

Review

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Review

Fishery Products Safety, Processing and Utilization

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Abstract: Global marine fish harvest has reached a plateau over the last decade. Efforts to increase aquaculture tend to face limitations in water resources and contamination problems. Of the current fish harvest at least 50% is discarded as waste. The chemical and microbiological contaminations limit utilization of harvested fish. There is a need to improve fish preservation to minimize spoilage, and process them into more appealing products. Instead of resorting to individual food processing methods, the efficiency of processing could best be increased by combination of conventional and modern processing methods, or combinations of modern processing methods. Fish waste is a rich source of oils containing essential fatty acids, polypeptides, and amino polysaccharides that could be utilized through upscaling of the current scientifically proven methods, to new processing technologies. Separation of collagens, gelatins, bioactive peptides, edible fish oils, and chitosan form the primary stages in utilization of fish waste. The products need purification to meet food quality and safety standards, and desirable industrial characteristics. The diversity of information and products generated through new methods requires advanced data handling and prediction systems, such as artificial intelligence, to address food safety and get the best out of fish processing and utilization.

Keywords: fish spoilage; safety of food-fish; fish processing; waste utilization; novel food additives and pharmaceuticals; artificial intelligence

Introduction

Fish are a large group of vertebrates living in marine and freshwater environments. They consist of approximately 34,000 species. Fish and fish products form a valuable component of the human diet as a source of essential amino acids, highly unsaturated fatty acids, vitamin A rich oils, and micronutrients [1]. The presence of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) make fatty fish healthy foods, with capacity to prevent cardiovascular diseases [2]. Fish are harvested as a natural product or a farmed product from the marine and inland aquatic sources.

The total global marine fish capture has remained between 78 to 84 million tons of live weight from 1990 to 2020 with no signs of increased capture, suggesting the needs for more efficient utilization of the marine fish harvest at current level. The total capture of inland fisheries remained 11-12 million tons between 2010 to 2020 indicating the limitations that may arise on availability of fish in the future [3]. Sustainability issues due to overfishing in the natural fisheries are bound to occur, unless fish production is increased expanding into more inland waters, or scientifically increase the marine fish production. China and India play a major role in increasing the inland fisheries, with some African countries joining production. There are efforts to increase fish production through marine farming, with high yielding and fast-growing species to meet the global demand, and through genetic engineering of fish muscles [4,5]. The post-harvest loss of fish due to quality and safety problems is around 35% with a range of 20-75% [6]. Reducing the losses is one approach to ensure meeting global food-fish demand. With 89% of the edible quality food-fish consumed by humans in 2020, there is 11 % diverted mainly for feed. Of the total harvest, about 40% is lost as of non-edible components [3]. Amidst the efforts to increase the harvest and farming of fish, both marine and inland, there is room for more effective utilization of fish and fish products through improved processing, preservation, and converting of hitherto unused fish components to foods. Effective utilization requires technologies to convert less attractive fish components into new foods,

carrying appealing qualities to the consumer. Meeting the future global food targets requires reduction of waste and generation of new processed fish products.

In natural and farming systems, fish tend to get exposed to contaminants in the water, impairing product safety. The contaminants may be microorganisms proliferating in the environment, or chemicals entering the waters naturally or due to anthropogenic activities. Foodborne illnesses associated with fish during 2011 to 2018 are reported to be 6-8 % against 1.6% for beef, and 3.6% for chicken, as reported in 2018 by the Center for Disease Control and Prevention, USA [7]. Environmental stresses and contaminants reduce the sensory qualities of fish muscles, lowering the food values due to lipid oxidation, breakdown of proteins, and other unexpected biochemical reactions [8]. Improved processing to ensure food-fish safety is continuously addressed through research. Microbial contaminations increase perishability of fish, further reducing the food value through biochemical changes in fish muscles, unless preserved or processed [7]. In domestic handling, only 50 - 60% of the fish components, consisting mainly of the edible muscles, are used and the rest is discarded. The challenge is to preserve the captured fish, minimizing spoilage and extending their shelf-life. The fish waste accounting for up to 50% losses could be converted to novel products through value addition [9]. Processing of fish waste carries a high potential to meet increasing food demands and reach profits in the fish industry. Utilizing the fish waste requires new management strategies that would contribute to effective environment mitigation too. Waste utilization is an important step in sustainable circular economies, a need identified in the current goals of the United Nations policies. There is much research in the recent decades for better utilization of the fish muscles, and currently unutilized components in fish, by converting to commercial products carrying sensory attributes preferred by the consumers. This review examines the new avenues for making the fish safe for human consumption, processing and preserving to retain the quality, and utilization of fish waste by converting them to rich nutrient sources.

2. Safety of Fish and Fishery Products

Fish become unsafe for human consumption due to natural and man-made activities. The causes could mainly be of microbial or chemical origin. The changing microbial population in the environment affects the microbiota of fish prior to harvest. High vulnerability of fish to microbial activity coupled with natural degradation of muscles at post-harvest, increase the food safety hazards from fish.

2.1. Autolysis of Fish Components

Perishability of fish begins with the natural degradation of the muscles after harvest, due to autolysis by endogenous enzymes and chemical oxidation of lipids [10]. The high-water activity of 0.98 – 0.99 makes fish muscles vulnerable to microbial growth, and release of harmful microbial metabolites. During autolysis, the odor, flavor, and texture in the fish muscles change, due to reactions triggered by endogenous enzymes. The dominance of spoilage bacteria in fish is reported to occur even before the muscles become unacceptable sensorily, with each species showing specific and predominant degradative biochemical reactions [11]. The outcome of autolysis and oxidation producing unappealing constituents in fish is summarized in Table 1.

Table 1. Unappealing constituents and properties caused during autolysis of fish components [10,12,13].

Fish component	Degrading enzyme / catalyst	Outcome
Glycogen	Glycolytic enzymes	Lactic acid and reduced pH
Proteins and peptides	Chymotrypsin, trypsin, calpains cathepsins, carboxypeptidases	Amino acids and softening of tissues, belly burst
Collagen and connective tissues	Collagenases	Proteins, glycopeptides and softening of tissues

Nucleotides	Nucleases	Purines, pyrimidines, hypoxanthine
Trimethylamine oxide	TMAO demethylase	Amines, formaldehyde
Lipids	Triacyl Lipases, phospholipases, lipoxygenases, peroxidases	Free fatty acids, glycerol, oxides, peroxides
Lipids	Light UV, copper, iron catalyzed	Hydroperoxides, aldehydes, ketones, alcohols

Some of the metabolites produced during fish spoilage serve as substrates for easy growth of pathogenic microorganisms or toxin producers, rendering food-fish unsafe.

2.2. Microbial Food Safety Hazards

The pathogenic microorganisms are present in the marine environment to a lesser extent due to saline environment, than in inland reservoirs and waterways. However, the pathogens tend to grow more commonly in the coastal waters of the sea supported by the nutrients discharged by the inland waterways. The common microorganisms associated with the pre-harvest and post-harvest environment of fish, and the toxins produced by them rendering the fish unsafe as a food are presented in Figure 1.

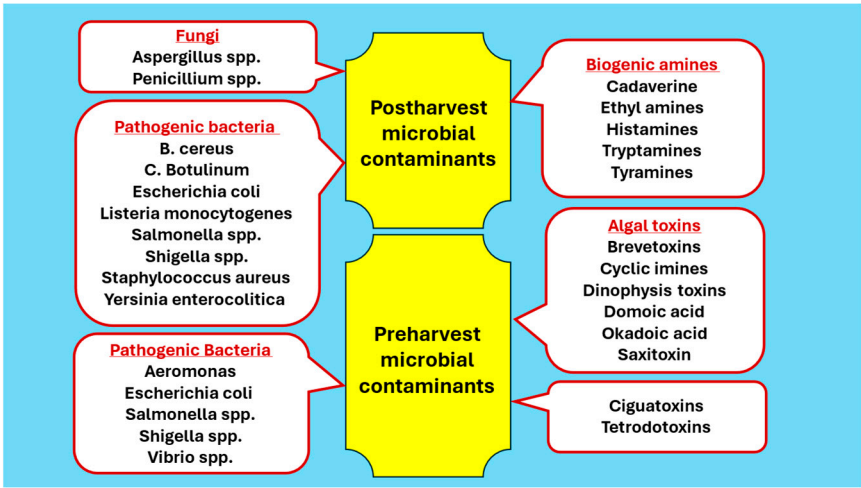


Figure 1. Pre-harvest and post-harvest microorganisms and toxins render the fish unsafe as food.

