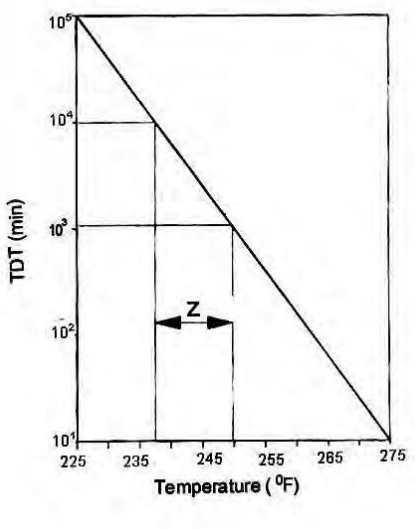


Fig. 2 TDT Curve

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

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

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

2.0 Thermal Process Severity or F_0 value

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

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

where, t = time required to achieve commercial sterility

This $\log No/N_t$ $F_0 = mD_{121.1°C}$

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

$t = 0.25 (\log 1 - \log 10^{-12})$, i.e., $t = 0.25 \times 12 = 3.00$ min

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

3.0 Commercial sterility

Commission (1983) defines, commercial sterility as the condition achieved by the application of heat, sufficient alone or in combination with other appropriate treatments, to render the food

free from microorganisms capable of growing in the food at normal non-refrigerated conditions at which the food is likely to be held during distribution and storage. Apart from this concept there are circumstances where a canner will select a process which is more severe than that required forcommercial sterility as in the case of mackerel and sardine where bone softening is considered desirable.

4.0 Mechanism of heat transfer

Understanding the mechanism of heat transfer is very important for thermal processing. Normally, there are three different modes of heat transfer: conduction, convection and radiation. Conduction is the transfer of heat by molecular motion in solid bodies. Convection is the transfer of heat by fluid flow, created by density differences and buoyancy effects, in fluid products. Radiation is the transfer of electromagnetic energy between two bodies at different temperatures.In thermal processed foods, the mechanism of heat transfer is either by conduction, convection orby broken heating (combination of conduction and convection). The factors which determine the mode of heat transfer are nature or consistency of a food product, the presence of particles, and the use of thickening agents and sugars. The heating modes in the thermal processing are first by heat transfer to the container or packaging material from heating and cooling media, second through the container wall and third is into the product from container wall. Convective-heat transfer rates depend largely on the velocity of flow of the media over the container, and this is animportant factor to be controlled in all processing operations. In conduction heading method, energy transfer takes place when different parts of a solid body are at different temperatures. Theslowest heating point or cold point in cylindrical metal containers is at its geometric centre for food products heated by conduction method. Convection heat transfer involves the transfer of heatfrom one location to the other through the actual movement or flow of a fluid. The slowest heatingpoint for convection heated products in cylindrical metal container is approximately $1/10^{th}$ up fromthe base of the container. Packaging material forms the most important component of thermal processed foods. It should be able to withstand the severe process conditions and should prevent recontamination of the product.

5.0 Containers for thermal processing

Containers used for thermal processing should have special properties like it should withstand high temperature and pressure. Tin cans are commonly used in the canning industry andcans

are denoted by trade name. First digit represents diameter of can (in inches) and next two digits represent measurement in sixteenth of inches. Apart from OTS cans, other container used in canning are: aluminium cans, tin free steel (TFS) cans, glass containers, retort pouches and semi-rigid containers.

5.1 Glass containers

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

Glass jars for food packing has the advantages of very low interaction with the contents and product visibility. However, they require more careful processing and handling. Glass containers used in canning should be able to withstand heat processing at high temperature and greater significance in caning than otherreasons of breakage. Thermal shock is due to the difference in the temperature between the insideand the outside walls of the container giving rise to different rates of expansion in the glass wall producing an int large cracks and container failure. Thermal shock will be greater if the wall thickness is high. Therefore, glass container in canning should have relatively thin and uniform walls. Similarly thebottom and the wall should have thickness as uniform as possible. More failures occur at sharp containers and flat surface and hence these should be avoided. Chemical surface coatings are oftenapplied to make the glass more resistant t seals are available, including venting and nonventing types, in sizes from 30 to 110 mm in diameter, and made of either tin or tin-free steel. It is essential to use the correct overpressure during retorting to prevent the lid being distorted. It is also essential to preheat the jars prior to processing to prevent shock breakage.

5.2 Metal containers

Metal cans are most widely used containers for thermal processed products. Metal containers are normally made of tin, aluminium or tin-free steel.

5.3 Tin plate cans

Tinplate is low metalloid steel plate of can making quality (CMQ) coated on both sides with tin giving a final composition of 98% steel and 2% tin. Thickness varies from0.19 to 0.3 mm depending on the size of the can. Specifications with respect to content of other elements are: Carbon (0.04 - 0.12%), manganese (0.25 - 0.6%), sulphur (0.05 % max), phosphorus (0.02 % max),silicon (0.01% max) and copper (0.08% max). Corrosive nature of tin plate depends principally onthe contents of copper and phosphorous. The higher the contents of these metals, greater the corrosiveness of steel. However, higher phosphorous content imparts greater stiffness to steel plate which is advantageous in certain applications where higher pressure develops in the container, eg;beer can.

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

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

5.4 Aluminium cans

Pure aluminium of 99.5 to 99.7% purity is alloyed with one or more elements like magnesium, manganese, zinc, copper etc. to obtain the desired composition. Aluminium alloyed with mangnesium is the most commonly used material. Alloyed aluminium is first given an anticorrosive treatment; usually anodising in dilute sulphuric acid. The thin layer of oxides formedprovides corrosion resistance. To enhance this, the sheet is further coated with a suitable lacquer.

Advantages of aluminium cans

- Light weight, slightly more than 1/3 of the weight of a similar tinplate can
- Nonreactive to many food products
- Clear, bright and aesthetic image

- Not stained by sulphur bearing compounds
- Nontoxic, does not impart metallic taste or smell to the produce
- Easy to fabricate; easy to open
- Excellent printability
- Recyclability of the metal

However, aluminium cans are not free from some disadvantages

- Thick gauge sheet needed for strength
- Not highly resistant to corrosion, acid fruits and vegetables need protection by lacqueringor other means
- Special protection needed during heat processing to avoid permanent distortion
- Aluminium has great tendency to bleach some pigmented products
- Service life is less than that of tinplate for most aqueous products

5.5 Tin free steel containers

Tin free steel (TFS) apart from aluminium, is a tested and proven alternate to tinplate in food can making. It has the same steel substitute as the tinplate. It is provided with a preventive coating of chromium, chromium oxide, chromate-phosphate etc. TFS is manufactured by electroplating cold-rolled base plate with chromium in chromic acid. This process does not leave toxin substrate such as chromates or dichromates on the steel and it can be formed or drawn in thesame way as tinplate.

Advantages:

- The base chromium layer provides corrosion barrier
- The superimposed layer of chromium oxide prevents rusting and pick up of iron taste
- Provides an excellent base for lacquer adhesion
- Good chemical and thermal resistance
- Tolerance to high processing temperature and greater internal pressure
- Improved and more reliable double seam

Disadvantages:

- Low abrasion resistance; hence compulsory lacquering
- Difficulty in machine soldering
- The oxide layer needs removal even for welding
- Limitations in use for acid foods

An important problem associated with TFS can ends is scuffing of lacquer on the double seam.This may occur at the seamer or downstream at different stages of lacquering. TFS cans have been found quite suitable for canning different fish in various media. Thus, it holds good scope as an important alternate to tinplate cans.

5.6 Rigid plastic containers

The rigid plastic material used for thermal processing of food should withstand the rigors of the heating and cooling process. It is also necessary to control the overpressure correctly to maintain a balance between the internal pressure developed during processing and the pressure of the heating system. The main plastic materials used for heat-processed foods are polypropylene and polyethylene tetraphthalate. These are usually fabricated with an oxygen barrier layer such as ethylvinylalcohol, polyvinylidene chloride, and polyamide. These multilayer materials are used tomanufacture flexible pouches and semi-rigid containers. The rigid containers have the advantage for packing microwavable products.

Retortable pouches

Retort pouch can be defined as a container produced using 2,3 or 4-ply material that, whenfully sealed, will serve as a hermetically sealed container that can be sterilized in steam at pressure and temperature similar to those used for metal containers in food canning. Retort pouch has the advantages of metal can and boil-in plastic bag. Configuration of some typical pouches are:

2 ply 12µ nylon or polyester/70µ polyolefin
3 ply 12µpolyester/9-12µ aluminium foil/70µ polyolefin
4 ply 12µ polyester/9-12µaluminium foil/12µpolyester/70µ polyolefin

3-ply pouch is most commonly used in commercial canning operations. This is a three- layer structure where a thin aluminium foil is sandwiched between two thermoplastic films. The

outer polyester layer provides barrier properties as well as mechanical strength. The middle aluminium foil provides protection from gas, light and water. This also ensures adequate shelf lifeof the product contained within. The inner film which is generally polyproplyline, provides the best heat sealing medium.

The normal design of a pouch is a flat rectangle with rounded corners with four fin seals around 1 cm wide. A tear notch in the fin allows easy opening of the pouch. The rounded cornersallow safe handling and help to avoid damage to the adjacent packs. The size of the pouch is determined by the thickness that can be tolerated at the normal fill weight. The size ranges (mm)available are:

A_1	130 x 160
A_2	130 x 200
A_3	130 x 240
B_1	150 x 160
B_2	150 x 250
B_3	150 x 240
C_1	170 x 160
C_2	170 x 200
C_3	170 x 240
D_1	250 x 320 (Catering pack)
D_2	250 x 1100
D_3	250 x 480

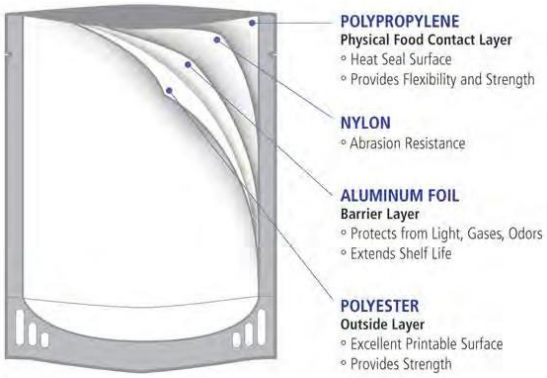
Advantages

- Thin cross- sectional profile – hence rapid heat transfer – 30-40% saving in processingtimes – no over heating of the product near the walls
- Better retention of colour, flavour and nutrients
- Shelf life equal to that of the same product in metal can
- Very little storage space for empty pouches – 15% of that for cans
- Easy to open

Disadvantages

- Pouches, seals more vulnerable to damage, can be easily damaged by any sharp material, hence necessitates individual coverage
- With an over wrap cost may go up above that of cans
- Slow rate of production, 30 pouches in place of 300-400 cans per minute
- Needs special equipment
- Higher packaging cost and low output push up the cost of production

Containers used for thermal processing



Composition of Retortable pouch

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

- Should withstand the sterilisation pressure and temperature
- Should be impervious to air, moisture, dust and disease germs once the can is sealed airtight
- Internal lacquer should not impart toxicity to the contents
- Strong enough to protect the contents during transportation and handling
- Inexpensive, preferably cheap enough to discard after use
- Capable of sealing at high speed
- Pleasing and sanitary appearance

6.0 Thermal Processing of Fishery Products

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

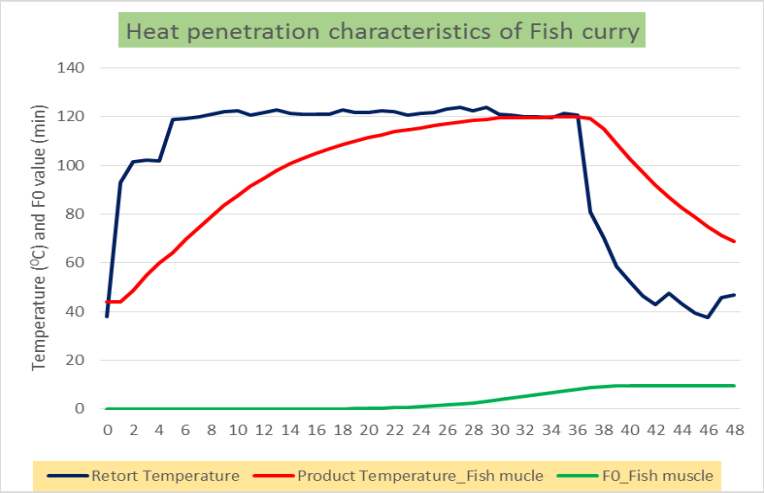
Fishery products, being categorized as low acid foods require heat processing severity with respect to *C botulinum* and F_0 value recommended is 5-20 min. Thermal processing of fishery products include various steps. These steps include, preparations like washing, beheading, gutting,removing scales / fins, cutting into required size, blanching (hot / cold), pre-cooking, filling fish pieces into containers, filling content or medium, exhausting to remove air, sealing, loading into the retort or autoclave, sterilization, washing and storing. Various packaging materials have beenused from historically starting from glass container to metal container, flexible retortable pouchesand rigid plastic containers. The sterilization process in the canned product can be subdivided intothree phases. First one is heating phase, in which the product temperature is increased from ambient to the required sterilization temperature by means of a heating medium (water or steam).This temperature is maintained for a defined time (phase 2 = holding phasing). In (phase 3 = cooling phase) the temperature in the container is decreased by introduction of cold water into theautoclave. In order to reach temperatures above 100°C (sterilization), the thermal treatment has tobe performed under pressure in pressure cookers, also called autoclaves or retorts. Simpleautoclaves are generally vertical ones with the lid on top. Through the opened lid, the goods to besterilized are loaded into the autoclave. The cans are normally placed in metal baskets. The autoclave and lid are designed to withstand higher pressures up to 5.0 bar. These types of autoclaves are best suited for smaller operations as they do not require complicated supply lines and should be available at affordable prices. Larger autoclaves are usually horizontal and loaded through a front lid. Horizontal autoclaves can be built as single or double vessel system. The doublevessel systems have the advantage that the water is heated up in the upper vessel to the sterilization temperature and released into the lower (processing) vessel, when it is loaded and hermetically closed. Using the two–vessel system, the heat treatment can begin immediately without lengthy heating up of the processing vessel and the hot water can be recycled afterwards for immediate usein the following sterilization cycle. In rotary autoclaves, the basket containing the cans rotates during sterilization which enhances the heat penetration resulting in reduced process time. This technique is useful for cans with liquid or semi-liquid content as it achieves a mixing effect of theliquid/semi-liquid goods. Water immersion retorts are also used in the industry for thermal processing which is advantageous over steam retorts due to its uniform temperature distribution asthere is no possibility of forming air pockets in the retort which limits the heat transfer in steam retorts.

At the final stage of the sterilization process the products must be cooled as quickly as possible by introducing cold water. The contact of cold water with steam causes the latter to condense with a rapid pressure drop in the retort. However, the overpressure built up during thermal treatment within the cans, jars or pouches remain for a certain period. During this phase, when the outside pressure is low but the pressure inside the containers is still high due to high temperatures there, the pressure difference may induce permanent deformation of the containers. Therefore, high pressure difference between the autoclave and the thermal pressure in the containers must be avoided. This is generally achieved by a blast of compressed air into the autoclave at the initial phase of the cooling. Sufficient hydrostatic pressure of the introduced cooling water can also build up counter pressure so that in specific cases, in particular where strong resistant metallic cans are used, the water pressure can be sufficient and compressed air may not be needed unlike in flexible retortable pouches. After thermal processing, the containers are washed with chlorinated potable water and stored for conditioning for 2 – 4 weeks. Conditioning helps in proper mixing of the ingredients with the fish products and helps in assessing the extent of thermal process severity. If the containers do not show any deformation, it indicates the effectiveness of the thermal processing.

The important steps in canning process are:

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

Steam retort and water immersion retort



Typical heat penetration curve of fish curry in retortable pouches

Non-Thermal Processing Techniques

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Non-thermal processing techniques

The demand from consumers for safe and nutritious food products has promoted the rapid development of non-conventional processing technologies. Non-thermal food processing simply refers to methods where the food materials receive microbiological inactivation without the direct application of heat. They are relatively young technologies, which use mechanisms other than conventional heating to reduce or eliminate microorganisms.

1. High-pressure processing

High-Pressure Processing is also known as high hydrostatic pressure (HHP) or ultra-high pressure (UHL) processing. It is a non-thermal, cold pasteurization technique, which generally consists of subjecting food, previously sealed in flexible and water-resistant packaging, to a high level of hydrostatic pressure (pressure transmitted by water) up to 600 MPa / 87,000 psi for a few seconds to a few minutes (1 – 20 min). HHP utilizes a very common medium, i.e., water, to apply the pressure on the product to be treated. HHP transmits isostatic pressure (100–1000 MPa) instantly to product at low temperature and might have comparable preservation effect as thermal processing through inactivating undesirable microorganisms and enzymes. An HPP unit consists of a pressure compartment in which food is kept and water is introduced into the chamber. Food is then pressurized using this water. HPP compromises cellular functions such as DNA replication, transcription, translation already at lower pressures (≤100 MPa) which impairs bacterial growth. At higher pressures, microorganisms start suffering lethal injuries due to loss of cell membrane integrity and protein functionality. The most sensitive to pressures are moulds, yeast and parasites.

Studies at ICAR-CIFT, Kochi

Ginson et. al. (2015) studied the effect of high-pressure treatment (250 MPa for 6 min at 25 °C) on microbiological quality of Indian white prawn (*Fenneropenaeus indicus*) during chilled storage. All microbes were reduced significantly after high pressure treatment and there was significant difference in microbial quality of control and high pressure treated samples in the entire duration of chilled storage. Kunnath et. al. (2020) reported that synergistic effect of high pressure and microbial transglutaminase (MTGase) could enhance the textural and functional properties of

fish gels, when compared with conventional cooking. MTGase enzyme along with pressure treatment enhanced the conformational stability and produce stronger networks through the formation of non sulfide bonds between proteins and setting reinforced these networks. Devatkal et. al. (2015) employed high-pressure processing (300 MPa for 5 min) as a non-thermal post-processing intervention to improve the shelf life and quality of cooked refrigerated chicken nuggets. Kundukulangara Pulissery et. al. (2021) compared the textural and nutritional profile of high pressure and minimally processed pineapple. On the basis of microbial quality and sensory assessment, high pressure treatment at 300 MPa for 10 min was found to be suitable for preserving the quality of pineapple up to 16^{th} day in refrigeration condition. Ginson et. al. (2020) investigated the piezotolerance and diversity indices of microflora of Indian white prawn (Fenneropenaeus indicus) after high pressure (HP)-treatment. *Arthrobacter spp., Listeria grayi* and *Corynebacterium spp*. were the most piezo tolerant bacteria in HP-treated samples.

2. Pulsed electric field (PEF) processing

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

3. Irradiation/Radiation processing

Irradiation refers to the process by which an object is exposed to radiation (A deliberate exposure to radiation). There are two forms of radiation: Ionizing radiation (IR) and non-ionizing radiation (NIR). IR includes high-energy electron beam, X-rays and γ-rays. IR leads to the production of charged particles or ions in material it comes in contacts with. Irradiation is a process of applying low levels of ionizing radiation to food material to sterilize or extend its shelf life. Radiation inactivates food spoilage organisms, including bacteria, moulds, and yeasts. It is effective in lengthening the shelf-life of fresh fruits and vegetables by controlling the normal biological

changes associated with ripening, maturation, sprouting, and finally aging. Radiation also destroys disease-causing organisms, including parasitic worms and insect pests, that damage food in storage. Irradiation is harmful or noxious to humans. However, the dose for seafood pre-treatment is low, therefore making it safe for consumption. Food irradiated under approved conditions does not become radioactive.

Studies at ICAR-CIFT, Kochi

Annamalai et. al. (2020) assessed the effect of electron beam irradiation ((0, 2.5, 5.0, 7.5 and 10 kGy) on the biochemical, microbiological and sensory quality of vacuum packed headless *Litopenaeus vannamei* during chilled storage (2 °C). There is a significant ($p<0.05$) reduction in *Brochothrix thermosphacta* and *lactobacillus* count in the irradiated sample. Based on the microbial and sensory analysis control had a shelf life up to 12^{th} day. However, electron beam irradiated sample had an extended shelf life of 15-23 days with respect to dose level.

4. Ultraviolet (UV) Radiation

UV radiation is a form of energy considered to be non-ionizing radiation having in general germicidal properties at wavelengths in the range of 200–280 nm (usually termed UV-C). UV irradiation has demonstrated to be effective not only in reducing microbial load but also inactivating enzymes activity in plant products. When food is exposed to UV-C, with 200–280 nm, these short wavelengths are absorbed by the microbial cell nucleic acids. These absorbed photons cause the breakage of the bond and interlinking between thymine and pyrimidine of different strands and the formation of dimers of pyrimidine. These dimers (Photo products) prevent DNA transcription and translation, thus leading to the malfunctioning of the genetic material, which causes microbial cell death. In principle, the UV radiation operates by destroying the genetic constituent of the pathogen to prevent division, multiplication and subsequently hinder its propagation. Usually, different kinds of food products require different doses of UV radiation (termed as UV-inactivation dose measured in mJ/cm^2) to inactivate different kinds of pathogens.

5. Pulsed Light (PL) Preservation

Pulsed light (PL) is an alternative technique to continuous ultraviolet treatment for solid and liquid foods. PL consists of successive repetition of high-power pulses of light/short time high-peak pulses of broad-spectrum white light. Comparatively, PL has a thousand times strength greater than the normal UV light which is quite continuous. Pulsed xenon UV uses the full spectrum of ultraviolet light to disperse germ-killing energy. The light spectrum includes wavelengths from

180 to 1100 nm with a considerable amount of light in the short-wave UV spectrum. Similar to other non-thermal food processing technologies, PL also has potential in the inactivation or elimination of microbes in food. Specific examples of foods processed by PL include fish, vegetables, fruits, and meat. PL can be used alongside other novel technologies as a hurdle in the inactivation of microbes on the surfaces of foods.

Studies at ICAR-CIFT, Kochi

Ananthanarayanan et. al. (2019) studied the effect of pulsed light (PL) treatment on the shelf-life extension of yellowfin tuna (*Thunnus albacares*) steaks stored at 2±1 ^{o}C. Tuna steaks of 1 cm thickness weighing 80 g packed in 300-gauge cast polypropylene pouches were subjected to PL treatment using Xenon pulse light machine RC-847. Shelf-life studies were carried out in terms of reduction of aerobic flora as inferred from the total plate count (TPC) and the psychrophilic count. An overall extension of 13 days of shelf life was achieved for PL treated samples.

6. **Ultrasound (US) processing**

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

7. Cold Plasma (CP) Technology

Ionization of gas molecules gives rise to plasma. Cold plasma is a non-thermal treatment that works in the temperature range 25–65 ^{o}C. Cold plasma has high antimicrobial activity and efficient enzyme inactivation capacity. The composition of the plasma reactive species largely depends on the composition of gas which is ionized. The gases commonly used for the generation of plasma include argon, helium, oxygen, nitrogen and air. The gases are subjected to any of the types of energy like thermal, electrical, magnetic field, etc., to generate plasma containing positive ions, negative ions, and reactive species like ozone and singlet oxygen.

8. Ozone treatment

Ozone is extensively employed as an effective antibacterial against many bacteria in food. Due to its high oxidizing potential and the ability to attack cellular components, ozone has broad-spectrum of disinfection. Ozone treatment is a chemical method of food decontamination that involves exposing contaminated foodstuffs (fruits, vegetables, beverages, spices, herbs, meat, fish, and so on) to ozone in aqueous and/or gaseous phases. Ozone alters the permeability of cells by damaging the microbial cell membranes. Ozone is also known to damage the structure of proteins, leading to the malfunctioning of microbial enzymes, which affects the metabolic activity and finally results in microbial cell death. Chemical composition, pH, additives, temperature, initial bacteria population, and ozone contact time with food and food surface type are factors determining the efficiency of ozone treatment on microbial reduction in seafoods

Conclusion

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

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Packaging of Fish and Fishery products

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1.0 Introduction

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

2.0. Types of Packaging Material

2.1. Glass

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

2.2. Cans

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

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

2.3. Paper

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

2.3.1. Paper board

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

2.4. Polymer Packaging

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

2.4.1. Low Density Polyethylene (LDPE)

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

2.4.2. High Density Polyethylene (HDPE)

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

2.4.3. Linear Low-Density Polythene (LLDPE)

Linear low-density polythene is low density polythene produced by a low-pressure process. Normal low-density polythene has many $-C_5H_{11}$ side chains. These are absent in LLDPE, allowing the molecules to pack closer together to give a very tough resin. It is virtually free of long chain

branches but does contain numerous short side chains. Generally, the advantages of LLDPE over LDPE are improved chemical resistance, improved performance at both low and high temperatures, higher surface gloss, higher strength at a given density and a greater resistance to environmental stress cracking. LLDPE shows improved puncture resistance and tear strength. The superior properties of LLDPE have led to its use in new applications for polyethylene as wellas the replacement of LDPE and HDPE in some areas.

2.4.4. Polypropylene (PP)

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

2.4.4.1. Cast polypropylene (CPP)

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

2.4.4.2. Oriented, Heat set Polypropylene (OPP):

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

2.4.5. Polystyrene

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

2.4.6. Polyester

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

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

2.4.7. Polyamides (Nylon)

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

2.4.8. Polyvinyl Chloride (PVC)

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

2.4.9. Copolymers

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

2.4.10. Aluminium foil

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

3.0. Packaging of fresh fish

A suitable package for fresh fish should keep the fish moist and prevent dehydration, retard chemical and bacterial spoilage, provide a barrier against moisture and oxygen to reduce fat oxidation and prevent permeation of external odors. Generally baskets made of split bamboo, palmyrah leaf and similar plant materials were traditionally used for packing fresh iced fish. However, they do not possess adequate mechanical strength and get deformed under stacking. The porous surface of these containers tends to absorb water and accumulate slime, creating an ideal breeding ground for spoilage bacteria, which can contaminate the fish. Even though washing cleans the contaminated surfaces of the container it has been shown to be ineffective in reducing the bacterial load significantly. Sharp edges of bamboo also cause bruises on the skin of fish. Used tea chests provided with 2.5 cm thick foamed polystyrene slabs inside have been found extremely beneficial for transport of fish over long distances up to 60 h duration.

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

3.1. Packaging of frozen fish

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

3.1.1. Primary wrap for block frozen products

The material used as a primary wrap for contact with the food is mainly Low-density polythene (LDPE). This can be in the shape of a bag or a film. Usually 2 kg or 5 lbs fish is packedalong with 10-20 % glaze. Glazing should be optimum at the recommended level, since this will add to cost

and weight during packaging and transportation. Alternately, films of high molecular weight high-density polyethylene (HM-HDPE), which is not as transparent as LDPE film are alsoused being more cost effective. 100-gauge LDPE is used for wrap while 200 gauge is used for bag.The corresponding values for HDPE are 60 and 120 gauge. Polythene films should be of food grade conforming to IS: 9845 specifications.

3.1.2. Duplex carton/ Inner carton

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

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

3.1.3. Master carton

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

3.1.4. Strapping and tying

Boxes are now mainly closed at the top and bottom by using cellophane tapes. They are also stapled or strapped by using polypropylene / high density/ rayon extruded straps. The straps are clipped or heat-sealed. The tensile strength must be great enough to withstand the load. For

polypropylene the fluctuations in the tensile strength and elongation at break (%) at –20°C are comparatively less. Hence this material is most suitable when compared to HDPE where the tensile strength and elongation at break vary.

3.1.5. Packaging of Individually Quick Frozen (IQF) Products

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

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

IQF shrimps are filled in primary containers along with code slip and weighed. Bar codingis nowadays adopted which will depict various product and inventory details through a series of bars. Bar coding is compulsory for products imported to the EEC and US markets. The product is filled into primary pack which heat sealed and further it is packed in master cartons for storage and transportation. The primary pack may be plastic film pouches (monofilm co-extruded film or laminated pouches). The unit pouches may be provided with unit/intermediate cartons or directly packed into master cartons. The unit/intermediate cartons are made of duplex or three ply corrugated fibreboard laminated with plastic film on the inside and outside to improve the functional properties as well as aesthetic value of the pack. The most functional cost-effective film has been identified as 10 μ biaxially oriented polypropylene (BOPP). Some duplex cartons are also wax-coated. One major requirement of the master carton is high compression strength to bear weight without damage to the product. Compression strength of 500 kg is the minimum recommended specification, which might give reasonable safety to the product. The cartons are made of 5 or 7 ply corrugated fibreboard.

