QUALITY ISSUES ASSOCIATED WITH FISHERY BY-PRODUCTS

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While cutting and pre processing of fish, parts such as head, bones, frames, tails, skin and viscera, are considered as waste and of low value. Such parts of the fish which are considered to be by-products which may constitute as much as 70 percent of the fish depending on the species of fish and the final product envisaged. Most of the time accumulation of fish waste is an environmental problem, but scientific intervention on the use of these by-products for human/animal consumption would improve the nutritional value of the diet, could reduce waste generated by fish processing and provide greater economic sustainability for fish processors.

The shortage of fishery resources calls for the development and adoption of new technological processes for better utilization of waste and by-products from fish processing activities. Some quantity these by-products are currently used as raw materials for animal feed, as fish meal or as fertilizer. It is estimated that their utilization in human foodstuffs, nutraceuticals, pharmaceuticals, or cosmetics would increase their value many folds. It has been estimated that apart from the quality losses in the supply chain, worldwide, around 130 million tonnes of fish waste is produced each year. Globally around 17.9 to 39.5 million tonnes of whole fish is discarded each year by commercial fishing operations. The value of loss through wastage could be as large as US$ 50 billion each year due to poor management of seafood resources. The strict environmental regulations for the disposal of fish processing waste add to the operational cost of seafood industry. The recovery of compounds from fish processing wastes would serve the dual purpose of obtaining these valuable biomolecules as well as controlling the environmental pollution problems associated with the disposal.

The remains of the fish are commonly called by-products and if treated correctly, classified as category 3 by-products according to EU regulation, meaning parts of animals that are fit for, but not intended for human consumption (EC No 1774/2002). An essential step in up-grading by-products to co-products for human consumption is that systems such as Good Manufacturing Practice (GMP) and the Hazard Analysis and Critical Control Point (HACCP) used in food production, are applied.

The diversity of species processed and the heterogeneity of the waste generated during the cutting operations has manifold implications. Low value realisation of fish waste due to non adaption of various technologies is the key factor on quality issues of fish waste as a raw material. In fact, the quality issues of any product derived from fish waste relate to the quality of raw material. Diversity and mixing up of waste, unscientific handling leading to oxidation and bacterial degradation are the major issues related to raw material.

By products derived from fishery discards

Product Category	Potential Products
Edible products	Fish bone broth/soup, Fish head stock, Products from recovered meat from waste
Industrial products	Fish oil, Fish meal
Bio-functional/ health products	Chitin derivatives, Collagen derivatives, Fish protein hydrolysate, Glucosamine, Chondroitin sulphate, Squalene, Sulphated polysaccharides, Hyaluronic acid, Fish bone calcium, Collagen surgical sutures
Feed and fertiliser	Fish silage, fish meal, fish manure, compost, foliar spray

Fish meal

In most animal diets, protein is the most expensive portion and is usually the first nutrient that is computed in diet formulation. Fish meal is produced by removal of 90 to 95% of water and fat present in the raw material which is highly concentrated nutritious feed supplement consisting of high quality protein, minerals, vitamins of B group and other vitamins, essential minerals, namely phosphorus, calcium and iron and other unknown growth factors. When fatty fish is used for making fish meal; which is carried out normally by wet rendering process, fish oil is also produced simultaneously.

Table Specifications for different grade fish meal

		Grade 1	Grade 2
1	Moisture% by mass maximum	10	10

2	Crude Protein% by mass minimum	60	50
3	Ammoniacal nitrogen% by mass Maximum	0.5	0.5
4	Crude Fat or Petroleum Ether Extract% by mass maximum	10	10
5	Acid Insoluble Ash% by mass maximum	3	3
6	Chloride (as NaCl)% by mass max.	4	5

Presence oil in excessive level in the fish meal is considered as disadvantage due to a possible danger of imparting off-flavour to animal products. However, a total content of fish oil of 1% or less in the feed mix, no harm will be off to poultry products. In excess level, the oily residue of the meal gradually oxidizes on storage. During this reaction the iodine value decreases from 130 to 90-95 units, the oil becomes less readily soluble

During this oxidation, a certain amount of fatty acids, peroxides and hydroperoxides are formed, which may be considered harmful. Freshly produced meal contains about 75 units of peroxides. The value decreases rapidly on storage and becomes the peroxide value insignificant.

Oxidation of fat present in the meal has also been identified as the cause of spontaneous heating which may cause danger during shipments and/or storage. Even when no direct fire hazard existed, the quality of the meal is seriously jeopardized. And such oxidative heating is responsible for much inferior quality meal. Fish meal can be stabilised by means of incorporation of antioxidants ethoxyquin or BHT immediately after manufacture.