Symbiosis among the spoilage bacteria in fish appears to enhance spoilage and spread of pathogenic bacteria. The spoilage bacteria appear to communicate with each other through signaling mechanisms during fish spoilage for mutual benefits [13]. Food safety hazards and quality changes in fish and fish products arising from biotoxins due to algal blooms in the oceans [14,15], diatoms releasing toxins [16], diarrhetic shellfish poisoning and azidinium poisoning [17], paralytic shellfish poisoning [14], ciguatoxin poisoning [18–21], and clupeotoxin [22] are reported among incidences of pre-harvest contamination of fish from different parts of the globe. FAO [3] has observed increasing appearance of the hazardous biotoxins from the tropical regions, predominantly from the upwelling regions of the Indian Ocean. The increased incidences from biotoxins are believed to be associated with increased contaminations in the coastal areas, due to discharge of nutrient rich pollutants to the seas. The increase of food safety hazards through fish associated with harmful algal blooms (HAB) is beyond control. Avoiding fishing in the infected marine locations practiced currently in temperate regions, prevent exposure of humans to biotoxins. Spreading biotoxin production into new fishing zones is a challenge beyond control, especially considering seasonal changes, global warming, and oceanic currents. The movement of ships and discharge of ballast water add to the spread of biotoxins

[23]. Preventive control could be implemented based on digital satellite imaging of the ocean surfaces to recognize spread of algal blooms [16].

While the biotoxin contamination of fish is regional, the contamination by pathogenic microorganisms tends to be more local, triggered by coastal contaminations. Pre-harvest contaminants *Vibrio parahaemolyticus* producing virulent thermostable hemolysins, have been reported in shellfish from marine harvests in Italy [24], in mussels and oysters from commercial shellfish farms in New Zealand [25], in retail shrimps in Malaysia [26], and shrimp and fish from retail markets in North China [27], among other reports. There is evidence of the increased spread of *V. parahaemolyticus* with summer temperatures. *Vibrio cholerae* producing exotoxins and a blood coagulation factor, is detected in marine environments [28,29]. *V. cholerae* is reported to be initially of marine origin, later adapted to freshwater environments [30]. *V. cholerae* is therefore more associated with fish from rivers and reservoirs undergoing eutrophication. However, capability of *V. cholerae* to contaminate fish in coastal areas cannot be ruled out. *V. cholerae* forms biofilms on fish skins pre-harvest, getting detected later as a post-harvest contaminant. In a rare situation, *Campylobacter jejuni* has been reported pre-harvest in marine fish from Kerala in India [31]. *Aeromonas* producing enterotoxins and hemolysis are associated more with locations of seawater mixing with river waters [32]. *Aeromonas* sp. are of low food safety risk.

Microbial pathogens of pre-harvest origin in fish may also appear as post-harvest pathogens, since they are carried with the harvest into the markets. *Escherichia coli*, *Salmonella* species, *S. aureus*, *C. botulinum*, *L. monocytogenes*, *Shigella* species, *B. cereus*, *C. jejuni*, *Y. enterocolitica* and histamine producers are identified as common post-harvest pathogens affecting fish [33,34]. *E. coli*, being a common bacterium of fecal origin, tends to be present more often with poorly handled fish. *Salmonella* species and *Staphylococcus aureus* originating from human contacts at handling, are found more frequently than other pathogens in market food-fish. Spoilage of fish and food safety hazards originating from post-harvest microbiological contaminations is reviewed by Sheng and Wang [7]. The authors have identified separately the contaminations originating at handling, storage, and transport of harvested fish. High genotypical and phenotypical diversity of *Escherichia coli* has made it possible for the bacteria to contaminate fish in marine and freshwater environments pre-harvest and appear in the market products as post-harvest contaminants releasing enterotoxins and Shiga-toxins [35–37]. Though *Salmonella* is predominantly a freshwater microorganism, its presence in coastal waters, and appearing as a post-harvest contaminant after pre-harvest entry on to fish is noted. *Salmonella* could be present in high populations in fish, though the populations are low in seawater [38]. *Clostridium* is mostly a post-harvest contaminant capable of withstanding thermal processing below 100 °C causing food safety hazards through canned fish [39].

Listeria monocytogenes is commonly present in food processing facilities, and it survives through a wide temperature range, high salt content, and wide pH range posing problems with post-harvest fish handling and processing [40]. *Listeria* is reported in fish pastes and ready to consume smoked fish [41]. Food safety hazards from *Listeria* in fish and fish products is a considerable problem, due to the ability of the microorganism to survive under a range of environments and adhere to surfaces of food processing plants and food storage utensils.

During post-harvest handling, fish get exposed to pathogenic *Staphylococcus aureus* and its heat stable enterotoxins. Enterotoxins withstand processing of fish leading to food safety hazards. Sivaraman et al. [42] has observed notable contaminations of *S. aureus* after primary processing and at storage, with some strains exhibiting resistance to methicillin. There is increasing evidence of the presence of microorganisms resistant to a variety of antibiotics in fish [43]. Consumption of fish carrying antibiotic residues tends to create antibiotic resistance in humans, leading to unforeseen health problems. Exposure of humans to post-harvest microbial contaminants is a continuing food safety hazard, needing controls through more effective and timely processing and preservation methods.

In view of the continuous food safety hazards associated with a variety of fish due to formation of biogenic amines, especially histamines, more stringent guidelines and measures associating histamine production to unhygienic fish handling and temperature controls are established by food

authorities [3,33]. The guidelines help to minimize consumption of food-fish contaminated by a variety of microorganisms ensuring food safety. Prevention of microbial contaminations in food-fish would save at least 25% harvested fish that gets rejected.

2.4. Chemical Food Safety Hazards

Chemical pollutants tend to accumulate continuously in marine and inland waters and enter fish muscles, reaching concentrations hazardous to humans. Based on facts and figures on marine pollution report of UNESCO, disposals from the lands contribute to 80% of the sea pollutants, whereas disposals from ships, and mineral explorations at sea bottoms account for the balance [44]. The accumulating pollutants create a negative impact on the marine and freshwater biota, threatening the existence and safety of food-fish. The common chemical and physical contaminants affecting the safety of food-fish are presented in Figure 2.

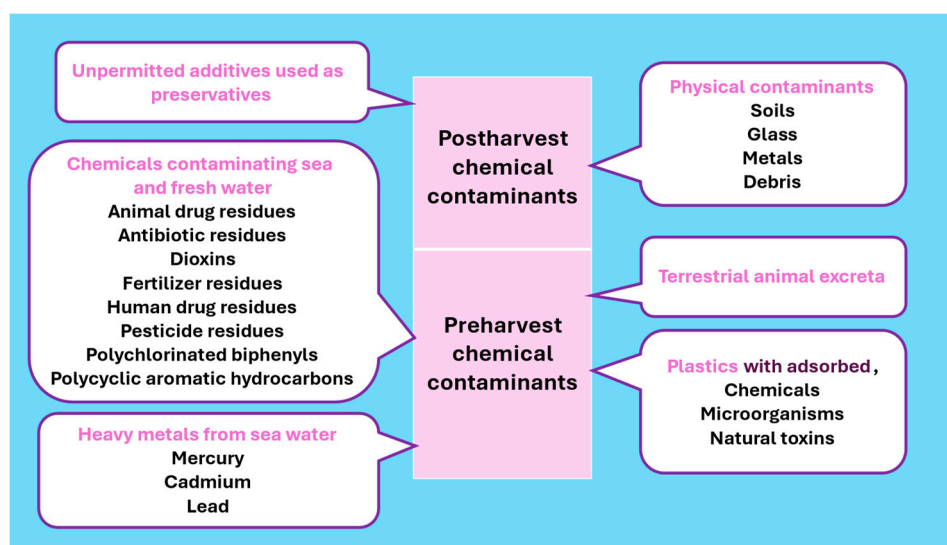


Figure 2. Chemical and physical contaminants affecting the safety of food-fish.

