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REVIEW ARTICLE

ENZYMES FROM WASTE PRODUCTS AFTER FISH PROCESSING AND THEIR USES

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ABSTRACT

Fish processing sector is the main exporter of marine and seafood products in various countries. Almost 70% of fish is being processed before sale. Fish processing comprises grading, stunning, slime removal, washing, deheading, gutting, scaling, cutting of fins and meat bone separation, fillets and steaks. Depending on the type of fish and degree of processing, a significant amount of waste (roughly 20–80%) is generated during this process which is abundant in enzymes including acetyl glycosaminidase, proteases, chitinase, alkaline phosphatase, hyaluronidase, transglutaminase, lipases, and many others for instance, proteins, minerals, oil, bioactive peptides, amino acids, gelatin and collagen. Moreover, all these enzymes have immense applications in the seafood industry such as cheese production, collagen extraction and utilized as a clarifier, stabilizer and in production of glucosamine and chitosan oligomers as well as an enhancer of cream flavor. Thus, enzymes have a great interest because of their high activity in a small concentration and under mild conditions of temperature and pH. Therefore, there is a growing demand for specific, reactive and novel enzymes. This article provides an overview with a focus on fish processing waste as an enzyme source and its applications.

INTRODUCTION

Enzymes are an essential tool in biotechnology and related sciences because of their catalytic nature. (Fraatz et al., 2014; Jemli et al., 2016). As a result, enzymes have been widely utilized for millennia in the production and processing of food for millennia, albeit in somewhat empirical way that has recently given way to a rational approach (Whitaker, 1994; Whitaker et al., 2002; Fraatz et al., 2014). In recent years, emphasis has shifted from legal and regulatory problems to practical and systematic issues (such as improvement of process, enzyme formulations, improvement of enzymes at molecular level, searching for new/enhanced enzymes through metagenomics and conventional approaches), all of which have an impact on the food industry (Fraatz et al., 2016; Li and Cirino, 2014; Alma'abadi et al., 2015; Jemli et al., 2016). Fish and shellfish make up a substantial portion of the market in this sector (Morrissey and DeWitt, 2014), where enzymatic activity is considered crucial.

Many of the enzymes found in the internal organs of fish have significant catalytic activity even at very low concentrations. Twenty million tons, or 25% of global production from marine capture fisheries, are lost in the waste streams of processing units AMEC (2003). These wastes may be utilized to produce value-added products including fish oils, fish protein concentrate and enzymes like trypsin, chymotrypsin, collagenase, and pepsin. Large quantities of these enzymes are commercially recovered from fish viscera and have better catalytic characteristics, efficiency at lower temperatures, increased resistance to the concentration of substrate and increased consistency over a larger pH range (Kim and Mendis, 2005;Byun et al.,2005;Zhou et al., 2011). Biodiesel, margarine and Omega-3 fatty acids are all manufactured from fish oil. Both human and animal feeds use the fish protein concentrate. Moreover, Fish protein is also rich in amino acids that are beneficial for human consumption (Murrey and Burt, 2001).

The food industry uses marine enzymes because of the possibility that they are new protein molecules, not present in any terrestrial creature or that they are well-known enzymes with novel properties. They are distinguished from homologous proteases from warm-blooded species because of their resistance to high salt concentrations, low or high temperatures, high pressure, and inadequate nutrition availability. These properties of enzymes are attributable to typical circumstances in their environments, including oceanic waves and hydrothermal vents. An attempt is made to analyze the types and potential industrial applications of enzymes found in seafood and their by-products because seafood and their processing wastes can be commercially viable sources of enzymes (Likhar and chudasama, 2021).

SEAFOOD PROCESSING WASTE ENZYMES

The vast variety of enzymes found in marine habitats provides a source of enzymes with potential biotechnological uses. Broad categories can be used to classify seafood enzymes (Likhar and chudasama, 2021).

1. Protein-degrading enzymes
2. Lipid-degrading enzymes
3. Carbohydrate-degrading enzymes
4. Other enzymes

Protein degrading enzymes

Proteases are classified as endopeptidases or exopeptidases based on whether they will hydrolyze side chain or interior of protein molecule. There are four types of proteases found in fish and aquatic invertebrates: cysteine proteases (cathepsins L, H, and