3.2. Battered and Breaded fish products

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

3.3. Dry fish

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

Table 1. Bulk packaging materials and their properties

Type	Merits	Demerits
Waxed corrugated cartons	Handy, light , hygienic and presentable	Very delicate, Not foolproof against insects, rodents, moisture, breakage
Dealwood or Plywood boxes	Compact and strong, Largerquantities can be packed, handling , transportation and stacking are easy, Can be reused, Protection against damage	Comparatively heavy, Cost is high, Cheap wood not easily available
Bamboo baskets	Handy, light, Not costly	Very delicate, Not foolproof against insects, rodents, moisture, breakage
Gunny bag	Light ,handy, cheap , proof against breakage	Not foolproof against insects, rodents, moisture, Not hygienic
Dried palmyrah and coconut palm leaves	Cheapest of all and readily available in the coastal regions of India	Not foolproof against insects, rodents, moisture, Nothygienic and does not give good appearance, Packing is laborious
Multiwall paper sack lined with 300 gauge LDPE	Hygienic, presentable and can be printed	Costly, polythene lining may break during handling and hence is not foolproof against insects, rodents, moisture
HDPE woven gusseted bags laminated with100 gauge LDPE	Hygienic, presentable and can be printed, Stackable, can be packed uniformly	

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

Table 2. Consumer packaging of dry fish

Material Composition	Merits	Demerits
250 gauge low density polyethylene film	Cheap, readily available, good bursting and tearing strength and heat sealability	High water vapour and gas transmission rate, easy to puncture due to sharp spines, smell comes out. Shelf life limited.
250 gauge polypropylene film	Cheap, readily available, good bursting and tearing strength and heat sealability	High water vapour and gas transmission rate, easy to puncture due to sharp spines Shelf life is limited.
300MXXT Cellophane/150 gauge LDPE	Very low water vapour and gas transmission rate, transparent, good bursting and tearing strength , heat sealability and long shelf life.	Prone to easy attack by insects, costly.
12 micron plain polyester/150 g low density polyethylene	Very low water vapour and gas transmission rate, transparent, good bursting strength, puncture resistance & heat sealability. No insect penetration	Costlier
20micron Nylon laminated with 150 gauge polyethylene	Very low water vapour and gas transmission rate, transparent, good bursting strength, puncture resistance & heat sealability. No insect penetration	Costlier

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

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

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

3.4. Accelerated freeze dried (AFD)

AFD products demand a very high price in the export trade. The final moisture content ofAFD products generally is about 2 %. Low moisture content and large surface area make these foods extremely hygroscopic. Most dried products deteriorate when exposed to oxygen. Changesin colour may also take place as a result of bleaching. Light accelerates oxidative reactions and hence contact with light should be prevented. If proper packaging materials are not used there is every chance that the materials may undergo flavour changes due to the oxidation of the product and also migration of flavour from the packaging material. Since, fish contains fat there may be also a chance of it taking up the taints from the packaging material. The particular structural properties of freeze-dried products lead to damage by mechanical means. The light porous naturecauses them to be very fragile and easily prone to breakage during handling and transportation. Freeze dried products are also liable to damage caused by free movement within the package. Measures must be taken to fit the product compactly in the container, while leaving the minimumheadspace for filling inert gas. Rigid containers both glass and cans were used earlier for packaging of freeze dried products. However, now metallised polyester laminated with polythene or aluminum foil

/paper/polythenes are used since they have low oxygen transmission rate and water vapour transmission rate. Most of the packages are filled with an inert gas. The product can also be packed under vacuum to give better protection against damage.

3.5. Packaging of thermal process fish products

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

3.6. Fish pickles

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

on the reverse side of the polyester film.

3.7. Fish soup powder

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

3.8. Extruded products

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

3.9. Surimi and surimi-based products

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

3.10. Fish Sausage

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

3.11. Glucosamine hydrochloride

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

3.12. Chitin and Chitosan

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

3.13. Fish Hydrolysate

Fish Hydrolysate is prepared from fish mince which has contain oil and is undiluted, and so is a richer food source for beneficial microbes and especially beneficial fungi in the soil. It is generally cold-processed and hence retains the amino acids and protein chains as such. Fish hydrolysate is concentrated, and when diluted can be used ideally as soil fertliser, and is suitable for all soils,

crops, ornamentals, trees and vegetables It contains a wide spectrum of major nutrientsand trace elements in organic, plant available form. It can be used as a foliar spray, but since the oil is present it may show patches on the leaves. The liquid is generally packed in jars or cans which are made of polypropylene or HDPE.

3.14. Fish Meal

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

3.15. Fish oils

Fish oils are highly unsaturated and easily susceptible to oxidation when exposed to air. Hence, they have to be packed in containers which have high barrier properties which are moisture proof, oil resistant and impermeable to oxygen. Larger quantities of fish oil are mainly packed in LLDE/Nylon films or in glass bottles. Bulk transportation food grade flexitanks made of 4 layered polyethylene and tubular PP. Advantages of using flexitanks are that they can carry 50% more than bottles and therefore will save on storage space, packaging and transportation cost. Fish oil is also marketed for regular oral dosage in the form softgel capsules. The shell is made of gelatin, water, glycerol or sorbitol. The process of encapsulation is by using the rotary die encapsulation process. The encapsulation process is a FFS operation. Two flat gelatin ribbons manufactured on the machine are brought together on a twin set of rotating dies that contain recesses in the desired size and shape, these cuts out the ribbon into a two-dimensional shape, and form a seal around the outside. At the same time a pump delivers a precise dose of oil through a nozzle incorporated into a filling wedge whose tip sits between the two ribbons in between two

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

3.16. Fish silage

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

3.17. Shark fin rays

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

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MAP, Active and Intelligent Packaging

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1.0 Introduction

Food packaging plays a very important role in the food supply chain, which can be used as a protective layer to prevent contamination of food and maintain food quality. However traditional packaging system only isolates food from the external environment and cannot provide food freshness information for producers, sellers and consumers. The presence of O_2 in standard air packing will cause the spoiling process to proceed more quickly. Advanced food packaging techniques refer to innovative methods and technologies used to package and preserve food products. These techniques go beyond traditional packaging approaches and aim to enhance food safety, extend shelf life, improve convenience, and reduce environmental impact. By using vacuum packaging or packaging with a changed atmosphere, it is possible to overcome the issue of shelf life. In reduced oxygen packaging, the oxygen levels inside the package are significantly reduced, typically by displacing oxygen with other gases or by creating a vacuum. The reduced oxygen environment can be achieved through various methods, including modified atmosphere packaging (MAP) and vacuum packaging. Smart packaging such as active and intelligent packaging technologies, is considered as an innovative packaging technique for the development of wide variety of products with competitive cost and achieved a great position in the preservation of different food systems. It is a fast-growing technology. The global smart packaging market size was estimated at USD 35.92 billion in 2022 and is expected to reach around USD 60.49 billion by 2032 with a registered CAGR of 5.4 % from 2023 to 2032.

2.0 Modified Atmospheric Packaging

Modified Atmosphere Packaging (MAP) can be defined as the enclosure of food in a package in which the atmosphere inside the package is modified or altered to provide an optimum atmosphere for increasing shelf life and maintaining food quality. MAP is the imposition of a gas atmosphere, typically containing an inert gas, such as nitrogen combined with an antimicrobially active gas, such as carbon dioxide, upon a packaged food product to extend its shelf life. MAP can

significantly extend the shelf life of food products, thus prolonging the distribution chain and diminishing the need for centralized production. MAP provides an added barrier against spoilage, and can therefore improve shelf life and enhance product safety. MAP is inexpensive, easy to apply, and suitable for a wide range of packaging machinery and production venues. However, food manufacturing and transport to the end user is a complex process to which MAP contributes only a partial solution regarding shelf-life problems: a constant chill chain and good hygiene in manufacture remain keys to maintaining the efficacy of the MAP process. Applying MAP to ready meals, in fact, presents considerable practical difficulties. Modified atmosphere packaging (MAP) combined with refrigerated storage is an efficient preservation method to extend the shelf life of fish products. The application of such "soft hurdles" reduces the rate of fish deterioration and spoilage caused by microbial growth. MAP offers many advantages to consumers and food producers. To the consumer, it offers convenient, high-quality food products with an extended shelf life. It also reduces and sometimes eliminates the need for chemical preservatives, leading to more "natural" and "healthy" products. At the same time, producers also enjoy the benefits of increased shelf life. By using MAP many products can be packaged centrally, and their distribution cost is reduced because fewer deliveries over longer distances become possible. Moreover, because of the extended shelf life, MAP allows transportation of foods to remote destinations and increases product markets. MAP also has several disadvantages. Usually, each MAP product needs a different gas formulation. This requires the use of specialized and expensive equipment. At the same time, production staff must receive special training. For most products, storage temperature control is required and product safety must be established. Furthermore, MAP causes larger package volumes, which leads to increased transportation and retail display space needs. All the above add a noticeable cost, which must be paid by the consumers. Finally, another disadvantage of MAP is that it loses all its benefits once the consumer opens the package.

3.0 Smart Packaging

Smart packaging provides a total packaging solution that on the one hand monitors changes in a product or its environment (intelligent) and on the other hand acts upon these changes (active). Smart packaging utilises chemical sensors or biosensors to monitor the quality and safety of food all the way from producers to consumers. As with the previously discussed technology, smart packaging utilises a variety of sensors for monitoring food quality and safety, for example by detecting and analysing freshness, pathogens, leakages, carbon dioxide, oxygen, pH level, time or

temperature. The exact functionalities of specific smart packaging solutions vary and depend on the actual product being packaged, for example, food, beverages, pharmaceuticals, or various types of health and household products. Similarly, the exact condition to be monitored, conveyed, or adjusted vary accordingly. Smart packaging allows to track and trace a product throughout its lifecycle and to analyze and control the environment inside or outside the package to inform its manufacturer, retailer or consumer on the product's condition at any given time.

4.0 Active Packaging

Active packaging (AP) incorporates functional materials or additives that actively interact with the product and packaging environment to enhance food safety and quality while extending shelf life. O_2 scavengers and moisture absorbers are by far the most commercially important sub-categories of active packaging and the market has been growing steadily for the last ten years and is predicted to grow even further. All other active packaging technologies are also predicted to be used more in the future, particularly ethylene scavengers, CO_2 scavengers and emitters, and temperature control packaging.

O_2 Scavenger

An oxygen scavenger is a substance that reacts with oxygen chemically or enzymatically, thus protecting the packaged food against oxidative deterioration and quality changes due to oxygen. Most commercially available oxygen scavenging systems are based on the oxidation of iron powder or enzymes such as glucose oxidase, by oxygen present in the package's headspace. These reactions absorb most of the available oxygen in the package, minimize the interaction of oxygen with food, and thus prevent deterioration. Most often, oxygen scavengers are packed in a sachet highly permeable to oxygen and placed inside the package. Some safety concerns regarding the use of sachet-type oxygen scavenging systems in packaged foods are accidental ingestion of the sachet by consumers and potential spill/leak of the sachet contents into food. Careful research and development must be undertaken in selecting the appropriate sachet material and its seal integrity before commercial application, and appropriate warnings must be declared on the package.

CO_2-emitter

A CO_2-emitter is a device designed to release carbon dioxide (CO_2) within a controlled environment, particularly in the context of food packaging. The purpose of a CO_2-emitter is to enhance food preservation by increasing the concentration of carbon dioxide in the packaging. This technology is often employed in conjunction with modified atmosphere packaging (MAP),

which involves adjusting the composition of gases surrounding the packaged product to extend its shelf life. The CO_2-emitter typically contains substances such as sodium bicarbonate interacting with ferrous carbonate or ascorbic acid, or a combination with sodium sulfite deoxidizer. These interactions result in the gradual release of CO_2, influencing the atmosphere within the packaging. The role of CO_2 in food preservation is multifaceted. It helps to reduce the respiration rate of fresh produce, slowing down the aging process, and inhibits the growth of bacteria in meat products. CO_2 achieves these effects by impacting cell membrane functions, altering the physicochemical properties of proteins, and hindering enzyme activities. The concentration of CO_2 released by such emitters is carefully controlled to create an environment that is conducive to food preservation. However, challenges such as the solubility of CO_2 in both water and oil, which can lead to the collapse of packaging, as well as the permeability of plastic films to carbon dioxide, necessitate ongoing advancements in packaging materials to ensure optimal gas content and ratios. CO_2-emitters play a crucial role in maintaining food quality and safety throughout the storage and transportation of various perishable goods.

Moisture Absorbers

Moisture absorbers play a crucial role in food packaging by helping to maintain the quality and freshness of products. These substances, commonly made of materials like silica gel or clay, are integrated into packaging to absorb excess moisture, which prevent the growth of mould, bacteria, and other microorganisms. Moisture can adversely affect the texture, taste, and shelf life of various food items, making the use of absorbers particularly important in products prone to moisture-related issues, such as dried fruits, grains, and snacks. By reducing humidity levels within the packaging, moisture absorbers contribute to the preservation of product integrity, preventing clumping, staleness, and microbial spoilage. This not only enhances the overall consumer experience by ensuring the product's sensory attributes but also extends the shelf life of packaged goods.

Antimicrobial Packaging

Antimicrobial active packaging represents a cutting-edge solution in the realm of food packaging technology. With the incorporation of antimicrobial agents like silver nanoparticles, essential oils, and enzymes, this packaging strategy aims to combat microbial growth on the surface of packaged goods. The utilization of silver nanoparticles, for instance, leverages the well-known antimicrobial properties of silver to inhibit the proliferation of bacteria and other microorganisms. Similarly,

essential oils, such as those derived from oregano and thyme, bring natural antimicrobial benefits that can be harnessed when encapsulated within packaging materials. These innovations contribute to an extended shelf life for products by minimizing the risk of spoilage and enhancing overall food safety. Beyond shelf life extension, antimicrobial active packaging plays a pivotal role in reducing food waste. By creating a protective shield against harmful microbes, this packaging technology ensures that products remain fresh for a more extended period, reducing the likelihood of premature spoilage. Additionally, the enhanced safety and preservation of product quality are noteworthy advantages. This technology aligns with the broader goals of the food industry to deliver safe, high-quality products to consumers while addressing sustainability considerations and minimizing the environmental impact associated with food waste.

Antioxidant Packaging

Antioxidant packaging is a specialized form of packaging designed to protect products from oxidative reactions that can degrade their quality. Oxidation, driven by exposure to oxygen in the air, can lead to the deterioration of certain products, such as food items and pharmaceuticals. Antioxidant packaging incorporates materials or additives that help mitigate the impact of oxidation, preserving the freshness and integrity of the packaged contents. Commonly used antioxidants in packaging include natural compounds like vitamin C (ascorbic acid) and vitamin E (tocopherol), as well as synthetic antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT). These antioxidants function by scavenging free radicals and inhibiting the oxidation process. Antioxidant packaging is particularly valuable for products vulnerable to oxidation, such as oils, fatty foods, and pharmaceuticals containing sensitive active ingredients. The benefits of antioxidant packaging extend beyond preserving product quality. They contribute to extension of shelf life, reduction of food waste and maintains the nutritional value of the packaged items.

Active packaging systems with dual functionality

Active packaging systems with dual functionality represent an advanced strategy for enhancing the stability of packaged goods. Combining oxygen scavengers with carbon dioxide and/or antibacterial/antioxidant releasing systems offers a comprehensive approach to extending shelf life. The use of an oxygen scavenger alone may create a partial vacuum, risking the collapse of flexible packaging. Additionally, flushing a package with a gaseous mixture containing carbon dioxide can lead to CO_2 permeation through the packaging film, impacting product quality. To

address these challenges, self-working devices that absorb oxygen and generate sufficient carbon dioxide are promising, especially for items like fishery products, where preventing microbial growth and maintaining optimal storage conditions are critical for prolonged shelf life and product freshness.

5.0 Intelligent Packaging

Intelligent packaging is a new packaging technology that integrates intelligent functions with conventional packaging systems. Intelligent packaging can sense, detect and record external or internal changes of products, further to provide food quality and safety information, and therefore extending the information function of packaging. Intelligent packaging is a revolutionary concept that goes beyond traditional forms of packaging by incorporating smart technologies to provide real-time information about the condition and quality of the packaged product. These systems utilize sensors, indicators, or RFID technology to monitor factors such as temperature, humidity, and freshness. The collected data can be relayed to consumers or stakeholders, enabling informed decisions about product safety and quality. Intelligent packaging is particularly valuable in the food and pharmaceutical industries, where maintaining specific storage conditions is crucial. This technology not only enhances transparency in the supply chain but also contributes to reducing waste by helping consumers make more informed choices based on the real-time status of the packaged goods.

Time-temperature indicators

Time-temperature indicators (TTIs) are innovative devices incorporated into packaging to monitor and communicate the cumulative exposure of a product to temperature over time. These indicators are crucial in industries such as food and pharmaceuticals where maintaining a specific temperature range is vital for product safety and quality. TTIs work by employing chemical or physical mechanisms that react to temperature changes, providing a visual indication of the duration and extent of temperature exposure. This visual signal helps both consumers and stakeholders to assess whether a product has been subjected to undesirable temperature conditions during storage or transportation. Time-temperature indicators play a pivotal role in enhancing transparency along the supply chain, allowing for informed decisions regarding the suitability of a product for consumption or use based on its temperature history.

Freshness indicators

Freshness indicator (FI) is an indispensable part of intelligent packaging, mainly including two types: indicator card (label) and indicator film. The colour changes of FI caused by the characteristic volatile from food can directly reflect the freshness of food. FI can provide qualitative or semi-quantitative information about food quality changes caused by physiological changes or microbial growth without destroying food packaging, and therefore can help consumers intuitively and scientifically judge food quality. FI is very suitable for non-destructive testing of freshness of perishable foods such as meat products, seafood, dairy products, fruits and vegetables. Although FI has such advantages, the applications of FI as a new intelligent packaging technology in the food industry are still rare.

6.0 Future Aspects

Smart packaging systems proved to be an effective mechanism to improve the food safety and shelf-life extension of the packaged foods. However, these technologies are in development stage in the seafood sector and thus needs ongoing researches and continued innovations to anticipate future advancement in food quality, safety and stability.

High Value Secondary Products from Fish Processing Discards

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Processing of seafood for human consumption results in enormous quantity of waste in theform of skin, head, viscera, scales, bones, trimmings and frames. The quantity of waste generateddepend on the type and size of fish and the product manufactured out of it. Industrial fishprocessing for human consumption yields only 40% edible flesh and the remaining 60% is thrownaway as waste. Fishery waste is prone to faster spoilage since it contains easily digestible protein. The microbial population associated with the digestive process are the major reasons of spoilage. Since the processor does not bother to preserve the waste the problem of environmental pollution is enhanced. Accumulation of fisherywaste results in nauseating and obnoxious smell due to the release of volatile nitrogenous compounds during decomposition.

The immense scope for high end product from fishery waste has been realized and different technologies have been developed with a view to utilize processing waste, for converting them into products for human consumption, animal nutrients and products of pharmaceutical and nutraceutical significance. The fish waste utilization technology evolved by ICAR-CIFT helps to eliminate harmful environmental effects and improve quality in fish processing. Research carried out at the Central Institute of Fisheries Technology, Cochin, paved the way for production of valuable food and industrial products namely protein extract, chitin, chitosan and its derivatives and glucosamine hydrochloride from the head and shell waste of prawns, crab and squilla.

1.0 Nature and composition of secondary raw materials from seafood processing

In seafood industry, the general understanding is that the edible meat part constitute about main raw material and the remaining parts include head, trimmings, skin, viscera, scale, bone etc. Quantum of secondary raw materials generated in seafood industry depends on several factors, which may be broadly categorised into resource related factors and process related factors. The former category includes species, size, age, biological nature (including presence of toxins and allergens) and morphological features. Generally, 40- 70% of original raw material is discarded in

commercial processing operations depending on intended product, style of dressing, type of handling (manual/ mechanical), skill of handling person, intended use and to a greater extent on the quality of raw material. Largely, seafood processing operations generate both liquid and solid wastes; solid waste being the bulk ranging from 30% to 65% of the weight of the landed fish. Head, viscera, skin, fin, swim bladder, bone, frame meat, dark meat, scale, gills, shells (crustacean, mollusca), cephalopod pen, ink sac etc. are the major components of solid waste. The liquid effluents mainly consist of blood, slime, mucus, wash off and other soluble. In surimi processing, soluble proteins are washed off to a greater extent during repeated water washing steps.

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

Products	**Waste Generated (%; w/w)**
Shrimp products (peeled and deveined, peeled and undeveined, Headless etc.)	50
Fish fillets	70
Fish steaks	30
Whole and gutted fish	10
Surimi	70
Cuttle fish rings	50
Cuttle fish whole	30
Cuttle fish fillets	50
Squids whole cleaned	20
Squid tubes	50
Squid rings	55

Table 2. Seafood products and their respective waste

Major Sea food Products	Major Waste	Major Compounds
Fish based Product	Head, frames, skin, intestine, roe, tails, fins, scales, etc.	Protein, fat, Minerals, enzymes, Chondroitin, Fe
Shrimp based products	Carapace (head) Telson (tail) Rostrum Antennae Appendages Eggs Cook juice	Chitin, Protein, Pigments
Frozen Squid (Whole Cleaned, Fillet, Rings	head behind the tentacles Visceral mass Beak Ink sac and ink Squid pen Skin membrane	Protein, Enzymes, Melanin, Chitin
Cuttlefish (Whole cleaned, deskinned	head behind the tentacles Visceral mass hard Beak Ink sac and ink Cuttle bone Skin membrane	Protein, Enzymes, Melanin, Chitin
Lobster Meat	Lobster shell Appendages	Chitin
Pasteurized crab meat	Crab shell	Chitin Pigments
Fish products (fillet, surimi)	Head Frame/bone Skin Scales Gills Fins Visceral mass Wash water	Proteins Lipids Enzymes Minerals
Frozen clam/Mussels	Shells Shuck water	Calcium oxide Protein

2.0 Fish meal

Fish meal is highly concentrated nutritious feed supplement consisting of high-quality protein, minerals, vitamins of B group and other vitamins and other unknown growth factors. Fishmeal is rich in essential amino acids. It is produced by cooking, pressing, drying and grinding the fish, by-catch fish, miscellaneous fish, filleting waste, waste from canneries and other processing operations. The composition of fishmeal differs considerably due to the variations in the raw material used and the processing methods and conditions employed. Traditional fishmeal production in India was from the sun-dried fish collected from various drying centers and the products were mainly used as manure. Better quality fish meal has been a prominent item of export from the very beginning of this industry. BIS has brought out the specification for fish meal as live stock feed for facilitating proper quality control. As per FAO projection, by 2025, fish meal

produced from fish waste will represent 38% of world fish meal production, compared with 29% for the 2013 to 2015 average level.

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

Protein - 50-57%

Fat - 5-10%

Ash - 12-33%

Moisture - 6-10

2.1 Fish body oil

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

2.2 Fish liver oil

The therapeutic value of fish liver oil was discovered in 18th century and fish liver oil become a common medicinal product especially for Vitamin A and D. Cod, shark and haddock livers are the important sources of Vitamin A and D. The weight of liver, fat content and presence of vitamins are dependent on a number of factors like species, age, sex, nutritional status, stages of spawning, and area from where it is caught. In cod (Gadus collarius), coal fish (Pollahius vireus) and haddock (Melanggrammus aenglefinus), the weight of liver normally amount to 4-9% of whole fish and livers contain about 45% to 67% oil. The species of shark such as dog fish (Squalus acanthias), Greenland shark (Somniosus microcephalus) and barking shark (Certrohinus maximus) have large

fatty livers weighing up to 10-25% of the whole fish containing 60- 75% oil. But halibut, tuna, and whale have 1% liver having 4 to 25% oil with high vitamin A & D content. Depending on the oil content and vitamin A potency fish livers are generally classified in to three groups.

Low oil content - high vitamin A potency High oil content - low vitamin A potency High oil content - medium vitamin A potency

2.3 Squalene from shark liver oil

Liver oils of some deep-sea sharks mainly Centrophorus sp. It contains 85% – 90% unsaponifiable matter which contains the hydrocarbon squalene. Squalene and its hydrogenated product are used for several decades as base for cosmetic products. It also used as skin rejuvenating agent. It is mild on human skin and imparts softness without oily appearance. The demand of squalene by cosmetic and pharmaceutical industry is on increase. Realizing the importance, ICAR- CIFT has developed a method of extraction, isolation and purification of squalene from shark liver oil

2.4 Fish silage

The product of the process of preserving and storing wet biological material in a silo (a pit or airtight container) is called silage. Fish silage is a liquid product and it can be prepared from whole fish or fish waste by adding acid, enzymes, lactic acid producing bacteria or by naturally occurring enzymes in fish. Fish silage is rich in protein and aminoacids and it can be used as protein source for animal feeding. Production cost for fish silage is very cheap, cost effective and eco-friendly. Fish silage preparation usually depends on locally available raw materials and conditions (Hasan, 2003). Depending on the process employed, fish silage can be categorized into two methods, viz. acid silage and bio-fermented silage. Acid silage is produced by mixing fish waste with organic acid (formic acid, acetic acid, propionic acid), inorganic (sulphuric acid, hydrochloric acid) and or a mixture of both organic and inorganic acid. In case of bio-fermented silage, fermentation process is carried out by lactic acid bacteria (LAB) which are already present in a fish mass or added externally.

2.5 Protein Compounds from secondary raw material

2.5.1 Fish hydrolysates

Fish protein hydrolysate is a product prepared from proteins sourced from fish meat/fish processing by products via enzymatic or chemical process. Enzymatically produced hydrolysates are widely accepted which contain mixture of peptides of varying sizes and free amino acids. The process consists of chopping, mincing, cooking, cooling to the desired temperature, hydrolysis, sieving, pasteurizing the liquid, concentrating and vacuum drying or spray drying of the product. Enzymes like papain, nisin, trypsin, bromelein, pancreatin are used for hydrolysis of fish protein. Hydrolysates find application as milk replacer and food flavouring agents. 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. Fish protein hydrolysate are proven to have specific health role other than the nutritional benefit. Protein hydrolysates or peptides present in the hydrolysate have demonstrated to have antioxidant, anti-obesity, immune modulation, anticoagulation, anti-microbial, anticancer and antihypertension etc.

2.5.2 Fish Gelatin

Fish skin and scales which constitutes about 30% and 5% of the total seafood processing discards,respectively are considered as the richest source for collagen and gelatin, which have wideapplications in nutraceutical product development due to its biocompatibility, biodegradability,and bioactive properties like antioxidant, antimicrobial, antihypertensive. Skin of fish constitutenearly 3% of the total weight and is suitable for the extraction of gelatin. Bones and scales can alsobe processed into gelatin. The process involves alternate washing of skin with alkali and acid andextracting gelatin with hot water. Gelatin finds applications in pharmaceutical products asencapsulation and in food industry as gelling agent. Fish gelatin has better aroma and flavor with less inherent off-flavor and off-odor than a commercial pork gelatin.

2.5.3 Fish collagen

Collagen is a structural protein having a characteristics triple helix structure. Collagen is insoluble

in water and fibrous in nature. Approximate molecular weight of a collagen molecule is 300KDa. Collagen derived from fish is generally of Type I and Type III. Type I and Type III collagen are the building blocks for connective tissues, bones and skins. Collagen is not soluble in water. However, fish type I collagen is unique in its extremely high solubility in dilute acid compared to avian and mammalian collagen. The solubility of collagen is affected by the pH and NaCl concentration of the solution

2.5.4 Collagen and gelatin hydrolysates

Although collagen/gelatin has several functional properties, its bioactivity is lower due to its high molecular weight. Hydrolyzing will enhance the bioactivities of the collagen/gelatin. Collagen or gelatin hydrolysates are produced by controlled hydrolysis of collagen or gelatin. Acid, alkali, enzyme or heat may be used for hydrolysis. During hydrolysis the peptide bonds are broken down producing low molecular weight peptides. The molecular weight of hydrolysate is generally in the range of 5.0-25 kDa

2.5.5 Fish maws/ isinglass

The world isinglass is derived from the Dutch and German words, which have the meaning ' air bladder of deepwater hake is most suitable for production of isinglass. In India air bladders ofeel and catfishes are used for the production of isinglass. The air bladders are separated from fishand temporarily preserved in salt during transport. On reaching the shore they are split open, washed thoroughly, outer membrane is removed by scraping and then air dried. Cleaned, desalted, air dried and hardened swimming bladders (fish maws) are softened by immersing in chilled water for several hours. They are mechanically cut into small pieces and rolled or compressed between hollow iron rollers that are cooled by water and provided with scraper for the removal of any adhering dried material. The rolling process converts the isinglass into thin strips or sheets of 1/8 ¼" for the production of isinglass in powder form also. Isinglassdissolves readily in most dilute acids or alkalis, but is insoluble in alcohol. It is used as a clarifyingagent for beverages like wine, beer, vinegar etc.

2.5.6 Fish enzymes

Fish visceral waste contains rich sources of enzymes, which have potential applications in different sector includes food, biomolecule extraction, descaling of fish, stain removal and pharmaceutical

applications. It has been reported that fish visceral waste contains rich source of proteolytic enzymes namely, pepsin, trypsin, chymotrypsin and collagenases. Enzyme extracted from marine sources has found application in Fish curing and fermentation, hydrolysed products production, pigment extraction, wastewater treatment and meat tenderizing. These enzymes also used as a component of biosensor for rapid assessment of fish quality.

2.5.7 Hemoproteins and Carotenoproteins

Hemoproteins are complex proteins, composed of a protein molecule and a non-protein compound (prosthetic group). Hemoglobin and myoglobin belong 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 and carotenoids are other classes of compounds found in the flesh and skin of fishes and in the exoskeleton of shellfish. Astaxanthin, a ketocarotenoid pigment naturallyfound in crustaceans and represent 74 and 98% of the total pigments. It has found wide applicationin food, feed, pharmaceutical and cosmetic products. It is also used as dietary supplement with very potent antioxidant effect for human health.

