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REVIEW ARTICLE

Enzymatic approaches in the bioprocessing of shellfish wastes

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Abstract
Several tonnes of shellfish wastes are generated globally due to the mass consumption of shellfish meat from crustaceans like prawn, shrimp, lobster, crab, Antarctic krill, etc. These shellfish wastes are a reservoir of valuable by-products like chitin, protein, calcium carbonate, and pigments. In the present scenario, these wastes are treated chemically to recover chitin by the chitin and chitosan industries, using hazardous chemicals like HCl and NaOH. Although this process is efficient in removing proteins and minerals, the unscientific dumping of harmful effluents is hazardous to the ecosystem. Stringent environmental laws and regulations on waste disposal have encouraged researchers to look for alternate strategies to produce near-zero wastes on shellfish degradation. The role of enzymes in degrading shellfish wastes is advantageous yet has not been explored much, although it produces bioactive rich protein hydrolysates with good quality chitin. The main objective of the review is to discuss the potential of various enzymes involved in shellfish degradation and their opportunities and challenges over chemical processes in chitin recovery.

Keywords Deproteination · Proteases · Chitin · Shellfish waste

Introduction

Health benefits associated with seafood consumption have created a demand for consuming them, resulting in waste generation. Crustacean processing industries generate about 6–8 million tonnes of shellfish wastes annually (Yan and Chen 2015). Normally seafood wastes are dumped in the landfill sites inviting unwanted pests; burned or dumped into the water bodies, contaminating the ecosystem (Yadav et al. 2019; Xu et al. 2013). Over the years, chitin and chitosan industries are partly utilizing these shellfish wastes to produce chitin and chitosan using harsh chemicals like acids and strong bases (Casadidio et al. 2019; Santos et al. 2020). These chemical methods are not environmentally friendly as they release chemical effluents into the environment. Therefore, safe and eco-friendly methods are suggested for the management of these shellfish wastes (Mathew et al. 2020).

The shellfish wastes are comprised of calcium carbonate (20–50%), protein (20–40%), and chitin (15–40%), lipids, and pigments, which can be exploited for commercial applications (Yan and Chen 2015). Among them, chitin is the valuable polymer that is found in abundance, next to cellulose. They exist naturally in three forms based on their microfibril orientation as alpha chitin (antiparallel chains), betachains (parallel chains) and gamma chitin (mixture of parallel and antiparallel chains) (Kaya et al. 2015). Chitin is recovered from calcium carbonate in the shellfish by demineralization using acids like HCl, mild acids like citric acid, acetic acid, formic acid, lactic acid or by using microbes producing organic acids (Mathew et al. 2020; Mahmoud et al. 2007). The proteins are separated by deproteination, which is done

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with the help of strong chemicals like NaOH. However, the application of harsh chemicals for deproteination and demineralization affects the quality of chitin resulting in partial deacetylation and depolymerization of the chitin polymer and the release of highly acidic/basic effluents (Kumari and Rath 2012). Therefore, green technologies using enzymatic approaches or microbial fermentation are preferred due to environmental concerns.

Enzymatic deproteination is achieved using proteolytic organisms or commercial enzymes like alcalase, trypsin, delvolase, papain, pancreatin, etc. (Fernandes 2016). The steps of demineralization (DM) and deproteination (DP) can be reversed based on the accessibility of these enzymes to remove the protein, a process that gives maximum chitin yield with near-zero waste being produced (Yadav et al. 2019). In the process of deproteination, crude enzymes are obtained from the microorganism itself or from the viscera of fish (Sila et al. 2012a, b), or proteolytic microbes in the shellfish are used (Guo et al. 2019). The use of commercial enzymes can be expensive but greener compared to chemical methods. In enzymatic deproteination, about 5–10% of protein remains adhered to chitin and are not completely removed in comparison to chemical methods (Kaur and Dhillon 2015).

