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Seafood enzymes and their potential industrial applications

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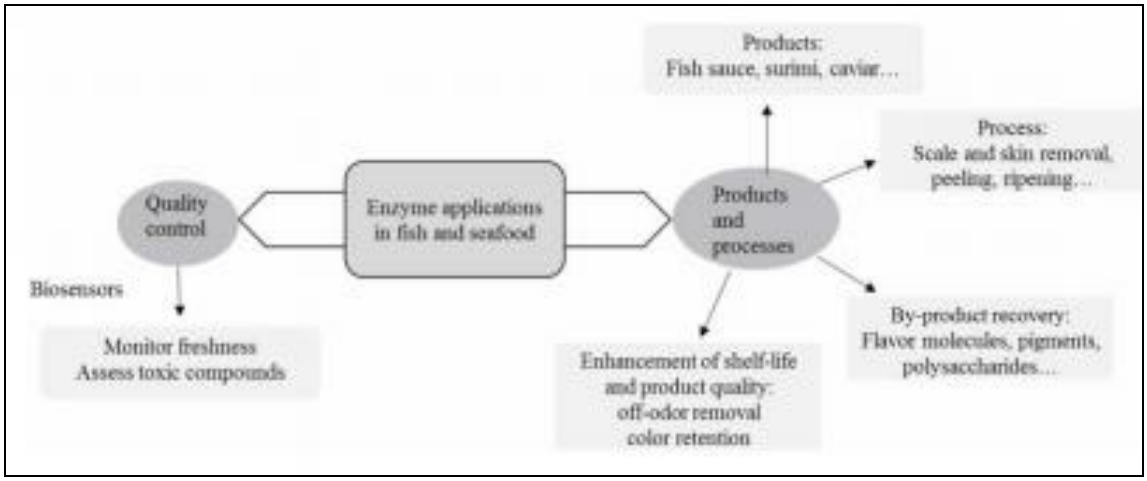
Abstract
Enzymes are key tools in biotechnology and related areas because of their catalytic nature. Accordingly, they have been extensively used in food production and processing for centuries, albeit in a rather empirical manner, which has been superseded by a rational approach in the last decades. In recent years, the focus has been on technical and scientific issues (enzyme formulations, molecular improvement of enzyme, screening for new/improved enzymes through traditional and metagenomics approaches, process improvement) as well as on legal and regulatory matters (definition of enzymes and technological purposes, procedures for safety assessment, harmonization of regulations, among others), all of these abridging the food industry. There is huge potential for their diverse applications such as food processing, biotechnology, clinical diagnosis, detergents, leather and fabric upgrading, organic synthesis, therapeutics, biosensors, among others.

Keywords: biotechnology, catalytic, enzymes, and food processing

Introduction
The global enzymes market is expected to grow at a compound annual growth rate of 7.1% from 2020 to 2027 to reach USD 14.9 billion by 2027, as predicted in the Enzymes Market Size and Share Industry Report, 2020-2027. Industrial enzymes area business worth US$ 2 billion, 50% of which is contributed by food enzymes. There is a steady increase in the number and applications, as well as annual turnover from food enzymes in the recent past. As at the beginning of 2001, the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) listed about 160 enzymes. Food enzymes as aids in food processing, including those from marine sources, have captured the interests of both regulators and food processors in most of the industrialized countries. In general, food enzymes are used to achieve desired properties in foods because they are more specific effective at low concentrations, active under mild conditions of pH and temperature, and are easy to inactivate after the desired transformations.

Underutilized fish and wastes from seafood processing are rich source of number of novel enzymes, which can be isolated employing conventional techniques. Because of their interesting properties, they can have several applications in food processing and other fields. These uses include modifications pf proteins, PUFA in lipids, shelf life extension and as components of biosensors and also for direct quality evaluation of fishery products.

Marine enzymes have application in the food industry, since they may be unique protein molecules not found in any terrestrial organism, or may be known enzymes from the 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.