Fish oil

Fish oil is a by-product obtained during fish meal production and then subjected through various steps in order to yield the final product. The oils contain mainly triglycerides of fatty acids (glycerol combined with three similar or different acid molecules) with variable amounts of phospholipids, glycerol ethers and wax esters. They are imparting positive effect in powerful metabolic and physiological regulators, which also influence the excessive fat deposition in the arteries. Fish body oil of fish is more important as an industrial product besides its limited use in human consumption. It Contains poly unsaturated fatty acids (PUFA), particularly n-3 PUFA n-3 PUFA which has beneficial effect in controlling heart ailments in humans. Fish oil is has use as carriers of fat soluble vitamins A and D.

Sardine oil is prepared from fresh or well preserved or frozen sound wholesome sardine fish either whole or dressed body portion. It is prepared by cooking, pressing and separating oil from press liquor by centrifugation or by any other suitable means. The requirements sardine oil includes: shall be free from foreign matters in settled or suspended condition, and separated water. The product shall be a bright and clear liquid when heated to a temperature of 40°C. (ii) it shall be free from any other kind of oil including mineral oils. It shall be free from foul and offensive putrefactive odour and should have only characteristic fish- oil odour. (iii) it shall be of greenish straw light golden yellow or light brown colour. (iv) product shall also conform to the requirement given in table below:

FSSAI requirements for sardine oil

Sr. No.	Characteristics	Requirements
1.	Free faty acids as percent oleic acid, w/w, max	1.0
2.	Moisture, percent by weight, max	0.5
3.	Iodine Value	145-180
4.	Saponification value	185-205
5.	Unsaponifiable matter, percent, w/w, max	2.0
6.	Refractive Index (40°C)	1.4739-1.47

Oxidative deterioration of fish oil

Due to its high content of polyunsaturated fatty acids, including EPA and DHA, fish oils are highly susceptible to oxidative spoilage and the rate of fish oil oxidation is significantly different from that of other oils. Various factors govern the oxidative reactions that occur at centres of unsaturation. Certain metals, visible light and light of shorter wavelengths, some oxidative enzymes, and other biological substances, accelerate oxidative deterioration. During the autoxidation of fish oils, undesirable flavours and odours develop at very low peroxide values at an early stage of oxidation, even during the induction period. A large number of saturated and unsaturated aldehydes, ketones, acids, and other products have been isolated from oxidised oils. Oxidation of lipids not only produces rancid odours and flavours, but also can decrease nutritional quality and safety by the formation of secondary products The products of lipid oxidation are known to be health hazards since they are associated with aging, membrane damage, heart disease and cancer

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

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

Undesirable substance	**Product**	**Maximum content in mg/ kg (ppm) relative to a feeding stuff with a moisture content of 12 %**
Arsenic	Feeding stuffs obtained from the processing of fish or other marine animals	15
Lead	Feeding stuffs obtained from the processing of fish or other marine animals	10
Fluorine	Feeding stuffs of animal origin with the exception of marine crustaceans such as marine krill	500
	marine crustaceans such as marine krill	3000
	calcareaous marine algae	1000
Mercury	Feeding stuffs produced by the processing of fish or other marine animals	0.5
Nitrite	Fishmeal	60 (expressed as sodium nitrite)

Cadmium	Feed materials of animal origin	2
Aflatoxin	complete feeding stuff	0.01
Dieldrin	Fish Feed	0.02
Camphechlor (toxaphene) —sumof indicator congeners CHB 26, 50 and 62	Fish, other aquatic animals, their products and by-products with the exception of fish oil	0.02
	Fish oil	0.2
	Feedingstuffs for fish	0.05
Chlordane	All feedingstuff	0.02
DDT (sum of DDT-, TDE- and DDE isomers, expressed as DDT)	All feedingstuff	0.05
Endosulfan	complete feedingstuffs for fish	0.005
Endrin	All feedingstuff	0.01
Heptachlor	All feedingstuff	0.01
HCB	All feedingstuff	0.01
HCH Alpha isomer Beta isomer gamma isomer	All feedingstuff	 0.02 0.01 0.2

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

Foodstuffs	**Maximum level**		
	Sum of dioxins (WHO-PCDD/F-TEQ)	Sum of dioxins and dioxin-like PCBS (WHO-PCDD/F-PCB-TEQ)	Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6)
Marine oils (fish body oil, fish liver oil and oils of other marine organisms	1.75 pg/g fat	6.0 pg/g fat	200 ng/g fat

intended for human consumption)			
Fish liver and derived products thereof with the exception of marine oils		20.0 pg/g wet weight	200 ng/g wet weight