2.6 Mineral compound from secondary raw material

2.6.1 Fish calcium

The recommended daily intake of calcium is 1000 mg for the adults, and 1300 mg for elderly women. Fish bones and scales are excellent source of calcium. Whole small fish or fish bone/scale can be used for calcium separation. The filleting frames of carps and other fishes can be used for extraction of calcium. The frames are washed and boiled to separate the adhering meat portions. It is washed again and treated with enzymes to remove the adhering connective tissue, washed, dried and powdered. Fish calcium is essentially dicalcium phosphate which has better nutritional qualities. The hydrolysis of collagen or gelatin yields bioactive peptides that have great potential in processing industries as natural preservatives. Collagen and gelatin peptides are known to have excellent antioxidant properties unlike its parent molecules. Recently gelatin hydrolysate has been

explored as plastisizer in protein film, identified as antihypertensive, cryoprotectant in additions to its wide known antioxidant activity.

2.6.2 Hydroxyapatite

The hydroxyapatite extracted from the scale are having uses as bioceramic coatings and bone fillers. The coatings of hydroxyapatite are often applied to metallic implants to alter the surface properties so as to avoid rejection by the body. Similarly, hydroxyapatite can be employed in forms such as powders, porous blocks or beads to fill bone defects or voids. For permanent filling of teeth hydroxyapatite is found to be a better option for import substitution.

2.7 Polymer compound from secondary raw material

2.7.1 Chitin

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

2.7.2 Chitosan

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

Chitosan finds extensive applications in following areas viz; food industries, pharmaceutical applications, chemical industries, dental and surgical uses as a haemostatic agent,wound healing,

biodegradable films as a substitute for artificial skins for removing toxic heavy metals, wine clarification, Industrial effluent treatment, agriculture, photography, cosmetic applications and textiles, and in nano applications

2.7.3 Glucosamine hydrochloride

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

2.7.4 Chitosan derivatives

Chitosan is not soluble in water but is soluble in dilute acid solutions like 1 % acetic acid. This has limited its applications in water soluble environments like human health and plant protection. Hence, the free amino and hydroxyl groups can be derivatized with new molecules to improve the functional properties of Chitosan. Advantages of Chitosan derivatives includes They are biodegradable and biocompatible; They are non-toxic and water soluble; They can be modified to impart special properties. Examples for chitosan derivatives are **N**-Trimethylene chloride Chitosan (TMC), Esters of chitosan with glutamate, succinate and phthalate Carboxymethyl chitosan (CM-Chitosan).

Major Applications of Chitosan derivatives includes 1) Controlled release and drugdelivery 2) Scaffolds for biomedical applications like stents, organs 3) Tissue engineering, woundhealing and regenerative medicine 4) Food supplements and natural preservatives 5) Anti-viral andanti-tumor applications 6) Bio-composite materials with functional properties

CIFT has developed technology for production of chitin, chitosan, glucosamine hydrochloride and carboxymethyl chitosan from prawn shell waste.

2.8 Other High value product from secondary raw material

2.8.1 Pearl essence

Pearl essence is the suspension of crystalline guanine in water or organic solvent. Guanine is an iridescent material found in the epidermal layers and scales of most pelagic species of fish likeoil

sardine, mackerel, herring etc. When guanine particles are deposited on the inside surface ofsolid beads, an optical effect similar to that of real pearl is obtained. It is used in the manufactureof artificial pearls. It is also used on diverse articles such as shoe, pencil, fishing rod and spectacle frame

2.8.2 Shark cartilage

The skeleton of shark is made of cartilaginous bones, which is about 10-15% of the body weight. Until recently, only very small quantity of these bones was made use of, that too from the small shark, for making buttons and necklaces. This cartilage is rich in chondroitin sulphate which has got application in medicine for treatment of atherosclerosis, blood vessel thrombosis and also to prevent infections. Now there is very good demand from Europe, USA and Australia for processed shark bones. The collected head and vertebral column of the shark are to be processed to a presentable and stable form before export. A procedure has been developed for the processing of the cartilage into a clean, dry, white, attractive material without any characteristic smell. The products are well accepted by the overseas buyers. The ban on shark fisheries is going to affect all these products, as mentioned above.

2.8.3 Shark fin rays

Shark fin rays are valuable products of export from India. Formerly, only shark fins were being exported. But now, fin rays are extracted and exported. CIFT has developed a technique for extracting rays from shark fins. The dried fins are soaked in dilute acetic acid for sufficient time to get the muscle and skin softened. The skin is then scraped off and the fins further treated with the dilute acetic acid when separation of the rays in clusters becomes easy. The rays are then dried and packed in polyethylene bags. The rays are utilised in the preparation of soup in many foreign countries. There is good internal demand also for shark fin rays especially in major star hotels.

2.8.4 Shark leather

Skin from both demersal as well as pelagic fish varieties are suitable for the leather production. Skins of shark can be processed into fine leather suitable for manufacture of fancy items. Leather tanned from Indian shark skin is about one and half times superior to that from cow hides in strength and durability. Shark skin has a protective coating of a calcareous deposit known as “Shagreen”. It is used as a suitable raw material for manufacture of suitcases, shoes, belts etc.

3.0 Conclusion

Seafood waste is prone to faster spoilage since it contains easily digestible protein and enzymes. The microbial population associated with the digestive process are the major reasons of spoilage. Accumulation of fishery waste results in nauseating and obnoxious smell due to the release of volatile nitrogenous compounds during decomposition. Hence utilization of seafood waste and development of high value product has high potential in recent years. A variety of by products can be developed which is found to have different applications in medical, food, and other fields. By simple cost-effective techniques, valuable products can be developed which will enhance the revenue of the fishermen and allied industries.

Chitin and its Derivatives

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

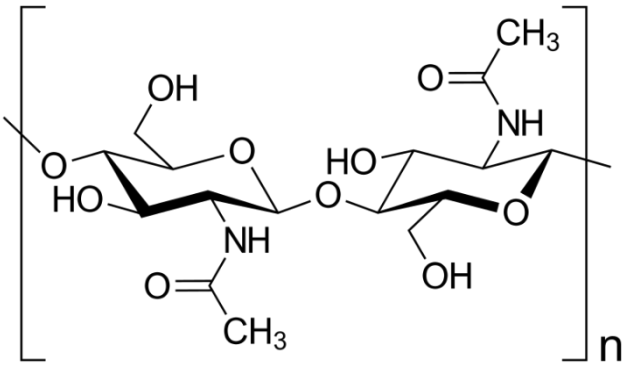

Fig. 1 Chemical structure of chitin

1.0 Biosynthesis pathway of chitin

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

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

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

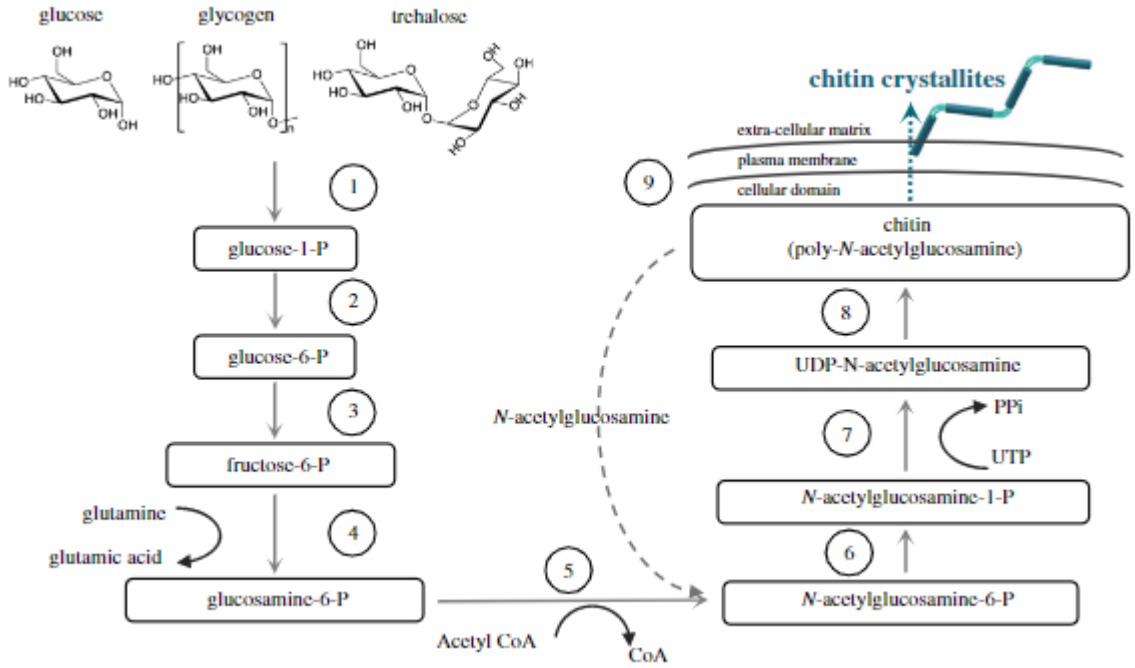

Fig. 2. Steps involved in biosynthesis path way of chitin in fungi and insects (Ref: Hou J,Aydemir BE,Dumanli AG. 2021)

2.0 Structure of chitin

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

- α-chitin (most common form) – Chains are parallel and adjacent polymer chains are always in the opposite direction. A strong network dominated by intrachain hydrogen bonds between the groups of C═O···NH and C═O···OH within a distance of 0.47 nm. Additional inter-chain hydrogen bonds bind the hydroxymethyl groups.
- β-chitin - all chains are parallel and in the same direction. The network is strong and dominated by intrachain hydrogen bonds. No additional inter-chain hydrogen bonds found in this conformation.

- γ-chitin – Two adjacent chains are parallel, and unidirectional while third one is in opposite direction.

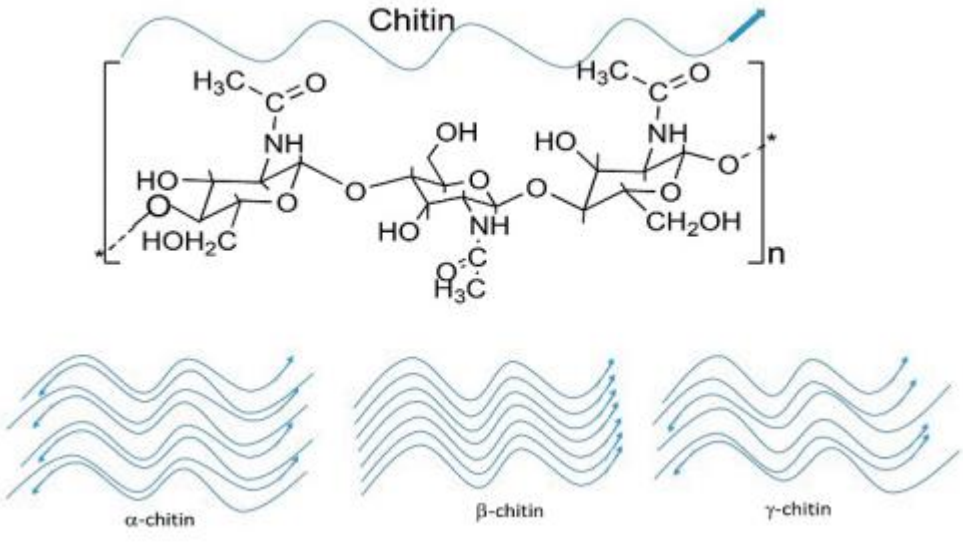

Fig. 3. Polymorphic forms of Chitin (Roy et al., 2017)

3.0 Major sources of chitin of aquatic origin

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

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

4.0 Composition of shell waste

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

Table 2. Proximate composition of shell waste

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

5.0 Shell structure – Inter linkage of the components

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

Epicuticle:

- Outermost layer which is thin and waxy
- Consists of long chain hydrocarbons, esters of fatty acids, and alcohols

Exo and endocuticle:

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

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

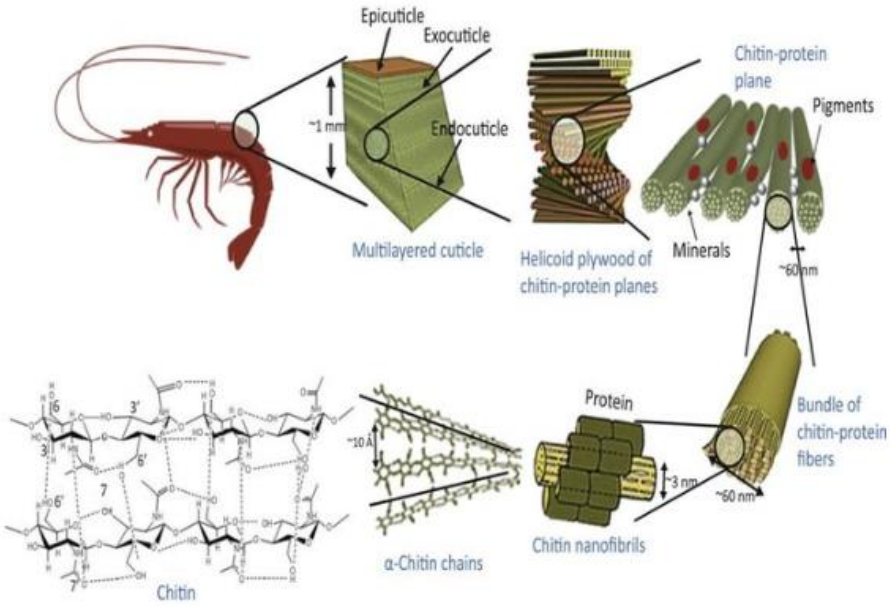

Fig.4 Shell structure and chemical composition (Adapted from Bradic, 2020)

6.0 Chitin process

The process for chitin production basically aims to eliminate other chemical constituents like proteins and minerals. For removing these constituents, concventionally chemical process is employed using diluted acid and alkali for demineralization and deproteinisation, respectively. The general process flow is presented in the flow diagram.

Shell waste (Wet/dry)

↓

Demineralization

↓

Washing to near neutral pH

↓

Deproteinization (Bleaching is optional)

↓

Washing to near neutral pH

↓

Drying

↓

Chitin flakes

↓

Packing

Major unit operations in the chitin process

1. **Raw materials**

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

2. **Demineralization**

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

3. **Deproteinisation**

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

4. **Washing**

   Use of water in chitin and chitosan production is enormous. In between the demineralization and deproteinisation process many number of washing cycle is required to achieve the near neutral pH. The requirement of water depends on the initial quality of water indirectly the source of water. More alkaline water like bore-well is required in high quantity. Seawater

also can be used for washing the demineralized and deproteinised shell. Washing can also be performed after neutralization. However, one should take care to remove the salt formed by giving wash using fresh water to keep the residual mineral in the chitin and chitosan low

5. **Drying of chitin**

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

7.0 Chitin derivatives

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

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

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Fish Processing Waste: A Valuable Raw Material for Meal, Silage, Foliar Spray and Animal Feed

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Fish is a highly perishable food material because of its moisture and nutrient contents. It is undoubtedly one of the most nutritious of foods available for human consumption. Fish flesh on an average contains 15-20 percent protein. Processing of fish leads to enormous amounts of waste. It is estimated that fish processing waste after filleting accounts for approximately 75% of the total fish weight. The total fish weigh around 30% remains as waste in the form of skins, and bones during the preparation of fish sticks fish fillets and other products. This secondary row material is an important for the preparation of high-value products including protein foods. The important of secondary row materials helps to destroy harmful environmental aspects and improve quality in the fish processing industry. Fish processing generates solid wastes that can be as high as 50-80% of the original raw material. Skin and bone are sources of high collagen content. An important waste reduction strategy for the industry is the recovery of marketable byproducts from fish wastes. Hydrolyzed fish wastes can be used for fish or pig meal as well as fertilizer components. The three most common methods for utilization of aquatic waste (either from aquaculture or wild stock) are the manufacture of fishmeal, fish oil and healthy feed, the production of silage and the use of waste in the manufacture of organic fertilizer. The utilization of by-products is an important cleaner production opportunity for the industry, as it can potentially generate additional revenue as well as reduce disposal costs for these materials. The transportation of fish residues and offal without the use of water is an important factor for the effective collection and utilization of these by-products. Another important waste which can be used for industrial purpose is prawn shell waste and crab shell waste.

1. Fish meal

Fish meal is solid product which is obtained by grinding the fish and fish byproducts and removal of water and all/ some oil (Ruiter, 1995). Fish meal is generally sold as a powder, and is used mostly in compound foods for poultry, pigs and farmed fish; it is far too valuable to be used as a fertilizer. Fish meal is regarded as highly concentrated nutritious supplement in feeds which contains high quality proteins, vitamin-B, minerals, etc.

1.1 Raw material used for production of fish meal

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

1.2 Production of fish meal

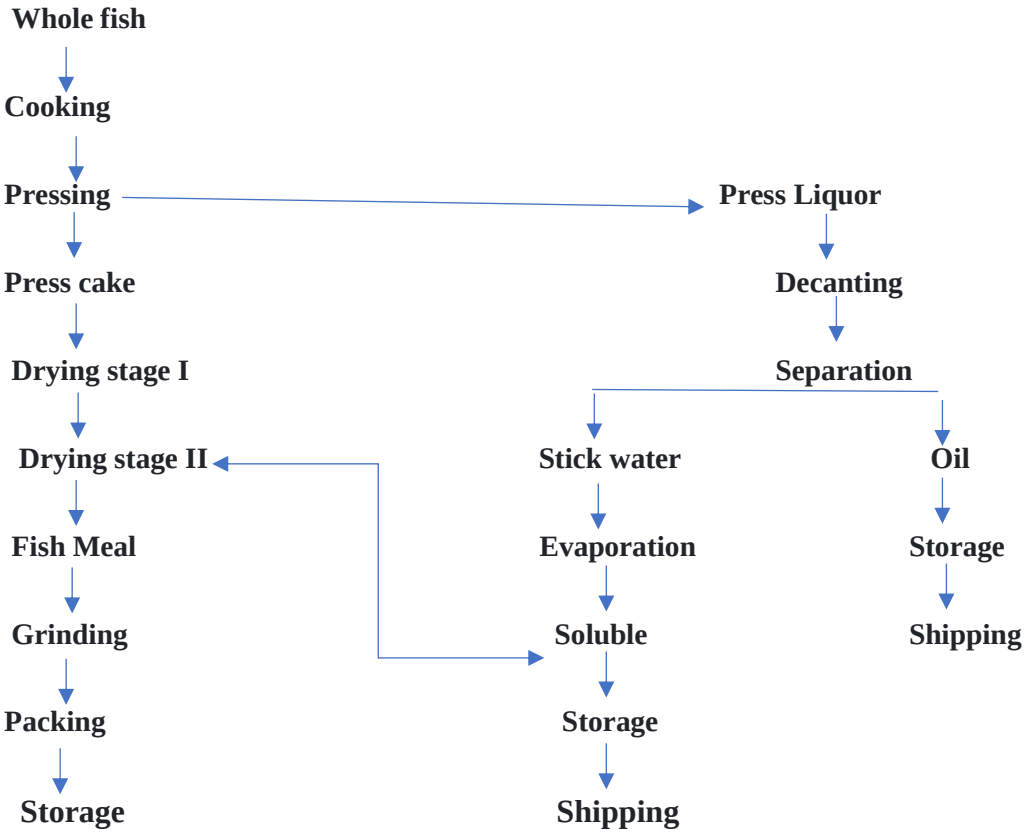
Methods of production of fish meal include; i) Dry rendering ii) Wet rendering

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

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

1. **Cooking**: after grinding, the material is cooked at a temperature of 100°C for 20 minutes in indirect steam. This process stops microbiological and enzymatic activity in the product and helps to separate the oil.
2. **Pressing**: In this process, mechanical pressing is done to separate the material into two types of phases. The liquid phase and the solid phase.
3. **Decanting**: In this stage, the liquid phase is decanted to recover more solid products and add them to the solid phase.
4. **Centrifugation**: In this procedure, the liquid phase is centrifuged. As a result, oil and water will be obtained.
5. **Evaporation**: the evaporation is done in the “tailwater” which is excess liquid, it is intended to reduce the volume of the product to concentrate it better and obtain solids.
6. **Mixing**: The solids remaining from centrifugation are mixed with the solid cake obtained from pressing until a paste is obtained.
7. **Drying**: Drying extracts more water from this mixture until the moisture content is reduced to 5-10%. This prevents bacteria growth and reduces chemical reactions.

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



Process flow diagram of fish meal and fish oil production

1.3 Chemical composition of fish meal

- In general, chemical components in fish meal are protein, fat, ash and moisture which are 50-70%, 5-10%, 12-33% and 6-10% respectively.
- Proteins in fish meal are rich in all essential amino acids which are not synthesized in body and need to be supplied from the diet. All essential amino acids present in fish meal makes it highly nutritive. Fish meal contains lysine in rich quantities which is deficient in cereals and legumes.
- Fish meal supplies vitamins such as riboflavin, niacin, pantothenic acid, choline, Vitamin B12 in addition to fat soluble vitamins such as Vitamin A and D. oil present in fish meal contributes to energy for fish and other animals.
- Average values of vitamins in fish meal are riboflavin – 7.3 mg/100 g of fish meal, niacin 126 mg/100 g of fish meal, pantothenic acid - 30.60 mg/100 g of fish meal,

Vitamin B12 – 0.25 mg/100g of fish meal, pyridoxine – 5.7 mg/100 g and choline – 4000 mg/100 g of fish meal. Fish meal also contains a significant quantity of Vitamin D due to residual oil in fish meal (5000 IU/ kg of fish meal).

- Inorganic constituents of fishmeal accounts for 11%. Indian fish meal exhibits higher proportions of phosphorus to calcium 1:1 against 1:2 proportions in other fish meals.
- Fish meal made from whole fish containing bones is rich in calcium, phosphorus and magnesium which are essential for growth. Mineral content in fish meal ranges from 25 to 30%. Mineral composition of fish meal involves zinc – 70 mg/kg, iodine – 70 mg/kg, iron – 250 mg/kg, copper – 7 mg/kg, manganese - 4 mg/kg, cobalt – 0.1 mg/kg
- Fish meal contains lower amounts of crude fibre in their diet which is good for proper digestion and absorption of nutrients in poultry and fish feeds.

1.4 ISI- Requirements for fish meal as poultry feed ingredient

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

ISI- REQUIREMENTS FOR FISH MEAL AS LIVESTOCK FEED INGREDIENT

Parameters	Grade I	Grade II
Moisture (%max)	10	10
Crude protein (% min)	60	50
Ammoniacal nitrogen (% max.)	0.5	0.5
Crude fat (% max.)	10	10
Acid insoluble ash (% max.)	3	3
Chloride (as NaCl) (% max.)	4	5

2. Fish Silage

Fish silage is a liquid product and it can be prepared from whole fish or fish waste by adding acid, enzymes, lactic acid producing bacteria or by naturally occurring enzymes in fish. Fish silage is rich in protein and aminoacids and it can be used as protein source for animal feeding. Production cost for fish silage is very cheap, cost effective and eco-friendly. Fish silage preparation usually depends on locally available raw materials and conditions (Hasan, 2003). Depending on the process employed, fish silage can be categorized into two methods, viz. acid silage and bio-fermented silage. Acid silage is produced by mixing fish waste with organic acid (formic acid, acetic acid, propionic acid), inorganic (sulphuric acid, hydrochloric acid) and or a mixture of both organic and inorganic acid. In case of bio-fermented silage, fermentation process is carried out by lactic acid bacteria (LAB) which are already present in a fish mass or added externally.

2.1 Fish silage preparation by Acid method

In this method, whole fish or fish waste are pulverized in mechanical mincer and then required quantity of acid is added and mixed well ensuring a pH of 4 for the acid silage prepared by using organic acid. Then it can be stored in containing with occasional stirring. During this process, the biomass gets liquefied by endogenous enzymes present in fish mass with the aid of added acid. Fish silage, a liquid product will be ready between 15-20 days. At 25^0C, the process takes two days for liquefaction, where as at 15^0C , it takes 5-10days. Moreover, if temperature $>40^0$C enzymes may get deactivated. Hence, ensilation process under higher temperature should be avoided. The degree of liquefaction in silage depends on the nature of raw material. The liquefied silage gets separate into 3-4 layers; an oily layer at top, liquefied protein at middle layer and undigested protein and sludge at the bottom. The incomplete protein digestion may depends on nature of raw material, pH, temperature and duration of ensilage. Moreover, all the layer can be separated and used as biofuels, agricultural fertilizers and feed for pet animals. Formic acid is most commonly used for silage production. Generally, 3% by weight of 98% formic acid is added to well pulverized fish waste. Silage prepared by using formic acid will have a shelf life of one year at room temperature in tropical countries.

2.2 Fish silage preparation by Fermentation

In this process, fermentation process is aided by addition of lactic acid bacteria and jaggery source. Since, the natural lactic acid bacteria in fish is limited, an external inoculum of lactic acid bacteria is needed. Molasses, a fermentable jaggery source are mostly used in fish ensilage

due to its low cost easy availability. The ratio of fish and molasses at 100: 10 will give stable silages with typical acid smell. The presence of sugar (jaggery), ensilation process gets initiate and prevents immediate deamination of amino acid by bacteria. Later, fermentation process dominated by lactic acid bacteria which results in pH reduction and it inhibits or reduces spoilage bacteria. The most commonly used lactic acid bacteria strains are *L. plantarum*, *L.acidophilus, Pedaucoccus halophilus* and *P.acidilactici*. The production of fermented ensilage depends on the in-situ production of lactic acid bacteria by added lactic acid bacteria to the fish or fish waste with a fermentable jaggery source. The concentration of lactobacillus used for fermentation is 10^6-10^8 cfu /ml.

2.3 Utilization of Fish Silage

2.3.1 Feed

Fish Silage are used for animal feeding, like powder fish silage is used to feed cattle, milk cow, swine, duck, sheep, mink and many other terrestrial animals (Rahmi et al., 2008, Al-Abri et al., 2014, Anuraj et al., 2014). Pigs resulting in higher growth rates improved health and reduced mortality. Fish silage rich in protein and it can be used as a protein source for broiler chicks alternative to fish meal to get increased weight and feed conversion ratio (Kjos et al., 2000). In many countries, it is used as bird feed (Arruda et al., 2007). It is also used as a feed supplement in aquaculture to convert nutrients into flesh. It has been reported that fish silage powder was found to give better growth than a fish meal in carp (Djajasewaka et al., 1986). Fish silage can be used directly as feed for pigs for improving higher growth rate, reduce the mortality and improving heath of animals. Fish silage can also mixed with other ingredients such as grains and dry flour for livestock feed. Since, fish silage contained hydrolysed protein it can be used as protein source by replacing fish meal at the level of 5-15% in fish feed preparation. It has been reported that inclusion of silage in pellet feed showed stronger, more resistant and reduce the waste during transportation and feeding.

2.3.2 Fertilizer

Fish silage is considered as potential source of Nitrogen, Phosphorus, Potassium, Calcium, magnesium and found to have application as a fertilizer. The quantity of nutrient present in fish silage differ depends on quality of raw material used and percentage of bones and fins. Moreover, adding 5-10 % liquid silage will meet the trace element required for plants.

2.3.3 Foliar spray

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

3. Feed from fish processing discards

Feed is considered as the major expense in fish farming, accounting for about 50– 60% of the total variable costs. Preparation of feed for aquaculture and poultry is an important option for utilization of general, unsorted waste from industry as well as fish markets. There is a growing demand for pellet feeds, due to the increase in aquaculture activity. Feed is also a major input affecting water quality and subsequently effluent quality in culture ponds. Fish feed management includes several factors viz. choosing the right feed, using a correct feeding method, calculating the feeding cost and ensuring the cost effectiveness of fish farm. Currently, aquaculture accounts for 40.33% of the world's fish production. Fish frames and other discards contain significant amounts of muscle proteins. They have a better balance of the dietary essential amino acids compared to all other animal protein sources. About, 25% of the protein requirement for feed is met from fish waste. The proximate composition and characteristics of many processing wastes suggest that it can be converted directly into feed. Most of these protein sources can be converted to fish flesh, which in turn provides quality protein for man. Utilization of these wastes can be direct or indirect. In direct utilization, either the wastes can be used as such as in the case of meals; cakes etc. or it can be used with some simple processes like fermentation, silage preparation etc. In indirect utilization, the wastes can be utilized as a substrate for the growth of single cell proteins for example, and these secondary products can be included in feed with or without primary substrate. Fish waste can be macerated into paste and prepared at farm site as meal and used for feed. Alternatively, fish waste may be initially converted to meal or silage, which later on can be made into feed after compounding with other essential nutrients like carbohydrate, fat, trace minerals and vitamins.