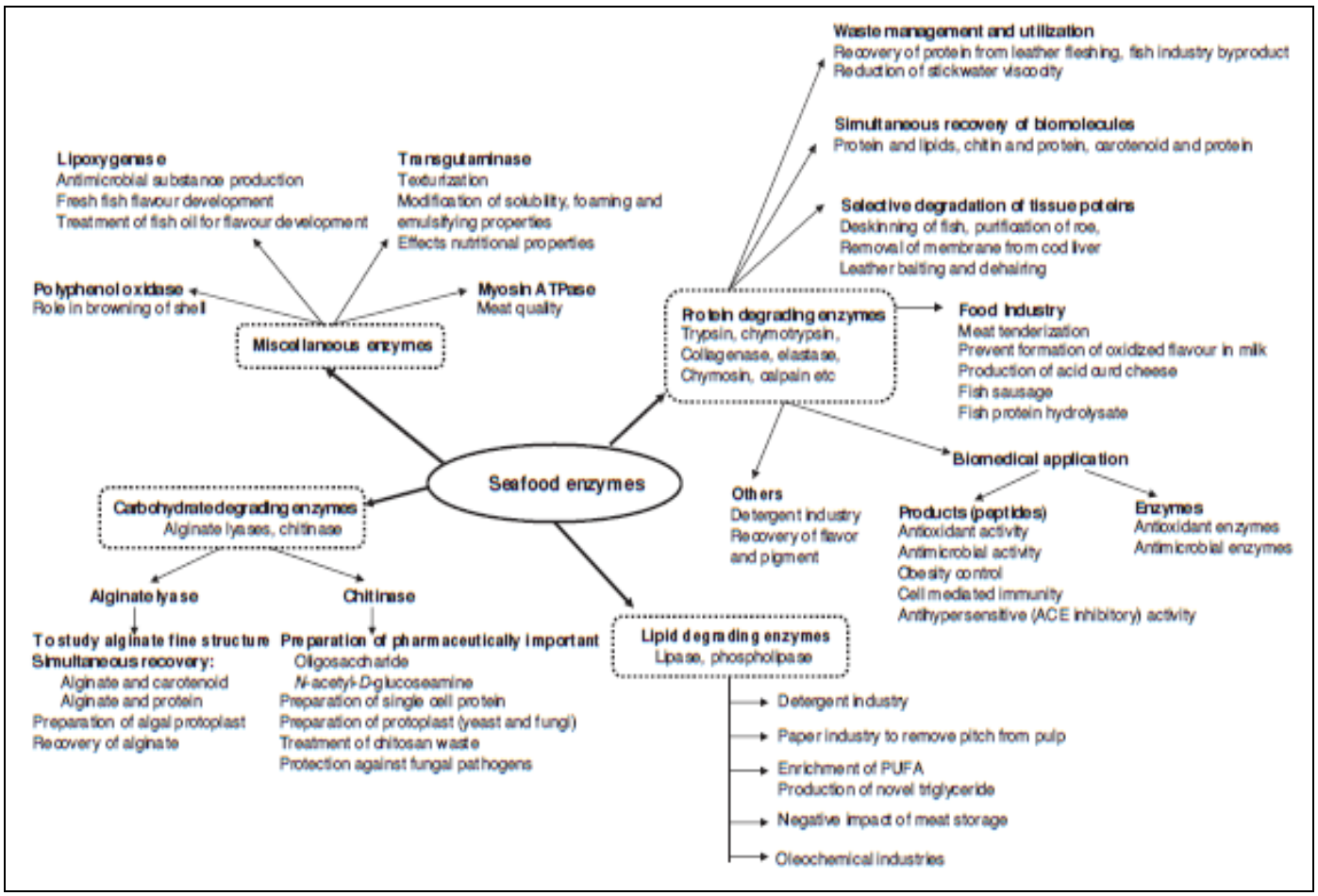
***Source*:** Trincone A. Enzymes in Fish and Seafood. Frontiers in Bioengineering and Biotechnology. 2016.

Fig 1: A schematic overview of enzyme applications in fish and seafood processing.

Enzymes from seafood processing wastes

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 of:

1. Protein-degrading enzymes;
2. Lipid-degrading enzymes;
3. Carbohydrate-degrading enzymes;
4. Nucleotide-Degrading Enzymes and
5. Miscellaneous enzymes.



Source: Hathwar SC, Rai AK, Nakkarike SM, Narayan B. Seafood enzymes and their potential industrial application. Handbook of Seafood Quality, Safety and Health Applications. 2010; 522-532.

Fig 2: Potential and scope for the application of seafood enzymes in various industries

1. Protein Degrading Enzymes

It includes Proteases. Proteases are widely used in fish and seafood processing (Diaz-López and García-Carreno, 2000; Suresh et al., 2015), covering a wide array of applications. Protein degrading enzymes hydrolyse 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. Endopeptidases contribute up to 48% of all industrial enzymes and are more important than exopeptidases. Proteases can be classified on

the basis of their optimal pH (acid, neutral, and basic), their similarities to well characterized proteases (trypsin-like, chymotrypsin-like, chymosin-like, and cathepsinlike, etc.), on the basis of substrate specificity or on the basis of their mode of catalysis (serine, cysteine, aspartyl, and metalloproteases).

Table 1: Occurrence, molecular weight, and optimum activity of different types of proteases.