Among the chemical contaminants, residues of agrichemicals, animal drug residues from the farms and animal excreta containing toxic chemical residues end up in the muscles of food-fish. With the rising sea levels more contaminants from land tend to move into fish muscles through increased pollution in seas [45].

Of the toxic residues, organochlorine (OC) pesticide residues in water are absorbed more through the gills, than through stomach depositing in fish muscles. With the ban on use of OC pesticides in the developed countries, decreasing OC concentrations in Pacific salmon species are reported for the period 2012 to 2018 [46]. However, OCs are continued to be used in some African and Asian countries, despite the knowledge on potential hazards through the food-fish.

Among the organic compounds directly polluting the oceans and reservoirs, contamination by polycyclic aromatic hydrocarbons (PAH) released from burned fuels into waterways is an increasing hazard. Of the PAH released, the low molecular compounds and the highly carcinogenic benzo[a]anthracene are reported from Nigerian waters. Uncontrolled release of petroleum products from refineries and other sources containing PAH, is a continuing food safety concern as they enter food-fish [47–49].

The toxic metal residues (arsenic mercury, cadmium, lead) enter the oceans due to weathering of rocks and anthropogenic activities. The toxic metals enter the fish chain starting from sea plants through benthic fish to pelagic fish continuously. Mercury gets converted to neurotoxic methyl mercury by marine flora, increasing the food safety hazards through food-fish. The ability of sword fish and tuna fish to accumulate toxicologically high concentrations of mercury and cadmium are indicated by high enrichment factors for them [50]. High cadmium content in sword fish is reported

in all oceans due to its bottom feeding nature. Cadmium gets deposited in many tissues of fish, leading to toxicities, and serving as a pathway to enter the human body [51]. Arsenic enters seawater from marine sediments. The concentration of arsenic in sea water is reported to be 100-fold higher than the US EPA human health criteria. However, the arsenates in seawater are converted through arsenites to less toxic arsenobutane, and other organic forms by marine algae, alleviating the dietary toxicity to humans through food-fish significantly [52]. Open sea carries higher concentrations of lead than the coastal areas, deposited into water from the atmosphere [50]. Lead in fish livers and muscles are reported to exceed the EU tolerance limit of 0.1 mg/kg [53]. Atmospheric transfer of lead to sea water is a continuing threat beyond control, with the emission of lead from fuels in engines globally. It is not easy to handle challenges associated with natural contamination of oceans by toxic metals, and their accumulation in fish muscles and viscera.

Nurdles and plastics of differing origin are released continuously to waterways. They settle partly in the ocean bottoms. Some plastics take about 1000 years to degrade, while others take a few hundred years [54]. Plastics account for 80% of marine litter [55]. Plastics themselves release carcinogens such as phthalates, bisphenol A, flame retardants, organophosphates, perfluorinated chemicals, and organo-tin compounds in the fish gut, when swallowed. The nurdles provide a base for the algae to grow, opening a pathway to enter fish. The hazards arising from swallowing the nurdles by fish is heavy. Nurdles carry the potential to adsorb algal toxins, microorganisms, toxic metals, and organic compounds. The adsorbed constituents tend to get equilibrated with the environment, getting released with change osmolarity and acidities [56]. The digestive environment in the fish gut tends to release organic pollutants, transporting them into fatty tissues, exposing humans to a variety of toxicants transmitted through fish [57].

Bioaccumulation of toxins transferred through nurdles may create food safety hazards to humans through food-fish. The plastics are also suggested to provide opportunities for horizontal gene transfer among the adhering microorganisms, creating new food safety hazards through food-fish [58]. Microplastics have been reported in muscles of canned predator fish, suggesting a possible route for entry of microplastics to human muscles [59]. Plastics bring in new and increasing hazards through food-fish, arising from a variety of toxicities that could be transferred to humans.

Use of unpermitted additives such as formaldehyde (formalin) for post-harvest preservation of food-fish happens in some markets introducing new food safety hazards [60,61]. At times, the inability to distinguish between formaldehyde naturally produced in fish as traces against added formaldehyde, make regulatory decisions difficult. Use of unpermitted chemicals for fish preservation is a problem in countries where the food regulatory measures are not strong.

The food safety hazards arising from microorganisms and chemicals tend to increase with natural and anthropogenic activities in oceans and waterways [62]. The increasing challenges in handling food safety hazards through food-fish are summarized in Figure 3 identifying the origins.

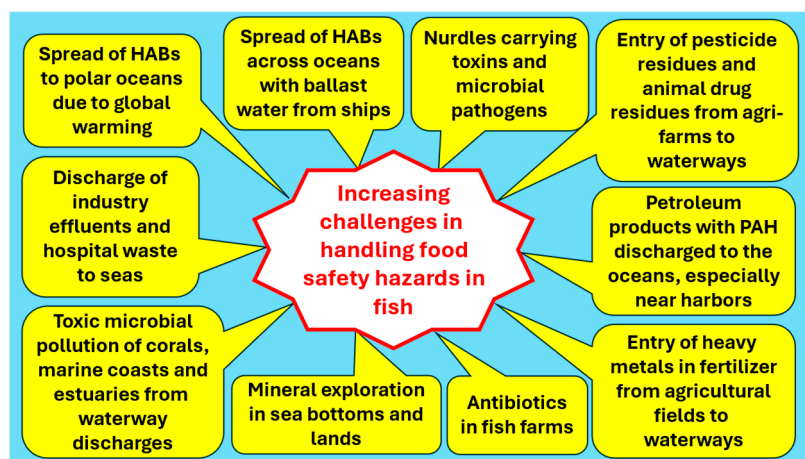


Figure 3. Increasing challenges and origins of food safety hazards through food-fish [62].

Public concern and responsible behavior by the industries, with understanding of the potential hazards aggravating continuously, is essential to protect the sources of food-fish and their environment. The oceans occupying 70% of the earth's surface and holding 97% of water are unfortunately considered a sink, to dispose of waste with low consideration of fish, a major source of food. The stagnant inland water reservoirs used for farming or harvesting fish tend to get polluted intensely due to eutrophication. The pollutants in the rivers get discharged into lagoons and coastal fishing areas. The above anthropogenic activities are a major challenge in maintaining a healthy food-fish supply in the human diet.

While it is possible to minimize the exposure of humans to pre-harvest microbial hazards in fish through appropriate handling and processing methods or avoiding harvests from contaminated waters. The same does not work with chemical contaminants. The chemical contaminants tend to spread across oceans and waterways unnoticed unless regular chemical monitoring of water quality is done. Being a difficult task, examination of water quality is unlikely to happen, except for known contamination patterns or incidences.

3. Processing and Preservation of Fish and Fish Products

Raw fish needs to be treated to make them appealing as food. Harvested fish are pre-processed, to get rid of adhering non-edible components and physical contaminants. Fish are processed to retain the organoleptic characteristics of muscles by freezing, heating, controlling water activity by removing moisture, using additives, or irradiation. Of the total capture of fish 60% is processed into different forms in the industrialized countries [63]. Processing aims in generating modified products of consumer appeal from fresh fish. The value addition associated with processing, brings increased profits and sustainability of the fish industry. From a consumer perspective, preserving fish to increase shelf life and prevent changes in their natural characteristics is not the top priority. Consumers mostly prefer fresh fish for the natural organoleptic characteristics. However, the need to make the food-fish continuously available as a rich nutrient source, has compelled the industry to preserve food-fish, especially to eliminate food safety hazards. Pre-process preservation of food-fish by storing in ice (chilling) between harvest and processing helps in retaining fish quality. Processing food-fish, which goes beyond preservation, carries the advantage of more efficient utilization of edible muscles, developing products appealing the consumers, and converting non-edible components to edible nutrient rich forms.

3.1. Conventional Fish Processing and Preservation

Preservation of fish goes back to the early history of food gathering period of humans. Fish muscle could contain up to 90% water which needs to be reduced to minimize microbial spoilage. Drying with salt, air drying, storing in ice, smoking and fermentation continue to be practiced in small and medium scale to preserve fish. The objectives of preservation were to prevent autolysis of muscles, and eliminate pathogenic microorganisms proliferating on the nutrients in fish. Two broad approaches to preserve fish are visible among the conventional methods. First, is to reduce the water activity by drying with different techniques with or without salt. The second is fermenting to competitively eliminate harmful microorganisms, allowing beneficial microorganisms and autolysis to bring about desirable organoleptic properties in the product.