3.1 Quality of animal feed

Apart from nutritional composition, the quality of animal feed may be expressed in terms of physical quality and microbial quality. Physical evaluation is easy but tough in nature. One must be highly trained to identify the changes in the nature of the raw materials/ feeds. This

primarily involves parameters such as such as bulk density, colour, odour, hardness (force at rupture), durability, pellet size and water stability. Handling practices followed presently for fish processing waste are not adequate and hence may harbour a number of microbial hazards including lethal toxins and metabolites. Salmonella is a major bacterial hazard in animal feed. E. coli also has been detected in animal feeds. Similarly, the contamination of foods and animal feeds with mycotoxins is a worldwide problem. Mycotoxins are fungal secondary metabolites that have been associated with severe toxic effects to vertebrates produced by many important phytopathogenic and food spoilage fungi including Aspergillus, Penicillium, Fusarium, and Alternaria species.

4.0 Conclusion

Fish processing waste from seafood Industry and retail market leads to major issue in waste disposal and environment pollution. The most important products prepared from the fish waste are fish protein hydrolysis, fish collagen/Gelatin, antioxidants, fish sauce, biogas, and biodiesel. Most of the byproducts prepared from fish waste are used for the pharmaceutical purpose or for food purposes. Apart from extraction of biomolecules from fish waste, it can also be converted to fish silage to preserve the bioavailability of nutrient in fishery waste and reduce the environmental pollution.

Marine Nutraceuticals from Fishery Waste

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

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

1. Options and opportunities

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

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

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

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

due to the unique combination or high proportions of certain amino acids such as arginine and taurine with low levels of branched-chain amino acids found in fish meat.

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

Collagen peptide from fish scale and Nutritional mix formulated by CIFT

***1.3 Chitin*:** The shrimp processing industry in India churns out more than 2 lakh tones of head and shell waste per annum, which can be economically converted to chitin and its derivatives. Chitin is the most abundant polymer next to cellulose. It is a linear polymer of N acetyl-D-glucosamine. Glucosamine hydrochloride can be produced from chitin by hydrolysis. Glucosamine hydrochloride and sulphate are at present marketed as food supplement for the treatment of osteoarthritis. It also possesses other beneficial actions in wound healing and skin moisturization. The deacetylated chitin is known as chitosan. Chitin and chitosan have various applications in agriculture such as in germination of seeds and enhanced protection against pathogenic organisms in plants and suppress them in soil to induce chitinase activity and protenase inhibition, antivirus activity, in micro encapsulation fertilizers and insecticides. The delivery of drugs and the interactions with living tissues seem to be the major topics of current research on chitosan. Other areas of interest are the antimicrobial action, nerve regeneration, cartilage and bone regeneration, skin and bone substitutes, oral delivery for wound healing etc. Carboxy methylation of chitosan imparts water-solubility to chitosan. ICAR-CIFT has recently standardised the methodology for production of chitin, glucosamine hydrochloride, chitosan and carboxymethyl chitosan. Similarly, collagen-chitosan film from fish waste, developed by CIFT has wide applications in wound dressing and dental surgery. The antioxidant chitosan derivative developed by CIFT recently was found to be useful in microencapsulating vitamins and ß carotene, so as to give a novel delivery system. Similarly, a biocompatible and biodegradable wound healing formulation, composed of microencapsulated curcumin and hydrogel composite (Succinyl chitosan-fish collagen-poly ethylene glycol) developed at ICAR-CIFT, showed significantly enhanced rate of collagen deposition and hydroxyproline content in wound tissue on 14th day of post wounding as compared to control and standard. Apart from that, free radical mediated grafting of gallic acid, ferulic acid, vanillic acid and coumaric acid onto chitosan were optimised. All the derivatives showed good antioxidant and antimicrobial activities.

***1.4 Fish calcium*:** In marine ecosystem, there is a large amount of calcium, mainly in the form of calcium carbonate and calcium phosphate, distributed as skeletal elements of teleosts, exoskeletal elements of molluscs or as coral deposits. Every year a considerable amount of total fish catch is discarded as processing left overs and these include trimmings, fins, frames, heads, skin and viscera The bone fraction, which comprises approximately 15-20% of the total body weight of fish has high calcium content. Calcium and phosphorus comprise about 2% (20 g/kg dry weight) of the whole fish. Generally, fatty fish have lower ash levels compared to lean

species. The filleting wastes of tuna and other bigger fishes are very good sources for calcium when the quantity of calcium is concerned. Also, the bone structure differs between species since a large number of teleosts have acellular bone (bone without enclosed osteocytes). Cellular bones are confined to only a few fish groups, e.g. Salmonidae. The higher surface to volume ratio in acellular fish bone is likely to increase the calcium availability compared to cellular bone. The ash content is highest in lean fish species with acellular bones. Apart from that exoskeleton of mollusks and coral deposits are excellent source of calcium. However, the calcium forms these deposits are mainly in the form of calcium carbonate. Central Institute of Fisheries Technology, Cochin has optimised the process to extract from fish bone which is mainly treated as processing discards during filleting operation of larger fishes, viz tuna, carps etc. The calcium powder was supplemented with vitamin D which is known to enhance absorption and bioavailability of calcium in the body. *In vivo* studies conducted at CIFT in albino rats have shown that fish calcium powder supplemented with vitamin D has improved the absorption and bioavailability.

Calcium extracted from Tuna bone

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

of the fish bone is composed of inorganic substances. Almost all these inorganic substances are hydroxyapatite composed of calcium, phosphorous, oxygen and hydrogen.

1.6 Squalene: Squalene is a highly unsaturated hydrocarbon present in the liver oil of certain species of deep sea sharks mainly Centrophorus and Squalidae spp. The liver oil of these species contain high percentage of squalene (90%) which can be isolated and purified and can be used as a dietary supplement. It belongs to a class of antioxidant molecules called isoprenoids. Squalene is found to be a proficient chemo preventive agent against lung metastasis in mice bearing lung carcinoma. Squalene revives damaged body cells and aids to revitalize cell generation. Its chief attribute is the protection of cells from oxidation reactions. Squalene assists to clean, purify, and detoxify the blood from toxins, facilitating systemic circulation. It purifies the gastrointestinal tract and kidneys, causes better bowel movement and urination. Squlalene helps in regulating the female menstrual cycle and also improves irregular and abnormal cycles.

1.7 Taurine: Taurine is a sulfur-containing non-protein amino acid (2-aminoethanesulfonic acid),with multiple functions like neurotransmission, cell volume regulation, stabilization of cellmembranes and in the transport of ions such as calcium, sodium, potassium and magnesium.Taurine is one of the most abundant amino acids in the brain, retina, muscle tissue, and organsthroughout the body, and taurine deficiency is associated with cardiomyopathy, retinal andtapetum degeneration, renal dysfunction, immune deficiency, muscle atrophy, developmental abnormalities, premature aging, and impaired reproduction. It can be synthesized frommethionine and cysteine with the help of vit B6. The osmoregulatory role of taurine in facilitating the passage of sodium, potassium, calcium and magnesium ions into and out of cells, thereby stabilizing the structural and functional integrity of cell membranes was

well discussed in earlier reports. It is involved in detoxification of xenobiotics and also essentially required for efficient fat absorption and solubilisation. Taurine has a protective effect on the tissue damagethat results from oxygen free radicals in mercury induced toxicity. It plays a crucial role in prenatal and infant development. Epidemiological studies have shown that increased taurine intake is associated with diminished risk of hypertension. The deficiency of taurine does not impose immediate health issues, however long-term deprivation can affect a multitude of metabolic pathways. It is a key ingredient of bile and has a major role in the maintenance of normal gastrointestinal development and functions. Taurine is found in greater concentrationsin all animal products. Meat, breast milk, dairy products, and fish are good sources of taurine.Shell fish contain higher concentration of taurine compared to that of fin fish. Zhao et al. (1998)determined the taurine concentration of a variety of common marine fish species and reportedthe highest content in crustacean and molluscs, ranging from 300-800 mg per 100 g meat. Apartfrom that red algae is considered as a good edible source of taurine. Eventhough, the cellular and biochemical mechanisms mediating the actions of taurine are not fully revealed, mounting evidences suggest that taurine might be a key functional ingredient for use as a nutritional supplement to protect against oxidative stress, neurodegenerative diseases, atherosclerosis and hypertension.

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

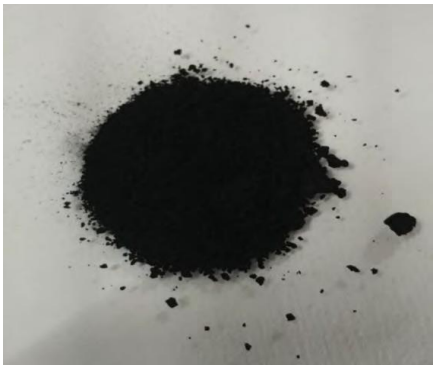
has a range of biological properties, although it has not yet been considered for therapeutic purposes. Marine heparin extracted from shrimp and sea squirt has proven anti-inflammatory properties.

1.9 Pigments*:* Astaxanthin, fucaxanthin, melanin etc. from different fish resources are found to have a variety of bioactive properties. The filleting discards of salmonids and the shell wastesof crustaceans contain significant amounts of carotenoid pigments such as astaxanthin and canthaxanthin. The protective role of carotenoids against the oxidative modification of LDL cholesterol could be explored by incorporating in health drinks. Carotenoids are also highly sought after as natural food colours. Cephalopod ink is another less tapped reservoir of a rangeof bioactives having therapeutic and curative values. It is an intermixture of black pigment melanin, glycosaminoglycans, proteins, lipids, and various minerals. Cephalopod ink has been reported to have anti-radiation activity, antitumor activity, immunomodulatory activity, procoagulant function and so on. The pigment melanin can be used both as a natural colorant as well as antioxidant, in addition to a number of other therapeutic and prophylactic propertiesincluding anticancer, antihypertensive, Anti IDA etc.

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

The basic structure of melanin comprises of covalently linked indole structure (Takaya and others 1994). Melanin performs a number of biological functions in the body, the main function being to protect the organism from harmful agents such as ultraviolet (UV) radiation; melanin is capable of dissipating over 99% of absorbed UV light. Besides, in the biological system, melanin plays a vital role in providing mechanical strength and protecting proteins from degradation. Numerous reports published in last thirty years reveal the therapeutic, prophylactic and curative value of cephalopod ink. Several researchers confirmed theeffect of

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



Melanin from cuttlefish ink

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

2.0 Challenges and way backwards

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

1. Consumer awareness and education is one such challenge. Without consumer acceptance offood waste reduction approaches, no sustainable eco-friendly food waste utilisation and management strategy can succeed. This demands proper extension efforts from the researchand extension organizations.
2. Seafood sector is a poorly organised sector. Highly scattered nature of seafood processing operations (across domestic market and processing acilities) poses problems in collection and processing.
3. Seafoods are highly perishable in nature owing to its unique richness in terms of protein, peptides, enzymes and microbial flora. This quite often leads to the mass resistance from public in starting up a business venture in the vicinity.
4. Inappropriate cold chain management from the source of generation to the point of conversion as the processors are least interested to invest further on discards

5. There is no baseline data on the availability and economics of production collected over thepast years, which poses uncertainty about economics and market demand of secondary products
6. Lack of clear legal classification of secondary products in the international market is yet another major challenge to the investors
7. Lack of unified protocols for quality assurance (such as HACCP) for secondary products leads to frequent rejections from the buyers.

Suggested Readings:

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Seaweed and its Nutritional Significance

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1.0 Introduction

Seaweeds serve as the primary source of biomass for the phycocolloid industry, producing agar, carrageenan, fucoidan, and alginates used in food and pharmaceutical applications. Additionally, these novel marine plants are globally traded as ready-to-eat food and functional food products, commanding a premium price. Seaweeds are an untapped source of health-promoting bio-molecules, including lipids, carotenoids, peptides, sulphated carbohydrates, dietary fibers, essential amino acids, vitamins, and trace minerals.

In addition to their therapeutic benefits, seaweeds are becoming increasingly valuable for their ability to modify textures in food. They can act as stabilizers, enhancers, modulators of viscosity, and gelling agents in a variety of food products. By incorporating seafood and seaweeds as "Green" additives in dishes made from meat, dairy, fish, vegetables, fruits, and other ingredients, they can boost the functionality of the food and improve its health-promoting properties. Furthermore, seaweeds are now being viewed as a potential source of vegan protein.

As the general public becomes more aware of the correlation between diet and overall health, the popularity of marine-sourced functional foods and nutraceuticals is steadily increasing (Granato et al., 2020). Among these, seaweeds are garnering attention as a potential food of the future, as they offer a promising source of unique molecules such as polyphenols, secondary metabolites, carotenoids, peptides, and sulphated carbohydrates that possess nutraceutical properties (Lafarga et al., 2020).

Seaweeds have a rich history of use as human food, both fresh and processed. They can be classified into three categories: red algae (Rhodophyta), brown algae (Phaeophyta), and green algae (Chlorophyta). In India, 770 different species of seaweed have been reported, with 184 species of green, 166 species of brown, and 420 species of red (D. Sahoo, 2010). While most seaweed in India is used as a raw material for the domestic hydrocolloids industry with a small portion being exported, there is significant potential for commercial cultivation of Indian seaweed for high-value food, functional food, and nutraceutical applications.

2.0 Biochemical composition and bioactive compounds from seaweed

Seaweeds are unique sources of bioactive phytochemicals. These marine plants are alternate rich source of protein with all essential amino acids. The protein content ranges from10–40% (w/w) dry weight basis and varies depending on the season and species, with the highest content found in red seaweed (Rawiwan et al. 2022). Taurine, alanine, amino butyric acid, ornithine, citrulline, and hydroxyproline are major free amino acids, found in appreciable quantities in seaweeds. Highest quantities of taurine have been reported from red seaweeds. Certain green seaweed species like Ulva, Caulerpa contains high level of arginine and glycine (Fouda et al. 2019).

Seaweeds are novel source of dietary fibre with prebiotic and antioxidant activity such as Fucoidan, Ulvan, and Galactans (Praveen et al. 2019). A serving of 8 g seaweed can provide around 12.5% of daily fibre needs and may exert several health promoting effects including anticoagulant, anti-inflammatory, antioxidant, anticarcinogenic, and antiviral activities (Huang et al. 2022). Cell wall of seaweed is mainly comprised of polysaccharides accounting for approximately 50% of the dry weight, which vary in the biochemical composition with species and environmental factors. Besides cellulose and hemicellulose, seaweed cell wall contains on an average 40% unique sulfated polysaccharides. These sulfated polysaccharides have different promising biological activities and finds application in food, nutraceutical, cosmetics, and pharmaceuticals. Among sulfated polysaccharides from brown seaweeds, fucoidan is most well studied for its range of bioactivities. The predominant sugar monomer in Fucoidans is always l-fucose over other sugar monomers such as galactose, mannose, glucose, and uronic acids. However sulfated galactofucans may have equal content of l-fucose and galactose. The position of the sulfation in the pyranose ring and branching of polysaccharide chain is responsible for certain bioactivity and vary widely from species to species. Laminaran is another type of prominent sulfated polysaccharide from brown seaweed Laminaria spp. Red seaweeds Gracilaria spp., Kappaphycus alvarezii, and Porphyra spp. are source of unique sulfated galactans such as agarans, carrageenans, and porphyrans respectively. The α-galactopyranose repeating unit in agarans have an L-configuration, while the carrageenans have D-configuration. Porphyran is a type of agaran with a linear structure consisting of alternating 3-linked residues of β-d-galactose and 4-linked residues of α-l-galactose-6-sulfate or 3,6-anhydro-α-l-galactose. Green seaweeds are source of bioactive sulfated polysaccharides such as ulvans, mannans, and xylans. Ulvans are most well studied sulfated polysaccharide from green seaweeds, consisting of sulfated glucuronoxylomannan

units with d-xylose, d-glucose, d-glucuronic acid, l-iduronic acid, with sulfation commonly found at the C-3 or C-2 positions (Olegovna et al. 2022). An infographic of different types of sulfated polysaccharide from different classes of seaweeds along with their bioactivity has been presented in Fig. 1.

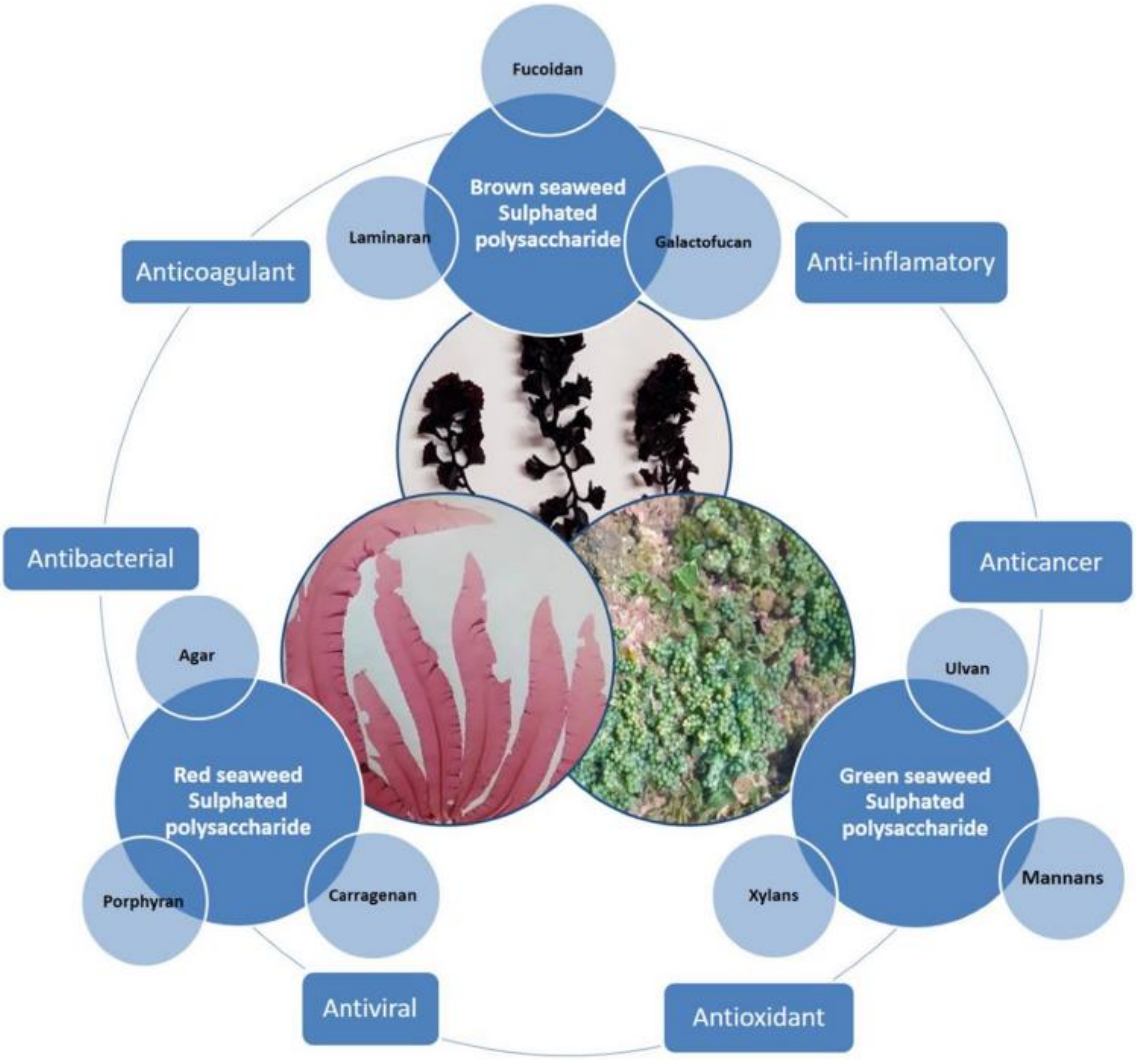

Fig. 1 Bioactive compounds from seaweeds (Reproduced with permission from Pandey et al., 2023)

3.0 Green chemistry approach for extraction of bioactives from seaweed biomass

The extraction of bioactive compounds in an economic and nature-friendly approach is a big dilemma. Traditional methods comprise Soxhlet extraction, hydro distillation, maceration, decoction, infusion, pressing, percolation, *etc.* Although they have been used since ages, they are very often time-consuming and require relatively large quantities of polluting solvents,

which can lead to sample contamination, losses due to volatilization during concentration steps, and environmental pollution from solvent waste **(**Zhao et al, 2009**).** Most of the commonly used organic solvents are harmful to the living system on earth. Because of the known hazards associated with many solvents, the transition to green alternatives has already begun to be popular in the laboratories and caught the attention of industrial project managers. Green chemistry aims to minimize the environmental impact of the chemical industry. This includes shifting away from oil to renewable sources where possible. Green chemistry also prioritizes safety, improving energy efficiency and, most importantly, minimizing (and ideally) eliminating toxic waste from the very beginning **(**Bissember, 2017**).** The principles of green chemistry are depicted in the following Figure 2. More recently, multiple green chemistry process is being used sequentially in a bio-refinery approach that produce several bioactive material in different steps from the same starting biomass.

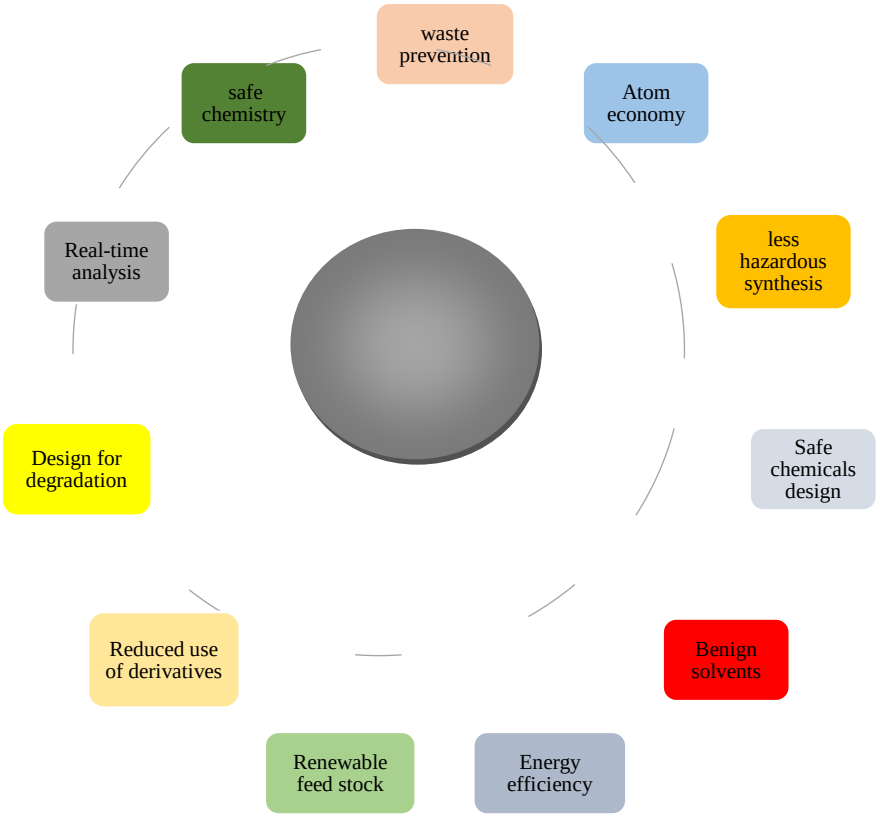

Fig. 2. Principles of Green Chemistry

There are several recent research that highlights the use of green chemistry extraction technologies for extraction of bioactives from seaweed biomass. A summary of major green chemistry extraction techniques used for seaweed extraction is presented in Table 1.

Table 1. Green extraction techniques used for extraction of bioactive compounds from seaweed

S.No.	Sample	Extraction aid adopted	Reference
1	*Eisenia bicyclis*	Pressurized liquid method	Shang et al. 2011
2	*Phaeodactylum tricornutum*	Pressurized liquid extraction and microwave assisted extraction	Gilbert et al. 2017
3	*Laminaria saccharina, Sargassum muticum, Fucus distichus*	Dimethyl Sulfoxide	Seely et al. 1972
4	*Phaeodactylum tricornutum*	Maceration, Ultrasound-assisted extraction, Soxhlet extraction, and Pressurized liquid extraction	Kim et al. 2012
5	*Eisenia bicyclis*	Pressurized liquid method	Shang et al. 2011
6	*Phaeodactylum tricornutum*	Ethyl acetate, Pressurized liquid method, Supercritical Fluid Extraction	Camargo et al. 2017
7	*Cylindrotheca closterium*	Microwave assisted Extraction	Pasquet et al. 2011
8	*Undaria pinnatifida*	Supercritical carbon dioxide Extraction	Roh et al. 2008
9	*Laminaria japonica, Undaria pinnatifida, Sargassum fusiforme*	Microwave assisted extraction	Xiao et al. 2012
10	*Isochrysis galbana*	Ethanol extraction	Kim et al. 2012
11	*Undaria pinnatifida*	Enzyme assisted extraction, Dimethyl ether and ethanol	Billakanti et al. 2013
12	*Saccharina japonica and Sargassum horneri*	Supercritical carbon dioxide Extraction	Sivagnanam 2015
13	*Sargassum muticum*	Supercritical carbon dioxide Extraction	Conde et al. 2015
14	*Undaria pinnatifida*	Supercritical carbon dioxide Extraction	Quitain et al. 2013
15	*Undaria pinnatifida*	Supercritical carbon dioxide Extraction and Subcritical Dimethyl ether	Goto et al. 2015
16	*Saccharina japonica*	Supercritical carbon dioxide Extraction	Saravana et al. 2017
17	*Undaria pinnatifida*	Dimethyl ether Extraction	Kanda et al. 2014

ICAR-CIFT, has established several green chemistry processes for extraction of bioactive compounds from seaweed in a biorefinery approach. Enzyme assisted extraction and supercritical carbon dioxide extraction in tandem, have been optimised for extraction of

fucoidan and fucoxanthin from brown seaweed. Both, fucoidan and fucoxanthin are generally considered as GRASS or novel food by international regulatory agencies. Both of these phytochemicals from seaweed are being commercially exploited as high value nutraceuticals all over the world. The ICAR-CIFT technology for extraction and formulation of these bioactives have been commercialized to domestic industry who are successfully producing and marketing the products. Food products incorporating these seaweed extracts have been developed by ICAR-CIFT. For example, fucoidan incorporated curd, fucoidan incorporated bakery products, seaweed dietary fibre incorporated ready to eat products. All of these products are successfully transferred to different entrepreneurs. Some of the seaweed-based products from ICAR-CIFT are depicted in Figure 3.

Fig.3 Seaweed incorporated functional food and nutraceutical products from ICAR-CIFT

4.0 Foodomics approaches of high throughput identification of bioactives in seaweed

Foodomics has been defined as a new discipline that studies the food and nutrition domains through the application of advanced omics technologies to improve consumer's well-being, health, and confidence. The research and interest around this new discipline has grown exponentially during the last decade. Still, our understanding of how diet affects health is limited to 150 key nutritional components that are tracked and catalogued by the United States Department of Agriculture and other national databases. These nutritional components represent only a small fraction of the more than 26,000 distinct, definable biochemicals present in our food—many of which have documented effects on health but remain unexplored systematically. A 2020 publication in "Nature Food" termed these unknown metabolites as

"Dark Matter of Food". The advances in high resolution mass spectrometry have made it possible to decode this dark matter and solve many issues of societal, health, and economic importance. The Foodomics approach has tremendous potential in high throughput identification several bioactive compounds in one go in seaweeds and can establish comprehensively the food value of the seaweed. In ICAR-CIFT, we are carrying out high throughput profiling of seaweed using high resolution mass spectrometry and chemoinformatics technology. This allows us to identify several bioactive compounds in one go and evaluate several seaweeds as potential source for those bioactives. For example, our study revealed that *Sargassum cinereum* is richer source of Fucoxanthin and isomers as compared to other brown seaweed species. Figure 4. Presents a comparative profile of fucoxanthin and isomers in several brown seaweed species.