Proteases	Body parts	Molecular Weight (kDa)	Optimum activity
Trypsins	Pyloric caeca, pancreatic tissue or intestine	22.5–24	pH 7.5–10, 35–45◦C
Chymotrypsins	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
Gastricin	Gastric juices	32.3 and 33.9	pH 3

Source: Hathwar SC, Rai AK, Nakkarike SM, Narayan B. Seafood enzymes and their potential industrial application. Handbook of Seafood Quality, Safety and Health Applications. 2010; 522-532.

Proteases are ubiquitous in bio-systems and are associated with biochemical, physiological, and regulatory functions in cells and organisms. They play vital role in digestion of food. Proteases from fish and aquatic invertebrates can be classified into four major groups, namely, acidic/ aspartic (formerly known as carboxyl) proteases, serine proteases, thiol or cysteine proteases and metallo-proteases. Proteases including pepsine, trypsin, chymotrypsin, gastricin, elastase, catharsis and other variedly distributed in the muscle, gastric mucosa, hepatopancrease, pyloric caeca, digestive organs and intestines of fishery products.

Fish pepsins, which may exist in isoenzymes forms, belong to the aspartic endopeptidase family. They have been isolated from the gastric mucosa of various marine and freshwater fish species. Collagenases, elastases and carboxypeptidases have been found in fish intestine.

Fish serine proteases are generally similar to those of warm blooded animals in their properties, which generally possess high activity under alkaline conditions rather than neutral pH. Two types of serine proteases have been recovered from fish pyloric caeca, namely, trypsin and chymotrypsin.

2. 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. For the purpose of this review, these enzymes have been classified under the two headings of lipases and phospholipases.

2.1 Lipases

Lipases, triacylglycerol acylhydrolases (EC 3.1.1.3), promote the hydrolysis of tri-, di-, and monoglycerides to glycerol and fatty acids, in the presence of excess water, while in water-limiting conditions they promote ester synthesis. They often express other activities, namely phospholipase or other esterase type of activity, all of which have acknowledged industrial relevance (Venugopal et al., 2000; Verma et al., 2012) [16, 17]. Lipases are of particular interest for the isolation of oil and fats from seafood byproducts as well as in the preparation of ω-3-poly-unsaturated fatty acids (ω PUFAs) and enriched marine oils, given the nutritional value of these compounds (Chen et al., 2012; Walker et al., 2015) [3, 20]. Lipases are active over a wide range of temperature ranging from – 20 ^{0}C to 65 ^{0}C (Shahidi and Kamil, 2001) [13].

2.2 Phospholipases (PL)

PL are lipolytic enzymes that hydrolyze phospholipids and are grouped into two categories, acyl hydrolases and phosphodiesterase. Unlike classic esterase, their natural substrate is insoluble in water and their activity is at maximum only when the enzyme is adsorbed onto a lipid water interface. The most studied types of phospholipids are PLA1, PLA2, and PLC. PLA1 plays an important role in wax ester synthesis, PLA2 plays a role in wax ester synthesis using carbohydrate and amino acids as precursors, and PLC plays an important role in signal transduction and provides potential secondary messengers. PLA2 hydrolyzes essential dietary phospholipids in marine teleosts, regulates membrane lipids modification as a response to environmental changes, and provides fatty acids as substrates for metabolic energy and biosynthesis of prostaglandins. Generally PLA2 is Ca2+ dependent and has an optimum pH in alkaline condition and is stable against acid and heat treatment. PLA2 from hepatopancreas (Isoform DE-1 and DE-2) and calcium dependent PLA2 from pyloric ceacum of red sea bream (*Pagrus major*) have been purifie and characterized. PL have been studied from various fish such as pollock, *Pollachius virens*, pyloric caeca and hepatopancreas of red sea bream, liver of trout, and cod muscle. The optimum temperature and pH ranges of these PL are reported to be 30 to 45 ◦C and 8 to 10, respectively. However, lysosomal PL of cod (pH 4) and PLA1 of Bonito (pH 6.5–7; temperature 20–30 ◦C) are exceptions to this. Furthermore, unlike porcine lipase, BAL (a carboxyl ester lipase) purifie and characterized recently from hepatopancreas of red seabream (*Pagrus major*) is reported to efficientl hydrolyze ethyl esters of polyunsaturated fatty acids (PUFA) such as arachidonic acid (AA) and eicosapentaenoic acid (EPA).