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B, calpain) or thiol, acidic/aspartic proteases (cathepsin D, pepsin), metalloproteases like collagenases having metal in the active site and serine proteases (chymotrypsin, trypsin) (Shahidi and Kamil, 2001; Sriket, 2014). Shellfish/ Fish trypsins have strong catalytic activity over a wide range of temperature and pH range (Balti et el., 2009; Bougatef, 2013; Bustos et al., 1999; Klomklao et al., 2012; Silva et al., 2011). Fish visceral alkaline proteases have an ideal pH of 10. Rennin, also referred as chymosin, is an acidic protease that is highly active in clotting milk and is found in carp and seals. Fish pancreatic tissues secrete chymotrypsin, an endopeptidase with greater specific activity than bovine chymotrypsin. Sardine has been used to characterise new acidic proteases (Castillo-Yanez et al., 2004; Khaled et al., 2011). Fish lysosomal cathepsins have been extracted from a variety of fisheries products (Chere et al., 2007; Sriket, 2014). In addition to epithelium, cartilaginous, and bony structures, shellfish and fish digestive tracts also contain collagenases (Amesen and Gildberg, 2006; Daboor et al., 2012). The hepatopancreas of squid has been shown to have an acidic serine carboxypeptidase (Komai et al., 2007). Table 1 lists several proteases that have been isolated from various fisheries products along with some of their prominent characteristics.

Lipid degrading enzyme

The lipase enzyme catalyzes the hydrolysis of ester bonds in numerous substrates, including as cholesteryl esters, triglycerides (TGs), vitamin esters and phospholipids. Most living things have lipases, which are essential for the digestion of lipids. In the past, lipases were employed in the processing of milk-based goods. Lipases help to improve flavor in cream, cheese, and milk products by hydrolyzing milk fat. In recent years, lipases have been used in a variety of manufacturing processes, including the production of laundry detergents and baking (Reetz, 2002). Environmental chemical load has decreased as a result of lipase ability to eradicate lipid stains efficiently than that of conventional cleaning agents. Lipases have also been effectively employed in several synthesis processes as biocatalysts. Lipases, especially microbial lipases, can be used to inexpensively manufacture a variety of surfactants and emulsifiers. Numerous papers have analyzed and explored the industrial uses of lipases (Hasan et al., 2006). Commercially, ruminant pancreas and serous glands, particularly those of young calves and pigs, are used to extract lipase. Synthesis of industrial lipase employing various species of moulds, bacteria and yeasts have gained more interest as a result of the multiple benefits of microbial lipases (Hasan et al., 2006). Additionally, plants have lipases extracted from oilseeds, fatty fruits, and cereal grains. Aquatic animals have also been a potential source of lipases during the past ten years. Ten enzymes from fish processing waste materials and 190 from fish intestine and liver were discovered to be possible sources of

Table 1: The Isolation of Protease Enzymes from Fish Processing Wastes and Their Outstanding Properties.

Enzyme	Shellfish/Fish	Properties	References
Pepsin	Cod, tuna, salmon, mackerel, sardine, trout, harp seals, carp and capelin	Typically, low temperature and low activation energy are ideal.	(Sriket , 2014)
Alkaline proteinase	Monterey sardine's Viscera, aquacultured tilapia, white croaker, chum salmon, goby, tilapia.	Highly stable between pH values of 5–12 and barely susceptible to oxidizing agents.	(Sila et al., 2012)
Trypsin	Atlantic croaker, carp, aquacultured tilapia, Sardine threadfin hakeling,cut-le fish red snapper, Salmon, mackerel, anchovy, shrimp, bluefish and smooth hound	Having approximately 25 k Da molecular weight, ideal pH of 8–9, and temperatures of 50–60 °C. Compared to mammalian trypsin, The optimum temperature and thermostability of threadfin hakeling trypsin are lower.Trypsin from smooth hounds is very salt-tolerant.	(Balti et al., 2009), (Bougatef, 2013)
Gastricin	Atlantic cod, Hake	Greatest Activity at pH 3.	(Haard and Simpson , 2000)
Acidic protease	orange roughy, Sardine	Temperatures between 37 and 45 °C are optimal, acidic protease with pH 2.5	(Castillo-Yan˜ez et al.,2004; Khaled et al., 2011)
Lysosomal cathepsins type B, H, L	Crustaceans, tilapia, white croaker, dogfish,	Catalytic activity is achievable at low	(Shahidi and Kamil ,2001; Sriket ,

	squid, rainbow trout, herring, prawn muscle, carp and mollusks	temperatures and optimal pH is 5-7.5.	2014)
Chymotrypsin	Atlantic cod, capelin, Carp, rainbow trout, sardine, scallop, herring, spiny dogfish, prawn	Milk coagulation activity at an alkaline pH.	(Sila et al., 2012; Sriket, 2014)
Cathepsin D and other lysosomal cathepsins	White croaker, tilapia, cod, carp, white croaker, Pacific rock fish, capelin and squid.	Others are cysteine or serine proteases, with the exception of cathepsin D, an aspartic protease. degradation of fish myofibrils	(Aoki et al., 2004)
Collagenases	Crab,Carp, marine bivalve, pacific rock fish, cod and catfish.	Similar to mammalian metalloproteinase	`
Elastase	Sardine, Herring	Belly bursting	(Felberg et al., 2010)
Chymosin	Carp, Seal	Ideal pH is 2.0 to 3.5 and there is a lot of milk coagulation activity.	(Shahidi and Kamil , 2001)