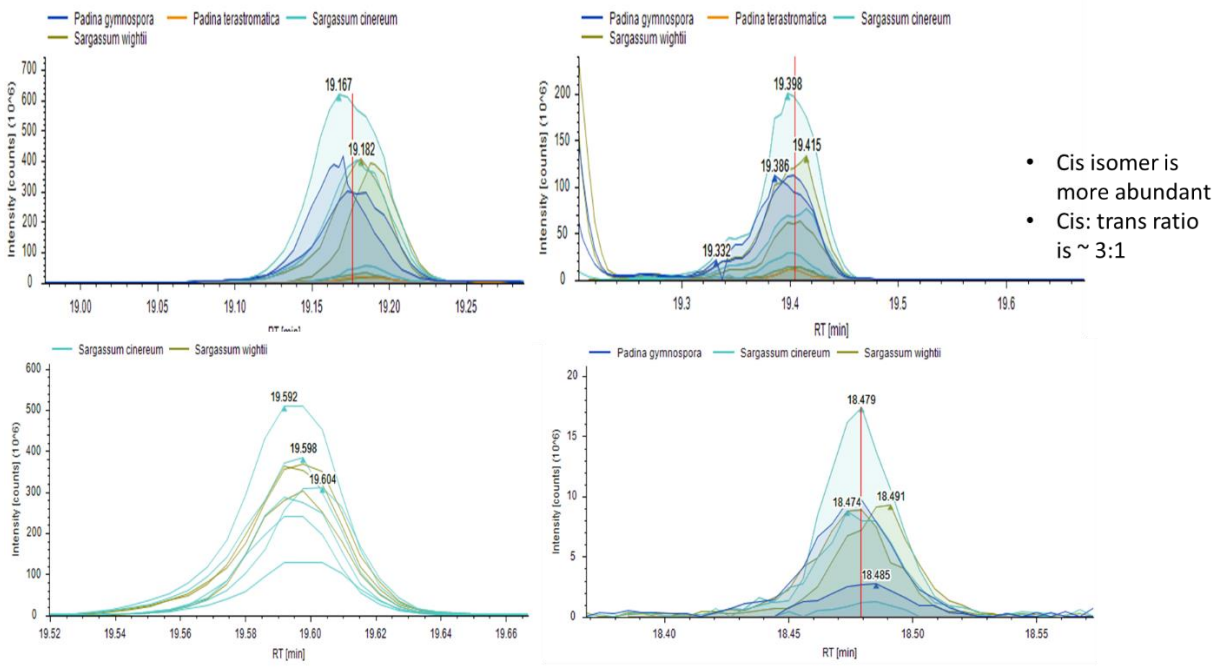

Fig. 4 Comparative phytochemical profile brown seaweeds in high resolution mass spectrometer

5.0 Quality issues and regulatory challenges in food use of Indian seaweed

For successful use of Indian seaweed as food, and functional food a quality system has to be established, same as any other food/novel food product. Unfortunately, the ecosystem for seafood quality control regime is not well developed at the moment. The seaweed raw materials used for food and feed purposes need to be tested for Iodine and Mercury. In 2006, the European Union (EU) for Scientific Committee on Food (SCF) established an upper limit of 600 µg/day for iodine intake for adults and 200 µg/day for children of 1-3 years of age. For

mercury in algae and prokaryotic organisms, a maximum residue level of 0.01 mg/kg is established according to Regulation (EC) No 396/2005. For arsenic, lead, cadmium, and mercury, the maximum levels in the feed are established under EU Directive 2002/32/EC of the European Parliament and the Council. As certain seaweed species are used as feed, the metal content of these species should also be investigated, both for animal health reasons and given the transfer of these metals to food products of animal origin. As per this EU directive 2002/32/EC Aldrin, Dieldrin, Toxaphene, Chlordane, DDT, Endosulfan, Endrin, Heptachlor, Hexachlorobenzene, and Hexachlorocyclohexane needs to be tested.

For polycyclic aromatic hydrocarbons and polychlorinated biphenyls such regulatory limits are not available. However, the presence of these organic pollutants is a possibility in seaweeds and should be monitored. In this case, a default regulatory limit of 0.01 ppm can be considered.

In India, as of now, there is no regulatory limit for heavy metals and persistent organic pollutants in seaweed for food supplement and feed purpose. The Food Safety and Standards (Contaminants, Toxins, and Residues) Regulations, 2011 mentions a regulatory limit for Mercury in non-specified food as 1 mg/kg and Methyl mercury in all food staff at 0.25 mg/kg. The same should be applied to seaweed-based food and supplements. More importantly, the Gazette of India Notification No. 465 on Food Safety and Standards (Health Supplements, Nutraceuticals, Food for Special Dietary Use, Food for Special Medical Purpose, Functional Food and Novel Food) Regulations, 2016 mentions only “Kelp” as an approved nutraceutical or supplement ingredient in India. No other edible Indian seaweeds are listed. This Gazette notification is being amended and may include Indian edible seaweed species as they have long history of use as food.

Regulatory limits for heavy metals have been mentioned in European Commission Regulation (EU) No 231/2012 of 9 March 2012 for high-value food additives from seaweed. Formaldehyde (50 mg/kg), Arsenic (3 mg/kg), Lead (2 mg/kg), Mercury (1 mg/kg), and Cadmium (1 mg/kg) should be monitored. E. Coli should be absent in 5 g, and Salmonella sp. Should be absent in 10 g. In India, the Food Safety and Standards (Food Products Standards and Food Additives) Regulation, 2011 mentions regulatory limits for Agar, Alginates, and Carrageenan. For Agar and Alginate, the Lead and Arsenic content should be no more than 5 and 3 mg/Kg respectively. For Carrageenan, regulatory limits of Cadmium (1.5 mg/Kg), Mercury (1 mg/Kg), Arsenic (3 mg/Kg), and Lead (5 mg/Kg) have been specified. E. Coli and Salmonella sp. should be absent.

6.0 Conclusion

Considering the rich biodiversity of seaweed, there is immense potential for the use of Indian seaweed as food, functional food, and nutraceuticals. However, there are many challenges and the quality regime of seaweed-based product to enable export in international markets is not so well developed. ICAR-CIFT has developed several technologies for value added products and nutraceuticals from seaweed. Five of the technologies have been transferred to industries and commercial production has started for four of them. ICAR-CIFT is the national reference laboratory of FSSAI for fish and fisheries products and will have important role to play for quality control of seaweed and seaweed products. The institute has pilot plant facility for process demonstration and have transferred the technology for solar dryers for hygienic drying of fish and fish products. However, a conducive policy environment needs to be formulated for achieving the true potential of seaweed value addition and processing in India.

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Encapsulation of Fish Based Bioactive Compounds

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Bioactive in food are physiologically active components that provide health benefits beyond their nutritional role. Bioactive ingredients include proteins, vitamins, minerals, lipids, antioxidants, phytochemicals and probiotic bacteria. These bioactives are very sensitive and their application in food is a great challenge to the industry without affecting their properties. Encapsulation technology has proven to be an excellent method to protect the sensitive food ingredients and to develop the novel foods formulations with improved properties. Encapsulation defined as a process of coating small particles of solids, liquids, or gaseous components, with protective coating material. In the food industry, the encapsulation process can be applied for a various purpose such as (i) to protect the core material from degradation and to reduce the evaporation rate of the core material to the surrounding environment; (ii) to modify the nature of the original material for easier handling; (iii) to release the core material slowly over time at the constant rate; iv) to prevent unwanted flavor or taste of the core material; v) to separate the components of the mixture that would react one another.

1.0 Overview of Encapsulation Technologies

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

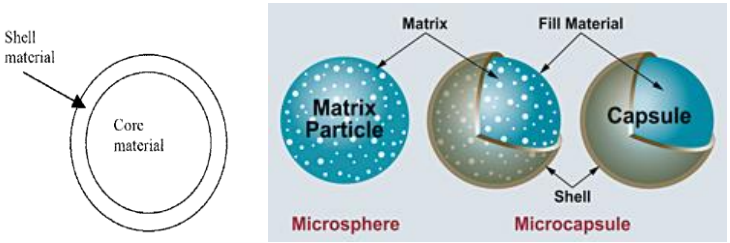

Fig. 1. Morphology of microcapsule

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

Nanoparticles can be prepared by two basic approaches: either by a ‘‘top-down’’ approach, in which nanoparticles are produced by means of physical processing of several materials, or alternatively by a ‘‘bottom-up’’ approach, in which nanoparticles are produced via self-assembly and self-organization of smaller molecules

Based on particle size they are categorized into i) Microcapsule: Particle size ranged from 0.2-5000 µm ii) Macrocapsules: Particle size larger than 5000 µm iii) Nano capsules/ nanoparticles/ nanospheres: Particle size smaller than 0.2 µm (200nm).

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

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

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

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

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

1.6 Liposome Entrapment: Major process involved are **i)** Microfluidization ii) Ultrasonication

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

2.0 Encapsulation of Fish Based Bio Active Compounds

A. Encapsulation of omega-3 fatty acids

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

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

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

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

B. Encapsulation of vitamins and minerals

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

Astaxanthin is a naturally occurring carotenoid pigment found in certain animals and plants. The main sources of astaxanthin are krill, algae, red trout, shrimp, crab and lobster. Astaxanthin possess various health benefits and is an important candidate for nutraceutical applications.

Methods and wall material used for encapsulation of vitamins and minerals

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

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

C. Encapsulation of calcium

Fish bone, is an important source of calcium in the form of dicalcium phosphate with high bio-availability. By encapsulating the calcium, provides possible to develop calcium enriched foods.

D. Encapsulation of enzymes

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

Methods and wall material used for encapsulation of enzymes

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

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

E. Encapsulation of protein hydrolysate and collagen peptide

Food protein hydrolysates and peptides are considered as a promising functional food ingredient. Peptide from fish scale, bone, skin exhibits various biological activities such as antioxidant, antihypertensive, anti-proliferative, anticoagulant, calcium binding, anti-obesity, anti-diabetic activities and postponement of age-related diseases. However, food application of peptides can be inhibited by their bitter taste, hygroscopicity and interaction with the food matrix. These problems can be solved by encapsulation.

Methods and wall material used for *encapsulation* of protein hydrolysate an peptide

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

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

3.0 Application of encapsulated bioactive ingredients in food and Pharmaceutical industry

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

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

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

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

4.0 Challenges and future prospects

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

Green technologies for isolation of marine bioactive compounds

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1.0 Introduction

Marine fauna and flora are reported to be richest repository of wider range of bioactive compounds. Polyunsaturated fatty acids, essential amino acids, unique proteins, vitamins, minerals, sulfated polysaccharides, pigments, etc., are present in marine organisms. It has been reported that marine bioactive compounds exhibit significant and biological properties contributing to their nutraceutical and pharmaceutical potential. By virtue of these properties, they are often reported to be safer alternatives to some of the synthetic drugs. Often marine bioactive compounds are reported to be extracted from various sources such as these compounds fish, shellfish, molluscs, crustaceans, echinoderms, seaweeds, microalgae and many other marine microbes. Further, there has been mounting evidence that the consumption of marine foods and biomolecules can reduce the incidence of many ailments. This coupled with the increased consumer awareness regarding the benefits of consumption of marine bioactive compounds has fostered its research. Accordingly, extraction of bioactive compounds is being attempted on a larger scale globally. Conventional solvent-based methods are often employed for extraction of bioactive compounds from marine sources. However, such methods are time consuming, require a longer duration and need further purification/concentration step. In addition to this, there is an increasing global concern on the use of organic solvents for extraction as they are not eco-friendly methods. Hence, innovative, sustainable and greener extraction technologies such as supercritical fluid extraction (SFE) were introduced as an alternative to conventional separation methods. SFE helps in the efficient extraction of thermolabile compounds as the process is often carried out at lower temperature. Apart from SFE, other greener technologies that are currently employed for extraction of marine bioactives include ultrasound-assisted extraction (UAE), microwave assisted extraction (MAE), eutectic solvents, deep eutectic solvents (DES), switchable solvents (SS), etc. Among all, SFE has garnered significant attention globally owing to its selectivity of extraction process.

2.0 Supercritical fluid extraction

Supercritical fluid extraction has emerged as one of the most feasible, economic, and environmentally friendly methods for extraction of commercially important bioactive

compounds from natural resources. Recently, a substantial amount of research has been carried out to establish the effectiveness of SFE in extracting biomolecules from marine sources/fish-processing discards. In general, supercritical fluid extraction can be defined as a green eco-friendly technology wherein a supercritical fluid (SCF) is used as solvent for extraction of natural biomolecules without affecting their bioactivity. Supercritical fluid can be any substance whose temperature and pressure should be above its critical point (CP) to form a homogenous phase possessing properties of both liquid and gas, commonly referred to as the mesophase. In simpler terms, supercritical fluid can be defined as that form of matter in which the liquid and gaseous phases are indistinguishable (Lekshmi et al., 2023). The properties of SCFs are listed below:

• Highly compressed gas having properties of both gas and liquids;

• Have low viscosity, high diffusivity and hence enhanced transport properties;

• Can fine tune the solvating properties, density, and solubility by changing the pressure/temperature accordingly.

The most commonly used solvent in SFE is carbon dioxide (CO_2) as its critical conditions (critical pressure, Pc – 74 bar, critical temperature, Tc – 31.1°C) are easily achievable at ambient conditions. Non-toxicity, safety, non-flammable, inexpensive, availability, inertness, high diffusion coefficient, lower density, increased mass transfer makes it as an excellent candidate for SFE. Furthermore, such lower temperatures make it favorable for extraction of many thermolabile compounds. Being non-polar in nature, supercritical CO_2 (SC-CO_2) can extract only non-polar compounds or substances of lower polarity. For this reason, certain solubility enhancers known as co-solvents/modifiers can be added to increase the polarity of SC-CO_2. Methanol, ethanol, chloroform, ethane, etc., are some of the co-solvents being used in SFE among others. Among the available co-solvents, ethanol is the most recommended as it is non-toxic, miscible in CO_2 and food-grade (Liza et al., 2010)

2.1 Supercritical Fluid Extraction- Instrumentation

The instrumentation required to perform a successful SFE is commercially available. The supercritical fluid extractor unit consists of fluid source (CO_2 cylinder), pumps, extraction chamber, heat exchangers, collection chamber/separator, restrictors. The process begins with a clean source of fluid, which in most cases is a high-pressure cylinder of CO_2. A pump is used to increase the pressure of the fluid above its critical pressure. The working extraction pressure is determined by the density required to dissolve the target analytes from the sample. The sample is contained in the extraction chamber, which is heated to the desired extraction temperature above the critical point. The pressurized fluid is brought to temperature by the

chamber and allowed to flow through the sample matrix to extract the analytes. After the sample, the analyte fluid flows to a restrictor, this controls the flow rate of the fluid. The restrictor maintains the high pressure of the fluid in the chamber. At the restrictor, the supercritical fluid loses its solvating strength as its pressure drops to atmosphere. After the restrictor, the analytes can be collected for analysis.

2.2 Advantages of SFE

SFE have several advantages over the conventional methods and some are listed below:

• **Lower viscosity and high diffusion coefficient attributes**, SCFs enhanced transport properties, facilitating efficient extraction;

• **Solvent strength of SCF** can be modified by changing the extraction pressure and temperature; • SFE leaves no chemical residue making the process sustainable;

• **Lower energy requirements**;

• **Highly efficient** process in terms of increasing yield and lower extraction times;

• **No degradative changes** to the bioactive compounds extracted using SFE

•**Environmental safety**: As carbon dioxide is easy to remove simply by reducing the pressure, the chances of having solvent residues in the final product is negligible only when ethanol is used as co-solvent making the process more environment friendly

•**Selectivity**: By changing the pressure and the temperature, the solvent strength of a supercritical fluid can be altered. For example, volatile oils can be extracted from a plant with low pressures (100 bar), whereas liquid extraction would also remove lipids. By SFE, lipids can be removed using pure CO_2 at higher pressures

•**Speed**: Being a diffusion-based process and with the enhanced transport properties associated with supercritical CO2, the whole process can be finished in 45 min – 6 h which is very less compared with the conventional extraction techniques.

•**Purity**: A supercritical fluid can be separated from an analyte by releasing pressure so that the product will be almost pure.

•**Recovery**: Recovery of analytes is simpler as compared to conventional techniques.

•Supercritical fluids are **cheap, inert and nontoxic**.

2.3 Efficiency in sample preparation

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

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

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

(3) In Super critical Fluid extraction, fresh fluid is continuously passes through the sample; therefore it can provide complete extraction

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

2.4 SFE for extraction of fish oil

The production of high-quality fish oil has assumed great significance in the recent past owing to its reported health benefits. Production of good quality fish oil depends to a greater extent on the type of raw material, quality of raw material, the type of extraction method and extraction conditions. Globally, there is an increasing trend to utilise the fish/seafood processing discards as raw material for production of high quality bioactive. It has been well documented that both fish and fish by-products can serve as excellent resources for extraction of omega-3 rich oil. SFE has evolved as one of the most feasible sustainable extraction methods for obtaining fish oil (Létisse *et al*., 2006). Rubio-Rodríguez *et al* (2012) have compared four different methods, such as cold extraction, wet reduction, enzymatic extraction and supercritical fluid extraction for obtaining oil from fish by-products. It was observed that among all methods, SFE can be a feasible method from the stability and safety point of view, as fish oil extracted by SFE had better oxidative stability and reduced arsenic content. Sahena *et al* (2010) has compared soxhlet extraction with SFE for obtaining oil from mackerel skin. It was observed that almost similar yield of fish oil was obtained using both the methods. Similarly, Sahena *et al* (2010) has analysed the fatty acid profile of oil extracted from various parts of Indian mackerel (*Rastrelliger kanagurta*) such as skin, flesh, viscera and head using various techniques of supercritical carbon dioxide (SC-CO_2), viz, continuous, cosolvent, soaking and pressure swing. All the extractions were carried out the same conditions (350 bar pressure, 60 °C temperature) and the efficiency of the process was compared with that of soxhlet. It was reported that among the different techniques employed, soaking and pressure

swing techniques were efficient in extracting major polyunsaturated fatty acids, EPA and DHA.

Generally, the extraction efficiency of SFE are reported to be affected by change of major factors such as pressure, temperature, time, co-solvents etc. (Plaza and Rodríguez-Meizoso, 2013). Several researchers have investigated the effect of these variables on the yield, quality and quantity of bioactives obtained. Létisse *et al* (2006) have optimised the extraction conditions for obtaining EPA and DHA rich oil from sardine and efficiency was compared with that of conventional solvent based extraction. It was reported that about 11% of EPA and 13% of DHA were extracted from sardine at the optimised extraction conditions (300 bar pressure, 75 °C temperature, 45 min time). Though the extraction yields were better with solvent based extraction, it was suggested that SFE can be more advantageous from a time and quality point of view.

3.0 Other emerging methods for extraction of marine biomolecules

Apart from the most commonly employed methods like SFE, there are other environment friendly extraction techniques such as ultrasound assisted extraction (UAE), microwave assisted extraction (MAE) etc. UAE has found wider applications in seaweeds for isolation of carotenoids like fucoxanthin and phenolic compounds such as phlorotannin, pigments such as phycoerythrin, phycocyanin, and biopolymers such as carrageenan, laminarin, alginate, etc. Apart from this, UAE are finding excellent applications in isolation of fish proteins. By employing microwave assisted extraction, a range of biomolecules such as chitin, chitosan, hydroxyapatite, fucoidan, etc. can be isolated. Other than this, there are other extraction techniques which employs ionic liquids, DES, switchable solvents as extraction medium. DES are extraction mixtures comprising of a hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD). Biodegradability, low toxicity, easiness in preparation, high thermal stability, low volatility, non-flammability, etc., makes DES one of the most efficient green extraction techniques (Xu et al., 2020). Das et al. (2016) have recently employed DES for extraction of kappa-carrageenan from red seaweed, *Kappaphycus alvarezii* and has reported that the hydrated DES were more effective in isolation.

4.0 Conclusions and future trends

Supercritical fluid extraction technology can offer attractive features for obtaining bioactive compounds and overcome many limitations that exist in other extraction methodologies. SFE allows the control of fluid density by changing its pressure and/or temperature thus providing

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

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Quality Control of Fishery Products

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Demand for seafood has consistently increased during recent years with fish protein being the major animal protein consumed in many parts of the world. According to the Food and Agriculture Organization, fresh seafood represents 40.5 % of the world's seafood production, while processed products (frozen, cured, canned, etc.) represent 45.9 %. To assure the quality of raw material used for processing, fish has to be treated carefully before and after harvest. The quality assessment of fish and other seafood is hampered by the immense variation between species and by the considerable variability between specimens of each species. The challenge, when assessing aquatic animals is that immediately after capture or harvest an alteration in intrinsic properties is initiated firstly by metabolic (autolytic) and secondly by microbiological processes, which last until the final state of spoilage is reached. Several analytical methods (chemical, physical, microbiological and sensory) can be used to determine spoilage depending on its state.

1.0 Freshness and quality of seafood

Freshness makes a major contribution to the quality of fish and of fishery products. For all kinds of products, freshness of the raw material used for processing is essential for the overall quality of the final product. It is impossible to process a high-quality product from raw material with poor freshness characteristics. Besides freshness, many other parameters also contribute to the complex quality of a seafood-based product such as the sensory properties (taste, odour, texture, appearance); the availability/disposability over a long time period; the safety; desired convenience characteristics; appealing packaging, colours and pictures; an attractive price; consistency, uniformity and conformity with standards and legislation; nutritional properties; ethical (stunning and slaughter); and ethnic (halal, kosher) constraints.

2.0 Indicators for freshness determination of fish

The properties, which wet fish in ice (fresh, unprocessed, not frozen) should have, when regarded as perfect fresh, can be considered as freshness indicators.

Rigor mortis: Fish regarded as fresh should be pre-rigor, in rigor or should just have passed rigor; it must be 'new', meaning that the time passed between harvesting or catch and marketing

(auction) and consumption or processing into final products (including freezing) should be as short as possible.

Sensory quality: The sensory assessment of a cooked sample of the edible part should give high scores for all attributes – appearance, flavour, odour, taste, colour and texture.

Volatiles: The concentrations of basic volatiles like trimethylamine (TMA), dimethylamine (DMA) or total volatile basic nitrogen (TVB-N) are low, and that of trimethylamine oxide (TMAO) is high. Fresh odours (of plants, cucumber and mushroom character) are high in concentration whereas spoilage odours (amines) are insignificant; typical fishy odour is lacking.

Physical methods: The pH is clearly below 7.0 and the impedance measured is high. The microstructure is intact. Mechanical properties of fresh fish are commonly measured post rigor. Elasticity decreases and flesh is softening.

Microorganisms: Only a few microorganisms are present on skin and gills, the muscle is sterile; the proportion and activity of specific spoilage bacteria in microbiological flora determines remaining shelf life (freshness).

ATP and ATP breakdown products: k-value should be low. k-value increases with time.

Proteins: Low proteolysis rate in proteins.

Lipids: The muscle is in a status, where lipid oxidation is inhibited.

3.0 Fish spoilage

Seafood deteriorates very quickly due to various spoilage mechanisms. Spoilage can be caused by the metabolic activity of microorganisms, endogenous enzymatic activity (such as autolysis and the enzymatic browning of crustaceans' shells) and by the chemical oxidation of lipids.

- Self-digestion by enzymes (Autolytic changes)
- Bacteria (Microbial changes)
- Oxidation & hydrolysis (Chemical changes)

Seafood flesh has a high amount of non-protein nitrogenous (NPN) compounds and a low acidity (pH > 6), which support the fast growth of microorganisms that are the main cause of spoilage. The growth and metabolic activity of the spoilage microorganisms, especially specific spoilage organisms (SSOs), result in the production of metabolites that affect the organoleptic properties of the product. Immediately following death, autolysis resulting from the action of endogenous enzymes, initially causes loss of the characteristic fresh odour and taste of fish and

then softens the flesh. The main changes that take place are initially the enzymatic degradation of adenosine triphosphate (ATP) and related products and subsequently the action of proteolytic enzymes. Enzymes are also responsible for colour changes. Chemical oxidation of lipids (oxidative rancidity) is one of the most important spoilage mechanisms, especially in fatty fish.

4.0 Quality assessment of seafood by chemical methods

The traditional methods, which have been using for evaluating the quality of seafood are as follows.

1. **TMA & TVB-N**: Formation of amines increases when spoilage microorganisms have entered the muscle tissue and microbial degradation of low-molecular substances and proteins starts. Depending on the fish species and on the storage temperature, it takes about 10 days before microbial action on muscle tissue leads to an increase of amines. From this point on, amine formation follows an exponential function and can be used as a measure for spoilage. TVB-N is still the most frequently used method for quality assessment of fish. Conway's microdiffusion method is the routine method used for checking the TMA and TVB-N limit.
2. **Biogenic amines/Histamine**: Histamine, putrescine, cadaverine, tyramine, tryptamine, β-phenylethylamine, spermine and spermidine are the most relevant biogenic amines in seafood. Quality assessment using biogenic amines content such as histamine, cadaverine, putrescine and agmatine was reported by many researchers. Histamine is determined by HPLC method/Spectroflurometric method.
3. **Indole**: Spectroflurometric/ Spectrophotometric method
4. **Hypoxanthine**: HPLC method/Enzyme immobilization technique
5. **K-value**: The extent of breakdown of ATP (adenosintriphosphate) and related products was expressed as K-value, defined as a percentage of the amount of inosin and hypoxanthin to the total amount of ATP-related compounds. HPLC method is used for K-value determination.
6. **Peroxide value**: Iodimetric titration
7. **TBA value**: Steam distillation/Spectrophotometric method

Physical and Instrumental methods for assessing seafood quality

1. Freshness meter: E.g., Torry meter, Intelectron fish tester. As Fish tester and Torry meter measurements are based on the existence of intact cell membranes, they fail when the cells are disrupted or broken (damage, bruising, frozen/thawed fish).
2. Texture measurement: Texturometers/ Universal testing machines
3. Colour determination: Colourimeter

4. pH: Measuring pH in an aqueous muscle homogenate or using an injection electrode directly in the muscle tissue is the simplest method for quality assessment of seafood. A general rule is that when a pH of 7.0 in fish muscle is reached or exceeded, the borderline of edibility is reached though it is considered by many as not a good indicator of freshness and/or spoilage.

Microbial methods for assessing seafood quality

- Determination of total bacterial count
- Determination of specific spoilage bacteria
- Determination of pathogenic bacteria

Sensory quality assessment

At present, the commonly used methods of sensory evaluation include Quality Index Method (QIM), Tasmanian Food Research Unit schemes, and the Torry schemes. Among them, QIM is the most commonly used, which is based on objective assessment of some attributes of raw fish.

Novel Methods for fish quality assessment

1. **Biosensors**
    - Amine gas sensor: Detect the volatile amine gas (ammonia, TMA and DMA) in a short time (60 s) from raw fish flesh
    - Electronic nose: Most widely used technique are based on metal oxide gas sensor
2. **Spectroscopic techniques**
    - Visible/near-infrared (Vis/NIR) spectroscopy: Analyse food quality due to the interaction between food components and electromagnetic radiation emitted from lights

5.0 Quality control programmes

The traditional quality control program was based on establishing effective hygiene control. Confirmation of safety and identification of potential problems was obtained by end-product testing. Control of hygiene was ensured by inspection of facilities to ensure adherence to established and generally accepted Codes of Good Hygiene Practices (GHP) and of Good Manufacturing Practices (GMP). Below are listed the most well-known methods to manage quality and/or safety.

- Good Hygienic Practices (GHP) / Good Manufacturing Practice (GMP) or Sanitation Standard Operating Procedures (SSOP) or prerequisite programmes
- Hazard Analysis Critical Control Point (HACCP)
- Quality Assurance (QA) / Quality Management (QM) - ISO standards
- Total Quality Management (TQM)

6.0 Food Safety and Standards Authority of India (FSSAI): Ensuring food quality

The Food Safety and Standards Authority of India (FSSAI) was established under the Food Safety and Standards Act, 2006 as a statutory body for laying down science-based standards for articles of food and regulating manufacturing, processing, distribution, sale and import of food so as to ensure safe and wholesome food for human consumption. Various central acts including the erstwhile Prevention of Food Adulteration Act (1954) were merged under this act. Ministry of Health & Family Welfare, Government of India is the governing Ministry of FSSAI. It has the main Headquarters in Delhi.

The FSSAI is responsible for maintaining the food quality & security in India. FSSAI role in food quality is crucial for food security. The FSSAI's role in food quality is to ensure safety and providing satisfaction to every customer. Food Testing and analysis is an essential part of the food safety ecosystem to assure that the food is safe to consume. For the same, FSSAI recognizes and notifies NABL accredited food laboratories under Section 43 of FSS Act, 2006. FSSAI approved notified laboratories as National Reference Laboratories (NRLs) and as ancillary facility of NRLs (ANRLs) for specific purpose.

1. **Primary food laboratories**: The Food Authority notifies food laboratories and research institutions accredited by National Accreditation Board for Testing and Calibration Laboratories or any other accreditation agency for the purposes of carrying out analysis of samples by the Food Analysts. Presently there are 187 notified food testing laboratories.
2. **Referral food laboratories**: The Food Authority recognizes referral food laboratories for the purposes of carrying out analysis of appeal samples. Presently there are 18 referral food laboratories.
3. **National Reference Laboratories**: FSSAI has recognised National Reference laboratory (NRL) to set up a country wide standard for routine procedures, validation of such standard procedure / testing methods, development of new methods and ensuring proficiency in testing across the food laboratories with special reference to the

risks or food categories. Either a primary food laboratory or a referral food laboratory can be considered for declaration as NRL. Presently there are 12 NRLs and 2 ANRLs.