3. Carbohydrate-Degrading Enzymes

Marine organisms feed on seaweed and produce a mixture of carbohydrate-degrading enzymes. In this section, we are restricted to occurrence of alginate lyases and chitinase occurring in seafoods and/or their by-products, and not from marine microbial sources. The sources and characteristics of alginate lyases and chitinase in seafoods are as follows:

3.1 Alginate lyases

Alginate lyases are important carbohydrate-degrading enzymes that allow marine organisms to efficiently harness all of the energy available. Alginate is a copolymer of alpha- *L*-guluronate (G) and its C5 epimer beta-*D*-mannuronate (M) arranged as homopolymeric G block, M block, alternating GM, or random heteropolymeric. Alginate lyases have been characterized based on their dominant cleaving action on M-rich or G-rich alginates as poly (M) lyases and Poly (G) lyases. They have been isolated from many sources, including marine algae, marine molluscs, and a wide range of micro-organisms. Although most marine organisms produce single alginate lyase with define substrate specificit, some produce two or more types. In marine molluscs, lyases has been isolated from gut, gland, style, or hepatopancreas

3.2 Chitinases

Chitin is the major shell component of crustacean and its deacetylated product, chitosan has several functional properties including antimicrobial and antitumour properties, which make them versatile tools in food, pharmacy and medicine (Venugopal, 2009). Chitinase hydrolysis (1-4)-β N-acetyl-D-glucosaminide linkages in chitin. Chitinases can be exo- or endo-types. Exo-chitinase catalyses diacetyl chitobiose units from non-reducing ends of chitin chains (Clark et al., 1988). Related enzymes are N-acetyl glucosaminidases, some of which can hydrolyze the terminal, non-reducing N-acetyl glucosamine residues of chitin; and lysozymes, which can act slowly and endohydrolases. Chitinases (Chitinase, chitobiase and lysozymes) are found not only in fishery products, but also in seaweeds, microorganisms and mammals. The enzymes may also present in gastro intestinal tract and also pyloric caeca of marine species including cod and sole. These enzymes have a wide range of molecular size from 5 to 20 kDa. The activity of enzyme depends on the nature of chitin. In marine species, chitinolytic activity is associated with the moulting processes of insects and crustaceans (Danulat and Kausch, 1984; Clark *et al*., 1988; Gooday, 1991).

Lysozymes, found in almost all-human and animal cells, is known to have antibacterial properties. Lysozymes has been recovered from commercial processing waste of arctic scallop and clam shell (Myrnes and Johanses, 1994).

4. Nucleotide-Degrading Enzymes

4.1 ATPase

ATPase activity is controlled within the muscle through the modulation of calcium. The most important form of ATPase in the muscle is actually a structural component of the major contractile protein, myosin. It is part of the globular head of the myosin molecule (called heavy meromyosin) and is only active when the sarcoplasm contains sufficiently high levels of soluble calcium. In the living animal, Ca+2-activated ATPase is responsible for the breakdown of ATP with the release of energy used for in the contractile process.

4.2 AMP Deaminase

In 1957, Jones and Murray discovered that IMP levels in postmortem cod muscle first increased and then subsequently decreased during iced storage. IMP has been recognized for many years as having flavor-enhancing properties. The production of ammonia as a spoilage compound also gives special significance to AMP deaminase. AMP deaminase was first reported in mammals during the 1920s but much of the work on this enzyme in the skeletal muscle of fish was reported during the 1960s.

$$2\ ADP \leftrightarrow ATP + AMP$$

The AMP deaminase reaction is almost nonexistent in the resting muscle due to the relative absence of AMP. The continuous removal of AMP keeps the myokinase reaction moving in a forward direction. The end products for the AMP deaminase reaction are IMP and ammonia (NH3). The IMP may be converted to Ino and then Hx, whereas the ammonia is transported to the gills through the blood. Excretion of the potentially toxic NH3 is carried out mainly in the gills with minor amounts being excreted in the urine. In marine elasmobranch fish such as sharks, which manufacture large quantities of urea for osmotic regulation, ammonia enters the urea cycle.

4.3 5′ Nucleotidase

There are in fact three possible enzymes responsible for the conversion of IMP to Ino in fish muscle: 5′ nucleotidase, alkaline phosphatase, and acid phosphatase. Of these three, 5′-Ntase has received the most attention and is probably the most important of the three in chilled postmortem fish. Perhaps the most remarkable feature of the 5′-Ntase reaction:

$$IMP \leftrightarrow Ino + Pi$$

4.4 Nucleotide Phosphorylase and Inosine Nucleosidase

The degradation of Ino to Hx is a critical step in the overall breakdown of ATP and its catabolites. This is because the formation of Hx is often an indication of the last stage in edible fish quality. Tarr suggested that this reaction was catalyzed by two enzymes, nucleoside phosphorylase (NP) and nucleoside hydrolase (sometimes referred to as inosine nucleosidase). It is reported that both enzymes were found in Pacific lingcod, but this is apparently not the case in all fish species. LeBlanc was able to demonstrate the presence of only minute quantities of NP or IN in the muscle tissues of freshly killed Atlantic cod (*Gadus morhua*). In fact, the levels of IN were more than 1000-fold less than the levels previously reported for Pacific lingcod by Tarr.