In the temperate countries fish are suspended in air, exposing to low humidity at subzero ambient temperatures over weeks, to reduce the water activity and to control microbial growth. Under tropical conditions, heat from solar radiation is applied to evaporate the moisture. Prolonged sun drying makes fish susceptible to microbial spoilage. The microbial activity is discouraged by reducing the duration of sun drying of fish using dryers, platforms above the ground and addition of salt to reduce water activity in fish muscles. The combinations of techniques are diverse in conventional fish preservation methods. Some of the basic practices of fish preservation and their effects on quality and safety of the products are summarized in Table 2.

Table 2. Conventional fish preservation methods and their effects on quality and safety.

Method	Action (Effect on quality and safety) [Reference]
Air drying (Wind drying)	Open air drying at 0-2 °C for 3 months. 75% moisture is lost. (Shelf life 12 months. Low temperature minimizes microbial growth; Anisakid parasites devitalized in 7.5 months) [64].
Salt drying (Osmotic dehydration)	Eviscerated fish is dipped in salt water, or sprinkled with salt and dried under sun, or with hot air drafts (Increased free fatty acids and partial loss of nutritional quality; amenable to mold attacks at low salt concentrations) [65].
Salt & ultrasound drying	Hybrid of ultrasound with ambient drying applied to salted cod fish (Ultrasound reduces drying time at low temperatures retaining quality) [66].
Sun & oven drying	Sun drying, solar drying and oven drying (The methods leave room for partial microbial spoilage affecting quality and safety; products are characterized by hard textures, protein leaching and degradation, and lipid oxidation as inherent enzymes continue to be active till the water activity becomes limiting [67].
Smoke curing	The preservatives carried with the smoke deposit low molecular acids, aldehydes, phenolics etc. Bring about surface preservation of fish and slow moisture removal retaining the texture. Partial cooking by low heat and burning of dripping fats occur. (The carcinogenic polycyclic aromatic hydrocarbons deposited on surface; change organoleptic properties for consumer preference) [62,68].
Fermenting	Mostly use undersized and low value fish. Autolysis and diverse microbial actions metabolize proteins and carbohydrates producing alkaline conditions, restricting spoilage organisms, and delivering soft solid texture, fish pastes, or fish sauces. Sodium chloride, nitrates/ nitrites and herbs when used protect against spoilage generating desirable organoleptic characteristics. (Biogenic amine formation impairs food safety) [69,70].

Conventional preservations reduce the nutritional quality of fish through changes in proteins, carbohydrates, and lipids due to heat and salt. The pathogenic microorganisms are controlled satisfactorily in the conventional methods, either providing an environment unfavorable to growth or allowing competition by non-pathogenic bacteria reducing the food safety hazards. Reduced water activity due to salt combined with drying, restricts microbial growth during preservation. However, halophilic bacteria and fungi may be active in salted dried fish, unless optimal salt concentrations are maintained. In dried fish, growth of halophilic fungi has been observed at salt concentrations below 30% combined with more than 30% moisture [71]. The limitation arising from improper combination of salt and moisture is visible in the poor quality of dried gray mullet carrying high moisture and low salt concentrations [72], by the presence mycotoxins [73], and in reviewing fish drying methods [67]. Combining conventional fish drying methods with other controls, botanicals, and chemicals, remains a possibility in ensuring product safety from microbial contaminants and mycotoxins. Spraying smoke liquid free of polycyclic aromatic hydrocarbons is practiced as a safe alternative to smoking imparting appealing sensory characteristics to preserved food-fish.

The conventional fish fermentations attract low value fish and undersized fish, as they do not find ready markets and advanced processing facilities. Fish fermentations are linked mostly with subsistent fishing. Fermentation carries the advantage of utilizing all the constituents of the fish including micronutrients, essential fats, and vitamins. Microbial activity adds to the value of vitamins, particularly vitamin B₁₂. Fermentation reduces waste and generates nutritious foods affordable to low-income populations in countries, where sophisticated processing and preservation facilities are scarce.

Combining permitted food preservatives with drying remains a mechanism to ensure product safety in conventional drying. Acetic acids, ascorbates, benzoates, and potassium lactate are already permitted in certain fish products [74]. Additives are an important component in preserving fish in

small and medium industries. Sodium chloride, essential oils (thyme, cinnamon, and clove extracts) were used conventionally in reducing microbial activity in fish aiming food safety. Natural additives are comfortably accepted by the consumers. With the identification of bio-preservatives as purified compounds, the use of nicin, pediocin, reutarin, bacteriocin, citric acid, and lactic acid became acceptable as safe in fish preservation. The chemicals BHA, BHT, EDTA, sodium nitrite, sulfites, and benzoates permitted for use in foods, also may be good candidates to be used in conventional drying of fish. The application of the preservatives continues in home and small-scale fish preservations in some countries. Adhering to minimum residue levels of the additives, is expected in preserving fish combined with conventional methods.

Conventional processing and preservation methods affect the nutritional quality of the end products, change the texture of fish, and introduce chemicals in certain processes raising questions on the safety of products. Thermal treatments tend to change the texture and generate new flavors, which are not preferred equally by consumers.

3.2. Emerging Food Processing and Preservation Technologies

The consumer demand is for fresh fish. Consumers seek fish which has undergone least interactions and no thermal treatments. This has resulted in interest on non-thermal processing methods to preserve fish. More recent technologies aimed irradiation and canning of fish to increase the shelf life. Canned fish is safe but is cooked changing the sensory appeal, though fish canning is a major industry. Present consumer trend is to seek non-thermally processed and preserved fish retaining sensory properties as close as possible to that of fresh fish. Emerging fish processing and preservation methods along with their food quality and safety outcomes are summarized in Table 3.

Table 3. Emerging processing and preservation methods and their effects on quality and safety of fish.

Method	Action (Food quality and safety outcome) [Reference]
High Pressure Processing (HPP)	Operate at pressure of 100 -1000 MPa on fish as a non-thermal process (Retains sensory and nutritional quality; inactivate microorganisms and spoilage enzymes) [75–77].
Ultrasound Technology	Use high frequency sound waves at 20 – 1000 kHz (Retains sensory and nutritional quality; inactivate microorganism and enzymes) [78,79].
Pulsed Electric Field (PEF)	Apply short bursts of electricity at high voltage at 20-80 kV/cm. Combine effectively with gas modifications and refrigeration. (Affects cell permeability inactivating microorganisms; lipid oxidation and high cost are limitations) [80–82].
Cold Plasma (CP)	Generate ions, charged particles, radicals and electrons in the gaseous environment around the fish muscles using an intense electric field (Oxidize cell membranes of microorganisms; oxidation of lipids and proteins in fish muscles limits the application even at atmospheric temperature) [82,83].
Low Voltage variable frequency electrostatic field & chemical preservatives	LVVFEF carries the benefit of combining the action of two methods, the voltage and chemical preservatives or low temperature treatment (LVVFEF interferes with biochemical reactions involving charged particles and formation of mini-ice crystals when combined with freezing. It inactivates spoilage bacteria <i>Pseudomonas</i> and <i>Bacillus subtilis</i>) [84]
Pulsed Light	Application of UV light pulses at high energy inactivating microorganisms on surface. (Prevents replication of DNA due to formation of dimers resulting in cell death; surface sterilization of fillet occurs with 300Jj/pulse; pigments lose color; low penetration; less effective on non-smooth skin surfaces) [85,86].