lipases by Nayak et al., 2003 in their examination of several tissues that might be exploited as a source of lipases. The amount and catalytic activity of lipases have been found to differ with origin and fish species. Fish with high levels of phospholipids and wax ester in their diets have relatively high levels of lipase activity, as do fish with active lipases like mackerel and scup. Grey mullet (Aryee et al., 2007), Sardine (Smichi et al., 2010), salmon, hoki and carp (Görgün and Akpnar, 2012) are all good sources of omega-3 fatty acids (Kurtovic et al., 2010). Each isolated enzyme had distinct physicochemical characteristics. Salmon and hoki lipases that had been isolated were stable in several water-impermeable solvents. Additionally, according to Rivera-Pérez et al., (2011), various crustaceans can be utilized for the extraction of digestive lipases. Commercial lipases have comparable activity to that of fish lipases because of its ability to release fatty acid and produce odor and flavor in dairy cream. Studies have showed that utilizing fish lipases, dairy products' flavor might be improved. The synthesis of concentrated -3 polyunsaturated fatty acids from marine oils is another intriguing use of lipases (Kahveci et al., 2010). Commercial lipases have high substrate specificity and are not appropriate to synthesize -3 concentrates because of utilization of long-chain PUFAs. Fish lipase-immobilized method, however, has been proven to be effective for the concentration of PUFAs with long-chain PUFAs. Fish tissue-isolated lipases along with fish digestive lipases both have been identified. Adipose tissue, liver and red muscle all are examples of potential tissues from which tissue lipases might be extracted (Nayak et al., 2003; Kurtovic et al., 2009).

Carbohydrate degrading enzymes

Galactosidase from tilapia, amylase from abalone, Chitinases (Proespraiwong et al., 2010), -1, 3-glucanase from sea cucumber are among the carbohydrases identified from fish and shellfish (Taniguchi and Takano, 2004). Chitin, a linear heterogeneous polysaccharide made of D-glucosamine and N-acetyl-D-glucosamine linked by (1, 4) glycosidic bonds and found in crabs and mollusks, is the most common and renewable polysaccharide on Earth after cellulose (Gortari and Hours, 2013). Chitin production is about a total of 10 billion tons per year (Zargar et al., 2015). Chitin's (1-4)-N-acetyl-D-Glucosaminide links are hydrolyzed by chitinase. Exo- or endo-type chitinases are both possible. Diacetyl chitobiose units are catalyzed by exo-chitinase from non-reducing ends of chitin chains. Since many microbial species may also produce chitinases, they can infect arthropods and cause diseases. Marine fish's digestive systems and related organs produce a variety of chitinases that have been identified and described (Gutowska et al., 2004; Ikeda et al., 2009). Chitinolytic enzymes have a wide range of potential uses, including the production of N-acetyl-D-glucosamine and chitooligosaccharides both are known to have a variety of biological activities (antitumor, antifungal , antimicrobial, immune-enhancers etc.) and are highly sought-after in the pharmaceutical industry (Tsai et al., 2000; Shen et al., 2009 ;Wen et al., 2002). Nearly all human and animal cells contain lysozymes, which are known to have antibacterial capabilities. Lysozymes have been extracted from commercially processed clam shell and arctic scallop waste (Myrnes and Johanses, 1994).

Other enzymes

Lignin is the most prevalent naturally occurring aromatic polymer in the world and ligninolytic systems found in plants, mammals, fungi and bacteria induce resistant aromatic polymer for degradation (Kirk and Farrell, 1987). An extracellular enzymatic complex called the ligninolytic system contains oxidases,

peroxidases and lactases (Ruiz-Duenas and Martinez, 2009). As a result, lignin enzymes may find use in a variety of industries, including those that produce chemicals, fuel, food, beverages, textiles, paper, cosmetics and clothing as well as in bioremediation processes (Rodr'guez and Toca, 2006).