4.5 Xanthine Oxidase

Xanthine oxidase (XO) has been found in a variety of species and catalyzes the final step in the nucleotide breakdown:

$$Hx + H2O + O2 \rightarrow Xa + H2O2$$
$$Xa + H2O + O2 \rightarrow \text{Uric acid} + H2O2$$

Hypoxanthine is not only an objective indication of spoilage in chilled fish but also contributes the off flavors typical of spoiled fish. Hx is itself bitter and contributes bitterness, but only under certain circumstances.

5. Miscellaneous Enzymes

5.1 Transglutaminase (TG)

TG (EC 2.3.2.13) is an endogenous fish enzyme that catalyzes acyl transfer reaction between γ carboxamide groups of glutamine residues in proteins, polypeptides, and a variety of primary amines. TG is a sulphydryl enzyme with a conserved pentapeptide active site sequence (Tyr-Gly Gln-Cys-Trp) and TG from seafood species are generally monomeric proteins. TG activity has been reported in the muscles of rainbow trout, red sea bream, atka mackerel, scallop, botan shrimp, squid, and walleye pollock liver. Postharvest TG activity rapidly decreases in fish and is completely inactivated by freezing. In absence of amine substrates, TGase catalyses the deamidation of glutamine residues during which water molecules are used as acyl acceptors. TGases are reportedly responsible for the regulation of cellular growth, differentiation and proliferation, blood clotting, wound healing, epidermal keratinization and stiffening of the erythrocyte membrane. TGases are widely distributed in most animal tissues and body fluids and have been isolated from plants, aquatic organisms and microorganisms.

5.2 Polyphenol oxidases (PPO)

PPO are a mixture of phenoloxidases (monophenol oxidase and cathecol oxidase), phenolase, cathecol oxidase, cresolase,

diphenol oxidase, and tyrosinase. They are generally found in shellfish and their by-products including shrimp, prawns, lobster, and cuttlefis. Stability of PPO varies over a number of factors such as temperature, pH, substrate, ionic strength, buffer system, and time of incubation, apart from source and environmental factors. Most of the PPO are heat-labile, except lobster PPO, which is reported to exhibit thermotolerance. PPO in crustacean species results in post-harvest melanosis and related quality problems.

5.3 Lipoxygenase (LOX)

LOX is a dioxygenase that catalyzes the oxygenation of PUFA containing a cis, cis-1, 4-pentadiene system to hydroperoxides. LOX activity is reported in a few marine organisms such as coral, sea urchin, grey mullet, skin and gills of trout, and eggs of starfish Although considerable amounts of LOX in the muscle or liver of various marine organisms is not reported, it was detected in large amounts in the light muscles of lake herring. LOX exists in multiple forms, as 5-LOX, 12-LOX, and 15-LOX, in the tissues of shrimp and grey mullet. Of these, 12-LOX has the highest activity and is in higher concentrations (almost 20-fold) than the other two. The suitable substrates for LOX are PUFA containing a series of methylene interrupted *cis* double bonds. LOX from shrimp has shown increased reactivity with PUFA substrate of increased unsaturation, while those from grey mullet act in an opposite manner.

Potential Industrial Application of Enzymes

1. Fish Sauce Production

Fish sauce is a traditional fermented fish product and is an important food ingredient on vegetable dishes in Southeast Asia. Fish sauce is made from small pelagic species such as anchovy and sardine. It is a matter of search to see whether endogenous fish enzymes or microbial enzymes are more important factors for the degradation of fish proteins during fish sauce fermentation. Most microorganisms except halophiles will not survive due to very high salt concentration in fish sauce (20-25%). The total bacterial count is drastically reduced after 3 weeks fermentation of anchovy and microbial enzymes did not contribute to the degradation of protein during fermentation of Arctic capelin. However, bacterial metabolites such as free fatty acids, amines, and various nitrogen-containing compounds are major contributors to fish sauce flavour.

Trypsin-like activities from fish intestine are significantly inhibited by salt, however the inhibition level varies considerably with different species. Activity of crude pepsin from Atlantic salmon (Salmo salar) was almost inhibited by 10% NaCl and completely inhibited by 15% NaCl concentration. Activities of trypsin and chymotrypsin from anchovy intestine were shown to have about 20% of their original activities for hydrolysis of casein in the presence of 15% NaCl. However, the activities of trypsin and chymotrypsin from anchovy intestine were displayed about 70% of their original activities for hydrolysis of anchovy myofibrillar proteins at same salt concentration. Chymotrypsin is more important than trypsin during anchovy sauce fermentation since chymotryptic activity is more active at neutral and weak acid conditions than trypsin. Furthermore, protein degradation during fish sauce fermentation was considerably reduced by addition of soybean trypsin inhibitor, which inhibits enzyme activity of trypsin and chymotrypsin. This suggests that trypsin and chymotrypsin in fish intestine are major factors for protein degradation and solubilization during fish sauce fermentation, even if their activity is partly inhibited by the salt.