	UV light emitting diodes with wavelength limitation of 365 nm are considered a safer technique in place of mercury lamps emanating UV light but carries more heat production than light (Causes lipid oxidation) [87].
Photodynamic inactivation	Activates internal cellular or external photosensitizers, light, and molecular oxygen generating Reactive Oxygen Species (ROS). ROS attacks proteins, lipids, and nucleic acids in microorganisms inactivating them (Microorganisms differ in their response to treatment; some microorganisms may generate tolerance; photosensitizers may continue to be active in foods entering human body; requires examining fish after treatment for safety) [88,89].
Microwave Heating	Electromagnetic waves of frequency 2.45 GHz -S band- having capacity to penetrate fish muscles is used to generate heat internally. More efficient combined effect from microwave assisted induction heating is gaining recognition (Possess advantage of pasteurization and cooking to preferred temperatures for varying consumer needs; heat would change the sensory characters of fresh fish) [90,91].
Microwave drying	Rapid fish drying compared with solar drying to prepare dry fish (Better microbiological safety, smooth texture; increase lipid oxidation and protein denaturation compared to air drying) [92].
Smoke technology	Smoke purified to eliminate polycyclic aromatic hydrocarbons is sprayed on fish in atomized gaseous form. (Inhibit bacteria, add smoky flavor and texture of consumer preference; products were darker than smoked fish; need to lower water activity and pH to make the treatment safe from microorganisms; sensorily atomized smoke treated fish is similar to traditionally smoked salmon) [93,94].
Ozone treatment	Sanitizing benefit from ozone is used by exposing fish to the gas, ozonized water, or ozonized ice slurries. (It inactivates microorganisms and suppress lipid oxidation) [95,96].
Vacuum cooking	Vacuum cooking in sealed plastic pouches retain sensory properties of fish. Acidic electrolyzed water retains the quality to a higher extent. (Carries the advantage of post-process contamination control, and longer storage life of omega-3-fatty acids; Carry the risk of proliferating anaerobic Clostridium spores) [97–99].
Edible coatings/films	They are biopolymers applied as thin films on fish muscles to protect from contaminants (May incorporate antioxidants, antimicrobials, monoterpenes, flavors, and vitamins; may contain constituents that cause microbial cell death; varying film forming abilities is a limitation; consumer awareness and safety are low) [100–102].
Active packaging	Active packages may carry oxygen scavengers, carbon dioxide absorbents or emitters, moisture regulators, antimicrobials, antioxidants (tea polyphenols), and flavor releasers for product safety and consumer preferences (Selective depending on the fish type and consumer demand; packaging mostly biodegradable) [103,104].
Intelligent packaging	These third-generation packaging screen the quality of fish and signal the changes. Packaging may response to moisture, light, pH, oxygen, heat and bacterial growth indicating loss of freshness and quality [105].

The methods summarized above provide zero heat, low heat, or cooking conditions to preserve fish. As against the emerging methods in Table 3, quick freezing, for core temperature to reach -18 °C rapidly continues to be the most widely used method to retain quality, controlling ice crystallization, and ensuring the safety of fish. Frozen fish may be stored for months at temperatures below -18 °C to prevent biochemical degradation and microbial spoilage. Fish stored above -10 °C tend to be vulnerable to slow enzymatic activity. In frozen fish, inoculated *Salmonella* is observed to survive -18 °C up to 90 days, indicating frozen fish may not remain safe if the initial microbial populations are

high [106]. Freezing carries the advantage of eliminating the risk associated with the parasites *A. simplex* and *P. decipiens* in preparation of the Japanese cuisines, sushi and sashimi. Fish to be used for Japanese cuisines are flash frozen at -37°C for 15 h or stored at -20°C for 7 days to ensure food safety [107].

Non-thermal fish processing and preservation is gaining consumer preference expecting to retain freshness in fish, while ensuring quality and safety. Fish preserved by physical methods carry an edge over conventionally preserved fish due to retention of freshness to a greater extent. Though there is no direct transfer of heat to fish muscles in the non-thermal processes, there is internal heat generation giving a cooked appearance and partial quality changes during microwave, high pressure, and pulse treatments. However, consumers and sensory analysis have not detected changes in odor and color, of fish muscles preserved using some of the novel methods, though laboratory testing instruments detected discolorations, whitening, increased hardness, and initiation of lipid oxidation [108]. The non-thermal processing possess advantage of retaining consumer preference of foods. However, emerging technologies possess limitations due to differential effects on microorganisms, and capacity of microbial spores to survive the treatments. The limitations compel the processing industry to be aware of the types of microorganisms in fish in advance, to manage processing conditions and hybrid processes.

With tuna fish, the HPP treatment is observed to be only bacteriostatic, leaving room for microbial proliferation during storage [109]. In view of observed occasional limitations in single treatments for fish preservations, hybrid treatments combining non-thermal and mild thermal applications are being examined for efficient microbial destruction, while retaining freshness of fish muscles. The outcomes of modern methods are yet to be fully acceptable, overcoming the drawbacks in them. The synergistic effects of multi-technology may result in consumer acceptance, as the quality changes may be minimal, compared to application of drastic conditions in a single processing technique. Synergistic effects of combining freezing overnight, spraying liquid smoke, together with HPP treatment at 200 MPa, are reported to inactivate *Listeria monocytogenes* on trout fillets [110]. The combination of low intense exposure by each of the methods in a combined treatment, may accrue benefits of quality retention together with improved food safety. While the multiple hurdles in multi-technology preservation results in effective for microbial controls, the additive effects on quality changes need to be established scientifically for each combination.

Though irradiation of fish as a non-thermal method, carrying proven potential to eliminate pathogenic microorganisms, the consumer acceptance is low due to several myths associated with irradiation, where knowledge between irradiation and radioactivity are mixed up [111]. The regulations requiring irradiated fish to be identified and labelled, has added to consumer concern.

The three major outcomes of applying the new processing technologies to increase shelf life of fish products are, the retention of sensory and nutritional quality arising from reduced lipid oxidation and other reactions, the low cost, and high consumer appeal. The cost continues to be a major deterrent in upscaling the new knowledge into industrial processes. Development of technologies to strike the optimum among the three concerns continue to be challenging. Combined treatments appear to meet the optimum processing requirements, but need to be identified for each fish size and type, and the microbial populations. Some of the fish products preserved using combinations involving chemical interaction by ozone or free radicals, need examination for quality and safety. In selecting combined treatments, more refined operational standards, deep scientific understanding on inhibitory mechanisms, unexpected biochemical reactions, and regulatory limitations need to be worked out, for methods describe in Table 3 for commercialization. The ultimate success in the new technologies would rest on consumer acceptance as safe, sensory satisfaction of the consumers and the product cost.

Fish processing has a vital role in improving utilization of fish components. With inadequacies in production to meet global demand, the need for better utilization of hitherto unused components in fish is a need. New processing technologies and fish waste utilization should bring about cost benefits and increase the market for fish products.

4. Fish Waste Utilization

The main edible component of fish is muscle. The muscles account for 30 to 56% of fish, of which the average consumption is about 49%. The balance 51% consisting of heads, viscera, skin, blood, belly flaps, trimmings, frame etc. are discarded as waste. There are notable variations in the percentage proportions of the waste material among different species of fish, and in the published analytical data. The ranges of constituents available from fish for direct consumption, for processing, and valorization are summarized in Figure 4.

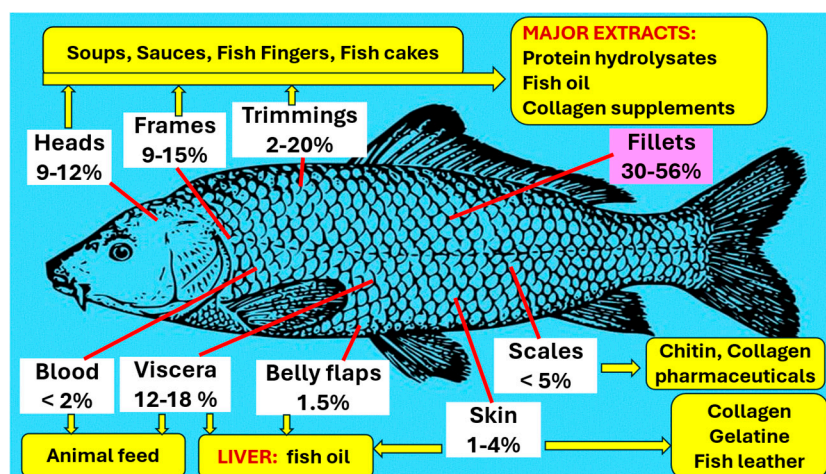


Figure 4. Percent ranges of unutilized or underutilized constituents in fish [112].