The extraction and characterization of collagenases from fish sources has been an intriguing area. Diverse forms of collagenases have been discovered in marine organisms. Rayfish, Carp, Atlantic cod and catfish are examples of marine and freshwater fish species that have had their extractable collagenases examined (Shahidi et al., 2001). Proteolytic enzymes are in high demand commercially, and there is an increasing focus on making the most of fish byproducts. Recently, interest has resurfaced in these topics. Overcooled acetone precipitation was shown to be an easy and long-lasting way to partly purify collagenase from rayfish viscera (Murado et al., 2009). Park and his research team used the same method ten years ago to separate collagenases from mackerel, *Scomber japonicus*. Collagenase may be isolated from fish waste by ammonium precipitation and chromatographic purification, as demonstrated by Kim et al. (2002). A similar technique was recently used to separate collagenases from the group metalloproteinase from a variety of fish waste, including flounder, haddock, ground fish and herring (Daboor et al., 2012). Marine fish waste has been used to isolate tissue collagenases in addition to digestive collagenases. The key factor contributing to the decline in fish tissue quality is collagenase, namely cathepsin B, which is found in fish tissue. It is considered vulnerable fish muscle waste. It suggests that collagenases can be extracted by the susceptible to the swiftly disintegration of fish muscle waste (Sovik and Rustad, 2006). Blue scads (Decapterus maruadsi) have optimal pH and temperature ranges of 5.5°C and 55°C, respectively, and their skeletal muscles have been found to contain the enzyme cathepsin L (Zhong et al., 2012). Furthermore, common carp gelatinolytic proteinases have been identified. These enzymes have the power to hydrolyze native type I collagen even at 4°C. These findings point out the possibility of extracting collagenase from digestive tissues , internal organs, cutoffs and fish processing waste (Wu et al., 2008).

Protein-glutamine-glutamyltransferases called transglutaminases (TGAs) (EC 2.3.2.13) enhance acyl transfer processes. Numerous major amines, such as-carboxyamide group of a peptide-bound glutamine residue as acyl donors and the -amino group of lysine, might be used as acyl acceptors. As a result of the simultaneous formation of intermolecular and intramolecular covalent bonds, named as -(glutamyl)-lysine, peptides and proteins are cross-linked and polymerization occurs. Since water serves as an acyl receptor in the absence of primary amines, glutamine residues' -carboxyamide groups deaminate into residues of glutamic acid (Diaz-López and Garca-Carreo, 2000; Zilda, 2014). The sulphydryl enzyme TG is a monomeric protein with the conserved pentapeptide active site sequence (Gln-Cys-Trp Tyr-Gly) found in seafood species. Walleye Pollock, atka mackerel, Rainbow trout, squid, red sea bream and botan shrimp liver muscles have all been found to have TG activity. Fish postharvest TG activity drops very quickly and is fully rendered inactive by freezing. In the absence of amine substrates, TGase uses water molecules as acyl acceptors to catalyse the deamidation of glutamine residues. According to reports, TGases control cellular proliferation, differentiation and growth as well as epidermal keratinization, blood clotting, hardening and wound healing of erythrocyte membrane. The majority of animal tissues and bodily fluids include TGases, which have also been identified from plants, aquatic species and microbes (Likhar and chudasama, 2021).

APPLICATION OF ENZYMES FROM FISH PROCESSING WASTES

Due to their powerful catalytic abilities, enzymes are now a necessary part of production processes for food, medications and cosmetics. Additionally, some enzymes have therapeutic potential or can be employed to treat diseases when the endogenous enzyme activity is compromised. Enzymes thus have a high economic value. Some of the possible uses for fish waste-derived enzymes are included in Table 2 (Kim and Depariya, 2014).

Proteases

Proteases have wide variety of uses during the processing of shellfish and fish (Diaz-López and Garca-Carreo, 2000; Fernandes, 2016).

Table 2: Possible uses of fish processing waste enzymes.

Enzymes	Area of Use	Applications
Pepsin	Fishery Dairy Cosmeceuticals, Pharmaceutical Leather	Caviar production, Descaled fish, Fish silage. Cheese production Therapeutic agents, Collagen extraction. To remove hair and remaining tissue.
Trypsin	Pharmaceutical Dairy Paint Food, fishery	Wound healing Production of cheese pearl essence production Production of seafood flavor, fish sauce

Chymotrypsin	Fishery Leather Detergent Food processing	Bone protein removal Dehairing, batting Cleaning agent Meat tenderizing
Collagenase	Fishery Food processing	Surimi processing, fish ripening Clarifier, stabilizer
Lipase	Food Detergent	Enhance flavor of cream cleaning agent
Chitinase	Nutraceutical, Pharmaceutical	glucosamine production, chitin production