2. Fish Protein Hydrolysate (FPH)

Fish protein hydrolysates are prepared by extensive digestion of inexpensive pelagic fish and fish processing wastes using proteolytic enzymes at adequate reaction conditions. The fish and shellfish protein hydrolysates have been mainly used as food or feed ingredients. The process to produce FPH consists of protease treatment, inactivation of the enzymes, and separation of soluble fraction, concentration and drying of the fraction. The main point of FPH processing is the breakdown the tissue proteins into smaller peptides or amino acids to obtain a water-soluble product.

Bitter taste is a common problem in preparation of FPHs, therefore, they are unsuitable for human consumption. Bitter taste is caused by peptides containing bulky hydrophobic groups in C terminal. The intensity of bitterness depends on the degree of hydrolysis and the specificity of protease. Bitter taste can be removed by selective separation with chromatography and by enzymatic treatment inducing plastein synthesis. Another method to prevent bitter taste in FPHs is to reduce the formation of bitter-tasting peptides by excessive protein hydrolysis to peptides and amino acids.

Cold-adapted proteases from fish have a relatively high specificity for peptide bonds and produce relatively high molecular activity. These enzymes have been suggested for the production of FPH, since this improves the level of pleasant-tasting active amino acid and eliminates the bitter taste in protein hydrolysis.

3. Seafood Flavorings

Seafood flavours are in high demand for use as food additives in artificial crab, kamaboko, fish sausage, and cereal-based extrusion products. Traditionally, seafood flavouring agents from various sources of raw material can be produced by extraction with water. The process for the recovery of organic materials from wastewater not only solves the water pollution problem but also maximizes the use of food processing wastes for ultimate human consumption. The potential for recovery of water-soluble organic components of discharge streams from shrimp processing plants has been demonstrated. Wash water from clam processing plants has been converted into a potentially marketable natural clam flavouring agent. Also, oyster shucking liquid waste, containing protein, nonproteinaceous nitrogen compounds and other organic materials, was concentrated and evaluated for human consumption as oyster soup.

Endogenous proteinases can aid the extraction of flavour compounds from fish and shellfish by-product. Enzymatic hydrolysis has been used to produce seafood flavorants from seafood processing by-products. A similar process to FPC preparation has been developed by a French company (Isnard-Lyraz) to recover seafood flavouring compounds from marine animals using whole animal or their by-product. The process consists of degradation of raw material by enzymatic hydrolysis, thermal inactivation of enzymes, separation of bone and shells, filtration or centrifugation, and concentration of flavour enhancers extracted from raw materials. The process of enzymatic degradation acts to fasten the liquefaction of raw material and to induce flavour precursors by hydrolysis of proteins and nucleotides. In the case of oyster or oyster cooker effluent, use of proteases and

amylases has potential since this material contains appreciable amounts of glycogen. A protease/ amylase process has particular appeal, since during thermal processing amino acids and peptides released by protease action can react with reducing sugars liberated by the amylase action to generate a cooked meat aroma. This technology has been used for the production of meat and savoury flavours.

4. Proteases As Fish Processing Aids

A. Caviar Production

Caviar refers only to the riddles and cured roe of the sturgeon, although the roe of sturgeon has been replaced with the roe of less expensive fish species. Less expensive caviar is made from the eggs of several fish: cod, catfish, herring, capelin, lumpfish, and skipjack. The riddling process in the preparation of caviar is a difficult task, which is either carried out mechanically or manually. One problem in the conventional process is that it is difficult to release the fish roe from the connective tissues of the roe sac without damaging the roe. The yield of intact roe is as low as 50% to 70%. Fish pepsin and collagenolytic enzymes from crab hepatopancreas, can be used to release fish roe from the connective tissues of the roe sac. A method for the enzymatic production of caviar from salmon roe has described in a US patent using proteinases at acid, neutral and alkaline conditions and enzymatic production of caviar from salmon and trout roe has been produced annually in Canada and Scandinavia. Several proteases have been used in the riddling process. Acidic proteinases from cold-adapted fish such as Atlantic cod and orange roughly have shown a good result in releasing of roe from sacs.

