AgriCos e-Newsletter
ISSN: 2582-7049
AgriCos e-Newsletter
Open Access Multidisciplinary Monthly Online Magazine
Volume: 04 Issue: 09 September 2023 Article No: 15

Enzymes from Fish Processing Waste and their Commercial Applications

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SUMMARY

Enzymes are key tools in biotechnology and related areas because of their catalytic nature. Accordingly, they have been extensively used in seafood production and processing for centuries. Commercial fishery processing results in discarding up to 50% of the raw material, consisting of scales, shells, backbones, viscera, head, liver, skin, dark muscle, roe, etc. Processing of the wastes has the potential to generate several valuable by-products, such as proteins, enzymes, carotenoids, fat, and minerals, besides addressing environmental hazards. Fishery wastes constitute good sources of enzymes such as proteases, lipases, chitinase, and alkaline phosphatase, etc. These enzymes have diverse applications in the seafood industry. Enzymes from fish and shellfish from cold habitats are particularly useful since they can function comparatively at lower temperatures, saving energy and protecting food products.

INTRODUCTION

Current global production of about 172 million tons of capture and aquacultured fishery products generate significant amounts of processing discards such as scales, shells, frames, backbones, viscera, head, liver, skin, belly flaps, dark muscle, roe, etc. The global enzymes market is expected to grow at a compound annual growth rate of 7.1% from 2020 to 2027, reaching USD 14.9 billion by 2027. Food enzymes as aids in food processing, including those from marine sources, have captured the interests of both regulators and food processors in most industrialized countries. Food enzymes are used to achieve desired properties in foods because they are more specifically effective at low concentrations and active under mild pH and temperature conditions. Under-utilized fish and seafood processing wastes are rich sources of novel enzymes, which can be isolated using conventional techniques. Because of their interesting properties, they can have several applications in food processing and other fields. These uses include modifications of proteins, PUFA in lipids, biosensor components, and for direct quality evaluation of fishery products. Marine enzymes have applications in the food industry since they may be unique protein molecules not found in terrestrial organisms or known enzymes from terrestrial sources but with novel properties. Their characteristics differ from homologous proteases from warm-blooded animals, such as tolerance to high salt concentration, low or high temperature, high pressure, and low nutrient availability. These characteristics of marine enzymes are due to the prevalent conditions in their habitats, such as hydrothermal vents and oceanic waves. As seafood and/or their processing wastes can serve as one of the economically viable sources of enzymes, an attempt is made to review the types and potential industrial applications of enzymes available in seafood and their by-products. A wide range of applications of enzymes in the bio-industry has considerably increased the global market for enzymes, and the production of industrial enzymes has become a cash cow for many companies.

Enzymes from Seafood processing waste:

The enormous pool of biodiversity in marine ecosystems offers a reservoir of enzymes with potential for their biotechnological applications. Seafood enzymes can be classified into broad categories:

- Protein-degrading enzymes
- Lipid-degrading enzymes
- Carbohydrate-degrading enzymes
- Nucleotide-Degrading Enzymes and
- Miscellaneous enzymes.

Protein degrading enzymes

It includes Proteases. Proteases are widely used in fish and seafood processing covering many applications. Protein-degrading enzymes hydrolyze peptide bonds that link amino acids together in the polypeptide chain, which form the backbone of protein molecules. Proteases are characterized as either exopeptidases or endopeptidases. Proteases can be classified based on their optimal pH (acid, neutral, and basic), their similarities to well-characterized proteases (trypsin-like, chymotrypsin-like, chymosin-like, and cathepsin-like, etc.), based on substrate specificity or based on their mode of catalysis.

Table 1. Protease enzymes - sources and molecular weight

Proteases	**Body parts**	**Molecular Weight (kDa)**	**Optimum activity**
Trypsin	Pyloric caeca, pancreatic tissue or intestine	22.5–24	pH 7.5–10, 35–45◦C
Chymotrypsin	Pyloric caeca	25–28	pH 9
Pepsin	Digestive glands, stomach tissue	27–42	pH 2–4, 37–55◦C
Chymosin	Gastric mucosa	33.8	pH 2.2–3.5
Gastrin	Gastric juices	32.3 and 33.9	pH 3

Lipid-degrading enzymes

Lipids are one of the major parts of the Earth’s biomass and lipid-degrading enzymes play an important role in the turnover of these water-insoluble compounds. These enzymes are classified as lipases and phospholipases.

Carbohydrate-degrading enzymes

Marine organisms feed on seaweed and produce a mixture of carbohydrate-degrading enzymes. eg: alginate lyase and chitinases. Chitinases can be endo- or exochitinases. Endochitinases randomly hydrolyze the internal β-1,4-N-acetyl-D-glucosaminide linkages of chitin, the major crustacean polysaccharide, generating soluble oligomers of GlcNAc.

Recovery of enzymes from seafood waste for seafood processing

Fishery wastes constitute rich sources of diverse proteases and other enzymes such as chitinase, alkaline phosphatase, and hyaluronidase, which are abundantly available in the intestines, followed by pyloric ceca, pancreatic tissues, hepatopancreas, shell, and other waste components. Being highly perishable, the wastes must be regularly available in good quality in relatively large amounts for the recovery of enzymes. Some of the commercially viable isolation processes for enzymes from crude waste extracts include precipitation by salts and polyacrylic acids, isoelectric solubilization/precipitation, ultrafiltration, pH shift, flocculation and membrane filtration, and overcooled acetone extraction.