The extractable unutilized nutritious fish constituents consist of proteins (collagen, gelatin, proteins, biopeptides, enzymes), fats (fish oils, squalene, vitamins), and amino polysaccharides (chitin and chitosan) in addition to bones (calcium, phosphates, carbonates). Most constituents can be processed into edible or medicinally valued products [113]. Current inability to meet the global demand for fish even with increased aquaculture, compels better utilization of hitherto unprocessed fish constituents into foods. Increasing the utility value of fish from the current 49% consumed directly, to about 65% through increased recovery of muscles, and value addition to components currently identified as waste, are priorities. In converting the unutilized components into novel products, the emphasis is expected to be on high nutritious values, economic benefits, value additions, and increased consumer acceptability while reducing environmental pollution. The byproducts of the fisheries industry could be purified to bio-functional ingredients including omega-3-oils, polysaccharides, gelatin, chitin, enzymes, and bioactive peptides (BP). Fish waste is a rich but unutilized source of nutrients and bioactive compounds exhibiting specific health benefits [114]. The major components to be separated from fish waste and possible approaches for better utilization of fish waste are discussed below.

4.1. Collagen

Collagen is a structural protein in skin, bones, ligaments, cartilage, and tendons performing structural functions, in the animals and humans. Collagen contains 30% protein. There are 5 main types of collagens identified by their locations and functions in the animal and human body. The types extend to at least 28 based on molecular properties [115]. Degradation of collagens in the human body tissues results in sagging skin, wrinkles, dry skin, and stiff joints. Chemically, collagen is a triple helix of 3 extended protein chains wrapped around each other. Collagen could be extracted from the skin, scales, cartilage, fins, bladder, and bones in fish sustaining industrial production [116]. Fish bone contains 30% of it as collagen [113]. Fish collagen possesses the advantages of low molecular weight, easy absorption by the human body, and biocompatibility with human collagen. Fish collagen could be processed at low cost. Low cost makes collagen an important material for biomedical applications

[117]. Fish collagen carries no risk of transmission of zoonoses during health care applications, compared with collagen from mammals. Collagens could be hydrolyzed to produce gelatin.

The collagens available in fish waste arise from skins (4.5%), scales (0.3%), and bones (0.6%) [115]. However, variations in the percentages of collagen are reported from different types and different organs of fish, even with the same extraction method. Collagens, being proteins of diverse characteristics arising from arrangements of amino acid sequences and amino acid proportions in their structures, show diversity in solubility and other properties. The applicable extraction methods may be specific to the source of collagens, using optimal pH, substrate–solvent ratios, the duration of heating, and temperature. Collagen specific extraction processes using acid, pepsin-hydrolytic acid, eutectic solvents, or supercritical fluid extractions are identified to suit the molecular characteristics of triple helix and the end use [115]. The extractions cause changes in the molecular structures of collagens, altering their biochemical properties. The effects of different extraction methods on the functional properties limit the utilization of fish waste as a common source of collagen.

The diversity of the structural, chemical, and morphology of extracted collagens provides opportunities for pre-determined biomedical applications. The applications include tissue engineering (cartilage and tissue regeneration), drug delivery, wound dressing, skin regeneration, and vascular tissue regeneration among several other potential applications under investigation. Low denaturation of fish collagen at temperature of 25–30 °C caused by low molecular weight amino acids, against denaturation of mammalian collagens at 39–40 °C, limits some biomedical applications of fish collagen. The ability of hydrolyzed collagen along with vitamin C to accelerate wound healing through proliferation and migration of fibroblast cells is reported [118]. The combination generates a functional ingredient, exhibiting nutraceutical properties for skin nourishment. Skin nourishment is an aim in cosmetics. While the potential for utilization of collagens in advanced biomedical applications is high, the sophistication needed to identify purpose focused extraction and purification methods, needs deep scientific basis. However, each application requires optimization of the functional properties of the extracted collagen. The functional properties are linked with the chemical and molecular characteristics of the extracted fish collagens. Matching the molecular structures with functionality of collagens, or even modification of collagens for targeted application is a research need. Fish collagen extracted with sophisticated methods to retain their original properties would find more applications based on their compatibility with human health needs.

Low-cost extraction is a need to utilize fish collagen for a wide range of purposes. A simple method to recover collagens by salt precipitation from salmon skin with low chemical interactions [119], and a method combining acid hydrolysis with ultrasonication are described [120]. The extraction method would alter the molecular composition of collagen extract. Correlation of fish species, tissues to be extracted, the extraction method, structural features of the extracted collagens, their suitability for different food and biomedical applications require large databases for predictive scientific decisions. Artificial intelligence (AI) may provide solutions for handling data aiming better utilization of collagens.

4.2. Gelatin

The predicted global market for gelatin is 5 billion USD by 2025 [121]. The high market potential provides a great opportunity to increase fish gelatin production from the current utilization of 1.5%. Gelatins are multi-functional products characterized by moisture contents slightly below 10%, protein contents reaching 85%, and ash content around 5%. The ash content in gelatin could be high when extracted from fish having heavy skeletons or crustaceans. Fish gelatin derived by partial hydrolysis of collagen proteins, carries high concentrations and diversity of amino acids. Fish gelatin predominantly contains lysine, and methionine but is low in tryptophan. The food industry does not target gelatin as a nutrient source, but a constituent providing multi-functional properties of consumer appeal in processed foods. Fish gelatin is nutritionally considered to be of low quality compared to mammalian gelatin, though the former exhibits diversity in the amino acids. The structural and physical properties of gelatin such as light penetration, mechanical strength, networking depend on the molecular weight and the cross-linking patterns of amino acids [122].

Additionally, the balance of hydrophilic and hydrophobic interactions in the peptides decide their network characteristics on food surfaces.

In foods, gelatins provide structural features to meet rheological and organoleptic properties to suit each situation, generating creaminess, mouthfeel, chewiness etc. Gelatin serves as ingredients in bakery products, candy, desserts, ice creams and meat products [123]. Gelatin extracted from skin and bone of fish has high utility value in the food industry for gelling, stabilizing, emulsifying, dispersing, encapsulating, and thickening the end products [116,124]. The properties of the gelatins would depend on the species of the fish and the conditions of extraction. Mild extraction conditions generate gelatins with higher molecular weight peptide fractions.

Comparing dry salting, wet salting, use of proteases, and heat-based methods to extract fish gelatin, enzymatic technique is reported to produce more robust sponge like gelatin, with high water absorption capacity of 10% and fat binding capacity of 10%, carrying better emulsifying properties [125]. The protease hydrolyzed gelatin from fish appears to carry high utilization potential for the food industry [122]. The results from different studies indicated the relevance of the extraction method to the required properties of the gelatin for varying food uses. Fish gelatin with a melting point about 10 °C below that of pork and beef gelatins, possess advantages as food ingredient with easy melting in the mouth. The proteins in gelatin from cannonball jellyfish exhibits antioxidant and antimutagenic properties. The antioxidant properties make gelatin a useful food preservative [126].

Fish gelatin is gaining recognition as a natural product against the petroleum-based packaging for foods [127]. Fish gelatin is combined with plant materials to modify their properties. Incorporation of extracts from the mangrove plants *Bruguiera gymnorhiza* and *Sonneratia alba* are reported to impart elongation, water vapor transmission, antioxidant properties and antibacterial activities to fish gelatin films used as active packaging materials [128]. The properties of gelatin are further modified combining chitosan, xanthan gum, and antioxidants. Chemical modifications with herbal extracts, essential oils, and enzymatic action of transaminases, modify the properties of gelatins for specific food uses. [121,128]. Gelatin based packages incorporating essential oils carrying antioxidant properties were found to be effective in quality retention of carp fish, by slowing lipid oxidation during refrigerated storage [129]. However, the treatment report setbacks due to color changes in the fillets. To suit the different food protection applications, gelatins are modified by enzymatic pretreatments during extraction, ultrasonication, and high-pressure processing [121]. Gelatin coatings are improved incorporating nano-capsulated essential oils and other additives to impart desirable properties as food coatings. Gelatin is used to coat nutrient food powders and pharmaceuticals as tablets and capsules. There is high potential for developing new processes and technologies for product specific modifications of fish gelatins. The new processes need to work on molecular rearrangement of amino acids and the cross links to generate gelatins with properties conducive for specific applications. Safety assessment of gelatines modified with additives needs research attention.

The main medical advantage of fish gelatin arises from the immunological safety of the proteins, allowing wide use in healthcare products [130]. Products containing fish gelatin are acceptable to groups having religious restrictions on use of gelatin from terrestrial animals. The low melting point of fish gelatin makes it suitable for micro-encapsulation of oils and drug delivery. Gelatin from fish in cold climates having low hydroxyproline content, are more suitable for the products requiring low gelling temperatures. Gelatin having location specific gelling properties, may meet specific processing demands.