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

Chitin and chitosan

Chitin is the second most abundant natural polymer available next to cellulose. It is composed of units of the amino sugar N acetyl glucosamine. It is the main source for the production of chitosan. In India, the major sources for chitin and chitosan is processing wastes of crustaceans like, shrimp, crab, lobster and squilla. Shrimp processing industries around the world turn out enormous quantity of head and shell as industrial waste. The head and shell constitute nearly 60% by weight of the whole prawn depending on the species and size of the prawn. In India its availability is estimated to be 100,000 tons annually and it is the single largest fishery waste of the country. Crab shell is yet another waste thrown out in large quantities from seafood processing centers. At present only a small portion of this finds use as ingredient in shrimp/poultry feed mix. The industry finds it extremely difficult to properly dispose of the same. Many a time this waste poses a problem of environmental pollution also.

Specification

In commerce chitin and chitosan with the following characteristics are acceptable to the end users.

Characteristics	**CHITIN**	**CHITOSAN**
Moisture %	<10	<7
Ash %	<2	<1
Protein %	<2	nil
Colour	off white	off white

Particle size	10-20 mesh	60-80 mesh
Solubility in 1% acetic acid	nil	soluble
Insolubles in 1% acetic acid	N.A	<0.5
pH	7.0-7.5	8-9
Nitrogen %	6.5-6.8	7-7.5
Deacetylation %	N.A	>80
Viscosity (m pa s) in 1% acetic acid at 1% level at 28°C	N.A	<100

Silage

Fish silage may be defined as a liquid product, made from whole or parts of fish, to which no material has been added other than acid and in which liquefaction is carried out by enzymes already present in the fish. Fish silage is a nutritionally balanced diet extensively used in feeds in combination with other ingredients. It is found to be superior to other protein diet of plant or animal origin. The nutritional composition of fish silage is almost similar to fish except a slight increase in moisture content.

Ensiling can be achieved either by treating the fish directly with a mineral acid or organic acid or by lactic acid produced *in situ* by fermentation. The fish is partially digested and preserved by the acid. The most commonly used organic acids are propionic, acetic and formic acids. A 3% by weight of 98% formic acid is added to the well ground fish mince and mixed well ensuring a pH around 4 to prepare acid fish silage using organic acid. The whole fish/waste is comminated in a mechanical mincer and the required quantity of acid or acid mixture is added and the slurry is mixed well. After this process the whole material becomes a good paste that can be stored in tanks with daily stirring. Within 15-20 days the silage is ready for use.

Lipid Oxidation

Unsaturated long chain fatty acids released from fish by lipid hydrolysis by lipases absorb oxygen and undergo rapid auto-oxidation, releasing a large number of volatile carbonyls and making the silage rancid. The rate of lipid oxidation is directly related to exposure to sunlight, presence of pro-oxidants, concentration of heavy metals, temperature, and other factors. Oxidised lipids are responsible for the poor nutritional quality of the silage. Hence, silage produced from fatty fish has shorter shelf-life than one produced from lean fish. Addition of antioxidants like BHA, BHT and ethoxyquin can substantially retard the development of rancidity but are seldom added in silage.

Fish silage has an inherent defect, its liquid consistency, which makes it difficult to transport to distant places and to store. Feeding experiments in India (Anon, 1972-78) showed that it was extremely difficult to convince the farmers who rear poultry, pigs and cows about the efficiency of fish silage as a protein supplement because of this disadvantage. To overcome this problem, a solid feed mix was compounded out of boiled fish silage and rice bran powder in the ratio 21% protein. It is easily transported and has extended shelf-life at ambient temperatures in the tropics. The rice bran contains all vitamins, particularly the B group, and many other micronutrients required for animals.

Fish Protein Hydrolysate

Protein hydrolysates are the breakdown products of proteins viz., smaller peptide chains with 2-20 amino acids obtained by hydrolysis either chemically or enzymatically. This process facilitates recovery of essential nutrients viz., amino acids as well as has immense scope in food, nutraceutical and pharmaceutical industry on account of the excellent physicochemical, functional as well as bioactive properties they possess. Based on the extent of hydrolysis that the parent protein undergoes, the properties exhibited by the hydrolysates vary considerably.