7.0 FSSAI Notified Referral Laboratories under section 43 (2) of FSS Act, 2006

1. Central Food Laboratory, 3 Kyd Street, Kolkata- 700016

2. Food Safety & Analytical Quality Control Laboratory, C/o Central Food Technological Research Institute, Mysore-570013

3. State Public Health Laboratory, Stav ely Road, Cantonment Water Works Compound, Pune-411001

4. National Food Laboratory, Ahinsa Khand-II, Indirapuram Ghaziabad-201014

5. Indian Institute of Horticultural Research, Hessaraghatta lake post, Bangalore-560089

6. Quality Evaluation Laboratory, Spices Board, Palarivattom P.O. Kochi-682025

7. Quality Evaluation Laboratory, Spices Board, Chuttugunta Center, GT Road, Guntur-522004

8. Quality Evaluation Laboratory, Spices Board, Plot No. R-11, Sipcot Industrial Complex, Gummidipoondi, Thiruvallur Dt., Chennai-601201

9. Quality Evaluation Laboratory, Spices Board, First Floor, Banking complex II, Sector 19A, Vashi, Navi Mumbai-400703

10. Centre for Analysis and Learning in Livestock in Food (CALF), National Dairy Development Board (NDDB), Anand-388001, Gujarat

11. CSIR-Indian Institute of Chemical Technology, Uppal Road, Tarnaka, Hyderabad - 500007

12. National Research Centre on Meat, Chengicherla, Buduppal, Hyderabad – 500092

13. Indian Institute of Food Processing Technology, Food Safety and Quality Testing Laboratory, Pudukkottai Road, Thanjavur – 613005, Tamil Nadu

14. ICAR- Central Institute of Fisheries Technology, Indian Council of Agricul tural Research, Willingdon Island, CIFT Junction, Matsyapuri P.O., Cochin – 682029, Kerala

15. ICAR-National Research Centre for Grapes, P.O. Manjiri Farm, Solapur Road, Pune – 412307

16. Pesticide Formulation and Residue Analytical Centre, National Institute of Plant Health Management, Rajendranagar, Hyderabad - 500030

17. Punjab Biotechnology Incubator, Mohali SCO7 & 8, Phase-5, SAS Nagar, Mohali 160059, Punjab

18. CSIR-Indian Institute of Toxicology Research, Vishvigyan Bhawan, 31, Mahatma Gandhi Marg, Lucknow - 226 001, Uttar Pradesh, India

8.0 Referral Food Laboratory

The Laboratory having competence to carry out the analysis as per "The Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011" and "Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011". In addition, the Referral laboratory must have the competence to meet the following requirements:

- **R & D Capabilities**: Laboratories having documentary evidence for carrying out R&D in food sector
- **Training Facilities**: The laboratory should have training centre for capacity building by way of organizing professional training, workshops and seminars.
- **Other Facilities**
    1. Analysis of samples of food sent by any officer or authority authorized by the Food Authority for the purpose and submission of the certificate of analysis to the authorities concerned;
    2. Investigation for the purpose of fixation of standard of any article of food;
    3. Investigation in collaboration with the laboratories of Food analysts in the various States and such other laboratories and institutions which the Food Authority may approve on its behalf, for the purpose of standardizing methods of analysis.
    4. Ensuring that the laboratory follows the scientific protocols laid down for handling/testing the articles of food.
    5. Maintaining high standards of accuracy, reliability and credibility in the operation of the laboratory and achieving and maintaining the required levels of accreditation and reliability.
    6. Laying down mechanism for ensuring that personnel of the laboratory adhere to

high professional standards and discipline.

7. Such other conditions, as the Authority may lay down for Referral Laboratories such as coordinating proficiency testing programmes in the country etc.

9.0 Reference Laboratory

The Food Authority may recognise any notified food laboratory or referral food laboratory as a reference laboratory for the purpose of developing methods of testing, validation, proficiency testing, and training.

The reference laboratory shall carry out the following functions, namely

i. be a resource centre for provision of information for certified reference materials and reference materials
ii. develop standards for routine testing procedures and reliable testing methods
iii. provide technical support in the area of competence
iv. evaluate the performance of other notified laboratories
v. coordinate exchange of information amongst notified food laboratories
vi. collaborate for data generation among the network of notified food laboratories and referral food laboratories and collate the data related to their specific domain
vii. such other functions as may be specified by the Food Authority

10.0 Criteria for notifying and recognising food laboratories

For being recognised and notified, every food laboratory shall have-

- Accreditation against ISO/IEC 17025 by the National Accreditation Board for Testing and calibration laboratories or such other equivalent accreditation agency as may be approved by the Food Authority.
- Adequate capability and competence for testing of food safety and quality parameters as per the requirements of the Act.
- Person possessing qualification and experience required for being appointed as Food Analyst under rule 2.1.4of the Food Safety and Standards Rule, 2011: Provided that a food laboratory accredited by an accreditation body having authorised signatory designated by such accreditation body, shall also be considered for being notified subject to the condition that such authorised signatory shall, within one year from the date of such notification, acquire the qualification and experience required for being appointed as Food Analyst under the said rule.

- The infrastructure and facilities including equipment required for carrying out the analysis as per the scope applied for.

11.0 ICAR-CIFT: National Reference Laboratory for Fish and Fish Products

ICAR-Central Institute of Fisheries Technology, Cochin has been conferred with a status of "National Reference Laboratory (NRL) for Fish and Fish Products" by Food Safety and Standards Authority of India (FSSAI), Ministry of Health and Family Welfare, Govt. of India under Regulation 3 of Food Safety and Standards (Recognition and Notification of Laboratories) Regulation, 2018 on 19th March, 2019 vide Order No. 12013/02/2017-QA. ICAR-CIFT is the only research Institute under SMD (Fishery), ICAR to be adorned with such a high-profile recognition. The Institute had already been notified as National Referral Laboratory vide Government of India Gazette Notification S.O. 97(E) of Ministry of Health and Family Welfare (Food Safety and Standards Authority of India) dated 10th January, 2017. Under the NRL notification, ICAR-CIFT has earmarked with the following research activities on emerging issues pertaining to:

- Risk assessment of dietary exposure of persistent organic pollutants and emerging contaminants such as brominated flame retardants and pharmacologically active substances to Indian population from fish and fisheries products.
- Research on ingression of specific migration of chemicals from plastic packaging materials to fishery products
- Research on incidence of biotoxins in finfish/shellfis

Presently there are 12 NRLs. Including ICAR-CIFT, seven laboratories in Government sector and five laboratories in private sector have been given the status of National Reference Laboratory in specific areas.

12.0 List of national reference laboratories (NRLs) approved by FSSAI

Sl. No.	Name of the laboratory/ institution/organisation	Specific area for which declared as NRL
Government laboratories		
1.	Central Food Technological Research Institute, Mysore	Nutritional information and labelling
2.	Export Inspection Agency, Kochi	GMO (genetically-modified organism) testing (subject to implementation of GMO regulations)
3.	Punjab Biotechnology Incubator, Mohali	Sweets and confectionery, including honey
4.	ICAR-National Research Centre For Grapes, Pune	Pesticide residues and mycotoxins
5.	Central Institute of Fisheries Technology, Kochi	Fish and fish products
6.	Centre for Analysis and Learning in Livestock and Food-National Dairy Development Board, Anand	Dairy and dairy products
7.	CSIR-Indian Institute of Toxicology Research, Lucknow	Toxicological evaluation/risk assessment of nutraceuticals, functional foods and novel/emerging foods/food ingredients
Private laboratories		
8.	Trilogy Analytical Laboratory Pvt. Ltd., Hyderabad	Mycotoxins in cereals and pulses, spices and condiments and related PT activities
9.	Edward Food Research and Analysis Centre Limited, Kolkata	Veterinary drug residues, antibiotics and hormones
10.	Vimta Labs Limited, Hyderabad	Water, alcoholic and non-alcoholic beverages
11.	Fare Labs Pvt. Ltd., Gurugram	Oils and fats
12.	Neogen Food and Animal Security (India) Private Limited, Cochin	Food allergens

13.0 Conclusion

Seafood is a very perishable product and the risk of contamination of seafood products by biological hazards is very high. Processing is necessary to assure the prolonged shelf life and safety of seafood. The seafood processing industry currently has to face new challenges. Production has increased and seafood products need to be transported over long distances. Increasing demands from legislation and from the consumer for better quality and safer products have to be taken into account. Seafood now has to be high quality, nutritious, safe and have the convenience of an extended shelf life. To meet these criteria, seafood processing has had to assimilate all the new advances in food science and technology and in quality and safety assurance. Advanced quality and safety methods, such as modern and rapid techniques for assessing quality and safety, species identification techniques and risk assessment tools, all have significant applications in the seafood sector.

Food testing and analysis is an essential part of the food safety ecosystem to assure that the food is safe to consume. This includes strengthening the network of food testing laboratories, assuring quality of food testing, investing in human resources, carrying out surveillance activities and educating consumers. As an essential part of the food safety ecosystem, the Food Safety and Standards Authority of India has created a network of 232 laboratories to fulfil its mandate on food testing and analysis. The network comprises of 142 accredited primary food-testing laboratories from both government and private sphere, 72 state food testing laboratories and 18 referral laboratories of which two are under the direct control of FSSAI. FSSAI's role in food quality is important for smooth functioning. As a resultant of this, every customer receives an equal level of assurance of food safety.

ISO 22000/ HACCP in Seafood processing

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1.0 Introduction

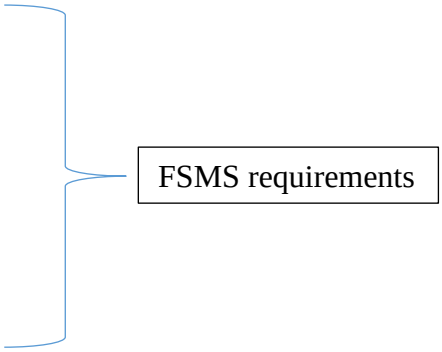
The fish processing industry is vital for providing a sustainable protein source globally, with production and consumption steadily increasing each year. To meet the demands of a growing population, ensuring the safety and quality of processed fish products is imperative due to the association of seafood with various foodborne illnesses worldwide. This concern becomes global with the rise in international fish trade. Achieving safety and quality in fish processing requires a systematic approach, and two key systems contributing to this goal are ISO 22000 and Hazard Analysis and Critical Control Points (HACCP). ISO 22000, an international standard for food safety management systems (FSMS), offers a comprehensive framework based on HACCP principles. HACCP, a scientific and systematic method, is effective when there's collaboration between governments, producers, and consumers. It identifies, assesses, and controls hazards, integrating food safety control into the process design. This chapter explores the application of these frameworks in the context of fish processing, emphasizing their role in preventive and cost-effective approaches to food safety.

2.0 ISO 22000

ISO (International Organization for Standardization) is a non-governmental organization established in 1947, headquartered in Geneva, Switzerland, with around 160 national standards institutes as members worldwide. ISO 22000, developed by a working group under ISO Technical Committee 34, is now managed by ISO sub-committee 17. Published in June 2018, ISO 22000:2018 replaces the 2005 version and aims to harmonize global food safety management requirements. It is applicable to all organizations in the food chain, contributing to food safety from farm to table. ISO 22000:2018 utilizes a process approach integrating the Plan-Do-Check-Act (PDCA) cycle and risk-based thinking. This approach allows organizations to plan and manage their processes effectively. The PDCA cycle ensures proper resource allocation, management, and continuous improvement, while risk-based thinking identifies potential deviations in processes and the Food Safety Management System (FSMS), enabling the implementation of controls to prevent or minimize adverse effects.

The main clauses of high-level structure are as follows:

1. Scope
2. Normative references
3. Terms and Definitions
4. Context of the organization
5. Leadership
6. Planning
7. Support
8. Operation
9. Performance evaluation
10. Improvement



ISO 22000:2018 utilizes a process approach with the Plan-Do-Check-Act (PDCA) cycle and risk-based thinking. This approach allows organizations to plan and manage processes effectively, ensuring adequate resources and continual improvement. The PDCA cycle ensures processes are well-managed and opportunities for enhancement are addressed. Risk-based thinking identifies potential deviations in processes and the FSMS, allowing organizations to implement controls to prevent or minimize adverse effects.

Plan-Do-Check-Act cycle:

The PDCA cycle can be described briefly as follows:

Plan: establish the objectives of the system and its processes, provide the resources needed to deliver the results, and identify and address risks and opportunities;

Do: implement what was planned;

Check: monitor and (where relevant) measure processes and the resulting products and services, analyze and evaluate information and data from monitoring, measuring and verification activities, and report the results;

Act: take actions to improve performance, as necessary.

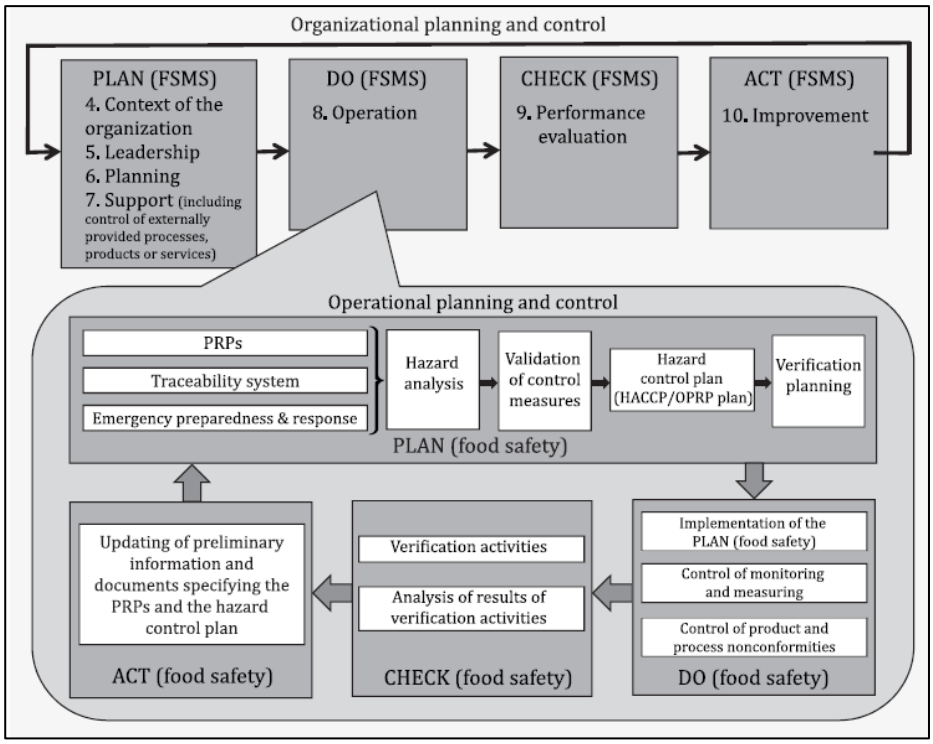


Fig 1. Organizational planning and control of ISO 22000:2018 (*Source: ISO 22000:2018- Food safety management systems)*

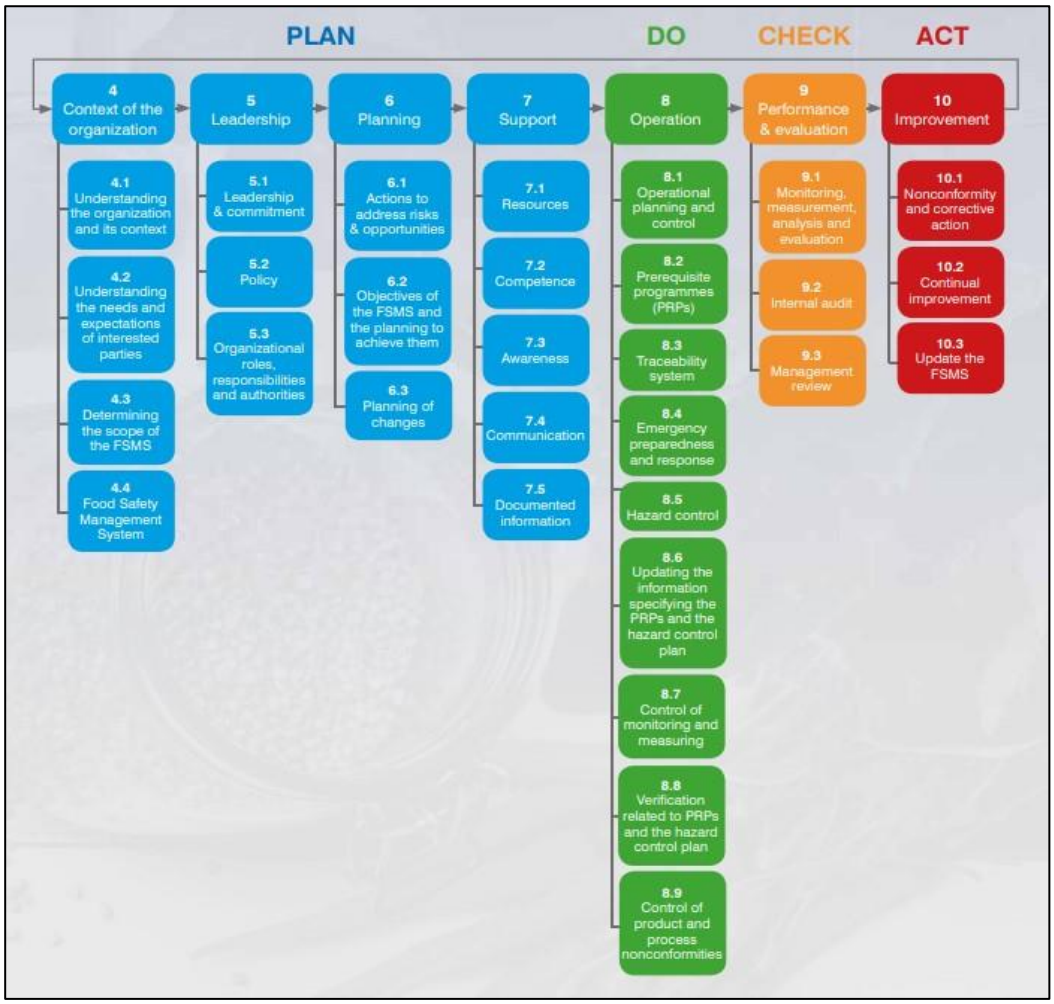


Fig 2. PDCA cycle of ISO22000:2018 (*source: NQA-ISO-22000-Implementation-Guide)*

Benefits of ISO 22000 and HACCP in Fish Processing:

1. ***Enhanced Food Safety*:** Both ISO 22000 and HACCP contribute to a systematic and science-based approach to food safety, reducing the risk of contamination and ensuring the production of safe fish products.
2. ***Compliance with Regulations*:** Adhering to these international standards helps fish processing facilities comply with regulatory requirements and gain consumer trust.
3. ***Continuous Improvement*:** The frameworks encourage a culture of continuous improvement, with regular assessments, reviews, and updates to the food safety management system.
4. ***Global Market Access*:** Certification against ISO 22000 provides a globally recognized assurance of food safety, facilitating access to international markets for fish products.

3.0 Food Safety Management System (FSMS)

Food Safety Management Systems are a set of practices and standards designed to ensure the safety and quality of food products throughout the entire supply chain. FSMS provides a systematic approach to identifying, preventing, and managing food safety hazards. In the context of seafood processing, this includes addressing risks associated with contamination, spoilage, and other factors that can compromise the safety of the final product.

FSMS principles

Food safety is related to the presence of food safety hazards at the time of consumption (intake by the consumer). Food safety hazards can occur at any stage of the food chain. Therefore, adequate control throughout the food chain is essential. Food safety is ensured through the combined efforts of all the parties in the food chain. The key elements of FSMS are interactive communication, system management, prerequisite programmes, and HACCP principles.

Key Benefits of FSMS in Seafood Processing:

- ***Compliance with Regulations*:** Implementing FSMS ensures seafood processors meet strict food safety regulations, crucial for gaining market access as countries and regions have specific standards for seafood processing.
- ***Risk Mitigation*:** Seafood processing, with its multiple steps from harvest to distribution, poses contamination risks; FSMS identifies and addresses hazards, lowering the chances of foodborne illnesses and product recalls.

- ***Enhanced Traceability*:** FSMS enhances supply chain traceability, crucial for swiftly and accurately identifying contamination sources in safety concerns to minimize impacts on consumers and the industry.
- ***Improved Product Quality*:** FSMS not only ensures safety but also enhances overall product quality by guiding proper handling, storage, and processing techniques, preserving the freshness, flavour, and nutritional value of seafood products.

Key components of ISO 22000 in fish processing include:

- ***Pre-requisite Programs*:** Good Manufacturing Practices (GMPs) and sanitation procedures are prerequisites mandated by ISO 22000. These lay the groundwork for maintaining a hygienic environment and minimizing the risk of contamination in fish processing facilities.
- ***Operational Pre-requisite Programs (OPRPs)*:** Operational controls at specific processing stages, known as OPRPs, are implemented to manage risks that might not be fully addressed by GMPs alone. These are critical measures integrated into the broader food safety management system.
- ***Integration of HACCP Principles*:** ISO 22000 incorporates HACCP principles, emphasizing the identification of Critical Control Points (CCPs) and establishing measures to control these points effectively.

4.0 The HACCP system

The HACCP system identifies, evaluates, and controls significant food safety hazards, requiring collaborative teamwork and firm commitment from top management for effective implementation. While HACCP doesn't guarantee zero risk, it systematically minimizes food safety hazards. The HACCP plan is not static; it requires modification as needed, emphasizing a continuous, risk-based process from farm to fork. The program encompasses all prerequisite programs, focusing on forecasting rather than reacting, getting the process right initially, and addressing potential food safety problems through proactive measures. It involves describing procedures, training personnel, implementing, recording, and ensuring food safety throughout the entire chain.

Pre-requisite programmes (PRPs)

PRPs, including GMPs and SSOPs, precede HACCP plans, addressing employee, facility, and equipment aspects. They encompass illness policies, cleaning procedures, pest control, equipment selection, and employee hygiene. PRPs extend to non-food-related elements like

water quality, transportation, storage, plant sanitation, and employee training, focusing on comprehensive plant management.

HACCP plan

A document, aligned with HACCP principles, ensures control of significant food safety hazards in the relevant food chain segment, following prerequisite programs. Prior to applying HACCP in a fish or seafood establishment, adherence to the Recommended International Code of Practice for Food Hygiene is crucial. Effective HACCP implementation requires management awareness, commitment, and ongoing training for employees. If expertise is lacking on-site, seeking advice from external sources is recommended. HACCP plan preparation involves two steps.

1. Conducts five preliminary steps
2. Applies the seven HACCP principles

Preliminary steps

Step 1. Assemble the HACCP team.

Step 2. Describe product.

Step 3. Identify intended use.

Step 4. Construct flow diagram.

Step 5. Confirm flow diagram.

HACCP principles

Principle 1. Conduct a hazard analysis and identify control measures

Principle 2. Determine CCPs

Principle 3. Establish validated critical limits

Principle 4. Establish a system to monitor control of CCPs

Principle 5. Establish the corrective actions to be taken when monitoring indicates a deviation from a critical limit at a CCP has occurred

Principle 6. Validate the HACCP plan and then establish procedures for verification to confirm that the HACCP system is working as intended

Principle 7. Establish documentation concerning all procedures and records appropriate to these principles and their application

HACCP plan is a final document that describes how a fish or seafood operation will manage the identified CCPs for each product under its particular environment and working conditions. The following are the details on how to apply the above sequence for the preparation of a specific HACCP plan.

1. ***Assemble the HACCP Team***

The HACCP Team, led by a coordinator, comprises diverse members—seven to eight for larger companies and two to three for smaller ones. The coordinator oversees the entire program, and the team, representing various fields, needs access to essential information and expertise in management, production, quality assurance, maintenance, marketing, and sales.

2. ***Describe the product:***

Create a detailed product description, noting safety factors like harvesting details, raw materials (with fish names), key parameters (water activity, pH, salt content), processing methods, packaging type, storage conditions, distribution methods, and specified shelf-life.

3. Identify the intended use:

Ensure product safety by aligning intended use with end-user expectations. The safety of certain products depends on user preparation, especially when natural organisms are present. Without a kill step in processing, safety relies on thorough heat treatment during preparation, like cooking. Identify usage patterns that may increase consumer risk or involve susceptible groups, such as the elderly or infants, particularly in settings like institutional feeding.

4. Construct a process flow diagram:

HACCP teams create a concise flow diagram outlining all operation steps, including receiving and storage. Consideration should extend to steps before and after the specific operation, with focus on time and temperature conditions during processing, especially in holding areas where delays or temperature issues may occur.

5. On site verification of the process flow diagram:

The HACCP team should confirm on-site the production operations against the flow diagram and amend it with information, such as correct durations, temperatures, and salt concentration, where appropriate. The site should be inspected during all hours (including night shifts and weekends) of operation to check for correctness and ensure that nothing crucial has been overlooked.

Principles of HACCP

1. Conduct a hazard analysis and identify control measures

A hazard is defined as a biological, chemical or physical agent in, or condition of, food (e.g. temperature abuse, insufficient thermal process), with the potential to cause an adverse health effect and harm. The HACCP team identifies hazards throughout production, processing, transportation, and distribution until fish consumption. Hazard analysis is the initial HACCP principle and a science-based component. Inaccuracies in hazard analysis can lead to an inadequate HACCP plan. The team determines critical hazards using a decision tree with specific questions. After hazard analysis, the HACCP team assesses available control measures for each hazard, recognizing that multiple measures may be necessary to manage a hazard effectively. These control measures aim to prevent, eliminate, or reduce hazards to an acceptable level.

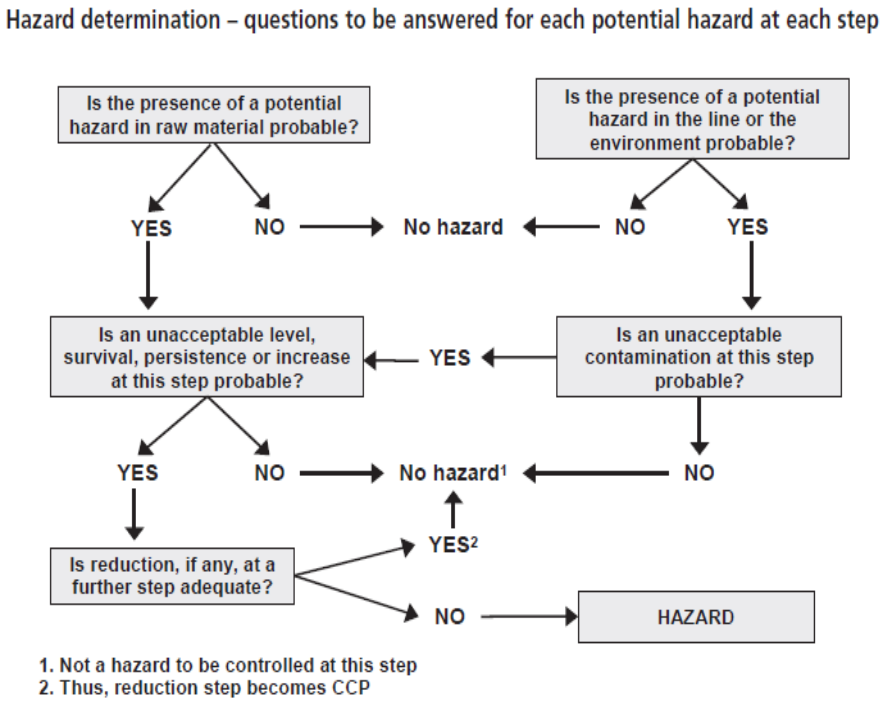


Fig 3. Hazard determination decision tree

USFDA suggested following control measure for seafood-borne hazards:

Pathogenic bacteria:

- Time/temp control, heating/cooking, freezing, fermentation, salt/preservatives.

Pathogenic viruses:

- Cooking, source control from acceptable region

Parasites:

- Cooking, freezing.

Chemical hazard:

- Source control (Biotoxins, contaminants), time-temp (histamine), labelling (allergens)

Physical hazard:

- Source control (metal/glass), metal detector (metal pieces), PRPs

2. Determine CCPs

A Critical Control Point (CCP) is a step essential for preventing, eliminating, or reducing a food safety hazard to an acceptable level. CCPs are specific to the product and process, and multiple CCPs may address the same hazard or control various hazards. Identifying all CCPs accurately is crucial for effective hazard control in HACCP. Using a decision tree can aid in determining CCPs, with flexibility based on the operation type. Alternative approaches may also be employed. If a hazard lacks control measures, the product or process should be modified at that step or earlier/later stages, ensuring this evaluation occurs for each hazard and step.