Fish protein hydrolyzate

One of the widest uses that have been proven effective is the synthesis of fish protein hydrolyzates (FPHs). FPH is produced by chemical hydrolysis of protein-rich byproduct waste or enzymes (exo-peptidases or endo-peptidases) from fish processing industry, including viscera of fish flesh, trimmings, bones, mince, head, skin and liver. This process produced peptides containing 2–20 amino acids, depends on the incubation time, the fish used as the source, degree of hydrolysis and the enzymes used. This means that for each hydrolyzed peptide link, a free -amino group is created (Nguyen et al., 2011; He et al., 2015). FPHs exhibit functional qualities that are useful for food formation, such as the ability to emulsify and create foam, the ability to make gel, the solubility of proteins in oils, and the ability to bind water (Kristinsson and Rasco, 2000; He et al., 2015). FPHs outperform protein hydrolyzate and chicken byproducts, both of which were produced by proteolysis using Alkalis; this consequence is explained by the different amino acid composition. Furthermore, they contained balanced amount of amino acids and have better gastrointestinal adsorption than that of free amino acids, FPHs are envisioned as viable sources of proteins for human nutrition (Clemente, 2000). As indicated by the existence of various commercially available products, Fish protein hydrolyzates also include antihypertensive, antioxidant, immunomodulatory, and antibacterial properties. As a result, their inclusion into health\functional and nutraceuticals foods has just lately become popular (Hu et al., 2015).

Fish sauce

Fish protein that has been digested and enzyme-solubilized produces fish sauce. Fish sauce is produced, preserved with salts, and added as an ingredient to vegetable dishes. Although it was immensely popular in Roman society, fish sauce has all but vanished from European cuisine since then and is now primarily linked with Southeast Asia (Gildberg et al., 2000). Exogenous enzymes can significantly accelerate the fermentation of fish (Aquerreta et al., 2001). But in order to maintain the essential functional and organoleptic features and quality of the final product, their use must be carefully examined. The application of exogenous enzymes, on the other hand, can result in a final product that performs the desired function, exhibits acceptable characteristics, and even shows potential for nutraceutical qualities (Aquerreta and coworkers, 2001).

Production of seafood flavorings

Demand for seafood flavors has increased the value of surimi-made seafood goods, such as crab meat and fish sausage. Extraction of taste components from crustacean shells and other materials can be aided by proteases (He et al., 2004). FPH can also serve as a model for taste systems by including substances like fish oil (Peinado et al., 2016).

Caviar production

Fish eggs from species including white sturgeon, salmon, trout and others are preserved to make caviar. While it is laborious to manually remove the roe sacks from eggs to make caviar, collagenases, can help by simple removal of supporting tissue (Gildberg, 1993).

Lipases

PUFAs Production

Nutritional benefits of DHA and EPA have encouraged research for new lipid sources (Venugopal, 2009). Fish oil EPA and DHA can be enhanced using lipases. The process involves transesterification, which involves exchanging the fatty acids of different monoesters and triglycerides or acidolysis, also known as interesterification, which involves exchanging the fatty acids of a single triglyceride or a mixture of triglycerides (Klinkesorn et al., 2004).

Flavor enhancement

Lipases impact the release of taste molecules along with aminopeptidases, muscle cathepsins, peptidases and calpains (Toldra, 2007).

Deacetylation of chitin to prepare chitosan

The conventional process of deacetylating chitin with concentrated sodium hydroxide at high temperatures is challenging for the production of chitosan (Venugopal, 2012). Chitinase deacetylation, on the other hand, is less expensive and safe for the environment (Kim and Rajapakse, 2005). Additionally, chitinase, lysozyme and cellulase have been used to create low molecular weight chitosan compounds (Lin et al., 2009).

OTHER APPLICATIONS

One of the most popular carbohydrates utilized in the food business to enhance sweetness, solubility, taste and digestibility is galactosidase (Husain, 2010). Aquafeed containing carbohydrases can lessen the negative effects of the feed's nonstarch polysaccharides. A key factor in the quality loss of meat foods is oxidative deteriorative processes, which can be reduced with glutathione peroxidase (Sinha et al., 2011).

CONCLUSION

The production of enzymes from the processing of aquatic species has unique properties, such as low temperature reactivity and stability. Their prospective applications include a wide range of industries, comprising biotechnology, drugs, clinical diagnostics, fabric improvement, detergents, biosensors, leather and organic synthesis. Novel peptides with intriguing clinically significant properties, including as antithrombotic activity, antihypertensive, antiamnesic, mineral-binding and antibacterial, immunodulatory, antioxidant activity can be created using proteases. Lipases have a wide range of uses, including as detergent and food additives, in molecular biology, environmental bioremediation and biotransformation. Other enzymes can also be used in food biotechnology in a huge variety of ways.

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