Although enzymatic hydrolysis of proteins develops desirable functional properties, it has the disadvantage of generating bitterness which is identified as a major hindrance regarding utilization and commercialization of bioactive. The mechanism of bitterness is not very clear, but it has been documented to be associated with the presence of bitter peptides mainly comprising hydrophobic amino acids. In addition to hydrophobicity of peptides, peptide length, amino acid sequence and spatial structure also influences the perception of bitter taste

Strict control of any hydrolysis experiment and termination at low degree of hydrolysis is a common and desirable method to prevent the development of a bitter taste and the retention of functional properties

As enzymes have different preferences for amino acids, choosing the most appropriate enzyme is the most widespread methodology for reducing bitterness. Enzymes with a high preference for hydrophobic amino acids such as alcalase are often preferred and frequently yield products of low bitterness

Oxidation products play a part in the development of bitter taste. A few suggested methods for bitterness reduction include treating hydrolysates with activated carbon that partly removes bitter peptides with absorption, extracting bitter peptides with solvents and by plastein reaction which is the formation of a gel-like proteinaceous substance from a concentrated protein hydrolysate. Masking can be performed by adding additives or molecules, e.g. cyclodextrin, to the hydrolysate to mask the bitter taste.

Although the specific health benefits from different hydrolysates are mostly supportable scientifically, the consistency of these benefits is debatable on account of the quality changes during storage on account of its hygroscopicity and other biochemical changes. Encapsulation may be adopted as an effective technique in this regard to improve the stability and delivery of these sensitive bioactive components by selection of suitable wall material.

Economic feasibility in upscaling is a major constraint with regard to the hydrolysate production. This can be effectively addressed by following standard protocols by using suitable inputs like raw material, enzymes, other hydrolytic conditions like time, temperature etc.

Surveillance and Monitoring

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

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

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

Gelatin

Recently fish skin, bone and scales has been identified as an important source for collagen and its derivatives. Evnthough there are many uses for gelatin in the food and pharmaceutical industries, apprehensions on the problems like allergenicity and odour development are to be addressed. Compared to bovine and porcine origin, the market share of fish gelatin/collagen/collagen peptide is still considered very small. Some limiting factors that hamper the large-scale development of the fish gelatin and collagen/collagen peptide industry include absence of internal quality assurance system, the difficulty to obtain certification on fish raw material etc. Certification is required for traceability, which has become an essential requirement for food additives, especially from animal sources. The intrinsic quality factors (such as odor, color, bloom strength, and viscosity of fish gelatin and collagen/collagen peptide) and quality variations from batch to batch and from species to species pose another major hindrance to the development of fish gelatin/collagen industry. The technical difficulties associated with producing fish gelatin and collagen/collagen peptide for human consumption generally surround the elimination of the unpleasant fish odor from the product. Persisting residual odor in fish gelatin and collagen can cause problems especially when it is intended for direct use in mildly flavored products and in cosmetic products. In cases, the product is odor free when produced, but when formulated into other products, the odor returns with generation of off-flavors

The allergenic activity resides in the meat of the certain fish, but recently concern has been raised as to whether products such as fish gelatin, which is derived from skin and bones, also may possess an allergenic potential. Recent interest in the labelling of foods derived from allergenic sources necessitates determination of the potential allergenicity of such food ingredients and pharmaceuticals including fish gelatin and collagen. The major allergen parvalbumin was purified from cod muscle tissues, and polyclonal antibodies were raised towards it. Washing of the skins, a common industrial procedure during the manufacturing of fish gelatin, reduced the level of parvalbumin about 1000-fold to 0.5 ppm.

There is an increasing concern about the heavy metal content of gelatin and collagen extracted from fish scale and fish skin. Heavy metals are considered as one of the most critical contaminants of the aquatic ecosystem because of their potential to enter water bodies and also their bioaccumulation and biomagnification in the food chain. Fish are commonly situated at the top of the food chain and are considered as a susceptible aquatic organism to toxics present in water. As regards heavy metals, fish gelatin/collagen shall not contain Arsenic in excess of 2 ppm, zinc in excess of 20 ppm, copper in exces of 30 ppm, and other heavy metals in excess of 50 ppm, chromium 10 (ppm) and lead 5ppm.

Presence of excess amount of heavy metals has also been reported from gelatin manufactured which needs serious attendtion in selecting the raw material. Even though some washing steps involved in gelatin/collagen manufacture can substantially reduce the pesticide incidence in the final product, there were several export rejections in the country on account of pesticide detection in the final product.

Even though fishery by products are utilised for the production of industrially and pharmaceutically important products, there are quite lot of quality issues specific to each product. Certification of the raw material, following of GMP and HACCP practices etc can reduce many of the quality issues with regard to such products.

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