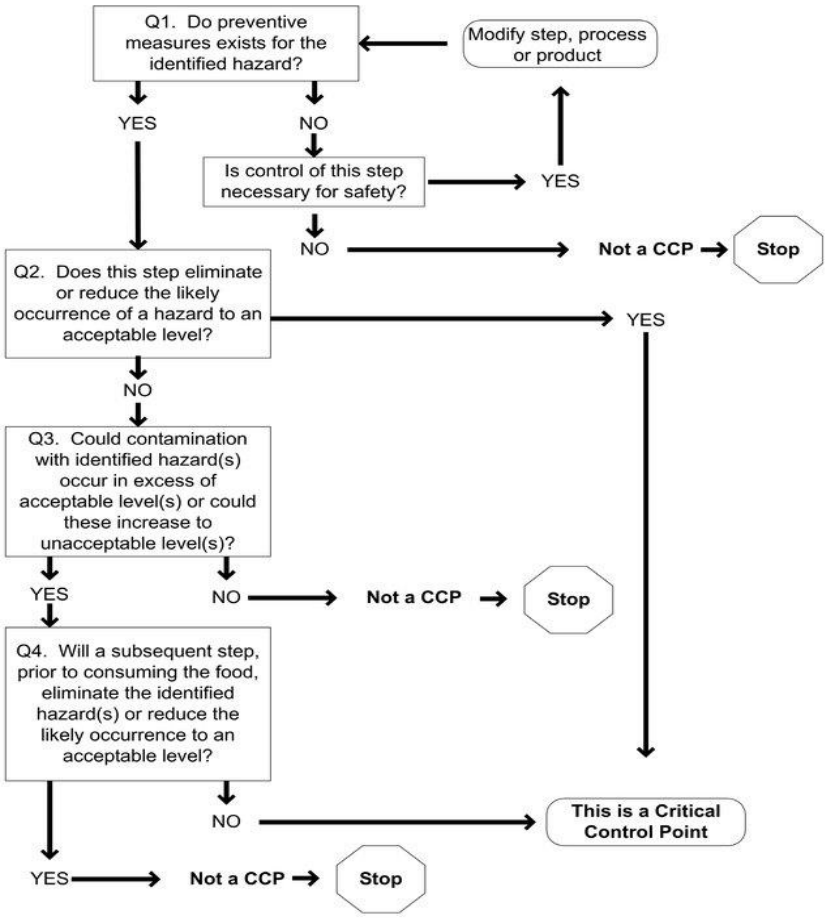


Fig 4. CCP decision tree

3. Establish validated critical limits

Critical limits are set criteria distinguishing acceptability from unacceptability, serving as boundaries to assess the safe production of products through proper control measures. Scientifically based and easily measurable factors like temperature, time, chlorine levels, water activity, pH, and others are used. Avoiding lengthy microbiological limits, rapid techniques are preferred when necessary. These limits must align with government regulations, company standards, or be backed by scientific data. Those establishing critical limits should possess knowledge of the process, legal requirements, and commercial standards. For instance, a cooking step (80°C for 2.5 min) in the process line establishes predefined time and temperature as the critical limit.

4. Establish a system to monitor control of CCPs

Monitoring is defined as the act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control. Monitoring procedures ascertain proper implementation of control measures and prevent exceeding critical limits at CCP. These procedures, which can be qualitative or quantitative, continuous or non-continuous, encompass sensory evaluation, physical measurements (pH, aw, humidity), chemical testing (e.g., chlorine level in water), and microbiological examination of raw materials and end products.

Components:

- What will be monitored?
- How the critical limit and control measures will be monitored?
- When (frequency)? and
- Who will monitor?

5. Establish the corrective actions to be taken when monitoring indicates a deviation from a critical limit at a CCP has occurred

Implementing HACCP aims to prevent issues, so predefined corrective actions are vital if monitoring at a CCP indicates a loss of control. Deviations from critical limits must be controlled by predetermined actions, including proper identification, control, and disposition of affected products. The establishment should have effective procedures for identifying, isolating, marking, and controlling products produced during the deviation period. Corrective action procedures are essential to determine the problem's cause, prevent recurrence, and reassess for effectiveness. Records of product control, corrective actions, and reassessment

must be maintained and available for verification, demonstrating control and corrective actions for deviations.

6. Validate the HACCP plan and then establish procedures for verification to confirm that the HACCP system is working as intended

Verification, which involves methods, procedures, tests, and random sampling, is used alongside monitoring to confirm HACCP plan compliance. The goal is to assess the plan's effectiveness and ensure adherence. Although careful preparation doesn't guarantee effectiveness, verification procedures help detect deficiencies. These activities should be conducted by qualified individuals, documented in the HACCP plan, and recorded with details such as methods, date, responsible individuals, results, and actions taken. Subsequent validation and verification are crucial with any changes in raw materials, product formulation, procedures, consumer practices, hazard information, complaints, recurring deviations, or indications of system failure.

7. Establish documentation concerning all procedures and records appropriate to these principles and their application

Records and documentation are essential for reviewing the adequacy of and adherence to the HACCP plan. Several types of records should be considered among those relevant in an HACCP programme:

- Support documentation, including validation records, for developing the HACCP plan;
- Records generated by the HACCP system: monitoring records of all CCPs;
- Deviation and corrective action records, verification/validation records;
- Documentation on methods and procedures used;
- Records of employee training programmes.

Records, whether in the form of processing charts, written procedures, or tables, can be stored in paper or electronic formats, ensuring record integrity. It's crucial to maintain complete, current, properly filed, and accurate records. Failure to document CCP control or corrective action implementation would be a critical deviation from the HACCP plan.

5.0 Conclusion:

In the dynamic realm of food safety, integrating ISO 22000 and HACCP into fish processing isn't just strategic but essential. These frameworks serve as crucial tools, ensuring safety and quality in the industry. Implementing them establishes robust food safety management

systems, meeting regulations and bolstering the industry's sustainability and reputation. This holistic approach, blending systematic risk management with international standards, is vital for securing the industry's future. With the growing demand for safe, high-quality food, adherence to these frameworks not only ensures fish product safety but also elevates the global standing of the fish processing industry. As consumers prioritize safe and high-quality food, embracing these standards becomes increasingly vital for success in the competitive global market.

Suggested Readings:

- ISO 22000:2018- Food safety management systems — Requirements for any organization in the food chain
- Food and Drug Administration. (2022). Fish and fishery products hazards and controls guidance. US Department of Health and Human Services Food and Drug Administration Centre for Food Safety and Applied Nutrition, pp 454.
- https://cdhd.idaho.gov/pdfs/food/HACCP%20worksheets.pdf
- https://southcenters.osu.edu/sites/southc/files/site-library/site-documents/abc/marketprocess_addresources/HACCP%20for%20fin%20fish%20.pdf
- https://www.fda.gov/food/hazard-analysis-critical-control-point-haccp/haccp-principles-application-guidelines

Seafood Export and Trade Issues

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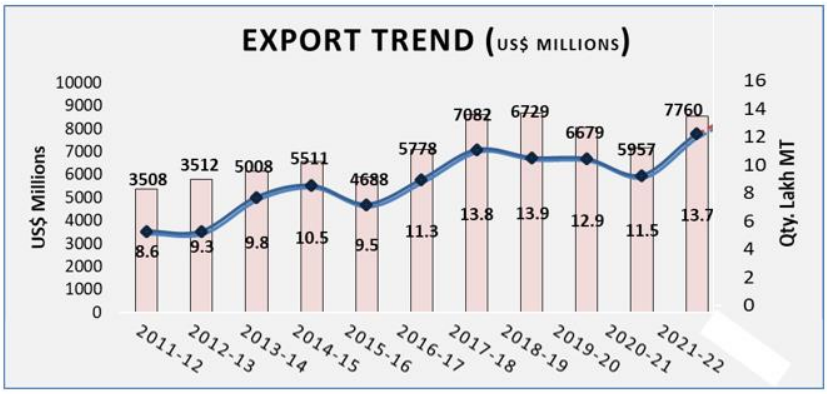
In 2021-22 India exported to 123 countries and the top 5 countries are USA, China, Japan, Vietnam and Thailand. The following figures illustrates the export performance of the marine products from India

Export Performance of Marine Products



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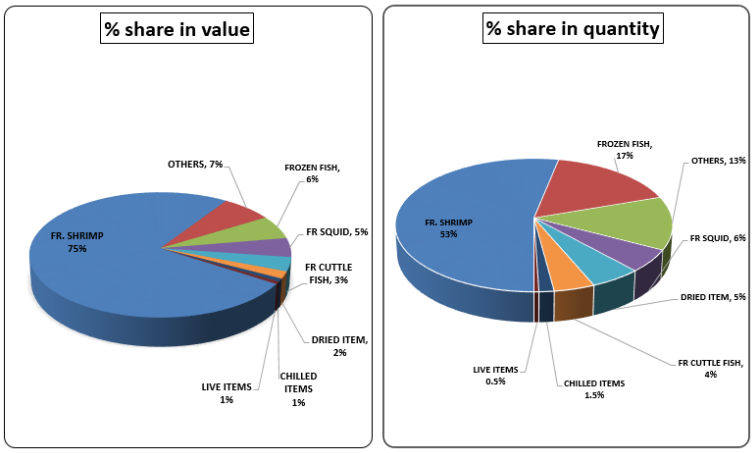

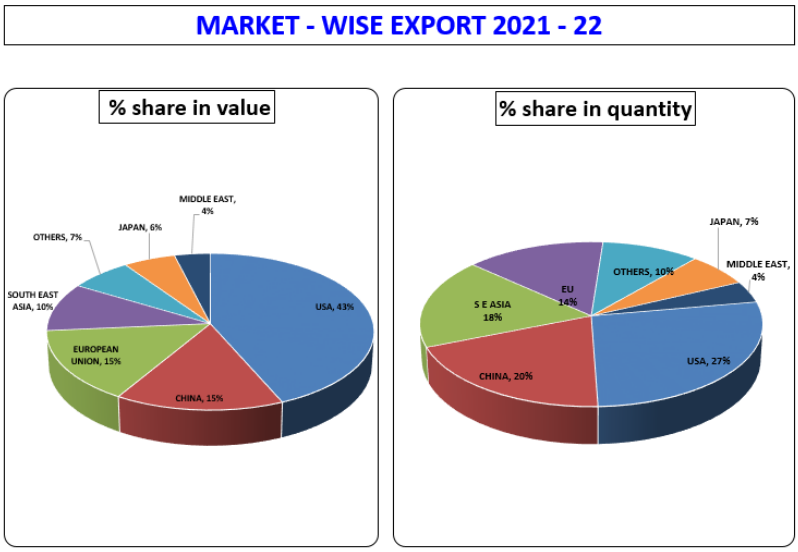

Sanitary & Phytosanitary measures

The word Sanitary means that measures relating to human or animal life or health while Phyto sanitary means those relating to plant life or health. There are three international standards setting bodies specifically mentioned in the SPS agreement. These are often referred as **"Three sisters"** and they are as follows:

1. **The International plant protection convention (IPPC) - dealing with plant health**
2. **The World Organization for Animal Health (OIE) – dealing with animal health**
3. **The Codex alimentarius Commission (CODEX) – dealing with food Safety**

World Trade Organization recommends that members must not use SPS measure that are Unnecessary, not science based, arbitrary or which constitutes a disguised restriction on International Trade. The key points of SPS are as follows:

- The SPS agreement recognizes the need for WTO members to protect themselves from the risk posed by the entry of pests and diseases but also seeks to minimize any negative effects of SPS measures on trade.
- The health aspect of SPS agreement basically means that WTO members can protect human, animal or plant life or health by applying measures to manage the risk associated with imports.
- The measures usually take the form of quarantine or food safety requirements.

Terms used in SPS agreement

Harmonization

WTO members are encouraged to base their SPS measures on international standards, guidelines & recommendations where they exist . The SPS Committee promotes and monitors international harmonization.

Equivalence

SPS agreement requires importing WTO members to accept the SPS measures of exporting WTO members as equivalent, if the exporting country objectively demonstrate to the importing country that its measures achieve the importing country's ALOP (Appropriate Level Of Protection).

Appropriate Level Of Protection (ALOP)

ALOP is the level of protection deemed appropriate by the WTO members to protect human, animal or plant life or health within its territory

Risk Assessment

The evaluation of livelihood of entry, establishment or spread of a pest or disease within the territory of an importing WTO member according to SPS measures which might be applied and of the associated potential biological and economic consequence.

OR

The evaluation of the potential for adverse effects on human or animal health arising from the presence of additives, contaminants, toxins or disease –causing organisms in food, beverages or feedstuffs.

Risk assessment is essentially the process of gathering scientific evidence and relevant economic factors on the risks involved in allowing a particular import to enter a country.

An importing member is likely to seek information on matters such as the pests or diseases that might be associated with the commodity for which permission to import has been sought, and if they are present in the exporting country, the type of question that might be asked as below:

- Does the pest or disease occur in your country?
- Have the pests or diseases been controlled?
- Are they restricted to particular parts of the country?
- How effective are procedures applied to ensure that the products for export are free from pests, diseases and other contaminants?

Regional conditions

WTO members are required to recognize the concepts of pest / disease-free areas and areas of low pests / disease prevalence.

Exporting WTO members claiming pest / disease-free areas or areas of low pests/disease prevalence must demonstrate to the importing WTO member that such areas are, and are likely to remain, pests / disease free areas of low pest / disease prevalence.

Transparency

SPS agreement requires WTO members to provide information on their SPS measures and to notify changes in the SPS measures. WTO members are required to publish their SPS regulations.

The notification requirements are met through a national notification authority. Each WTO member must nominate a national enquiry point to deal with SPS related queries from other WTO members.

The World Organization for Animal Health (OIE)

The objective of the world organization for Animal Health (OIE) include ensuring transparency in the global animal disease and zoonosis situation, publishing health standards for trade in animals and animal products, promoting veterinary skills, improving the safety of food of animal origin and promoting animal welfare through a **science based approach.**

OIE standards, guidelines and recommendations are contained in the **aquatic animal health code** and the **manual of diagnostic tests for aquatic animals.**

CODEX

- Codex has a dual mandate to protect the health of consumers and to ensure fair practices in the food trade.
- Codex develops and encourages implementation of standards, codes of practice, guidelines and recommendations covering all aspects of food safety, including handling and distribution.

SPS – Notification – Australia

Notification No. G/SPS/N/AUS/298 dt. 09/07/2012 (Bio security bill and the inspector general of Bio security bill)

As per Bio Security advice 2009/25, the imported prawns –

1. be sourced from a country or zone that is recognised by Australia to be free of WSSV, YHV, TSV and NHPB – Necrotising Hepetopancriatis Bacterium (the last disease agent, for unfrozen product only); or

2. have the head and shell removed (except for the last shell segment and tail fans) and, if not from a disease free source, have each batch tested on arrival with negative results for WSSV, and YHV; or

3. be 'highly processed', that is head and shell-off (except for the last shell segment and tail fans), and coated for human consumption by being breaded or battered, marinated in a wet or dry marinade, marinated and placed on skewers or processed into dumpling, spring roll, samosa, roll, ball or dim sum-type product; or

4. be cooked to a standard where all protein is coagulated and no uncooked meat remains.

Aquatic animal health certificate for import of seafood

The Canadian Food Inspection Agency (CFIA) brought out guidelines for import of aquatic animals on 10th December 2011 with amendments to Health of animals act. These new guidelines are operational from 10th Dec 2012.

As per this guidelines every consignment of aquatic animal into Canada must be accompanied with an aquatic animal health certificate by the competent authority for aquatic animal health services of the country of origin.

The aquatic animal health certificate must clearly certify **zoo sanitary requirements,** packaging and shipping requirements by competent authority.

Imposition of stringent standards for fish and fishery products by developed countries

The higher standards imposed by the developed countries becoming a major threat to exports of developing countries.

MRL of Ethoxyquin in shrimps under the Food Sanitation Law of Japan

Japanese authority responsible for ensuring the quality of imported food products into Japan has unexpectedly started examining the shrimp consignments from India for Ethoxyquin since August 2012. Japan have adopted the default standard as 0.01 ppm, as designated for parameters or residues that do not figure in the positive list introduced by Japan in 2006. Japan has fixed MRL for fish at **1ppm** whileNo MRL was fixed for shrimp.

Registration of Overseas enterprises and exporting companies in importing countries

The recent requirements of registration under US food safety modernization act and the AQSIQ of China is leading to procedural issues and adding documentation costs for seafood exporting countries in Asia including India. European Union, Russian Federation, Brazil, China etc. are following such kind of registration procedures in their countries to import the seafood from India. Some of these countries also reserve the right to inspect the establishments in India although stringent regulations are in place in India.

ITC HS

ITC-HS Codes or Indian Trade Classification based on Harmonized System of Coding was adopted in India for import-export operations. Indian custom uses an eight digit ITC-HS Codes to suit the national trade requirements. ITC-HS codes are divided into two schedules:

- Schedule I - Describe the rules and guidelines related to import policies
- Schedule II - Describe the rules and regulation related to export policies

The total number of chapters in the schedule I is 98. The chapters are further divided into sub-heading under which different HS codes are mentioned. Export Policy Schedule II of the ITC-HS code contains 97 chapters giving all the details about the guidelines related to the export policies. The marine products mainly come under chapter 03 and 16.

List of marine products coming under various HS code (4 digit level) is as below

Chapter	HS Code (4 digit level)	Description
03	0301	Live fish
	0302	Fish, fresh or chilled, excluding fish fillets and other fish meat of heading 0304
	0303	Fish, frozen, excluding fish fillets and other fish meat of heading 0304
	0304	Fish fillets and other fish meat (whether or not minced), fresh, chilled or frozen
	0305	Fish, dried, salted or in brine; smoked fish, whether or not cooked before or during the smoking process; flours, meals and pellets, of fish fit for human
	0306	Crustaceans, whether in shell or not, live, fresh, chilled, frozen, dried, salted or in brine; smoked crustaceans, whether in shell or not, whether or not cooked before or during the smoking process; crustaceans, in shell, cooked by steaming or by boiling in water, whether or not chilled, frozen, dried, salted or in brine; flours, meals and pellets of crustaceans, fit for human consumption
	0307	Molluscs, whether in shell or not, live, fresh, chilled, frozen, dried, salted or in brine; smoked molluscs, whether in shell or not, whether or not cooked before or during the smoking process; flours, meals and pellets of molluscs, fit for human consumption
	0308	Aquatic invertebrates other than crustaceans and molluscs, live, fresh, chilled, frozen, dried, salted or in brine; smoked aquatic invertebrates other than crustaceans and molluscs, whether or not cooked before or during the smoking process; flours, meals and pellets of aquatic invertebrates other than crustaceans and molluscs, fit for human consumption

Chapter	HS Code (4 digit level)	Description
05	0508	Coral and similar materials, unworked or simply prepared but not otherwise Worked; shells of molluscs, crustaceans or echinoderms and cuttle-bone, unworked or simply prepared but not cut to shape, powder and waste thereof
	0511	Animal products not elsewhere specified or included; dead animals of chapter 1 or 3, Unfit for human consumption: **Products of fish or crustaceans, molluscs or other aquatic invertebrates; dead animals of Chapter 3** ***(Fish nails, fish tails and other fish waste)***
12	1212	Locust beans, seaweeds and other algae, Sugar beet and sugarcane, fresh, chilled, frozen or dried, whether or not ground; fruit stones and kernels and other vegetable Products (including unroasted chicory roots of the variety cichorium intybus Sativum) of a kind used primarily for human consumption, not elsewhere specified or included : ***Seaweed***
13	1302	Vegetable saps and extracts; pectic substances, pectinates and pectates; agar-agar and other mucilages and thickeners, whether or not modified, derived from vegetable products : ***Agar-agar, Kappa Carrageenan***
15	1504	Fats and oils and their fractions, of fish or marine mammals, whether or not refined, but not chemically modified
16	1604	Prepared or preserved fish; caviar and caviar substitutes prepared from fish eggs
	1605	Crustaceans, molluscs and other aquatic invertebrates, prepared or preserved
23	2301	Flours, meals and pellets, of meat or meat offal, of fish or of crustaceans, molluscs or other aquatic invertebrates, unfit for human consumption; greaves : **Fish meal unfit for human consumption**
	2309	Preparations of a kind used in animal feeding: **Feeds for fish (prawn, etc.)**

Trade agreements: Review & Execution

Trade agreements	Way Forward
1. India - Japan CEPA 2. India - Korea CEPA 3. India - EU BTIA 4. India - Peru FTA 5. India - Mauritius CECPA 6. India - EFTA 7. RCEP Negotiation 8. India Australia Free Trade Agreement 9. India New Zealand Free Trade Agreement 10. India Canada Free Trade Agreement 11. India MERCOSUR Free Trade Agreement 12. India Eurasian Economic Union Trade Agreement	• **India EU FTA** : Early execution of India-EU FTA and reduction of tariff is urgently required for better market access of Indian seafood in EU. • **India-Korea CEPA**: It is understood that during previous review the duty for frozen shrimp was agreed to be reduced to 0% by South Korea with a quota restriction. This may be urgently brought into effect for getting duty benefit for exporting to South Korea. **This review and execution of FTA's on a fast track mode will facilitate India's export to these markets.**

Strategies to overcome SPS issues:

Various strategies to overcome the SPS agreement related issues are as follows:

- Prevent the usage of banned antibiotics like chloramphenicol, nitrofuran *etc.,* in food producing animals
- Competent authority needs to take steps to create aquatic disease-free areas/zones/region
- Use SPS as tool to counter the countries who are using SPS as a tool to restrict the trade
- Raise the SPS issues in bilateral trade meetings for market access
- Raise the SPS issue sin WTO SPS Committee meetings; and
- Active Indian participation in CODEX, OIE & IPPC proceedings

Microbial Safety of Fish and Fishery Products

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1.0 Introduction

Microorganisms play a vital and distinct role in the safety of fish and fish products. Ensuring the safety of fish products necessitates the effective control of both pathogenic microorganisms and spoilage bacteria due to their potential effect on human health. Bacteria are found on fish skin, gills, digestive tracts, and organs including the kidney, liver, and spleen. Fish and fish products, particularly raw or undercooked ones, have caused outbreaks of bacterial infections, biotoxins, histamine, viruses, and parasites. Fish have been identified as reservoirs of various bacterial pathogens that have been linked to human diseases. These pathogens include *Mycobacterium spp., Streptococcus iniae, Photobacterium damselae, Vibrio alginolyticus, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio cholerae, Erysipelothrix rhusiopathiae, pathogenic Escherichia coli, Aeromonas spp., Salmonella spp., Staphylococcus aureus, Listeria monocytogenes, Clostridium botulinum, Clostridium perfringens, Campylobacter jejuni, Delftia acidovorans, Edwardsiella tarda, Legionella pneumophila, and Plesiomonas shigelloides*. Furthermore, the existence of antibiotic-resistant genes (ARGs) within these microorganisms has raised concerns regarding the dissemination of antibiotic resistance (AMR) in both the environment and human populations. In addition to pathogens that impact human health, bacteria have been identified as the primary factor responsible for fish spoilage. Certain spoilage bacteria possess decarboxylase enzymes that can effectively convert free histidine into substantial quantities of histamine. The consumption of such food items can potentially result in scombroid poisoning, a significant concern in terms of food safety.

2.0 The infiltration of microorganisms:

In living conditions, when fish are first introduced to the environment, they are exposed to a wide range of microorganisms, but the fish's immune system keeps bacteria away from growing in the muscle of the fish. Once the fish dies, its immune system breaks down, letting bacteria grow out of control. During storage, they move between the muscle fibres and into the flesh. But, very few bacteria got into the meat when it was stored in ice. Since only a small number of organisms actually get into the flesh, and most microbial growth happens outside, spoilage is probably caused by bacterial enzymes getting into the meat and nutrients getting out. Still, the types of microbes usually found in fish and fish products fall into two groups: I.

microorganisms responsible for spoilage, II. microorganisms that cause disease, i.e., Pathogenic bacteria

3.0 Potential Spoilage bacteria of fish:

Fish and fish are often considered favourite foods due to their deliciousness, high protein content, unsaturated fatty acid content, and omega-3 fatty acid content. However, fish quickly spoils after being caught due to the biological and chemical components and microbial load. After twelve hours, the putrefaction process will start because of the metabolic activity of bacteria, the activity of endogenous enzymes (autolysis), and the oxidation of lipids caused by chemical reactions. After the catch, fish are particularly susceptible to spoilage, and it is essential for human health and safety that high standards of fish qality must be maintained at every stage of the food chain, from capture to consumption. Freshness and quality of fish at each step of the fish production chain can help manufacturers make safe, high-quality, and healthy fish meats, giving them an exceptional price in Global demand.

In spoilage, only a subset of these contaminants can colonize and multiply in large numbers. The spoilage association in aerobically preserved fish is generally composed of Gram-negative psychrotrophic non-fermenting rods. There are several bacterial species on the surfaces of fish. According to their development temperature range, all temperate water fish bacteria are classified as either psychrotrophs or psychrophiles. Psychrotrophs (cold-tolerant) bacteria can thrive at 0°C; their growth is most efficient around 25°C. Bacteria known as psychrophiles (cold-loving) thrive best at temperatures between 15°C and 20°C. The term "spoilage association" has been coined for such a microbial community, but the precise mechanism by which one bacterial group dominates another closely related group is not always fully understood. Thus, under aerobic iced storage, the flora is virtually entirely constituted of *Pseudomonas sp*. and *S. putrifaciens*. Gram-negative, fermentative bacteria (such as *Vibrionaceae*) are responsible for spoiling unpreserved fish. At room temperature (25^{o}C), the microflora is dominated by mesophilic *Vibrionaceae,* especially if the fish are taken in contaminated waters. Fish spoilage is mainly caused by microbial growth, which creates flavour-altering amines, biogenic amines, organic acids, alcohols, aldehydes, and ketones.

Internal fish tissue is often considered sterile, but the bacteria on the slime layer of the skin, gills, and gut would invade after the death of the fish. Factors such as high water activity and low acidity (pH > 6) of fish contribute to the rapid proliferation of microorganisms, which cause negative changes in the fish's appearance, texture, taste, and odour, diminishing its quality. Fish muscle consists of proteins, lipids, carbohydrates, water, and amino acid components, such as trimethylamine oxide (TMAO), urea, taurine, creatine, free amino acids,

and trace glucose. In addition to psychrotrophic, aerobic, and facultative anaerobic Gram-negative bacteria, such as *Pseudomonas, Moraxella, Acinetobacter, Shewanella putrifaciens, Vibrio, Flavobacterium, Photobacterium,* and *Aeromonas,* Gram-negative bacteria also contribute to fish spoilage.

Table 1. Specific spoilage Organisms in the fish and fishery products:

Storage temperature	Packaging atmosphere	Dominating microflora	Specific spoilage organisms (SSO)
0°C	Aerobic	Gram-negative psychrotrophic, non-fermentative rods *(Pseudomonas spp., S. putrefaciens, Moraxella, Acinetobacter)*	S. *putrefaciens* *Pseudomonas*
0°C	Vacuum	Gram-negative rods; psychrotrophic or with psychrophilic character (S. *putrefaciens, Photobacterium)*	S. *putrefaciens* *P. phosphoreum*
0°C	MAP	Gram-negative fermentative rods with psychrophilic character *(Photobacterium)* Gram-negative non-fermentative psychrotrophic rods (1-10% of flora; *Pseudomonas, S. putrefaciens)* Gram-positive rods (LAB 2)	*P. phosphoreum*
5°C	Aerobic	Gram-negative psychrotrophic rods *(Vibrionaceae, S. putrefaciens)*	*Aeromonas spp.* *S. putrefaciens*
5°C	Vacuum	Gram-negative psychrotrophic rods *(Vibrionaceae, S. putrefaciens)*	*Aeromonas spp.* *S. putrefaciens*
5°C	MAP	Gram-negative psychrotrophic rods *(Vibrionaceae)*	*Aeromonas spp.*
20-30°C	Aerobic	Gram-negative mesophilic fermentative rods *(Vibrionaceae, Enterobacteriaceae)*	Motile *Aeromonas* spp. *(A. hydrophila)*

(Courtesy: FAO fisheries technical paper – 348)

Microorganisms develop spoilage chemicals during the preservation of fresh fish. Bacterial proliferation results in the formation of a slime layer, the darkening of the gills and eyes (in whole fish), and the loss of muscle texture (softened due to proteolysis). The volatile molecules produced by protein putrefaction cause odours such as fishy (due to trimethylamine) and spoilage. Numerous proteolytic and hydrolytic enzymes are produced by *Pseudomonas*

putrifaciens, Pseudomonas fluorescens, and other spoilage bacteria when they proliferate and multiply fast. *Pseudomonas fluorescens* is responsible for fish's greenish-yellow hue, whereas Micrococcus, Bacillus and Sarcina are responsible for the yellow and red hues, respectively. Yeasts and moulds are responsible for the chocolate-brown hue, and Streptomyces for the musty stench.

4.0 Seafood borne pathogens:

In addition to human non-pathogenic bacteria species and the natural microflora of aquatic habitats, pathogenic bacteria are prevalent in fish. According to the European Food Safety Authority, *Campylobacter, Salmonella, Yersinia, E. coli, and Listeria monocytogenes* are responsible for significant foodborne outbreaks across the globe. However, not all bacteria are linked to outbreaks of foodborne illness caused by the eating of contaminated fish and fish products. Meanwhile, *L. monocytogenes, Vibrio spp., Salmonella, Yersinia spp., and C. botulinum* are particularly interested. These pathogens have a broad distribution in aquatic habitats and are associated with significant death rates in people due to illnesses such as listeriosis, botulism, and *V. vulnificus* infection. Thus, along with the nutritional advantages of consuming fish, there is also a possible danger to human health.