The caviar yield from rainbow trout (Oncorhynchus mykiss) roe is approximately 90% compared to 70% with conventional mechanical methods. There is a commercial production of cold-adapted pepsins from Atlantic cod from a Norwegian company (BIO TEC, Tromso). This type of production is also beginning in Iceland at the Icelandic Fisheries Laboratories. Commercial production is also being started in New Zealand. Industrial production of caviar with these proteinases has been started in Canada, The United States, Australia, and several Scandianvian countries.

B. Skin Removal

Mechanical removal of fish skin is laborious and may cause reduced yield and quality. Enzymatic method for deskinning of fish is based on the solubilization of collagenous skin tissue without degrading the muscle tissue. Enzymatic deskinning can be done by cold-adapted fish pepsin at low reaction temperatures. Proteases are usually mixed with carbohydrases to facilitate skin removal. An enzymatic deskinning method has been developed for herring in Norway. The whole herring is soaked in 5% acetic acid at 10 ºC for 5 min to loosen the scales and denature the skin collagen, and incubated with fish pepsin in 0.5% acetic acid at 20 ºC for 30 to 120 min depending on the size of fish and harvesting season. The skin is partially solubilized and washed off in cold water. This method has worked well on a pilot scale; however, uneven deskinning may be caused by different skin thickness and toughness at different parts of the body.

An enzyme for deskinning of squid rubbery membrane is commercially available by the Norwegian company (Biotec ASA). A production line for a similar process of squid enzymatic deskinning has been developed by Biotec Mackzymal in Norway and produced by Carnitech in Denmark.

C. Scale Removal

International market for fresh and frozen skin-on fillets of fish such as haddock, hake, ocean perch, redfish, and salmon are increasing. Mechanical descaling process has been used for a long time and the fish is exposed to a rough treatment that often results in lower fillet yield and quality reduction. Thus, biotechnological methods using cold-adapted enzymes have been developed in Iceland. Enzymatic descaling process is composed of mild acid treatment to denature the skin proteins and loosen the mucus layer, enzymatic degradation of the outer skin structures adhering the scales to the skin and washing off scales and enzymes by water jets.

The scales can be successfully removed without affecting the skin or flesh after incubating the fish in a special mixture of fish digestive enzymes at lower temperature. Incubation conditions depends on the characteristics of fish digestive enzymes should be confirmed for each fish species to obtain optimal results. An enzyme for descaling of fish is commercially available from the Norweigian Company (Biotec ASA).

Future Prospect

Nature is a rich source of several enzymes, which may have hidden potential for varied applications in human endeavors whereas only a few of them have been utilized in the past 100 years, and the rest remain unutilized. There are several reasons for this scenario, which may include factors such as that the source could be unreliable or undesired, such as pathogenic microorganisms, or from inaccessible environments, such as the deep sea; difficulties in large scale isolation and purification, commercial scale production, and downstream processing; requirements for activity under harsh conditions, etc. Hence, scientists and technologists are continuing their efforts in discovering new enzymes from new sources and experimenting with their possible utilization, meeting all challenges through research efforts on a laboratory scale. However, only a few of them have tasted success and reached commercial scale production and application. As a result, extensive application of enzymes in food processing industries happened in the latter half of the twentieth century, and this emerging trend opened up renewed interest in researchers to discover a new range of enzymes from unconventional resources and to describe enzymes for possible application. Consequently, now, enormous activities are going on in the search for novel enzymes and their novel applications.

As discussed earlier, advancements in molecular biology, instrumentation, biotechnology, bioinformatics, and nanotechnology have opened up new horizons in the design and development of artificial enzymes, hybrid catalysts, and tailor-made properties in enzymes for varied applications in food processing industries. Further, it is possible through biotechnology to isolate the gene coding for novel enzymes from organisms that cannot be cultivated on a large scale for deriving copious amounts of enzymes and cloning easily cultivable microorganism (s) for large-scale/commercial production. Hence, it is anticipated that future initiatives hold immense promise for valuable new enzymes, which are to be safe and commercially available at affordable prices. New enzymes through modern biotechnology will lead to enzyme products with improved effects at diverse physiological conditions, such as low or high temperatures, which may

allow various industrial processes to operate at low temperatures; less harm to the environment; greater efficiency; lower costs; lower energy consumption; and the enhancement of product properties. Further, environmental genomics and proteomics are anticipated to transform the enzyme industry with rich novel genes and novel enzymes from microbial resources of extreme environments and underexplored biological resources that remain hitherto unutilized for applications. Modern tools of biotechnology hold the key for efficient harnessing of enzymes from nature that are sufficiently robust to be useful under harsh processing conditions, such as extremes of pH and temperature, and thus hold great promise for replacing certain chemical processes in the future with much cleaner protein-catalyzed processes. Already there is a major drive for adoption of greener technologies that are ecofriendly, and the need for conservation of the environment and sustainable
utilization of natural resources have necessitated future initiatives that hold the key for solutions in the efficient management and valorization of enormous wastes and by-products disposed into the environment by the food processing industries.