In the cosmetics industry, gelatin is used for preparation of hair gels, creams, lotions, and shampoos. Gelatin plays an important role in protecting the human skin from UV irradiation, thereby minimizing oxidative stress [131]. Fish gelatin reduces peroxidation of lipids on the skin and possesses scavenging effect against reactive oxygen species. The skin protective properties of fish gelatins make it an important raw material in the cosmetic industry.

While fish is a rich source of gelatin for the food industry, the industry requires ingredients with standardized physical properties, to maintain market characteristics of consumer preference in foods. Identifying and converting the crude gelatin for industrial use requires modifications to produce

standardized purpose-oriented gelatins. Modifications may require further processing, incorporating additives such as glycerol etc. permitted in foods. Nevertheless, fish gelatin provides the opportunity to process products to be utilized in the modern food industry.

4.3. Other Fish Proteins and Derivatives

Fish proteins generate a range of valuable products such as hydrolysates, active polypeptides, enzymes, and other derivatives for sustainable utilization in addition to collagen and gelatin [132]. The distribution of proteins in the whole fish, and components of Pacific Ocean perch are 17.9% in whole fish, 15.2% in frames, 14.9% in heads, and 11.3% in viscera [133]. The authors indicate high lysine content in fish heads and frames, suggesting the quantitative and qualitative importance of extracting protein derivatives from perch fish. A considerable proportion of the proteins available in fish is lost quantitatively with the fish waste.

The proteins in fish waste are separated mainly by chemical hydrolysis, enzymatic hydrolysis, or fermentation. Ultrasound technology is adding to the efficiency of chemical extraction of the fish proteins. Of the different hydrolytic methods, enzymatic hydrolysis is the best option, generating structurally and functionally uniform hydrolysates. The uniformity is linked to the molecular nature of the derived polypeptides. Through selection of the enzymes for proteolytic and other biochemical reactions, bioactive peptides (BP) of specific characteristics could be generated. The extracted polypeptides are purified by membrane filtration and chromatographic methods, to separate BP of desirable and predictable properties, having high economic values. Extraction conditions specific to fish types and the properties expected in hydrolysates are established by different researchers.

BP imparts a positive impact on body functions and may influence human health positively or negatively, depending on the way BP is used. There is potential to use BP as food additives, and ingredients of pharmaceuticals designed to prevent lifestyle diseases obesity, hypertension, and type II diabetes [134]. However, retaining the activity of BP in commercial production demands rigorous scientific scrutiny. Much research is needed to understand the interactive behavior of polypeptides in treating humans. With many hundreds of BP generated from fish proteins, there is much room for future research correlating the molecular properties with activities as pharmaceuticals [135]. The use of BP in inhibiting angiotensin-converting enzymes (related to treatment for hypertension), antioxidant activity, antimicrobial activity, inhibition of dipeptidyl peptidase (in relation to diabetes control) and 49 possible other applications already recognized. This opens a vast field for biomedical use of fish BP. Biomedical applications need functionally specific BP derived through well-defined extractions.

Biotechnological processes, using pure enzymes with known biochemical activities provide opportunities to obtain BP of predictable characteristics. The process for by-catch (low value fish) using papain was found to extract BP efficiently [136]. Cryoprotective effects of fish hydrolysates generated with alcalase® and protamex® enzymes appear to differ from each other in performance on storage fish. The noted differences indicate diversities arising from the properties of generated polypeptides. The cryoprotective effects of fish-based gelatins appear to follow a mechanism different from that of the commonly used cryoprotective agent consisting of sucrose-sorbitol. The application of fish cryoprotectant in developing non-sweet surimi products is under investigation [137]. The diversity among BP provides opportunities for a variety of applications. BP consisting of 3-20 amino acids carry anti-inflammatory, antioxidant, anticancer, and antimicrobial activities, linked to their structural features [138].

BP from fish proteins carries the potential to generate novel compounds for biomedical and food industries. Sanapala et al. [138] highlights the importance of improving and refining extraction methods, to obtain BP in their natural forms as far as possible to increase utility value. Fish protein hydrolysates having known amino acid sequences, with potential to generate high value nutraceuticals and healthcare products industrially, are useful in gaining consumer acceptance and for treating several non-communicable diseases [139]. The potential to utilize hydrolysates for the benefit of human health, justifies development of processes, to generate purpose-oriented products

from low-cost raw materials from fish waste. Already there are commercialized drugs extracted from fish, prescribed for noncommunicable diseases.

Fish protein hydrolysates carry water holding capacity, oil absorption capacity, protein solubility, gelling activity, and foaming properties essential for application as food emulsifiers [140]. The properties of hydrolysates vary with the degree of hydrolysis, and the distribution of peptides and amino acids in them. The product diversity in protein hydrolysates could be retained using specific enzymatic hydrolysis mechanisms to generate function-oriented BP. Hydrolysis of fish proteins using purpose-oriented enzymes is an area to be explored.

A variety of proteolytic and other enzymes are used to extract protein based hydrolyzed fractions from fish tissues. Hydrolysates prepared using commercial enzymes alcalase®, and flavourzyme® on minced amur sturgeon skin are reported to generate products having retarded lipid oxidation and protein oxidation, when examined for their biochemical activities on fish mince. Controlled enzymatic hydrolysis appears to generate products with more specific properties, indicating the importance of refining the hydrolytic process [141]. Supplementation of poultry by-product meal with 10% fish protein hydrolysates, is reported to reduce gut enteritis caused by the poultry by-product meal alone. The fish protein hydrolysates restore gut microbial composition in farmed juvenile seabass fish, indicating improvements on aquafeed formulations. The anti-inflammatory effect imparted by fish proteins is noteworthy [142]. The anti-inflammatory feature gives a competitive marketing edge to fish protein hydrolysates over poultry hydrolysates.

While the fish protein hydrolysates could generate products carrying a variety of desirable properties, processing technology need to be tuned for handling different fish substrates aiming envisaged properties in the hydrolysates, targeting novel foods and medicines.

4.4. Enzymes from Fish

The fish carry endogenous enzymes having diverse biochemical efficiencies, each with distinct characteristics. They could be used to target specific biotechnological reactions industrially. Of the enzymes available, proteases, lipases and glutaminases are commonly utilized industrially. Borges et al., [143] describes successful use of proteolytic enzymes extracted from viscera of fish to hydrolyze fish proteins generating comparable results with alcalase®, though the antihypertensive potential of fish enzyme hydrolysate was less. The viscera could be a useful source of proteolytic enzymes for the detergent industries where high specific activity is a low requirement, and multiple effects of combined enzyme extracts may carry advantages.

Fish enzymes chitinases and collagenases are available from fish waste products. Enzymes obtained from fish living in a diverse range of temperature regimes may carry hitherto unexamined traits, to be harnessed beneficially for the industry [144]. Deep research into fish enzyme biochemistry may lead to new biotechnological openings to use fish waste.

4.5. Chitin and Chitosan

Chitins are linear amino polysaccharides, occurring in three forms as α -chitin, β -chitin, and γ -chitin. They are obtained predominantly from crustaceans. Living organisms in the ocean generate 10^2 - 10^4 tons of chitin annually [145]. The major marine waste products containing chitin are crustacean exoskeletons and finfish scales. Chitin is covalently bound to the proteins of shrimp [146]. Chitin is converted to chitosan, chito-oligosaccharides, and glucosamine for different applications [147].

Chitin is deacetylated to produce chitosan. Chitosan is extracted chemically (demineralization, deproteination and deacetylation) [147], biologically (enzymatic deproteination, fermentation) [147,148] or microwave irradiation combined with acid hydrolysis [146]. Chemical methods are least preferred as the processed chitosan are non-uniform in size, charge, molecular weight, and the degree of deacetylation, limiting their applications [147]. However, chemical extraction of chitosan of industrial quality from shrimp is described in Malaysia [149]. The viscosity of chemically generated chitosan is reported low [148] and the chemical processes generate hazardous chemical waste. Biological extraction of chitosan carries several advantages. Biological extraction gives the

opportunity for concurrent extraction of proteins, minerals, and pigments from fish waste with higher economic benefits. However, biological extraction requires well controlled fermentation conditions the pH, time, and temperature. Biological extraction needs to guard against possible microbial contaminations during processing. It may be necessary to follow up biological hydrolysis with mild chemical methods to remove residual proteins from the chitosan hydrolysates. Ploydee and Chaiyanan [148] reported production of high viscosity chitosan by chemical treatment of biotechnologically extracted chitosan. Waste heads and exoskeletons of shrimp are biotechnologically extracted by fermenting with *Lactobacillus pentosus* to convert glucose to lactic acid together with decalcification, and proteolysis by *Bacillus thuringiensis* to separate chitosan.