5.0 Factors contributing to the risk of seafood-borne diseases:

The increasing demand and consumption of seafood in various countries has led to a heightened susceptibility to bacterial and viral contamination in seafood products. The consumption of seafood may occur at various stages, including primary production, handling, transferring, and preparation. The incidence of disease related to contaminated seafood has significantly risen in the past decade. Consequently, there has been a global increase in awareness regarding seafood-related illnesses due to the growing threat they pose. Seafood-borne outbreaks can arise due to the presence of parasites, bacteria, and viruses. The resulting symptoms can range from mild gastroenteritis to severe, life-threatening infections. Shellfish can be susceptible to contamination by various viruses, including norovirus and hepatitis A virus, as well as bacteria such as *Vibrio spp*., Shigella spp., and *Salmonella spp.* Additionally, protozoan parasites like *Toxoplasma gondii, Cyclospora spp.,* and *Cryptosporidium spp.* have been identified as potential contaminants of shellfish. Additionally, both freshwater and marine environments can harbour various zoonotic pathogens, which can be found in finfish and cephalopods. There have been multiple instances of Vibrio spp. infection documented due to consuming shellfish, specifically oysters. Salmonella outbreaks are frequently linked to

sushi consumption, whereas contamination of smoked mussels, salmon, and other fish has been associated with outbreaks caused by *Listeria spp*. *Vibrio spp*. has been identified as the causative agent in numerous food-borne outbreaks. Disease transmission can occur through zoonotic bacteria, such as Salmonella, which can cause illness in aquatic species and humans. The potential for seafood contamination leading to illness can be classified as either high or low, although there may be differing opinions among authors regarding this categorization. Food products that are considered high risk include mollusks and shellfish, raw and lightly processed fish products, as well as fish products that undergo processing at low temperatures. Seafood options with a low risk factor encompass smoked dried fish, semi-preserved fish, fresh or frozen fish and crustaceans, as well as heat-treated fish that is canned. The consumption of dry and heavily salted fish presents minimal risk of infection or pathogen transmission. Despite the implementation of the Hazard Analysis Critical Control Point (HACCP) system aimed at mitigating seafood-borne diseases, the prevalence of contaminated seafood remains a significant contributor to food-borne infections in the United States. Interestingly, it has been observed that seafood-borne outbreaks are frequently associated with intoxication rather than infection. This can be attributed to the significant number of reported cases of histamine food poisoning. The presence of naturally occurring pathogens such as *Vibrio spp.* and *Aeromonas spp*. in sea water and sediment can pose a significant risk to consumers who consume seafood. This environmental contamination should be considered as an important factor to be addressed. Additionally, there is a potential for inter cross-contamination between different operations.

6.0 Methods for reducing microbial load:

Physical damage, such as scale loss, bruising, and gut bursting, increases the number of sites available for bacterial attack and spread. Furthermore, cortisol levels rise during prolonged stress, affecting fillet quality. After the catch, fish may be held in the vessel for a few hours or weeks in melting ice, cooled brine, or -2 °C saltwater. Inadequate circulation of chilled brines may lead to the localized anaerobic development of specific microbes and spoiling, accompanied by the formation of off-odours. Used refrigerated brines may be polluted with many psychrotrophic spoiling bacteria, and reusing them can enhance the cross-contamination of other fish with these microbes. Increasingly, and mainly when fish is held on board for extended durations, freezing facilities (-18°C) may be employed to preserve the harvest (if possible). Fish may be eviscerated before marine storage, which has pros and cons. Intestinal enzymes and gut bacteria may discolour, degrade, and off-flavour un-eviscerated fish. In eviscerated fish, the incisions reveal microbial-vulnerable flesh. When eviscerating at sea,

remove all stomach contents and wash the corpse before refrigerating, icing, or freezing. Whether to gut the catch at sea depends on its size.

7.0 Practices to control the pathogenic bacteria:

There are several bacterial pathogens that pose a microbiological hazard to seafood, including *Vibrio spp., Salmonella spp., L. monocytogenes, S. aureus, C. botulinum, Shigella spp.* and *Aeromonas spp.* These bacteria have the potential to contaminate seafood products at any stage of the supply chain, from farm to table. It is imperative to implement effective strategies for the control and prevention of bacterial hazards in the fish industry. To minimise bacterial hazards, it is important to maintain the microbiological water quality of domestic capture, practise proper post-harvest care, adhere to good manufacturing practises (GMP), follow good hygienic practises (GHP), and implement HACCP protocols. Foodborne intoxications can be effectively managed through the implementation of appropriate refrigeration practises for seafood and the consistent monitoring of the cold chain throughout the entire production process until consumption. Additional measures to mitigate the occurrence of food-borne outbreaks resulting from the consumption of seafood include educating consumers on appropriate food handling practises, ensuring proper preparation techniques, and implementing effective seafood storage methods. Maintaining regular surveillance is crucial for evaluating the efficacy of both present and future prevention strategies.

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CIFT Machineries in Fish Processing

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Major areas of technological interventions in the field of fishery engineering cover the design and development of fish processing equipment and machinery, energy-efficient and eco-friendly solar fish dryers, fuel-efficient fishing vessels and fibreglass 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, and development of tools and techniques for non-destructive evaluation fish quality and freshness sensor. Focused areas include the development of cost-effective solar dryers with LPG, biomass, Infrared or electrical backup heating systems, fish descaling 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. Solar dryers

Fisherfolks catch fish as major aquatic products to sell in the local market, and in case of over catch tremendous losses occur due to inadequate cold chain management facilities in the developing countries. Alternatively, the fisherman could convert the excess catch of fish into a value-added product *i.e.* dried fish. For example, In India, about 20-30% total catch of fish is dried for export and or local consumption. Drying preserves fish from decay by removing moisture from fish, thereby arresting the growth of bacteria, the action of enzymes, and the chemical oxidation of the fat. Open-air sun drying is the traditional method employed by fisherfolks in India to dry fish and fishery products. It denotes the exposure of a commodity to direct solar radiation and the convective power of the natural wind. This form of energy is free, renewable, and abundant in any part of the world, especially in tropical countries. However, it often results in inferior quality of product due to its dependence on weather conditions and vulnerability to the attack of dust, rain, insects, pests, and microorganisms. Also, it requires a longer drying time (Murali et al. 2019).

Solar drying is an alternative that offers numerous advantages over the traditional method and is environmentally friendly and economically viable in developing countries. In solar drying,

a structure, often of very simple construction, is used to enhance the effect of solar radiation. Compared to sun drying, solar dryers can generate higher air temperatures and consequential lower relative humidity, which are conducive to improved drying rates and lower final moisture content of the products. However, there exist some problems associated with solar drying i.e. reliability of solar radiation during a rainy period or cloudy days and its unavailability during nighttime. To overcome this limitation, an auxiliary heat source and forced convection system are recommended for assuring reliability and better control, respectively.

ICAR-Central Institute of Fisheries Technology (CIFT), Cochin, has already developed low-cost, energy-efficient, and eco-friendly dryers like Solar cabinet dryers, Solar tunnel dryers, Infrared dryers, etc for uniform and hygienic drying of fishes. These dryers are also suitable for drying agricultural products like fruits, vegetables, spices, and condiments.

1.1.Solar-gasifier hybrid dryer (50 kg)

In this dryer, water was utilized as a sensible heat storage (SHS) material as well as heat transfer fluid and biomass gasifier as an indirect backup heat source (Fig 1). In this dryer, during sunny days fish will be dried using solar energy and during off-sunshine hours i.e. cloudy/ rainy days, and night biomass-based gasifier unit will be fired to supplement the heat requirement (Murali et al. 2023). Water is heated with the help of solar vacuum tube collectors installed on the roof of the dryer and circulated through heat exchangers placed in the PUF-insulated stainless steel drying chamber. Thus, continuous drying is possible in this system without spoilage of the highly perishable commodity to obtain a good quality dried product. This dryer is ideal for drying fish, fruits, vegetables, spices, and agro products. This dryer is ideal for drying fish, fruits, vegetables, spices, and agro products. It helps to dry the products faster than open drying in the sun, by keeping the physicochemical qualities like colour, taste, and aroma of the dried food intact and with higher conservation of nutritional value.

Fig. 1. Photograph of Solar-gasifier hybrid dryer developed at CIFT, Kochi

1.2.Solar dryer with LPG backup (50-60 kg)

ICAR-CIFT designed and developed a novel system for drying fish using solar energy supported by environment-friendly LPG backup (Fig. 2). In this dryer during sunny days fish will be dried using solar energy and when solar radiation is not sufficient during cloudy/ rainy days, LPG backup heating system will be automatically actuated to supplement the heat requirement. Water is heated with the help of solar vacuum tube collectors installed on the roof of the dryer and circulated through heat exchangers placed in the PUF-insulated stainless steel drying chamber. Thus, continuous drying is possible in this system without spoilage of the highly perishable commodity to obtain a good quality dried product. A programmable logical controller (PLC) system can be incorporated for automatic control of temperature, humidity, and drying time. Solar drying reduces fuel consumption and can have a significant impact on energy conservation (Murali et al. 2020; Murali et al. 2021).

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

1.3. Solar dryer with electrical backup (20 kg)

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

of the product fetch higher income for the fisherfolk. The eco-friendly solar drying system reduces fuel consumption and can have a significant impact on energy conservation.

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

1.4. Solar dryer with electrical backup (40 kg)

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

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

1.5.Solar tunnel dryer

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

Fig. 5. ICAR-CIFT Solar-tunnel dryer

1.6. Less Emission Biomass dryer (20-30 kg)

The dryer consists of a drying chamber, blower, biomass furnace, and hot air recirculatory system. The capacity of the dryer is 30-40 kg with 10 trays. The tray dimension is 0.9m x 0.45m. The drying chamber dimension is 0.9 m x 0.9 m. The biomass furnace capacity is 25 kg (wood) with the dimension 0.77 m x 1.76 m x 1.42 m. It is provided with a blower of 0.5 hp and an axial fan of 0.25 hp. This dryer is suitable for drying all types of materials including fruits, vegetables, spices, and condiments. It will be highly economical to operate where biomass availability is abundant and free of cost. The cost is approximate Rs.1.5 lakhs.

Fig. 6. ICAR-CIFT Biomass dryer

2. Fish Descaling Machines

2.1.Fish descaling machine with variable drum speed

The fish de-scaling machine is designed and fabricated to remove the scales of fish easily. This equipment can remove scales from almost all types/sizes/ species of fishes ranging from marine to freshwater species like Sardines, Tilapia to Rohu. The machine is made of SS 304 and has a 10 kg capacity (Fig. 7). 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 the area of contact to the surface will be greater for the removal of scales. The water outlet is also provided to remove scales and water from the machine. An Electronic RPM meter was

attached to 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. 7. Fish de-scaling machine with variable drum speed

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

The fish de-scaling machine is designed and fabricated to remove the scales of fish easily. This equipment can remove scales from almost all types/sizes/ species of fishes ranging from marine to freshwater species like Sardines, Tilapia to Rohu. 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. 8). 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.

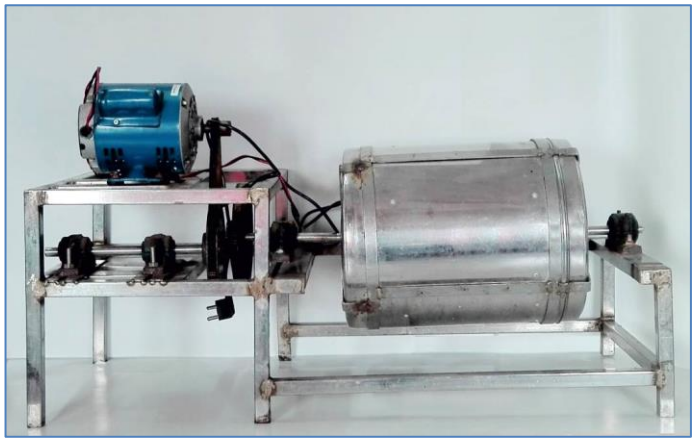

Fig. 8. Fish de-scaling machine with fixed drum speed

2.3. Hand operated Fish descaling machine

The fish descaling machine is designed and fabricated to remove the scales of fish easily. This equipment can remove scales from almost all types/sizes/ species of fishes ranging from marine to freshwater species like Sardine, Tilapia to Rohu (Fig. 9). This machine is made of SS 304 and has a 5 kg capacity. The body is fabricated by 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 on the side to rotate the drum manually (Delfiya et al. 2019).

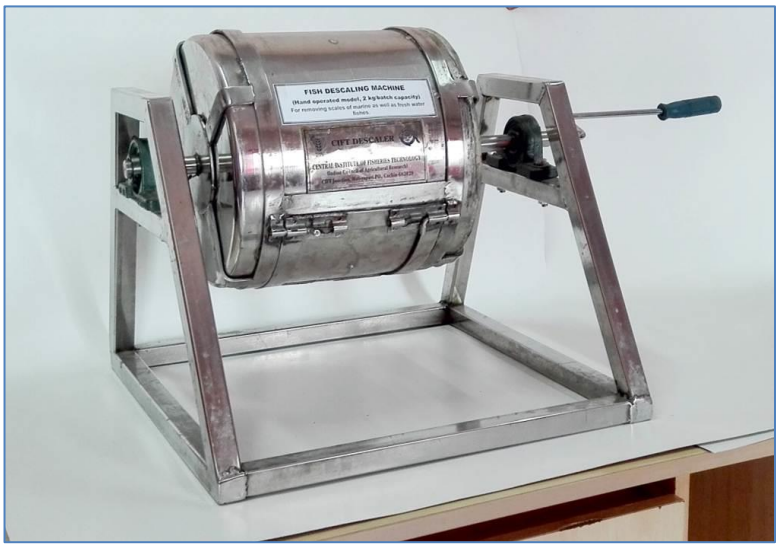

Fig. 9. Hand-operated fish de-scaling machine

3. 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. 9). This can be used at a range of 5-100 rpm. With a unique belt tighten system developed; the new machine can be easily adapted to any species and need not be customized for specimens during the design stage. In existing imported models, only two speeds are possible which restricts the yield efficiency in a single-span operation and also limits easy switching of the system for utilizing specimens other than

for which the yield has been originally customized. The meat yield of this machine was about 60% against 35% in imported models. The capacity of the machine is 100 kg/hour.

Fig. 10. Fish meat bone separator

4. 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's 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 a 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 fish coming out of the de-scaler is free of scales, dirt, or slime. It also reduces human drudgery and avoids cross-contamination, consuming less time. Fish dressing deck with washbasin is also designed conveniently to prepare fresh clean fish under hygienic conditions. The unit also extends the

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. 11. Refrigerated mobile fish vending kiosk

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Process and Product Development using Response Surface Methodology

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1. Introduction

The development of a new or modifying an existing product/process is designed with a purpose to solve/modify an existing problem. In most cases, any product/process development involves tests or experiments, since the product/process is not well understood, and the desired response can't be guaranteed. Experimental design and analysis has been used to improve the performance of product/process given the inherent noise in the various responses of interest. In development of new product/process, research & development groups conduct experiments, develop models and finally optimize the responses related to the performance of the new product/process being developed.

Response surface methodology (RSM) is such a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes. It also has important applications in the design, development and formulation of new products, as well as in the improvement of existing product designs. The most extensive applications of RSM are in the industrial world, particularly in situations where several input variables potentially influence some performance measure or quality characteristic of the product or process.

In general, suppose that the scientist or engineer is concerned with a product, process or system involving a response Y that depends on controllable input factors x_1, x_2,------x_p .The relationship between Y and the x's is defined as

$$Y = f(x_1, x_2, \cdots\cdots\cdots, x_p) + \varepsilon$$

Where the form of the true response function *f* is unknown and perhaps very complicated, and ε is a term that represents other sources of variability not explained or accounted by *f.* Thus ε includes effects such as measurement error on the response, other sources of variation that are inherent in the process or system, the effect of other variables, and so on. We treat ε as a statistical error term with mean zero and constant variance i.e. $\varepsilon \sim N(0, \sigma^2)$, then

$$E(Y) = E\left[f(x_1, x_2, \cdots\cdots\cdots, x_p)\right] + E(\varepsilon)$$

$$= f(x_1, x_2, \cdots\cdots\cdots, x_p)$$

Because the form of the true response function f is unknown, we must approximate it. In fact, successful use of RSM is critically dependent upon the experimenter's ability to develop a suitable approximation for *f*. Usually, a low order polynomial in some relatively small region of the independent variable space is appropriate. In many cases a first order or a second order model is used. The major objectives and applications of RSM are

1. To determine and quantify the relationship between response variables and settings of a group of experimental factors (independent variables) i.e. Mapping a response surface over a particular region of interest
2. To find the settings of experimental factors that produces the best value or the best set of values of the response variables i.e. Optimization of the responses

The major steps involved in RSM to improve an existing process/product or formulation of new product are

1. Formulation of experimental design in terms of independent variables
2. Formulation of hypothesis
3. Execution of experiments and generation of experimental data
4. Development of empirical model to predict the response variables in terms of independent variables
5. Model adequacy checking and testing of hypothesis
6. Optimization of response variables in terms of independent variables

2. Formulation of Experimental Design

Factorial designs are widely used in experiments involving several factors (independent variables) to investigate the main and interaction effects of the factors on response variables. The factorial designs can be classified into two groups viz: symmetrical and asymmetrical factorial experiments. A good response surface design should possess the properties *viz.*, detectability of lack of fit, the ability to sequentially build up designs of increasing order and the use of a relatively modest, if not minimum, number of design points. Examples on some experimental situations, where response surface methodology can be usefully employed are

Example 1: To Optimize the high pressure process parameters viz: pressure, ramp rate and holding time and to see its effect on high pressure treated Indian white prawn. The levels of various factors are

	Factors	**Levels**
1.	Pressure (MPa)	150, 250, 350
2.	Ramp Rate	300, 400, 500
3.	Holding Time(Min)	5, 10, 15

Example 2: For value addition to the agriculture produce, food-processing experiments are being conducted. In these experiments, the major objective of the experimenter is to obtain the optimum combination of levels of several factors that are required for the product. To be specific, suppose that an experiment related to osmotic dehydration of the banana slices is to be conducted to obtain the optimum combination of levels of concentration of sugar solution, solution to sample ratio and temperature of osmosis. The levels of the various factors are the following

	Factors	**Levels**
1.	Concentration of sugar solution	40%, 50%, 60%, 70% and 80%
2.	Solution to sample ratio	1:1, 3:1, 5:1, 7:1and 9:1
3.	Temperature of osmosis	25^0C, 35^0C, 45^0C, 55^0C and 65^0C

In this situation, response surface designs for 3 factors each at five equispaced levels can be used.

In general response surface methodology is useful for all the factorial experiments in agricultural experimental programme that are under taken so as to determine the level at which each of these factors must be set in order to optimize the response in some sense and factors are quantitative in nature.

Examples of experimental design setup for RSM

1. All the factorial experiments where the factors are quantitatively measured
2. Central Composite Design
3. Box-Behnken Design
4. Simplex lattice mixture design
5. Simplex centroid mixture design
6. D-optimal design

3. Development of Empirical Models

In practice the mathematical form of '*f*' discussed in the introduction is not known; we, therefore, often approximate it, within the experimental region, by a polynomial of suitable degree in variables x_{iu} (independent variables). The adequacy of the fitted polynomial is tested through the usual analysis of variance. Polynomials which adequately represent the true input-response relationship are called **Response Surfaces** and the designs that allow the fitting of response surfaces and provide a measure for testing their adequacy are called **response surface designs**. If the function '*f*' is of degree one in $x_{iu}{}'s$ *i.e.* the response can be represented as

$$y_u = \beta_0 + \beta_1 x_{1u} + \beta_2 x_{2u} + ... + \beta_v x_{vu} + e_u$$

And we call it a first-order response surface in $x_1, x_2, ..., x_v$.

The second-order (quadratic) response surface can be represented as

$$y_u = \beta_0 + \sum_{i=1}^{v} \beta_i x_{iu} + \sum_{i=1}^{v} \beta_{ii} x_{iu}^2 + \sum_{i=1}^{v-1} \sum_{i'=i+1}^{v} \beta_{ii'} x_{iu} x_{i'u} + e_u$$

This functional form has many applications in most of the agricultural experiments

The analysis of variance table for a second order response surface design is given below.

Analysis of variance for second order response surface

Source	d.f.	S.S.
Due to regression coefficients	$2v+\binom{v}{2}$	$\hat{b}_0\sum_{u=1}^{N}y_u+\sum_i\hat{b}_i\left(\sum_{u=1}^{N}x_{iu}y_u\right)+\sum_i\hat{b}_{ii}\left(\sum_{u=1}^{N}x_{iu}^2y_u\right)+\sum_{i\neq i'}\sum\hat{b}_{ii'}\left(\sum_{u=1}^{N}x_{iu}x_{i'u}y_u\right)-CF$
Error	$N-2v-\binom{v}{2}-1$	*By subtraction = SSE*
Total	$N-1$	$\sum_{u=1}^{N}y_u^2-CF$

In the above table CF = correction factor = $\frac{(Grand\ Total)^2}{N}$. For testing the lack of fit the sum of squares is obtained using (2.16) and then sum of squares is obtained by subtracting the sum of squares due to pure error from sum of squares due to error. The sum of squares due to lack of fit and sum of squares due to pure error are based on $N'-2v-\binom{v}{2}-1$ and $N-N'$ degrees of freedom respectively.

The lack of fit is tested using the statistic $F=\frac{SS_{LOF}/(N'-p)}{SS_{PE}/(N-N')}$

where N is the total number of observations, N' is the number of distinct treatments and p is the number of terms included in the model. SS_{PE} (sum of squares due to pure error) has been calculated in the following manner: denote the l^{th} observation at the u^{th} design point by y_{lu}, where $l=1,\ldots,r_u\ (\geq 1),\ u=1,\ldots,N'$. Define $\bar{y}_u$ to be average of r_u observations at the u^{th} design point. Then, the sum of squares for pure error is

$$SS_{PE}=\sum_{u=1}^{N'}\sum_{l=1}^{r_u}(y_{lu}-\bar{y}_u)^2 \qquad (2.16)$$

Then sum of squares due to lack of fit (SS_{LOF}) = sum of squares due to error - SS_{PE}

It is suggested that in the experiments conducted to find an optimum combination of levels of several quantitative input factors, at least one level of each of the factors should be higher than the expected optimum. It is also suggested that the optimum combination should be determined from response surface fitting rather than response curve fitting, if the experiment involves two or more than two factors.

4. Optimization of Response

The result of model-building procedure is an equation. Once the model is developed, the next stage is to optimize the process. Different type of optimization methods are

1. Method of steepest ascent/descent
2. Method of graphical evaluation of response surface plot
3. Method of desirability function analysis
4. Method of genetic algorithm

4.1 Method of steepest ascent/descent

The experimental design, model building procedure and sequential experimentation that are used in searching for a region of improved response constitute the method of steepest ascent. The method of steepest ascent contains the following steps to optimize the system in the initial region of $x_1,x_2,-------,x_p$

1. Fit a first order model (a plane or hyperplane) using either factorial or response surface design
2. Compute a path of steepest ascent if maximizing the response or steepest descent if minimizing the response. The path of steepest ascent is computed to get maximum increase in the response and path of steepest descent is computed to get maximum decrease in the response.
3. Conduct the experimental runs along the path. That is do either single response or replicated runs, and observe the response value and the result will normally show improving values of the response. At some region along the path the improvement will decline and eventually disappear.

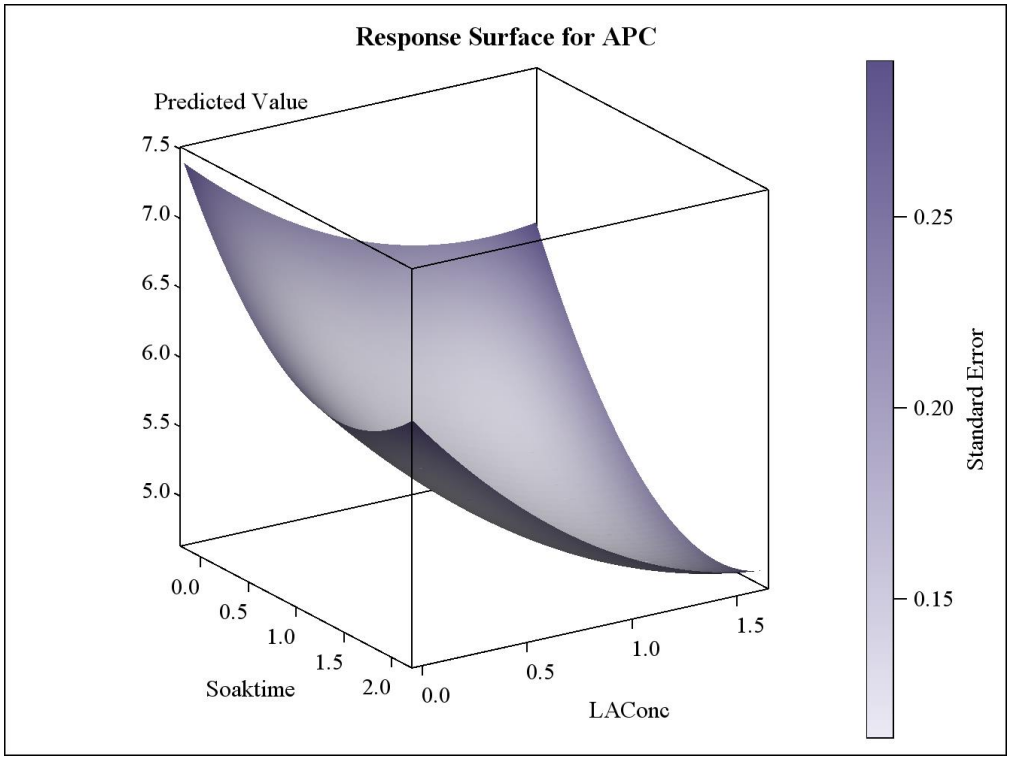
4. At point where an approximation of the maximum (or minimum) response is located on the path, a base for second experiment is chosen.

5. A second experiment is conducted, and another first order model is fitted to the data. A test of lack of fit is made. If the lack of fit is not significant, a second path based on the new model is computed. This is often called a mid-course correction. It is quite likely that the improvement will not be as strong as that enjoyed in the first path. After improvement is diminished, one typically has a base for conducting a more elaborate experiment and a more sophisticated process optimization.

4.2 Method of graphical evaluation of response surface plot

Evaluation of three dimensional response surface plot will help to identify the optimum values of input factors (say x_1 and x_2) that maximizes (minimizes) the predicted values of the response. Here, x_1 takes on X axis, x_2 takes on Y axis and predicted values of response variables takes on Z axis.

Example: An experiment was conducted to optimize the Lactic acid concentration and soaking time to minimize the APC in Tuna chunk. The second order response surface plot is given below. From the graph, it can be inferred that the minimum APC was observed at higher levels of Lactic acid concentration and soaking time.



4.3 Method of desirability function analysis

The desirability function approach is one of the most widely used methods in industry for the optimization of multiple response processes. It is based on the idea that the "quality" of a product or process that has multiple quality characteristics, with one of them outside of some "desired" limits, is completely unacceptable. The method finds operating conditions x that provide the "most desirable" response values. For each response $\boldsymbol{Y_i(x)}$, a desirability function $\boldsymbol{d_i(Y_i)}$ assigns numbers between 0 and 1 to the possible values of $\boldsymbol{Y_i}$, with $\boldsymbol{d_i(Y_i) = 0}$ representing a completely undesirable value of $\boldsymbol{Y_i}$ and $\boldsymbol{d_i(Y_i) = 1}$ representing a completely desirable or ideal response value.

A desirability function is a useful approach to optimize for simultaneous optimization of multiple responses. The general approach is to first convert each response y_i into an individual desirability function d_i that varies over the range $0 \leq d_i \leq 1$

Where if the response y_i is at its goal or target, then $d_i=1$, and if the response outside an acceptable region $d_i=0$. Then the design variables (input variables) are chosen to maximize the overall desirability$D = (d_1 d_2 \cdots\cdots\cdots d_m)^{1/m}$, where there are m responses

If the objective or target T for the response y is a maximum value,

$$d = \begin{cases} 0 & y < L \\ \left(\dfrac{y-L}{T-L}\right)^r & L \leq y \leq T \\ 1 & y > T \end{cases}$$

When the weight r=1, the desirability function is linear. Choosing r>1 places more emphasis on being close to the target value, and choosing 0< r <1 makes this less important. If the target for the response is a minimum value,

$$d = \begin{cases} 0 & y < T \\ \left(\dfrac{U-y}{U-T}\right)^r & T \leq y \leq U \\ 1 & y > U \end{cases}$$

The two sided desirability function when the target is located between lower (L) and upper (U) limits is defined

$$d = \begin{cases} 0 & y < L \\ \left(\frac{y-L}{T-L}\right)^{r_1} & L \leq y \leq T \\ \left(\frac{U-y}{U-T}\right)^{r_2} & T \leq y \leq U \\ 0 & y > U \end{cases}$$

4.4 Method of genetic algorithm

GA is an optimization technique based on the principles of genetics and natural selection. Genetic operators, such as selection, crossover, and mutation, are applied to repressor settings while searching for the optimum. In a GA, a search point, a setting in the search space, is coded into a string which is analogous to a chromosome in biological systems. The string/chromosome is composed of characters which are analogous to genes. In a response surface application, the chromosome corresponds to a particular setting of k factors (or regressors), denoted by $x = [x_1, x_2, ..., x_k]'$, in the design space and i^{th} gene in the chromosome corresponds to a x_i, the value of the i^{th} regressor.

Suggested Readings:

- Box, G.E.P. and Draper, N.R. (1987). *Empirical model building and response surfaces*. New York, Wiley.
- Khuri, A.I. and Cornell, J.A. (1987). *Response Surfaces: Designs and Analysis*. New York: Marcel Dekker.
- Myers, R.H. and Montgomery, D.C. (1995). *Response Surfaces Methodology: Process and product optimization using designated experiments.* John Wiley and Sons.

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