Application of Fishery enzymes in Seafood processing

Applications of enzymes, including fishery enzymes in industrial processing, have many advantages over chemical methods, such as better process control, environmental and toxicological safety, low energy requirement, and cost.

Recovery of Proteins from Fish Processing Waste:

Fishery wastes constitute rich sources of various proteins, including myofibrillar, sarcoplasmic, and collagen. Collagens from the fins, scales, skins, bones, head, and swim bladders of bighead carp have been extracted using collagenases, pepsin from tuna, or trypsin from cod or pyloric caeca. Pepsin-solubilized collagen (PSC) could be substituted for mammalian collagen. PSC and its hydrolyzed product, gelatin, have relevant applications in the food, pharmaceutical, and photographic industries.

Fish Protein Hydrolyzates

Fish protein hydrolysate (FPH) is a breakdown product of fish proteins containing smaller peptides and amino acids. FPH is obtained by treatment of fish meat with trypsin, alcalde, chymotrypsin, pepsin, or other enzymes under controlled conditions of pH and temperatures. Most FPHs are amorphous powders, hygroscopic in nature, containing 81–93% protein, less than 5% fat and 3–8% ash and 1–8% moisture. Lean fish species or their processing wastes are ideal raw materials for FPH, which can be used as food binders, emulsifiers, gelling agents, and nutritional supplements. Besides, FPH can function as a cryoprotectant and nutritional additive in liquid fertilizer and aquafeed. Seafood proteases from Atlantic salmon and trypsin from fish pyloric ceca have been used for FPH.

Fish sauce

Fish Sauce Fish sauce is a popular condiment due to its characteristic flavor and taste, prepared by autolysis of fish by in situ proteolytic enzymes. Exogenous proteases can accelerate their production process. The addition of squid hepatopancreas accelerated sauce production.

Ripening of Fermented Fishery Products

Ripening contributes to developing characteristic flavor and soft texture in fermented fishery products during storage, which may accelerate exogenous proteases.

Meat Tenderization

Postmortem toughening of meat is a problem that affects its consumer acceptability. Aoki et al. (2004) observed that shrimp protease can tenderize beef. The hard texture of squid rings can be softened by protease treatment after removing the tough outer proteoglycans layer of the rings. Curd formation in canned salmon can be controlled by protease treatment

Caviar Production

Caviar is cured fish eggs of white sturgeon, salmon, trout, etc. While manual removal of roe sacks of eggs to prepare caviar is cumbersome, proteases, including collagenases, can assist in easily removing the supportive tissue.

Extraction of Chitin

Shrimp shell discards are an excellent source of chitin, the major component of crustacean and cephalopod shells. Chitosan, obtained by partial deacetylation of chitin, is a valuable polysaccharide for its antimicrobial, antitumor, hypocholesterolemic, and immunostimulation, and other functions.

Production of Seafood Flavorings

Seafood flavors are in demand to enhance the appeal of surimi-based seafood items such as artificial crab meat and fish sausage. Proteases can aid the extraction of flavor compounds from crustacean shells and other materials.

Production of Aquafeed

Feed ingredients containing nutritionally important docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were made by enzymatic digestion of eye or brain tissues from dusky rockfish and salmon.

Shelf Life Extension of Fishery Products

Dipping fresh shrimp in lysozyme at a concentration of up to 150 μg/mL retards the growth of spoilage microorganisms due to the action of the enzyme on the mucopeptide structure of microbial cell walls. Combining lysozyme, glucose oxidase, and catalase can enhance fish quality.

Retention of colour of cooked and frozen shrimp

The characteristic yellow color of pre-cooked frozen shrimp or crab as a result of oxidation can be prevented by dipping the cooked shrimp in glucose oxidase. The surface pH is lowered thanks to the generated gluconic acid retarding bacterial spoilage.

Perhaps β-Galactosidase is the widely used carbohydrase in the food industry to improve sweetness, solubility, flavor, and digestibility.

CONCLUSION

Fish trypsin, chymotrypsin, and cold-active chlamysin (lysozyme) from a marine bivalve are available commercially. Other commercial products include cold tolerant protease from North Atlantic cod, designated as Penzim, Neptune Aquatein, krill extract, heat-labile shrimp alkaline phosphatase, cod uracil-DNA glycosylase from Atlantic cod, oyster peptide and protein blends from CIFT, Kochi, India, and other seafood protein extracts. Potential also exists for protein engineering to introduce novel and precise sequence specificity into marine enzymes that can result in interesting applications. Despite its health benefits, FPHs are not available in good quantities. Calcitonin, perhaps, is the only bioactive peptide hormone commercially produced from salmon to treat osteoporosis. Enzymes from aquatic animals can be useful for developing enzymes with novel features such as low-temperature reactivity and stability. There is immense scope for applications of enzymes in food processing and biotechnology.

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