In chemical extraction of chitosan, the ability of microwaves to generate heat inside the exposed fish waste carries an opportunity to increase the efficiency of thermal processes at low heat levels. Microwave heating coupled with chemical hydrolysis provide the advantages of rapid hydrolysis, with low concentrations of alkali, at low external heating [145]. In microwave heating, reduction of heating duration by 1/3, and achieving same degree of deacetylation as with chemicals, with no significant differences in the structures of chitosan compared to heating in water bath is reported [146]. Chitosan produced by microwave treatment coupled with alkali had higher crystallinity, lower molecular weight, and low zero-shear viscosity, compared extracts obtained by chemical treatment only. Laboratory experiments on different hydrolytic processes have generated a wealth of data that could be scaled up to industrial practices to generate purified chitosan from the fish waste. The process design needs to work on the properties of chitosan required for specific uses both in biomedical and food applications.

Chitosan is used in drug delivery, tissue engineering technology, wound healing in humans, and production of sensors for electrical appliances [145]. Biodegradability, biocompatibility, antimicrobial, anti-tumor, and antioxidant activities make chitosan a valuable compound for use in food, pharmaceutical, agrichemical, and wastewater treatment processes [150]. Of the different potential markets for chitosan, nutraceutical, and cosmeceutical appear to be the fast-growing, followed by therapeutical and biomedical markets. The latter two needs increased sophistication leading to products with well-defined characteristics.

4.6. Fish Oil and Associated Products

The benefits of fish oil stand next to the fish muscles as a rich nutrient carrying health benefits. Crude fish oils and fish liver oils constitute the fish oil group. Tuna, salmon, sardine, and mackerel are rich sources of fish oils containing essential fatty acids, though all fish oils contain essential fatty acids. Association of fish oils in preventing cardiovascular diseases, diabetes, cancer, vision health, improvements in the immune systems, and development of nervous systems are documented. The role of fish oils in managing health in the aging population has been identified by several International Organizations. The fatty acids EPA and DHA are precursors for production of mediators inhibiting synthesis of proinflammatory cytokines in human body [151].

With depleting of fish harvests, capturing of fish solely for oil is discouraged globally, compelling industries to extract fish oils from fish waste by novel technologies. Higher concentrations of fish oil appear to be in the discarded waste, than the muscles consumed. The lipid (oil) percentages in components of the Pacific Ocean perch were reported to be 7.8 in the whole fish, 10.5 in the frames, 9.3 in the heads, and 13.5 in the viscera, suggesting the high potential for oil recovery from fish waste [133]. Production of omega-3-polyunsaturated fatty acids through microalgae, though possible, is more expensive than extraction from fish waste [151].

The technologies for extraction of oils from fish waste has evolved beginning from wet pressing (cooking, pressing, decanting, centrifugation, and chemical purification), through solvent extractions using isopropyl alcohol, to more recent super critical fluid extraction. The latter can be manipulated to separate dissolving quality oil, rich in essential fatty acids [152]. Considering the health benefits associated with omega-3-fatty acids and the heavy global demand for it, utilizing discarded fish components, which amounts to 50% of whole fish weight, is an industry with massive potential. Microencapsulation providing oxidative stability appears to be the best option to market omega-3-

fatty acids from fish. After extracting fish oil, the left-over meal is available either for extraction of proteins or as an ingredient in the animal feeds beneficially. The potential for a multi-product processing system should be targeted for economic and nutritional benefits from fish waste [152].

Shark oil possesses outstanding moisturizing properties and the ability to penetrate human skin strengthening barrier properties. Consumption of shark oil for 6 weeks has shown significant decrease of C-reactive proteins in human blood sera, and intracellular cholesterol levels in peripheral blood mononuclear cells. Shark oil also improves human erythrocyte fatty acid composition and provides other health promoting consequences [153]. Shark liver oil is a rich source of squalene, at concentrations higher than in vegetable sources. Squalene, a polyunsaturated hydrocarbon, carries health benefits including imparting antioxidant properties to human skin. Squalene has high potential in pharmaceutical and nutraceutical preparations focusing on human health [154]. Demand for fish oils tends to increase with increasing population, and with the public interest in a healthy life. The increasing demand could be met only by using new sources of polyunsaturated fatty acids. Fish waste qualifies to be the main inexpensive provider of heart healthy fish oils.

Annual global fish waste is around 75 million metric tons. It is recognized as a resource to produce components beneficial to health and fuels. Fish oils possess potential for use as a biodiesel. Distillation and supercritical fluid extraction could separate fish oils from fish waste material, to be used as biodiesel [155]. Considering antibiotics are used in fish farming, which could lead to residues in foods from fish, conversion of fish waste from farmed fish to fuel is a safer option. However, the cost of supercritical fluid extraction may be a deterrent in producing fish oil-based fuels economically. Distillation may be the best option currently. With the impending fuel shortages globally, increased production of biodiesel is a beneficial proposition.

4.7. Conventional Uses from Fish Waste

Calcium from fish bones, DHA extracted from tuna eyes, shark fins and cartilages as a main ingredient in soup, fish albumins as a possible substitute for processes using egg albumen, fish meals as a source of B vitamins, and proteins as components in animal feeds are some of the end products that could be utilized with low-cost processing. However, the benefits from high scale processing of fish waste bypass the benefits from low-cost processing.

5. Discussion

In a situation where expansion of marine fish harvest is not possible, and potential food safety hazards from contaminants in aquaculture is high, efficient use of captured fish is essential. In the 'blue growth' concept of prevention of pollution, and the circular economy concept of the World sustainable goals for utilizing waste for human benefit, the utilization of fish waste forms a significant component. There are many methods published for efficient preservation and processing of fish products. The challenge lies in converting current scientific knowledge into cost effective technologies. The conversion may be through combinations of preserving and processing methods, to be energy efficient and cost effective.

Proteins are extracted from fish waste to generate collagen, gelatine, proteins, and biopeptides, listed in the decreasing molecular size. The processes discussed in the above sections utilize chemical or enzymatic hydrolysis as major methods. Chemical hydrolysis produces a mixture of products listed above in different ratios. The mixture of hydrolyzed products could be fractionated by a single process to separate each of the products in the mixture for appropriate end uses. Enzymatic hydrolysis could generate products of more uniform quality and uniform properties beneficial to more refined purposes, such as biomedical production. The vision should be to establish chemical and biotechnological industries for fish waste, targeting the properties expected by the users of end products.

The wealth of scientific data available on fish waste needs to be handled with a focus to select the best permutations and combinations of processes and treatments to arrive at best end use. AI provides a platform for taking a better approach. The prospects of early warnings and risk identification tools to handle food safety through AI are described [156]. The suitability of AI systems

to predict algal blooms and mycotoxin production in foods is already under consideration. Hydrolysis of waste fish components to extract collagens, gelatines, proteins, enzymes, bioactive peptides, chitin, chitosan, and fatty acids are done through several methods or combinations of them, each giving a different degree of hydrolysis yielding diverse products. The products extracted are also dependent on the differences among fish species and the differences in fish habitats, temperate or tropical etc. Against the diversity of the raw material quality and processed product characteristics, there is the need to select products meeting specific medical, food, and cosmetic applications. The future may lie in the application of AI to combine the knowledge on fish production, fish availability, fish spoilage, safety of food-fish and possible processing applications to predict the best means to utilize the fish capture. The beneficiaries will be the public especially through economic benefits, efficient food industry and a sophisticated biomedical industry minimizing environmental pollution. The amount of information appearing in the above areas demands high scale data retention and analytical tools, where AI has a role to play.

6. Conclusion

More effective preservation and processing of food-fish ensuring food safety, and efficient waste utilization applying the rapidly generating knowledge need to be converted to viable technologies for global benefit.

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