It is envisaged that the future could witness some major prospective initiatives listed below, which would transform food and beverage processing, significantly contributing to quality and healthy food, meeting consumers' ever-growing needs and demands, in addition to taking care of environmental, health, and socioeconomic considerations. Key market drivers for such initiatives would be consumer demands, large-scale availability of source materials and biocatalysts, optimal technologies, economic downstream processing, and overall production costs among others.

- Discovery of genes coding for novel enzymes with competent properties that will meet the Demands of the food and beverage processing industries.
- Understanding of structure, functions, and interactions of enzymes with other biomolecules and chemical species, which may pave ways and means to design and develop suitable biocatalysts for varied applications with high specificity, stability, improved catalytic activities, mode of action, optimal operational conditions, and for developing ideal enzyme combinations with which multienzyme complex(es) need to be used.
- Design and development of ideal commercial production process (es) for large-scale manufacture of enzymes, which are cost-effective and affordable. For example, solid-state fermentation using cheap agro-residues and wastes as substrates for production of fungal exoenzymes that are extensively used in the food and beverage industries.
- Development of cost-effective downstream processing of enzymes from production media that determine the yield and consequent cost of enzymes and properties of enzymes.
- Development of ideal immobilized biocatalyst(s) with suitable chemical, physical, and geometric characteristics, which can be produced under mild conditions, used in different reactor configurations, and comply with the economic requirements for large-scale application. Nanomaterials hold the key in this respect, and considering the recent developments in this field, this trend is foreseen to be further implemented.
- Newer applications of enzymes in production of modified starch, oligosaccharides, sugar, and sugar syrup, utilizing underutilized starch sources for use in the manufacture of a diverse range of products in bakeries, confectioneries, and prebiotics.
- New enzymes and improved enzymes for removing undesired chemical compounds and biomolecules from fruit juices, beverages, and fats and oils derived from vegetables.
- Development of enzymes for enhancing the final quality of meat and its products, which are much consumed worldwide. For example, the tenderness is the main characteristic considered by consumers; strategies to improve meat tenderness could increase the value of non-prime cuts, making possible the use of by-products from the meat industry and reducing the waste and environmental impact generated in this supply chain.
- In the seafood industry, enzyme technology is still in its early stages. Enzymatic approaches will become an alternative solution to circumvent the environmental problems caused by the mechanical and chemical processing of seafood.
- The enzyme-assisted extraction of biomolecules of interest and micronutrients from fruits, vegetables, and other plant materials of interest for use as nutraceuticals, functional foods, and pharmaceuticals.
- Evaluation of the effect of enzyme processing on the biological availability and effectiveness of nutraceuticals.
- Enzymic bioconversions or biotransformations of food processing by products and waste into functional compounds.
- Discovery and development of new enzymes that have the potential for use as additives in probiotics, prebiotics, symbiotics, and cobiotics manufactured in the future because the consumeroriented food and beverage industry is experiencing a global evolution focusing on safe health.
- Discovery and development of novel enzyme inhibitors from plants, seeds, and microorganisms for varied applications, such as regulating enzymes that lead to undesirable effects when used in excess as in the case of lipase or protease in food and beverage processing.
- Valorization of fruit and vegetable peels and pomace; visceral organs of animals and fishes, shellfish waste, etc., for deriving catalytic enzymes, pigments, flavors, functional ingredients, micronutrients, nutraceuticals, active pharmaceutical ingredients, phytochemicals, biofuel, and biomaterials employing enzyme processes.
- Many of the investigations and studies are conducted on a laboratory scale whereas successful commercialization of any bioprocess solely relies on appropriate pilot-scale studies that ensure success at industrial-scale production levels. Thus, the future could witness the development of ideal bioprocesses based on enzymes based on pilot-scale studies and subsequent technology transfer to industries.
- It is envisaged that there will be an increase in trained human resources capable of adopting and implementing enzyme-based food and process.

Conclusion

Enzymes from aquatic animals can be useful to develop enzymes with novel features such as low temperature reactivity and stability. There is huge potential for their diverse applications such as food processing, biotechnology, clinical diagnosis, detergents, leather and fabric upgrading, organic synthesis, therapeutics, biosensors, among others.

Proteases can be used to develop novel peptides having interesting therapeutically important functions such as antihypertensive, antiamnesiac, mineral-binding, immunodulatory, antioxidant, antimicrobial and antithrombotic activities. Lipases cover a broad spectrum of applications like additives in detergents and in food industries, environmental bioremediations, biotransformation and molecular biology. There is immense scope for applications of other enzymes also in food processing and biotechnology.

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