The shellfish wastes of crustaceans are comprised of chitin fibres adhered with proteins along with the attachment of minerals like calcium. The shell wastes contain carotenoid pigments like astaxanthin, proteins, chitin, calcium carbonate, and lipids. These constituents vary from species to species and between organisms of the same species depending on their habitat, climatic conditions, growth phase, and feeding. Proteins associated with the shellfish matrix acts as lower modulus matrix covering chitin. Microscopic studies and X-ray diffractive studies have confirmed that chitin and protein occur as distinct phases and are connected at the interphase (Díaz-Rojas et al. 2006). The proteins in the shell wastes prevent excess hydration and the degradation of chitin caused by chitinases by sclerotization caused by o-dihydric phenol cross-linking. It has been observed that the protein associate with chitin to form a chitin proteoglycan matrix with amino acids like aspartic acid and histidine (Guo et al. 2019). At least 55% of the proteins are attached to chitin via covalent bonds and the remaining soluble proteins are linked by Van der Waals' forces, hydrogen bond, and ionic bonds (Machałowski et al. 2020). Although chemical methods are effective in the removal of proteins from the shells, the extracted proteins are denatured and harmful in formulating animal feeds (Suryawanshi et al. 2019).

The proteins derived by enzymatic and microbial conversion of crustacean shell processing are employed as additives in animal feeds, as they are rich in essential amino acids with a rich nutritive value comparable to soybean meal (Mathew et al. 2020). Hence, enzymatic methods are highly preferred over chemical methods for shellfish waste degradation to obtain protein hydrolysate rich in essential amino acids (Likhar and Chudasama 2021; Sumardiono and Siqhny 2018; Yan and Chen 2015). There are no specific reviews on the utilization of enzymes in shellfish waste degradation, though many reviews have discussed the chemical and microbial fermentation of shellfish wastes. Therefore, this review aims to discuss the different types of enzymes involved in shellfish degradation, their opportunities, and challenges over conventional methods.

Conventional process for shellfish waste processing

The traditional process of recovering chitin includes demineralizing the shellfish wastes using harsh acids like HCl, HNO_3, and H_2SO_4 (Gadgey and Bahekar 2017) or organic acids like formic acid, acetic acid (Regis et al. 2015), and lactic acid. The protein removal is achieved by hydrolyzing the waste with strong bases like NaOH, Na_2CO_3, $NaHCO_3$, KOH, K_2CO_3, $Ca(OH)_2$, Na_2SO_4, $NaHSO_4$, $CaHSO_4$, Na_3PO_4, and Na_2S (Younes and Rinaudo 2015). The extracted chitin contains pigments like astaxanthin, which are separated using hydrogen peroxide, and other strong oxidizing agents to obtain colourless chitin (Arnold et al. 2020). However, this chemical process is disadvantageous resulting in the release of hazardous chemicals along with essential minerals and amino acids that are impossible to be recycled. It also affects the crystallinity of the recovered chitin causing low molecular weight (Mathew et al. 2020).

Chitin is deacetylated with NaOH concentration ranging between 25 and 50%, to obtain chitosan, a valuable and desired biopolymer used in versatile applications like food packaging, agriculture, wastewater treatment, biomedical applications, and wound healing (Suryawanshi et al. 2019; Shamshina et al. 2019; Priyadarshi and Rhim 2020; Satitsri and Muanprasat 2020). Chitosanase converts chitin to chitooligosaccharides, which have wide applications in medicine (Kaczmarek et al. 2019; Aam et al. 2010). Depending on the nature of chitosan required for various industrial applications, chitin is deacetylated to chitosan at room temperature (homogenous deacetylation) or at higher temperatures (heterogeneous deacetylation) to attain chitosan with varying molecular weights (Tharanathan and Kittur 2003). However, excess alkali has to be thoroughly washed from the chitosan flakes, which is time-consuming (Weinhold et al. 2009). El Knidri et al. (2016) established a thermo-chemical process in chitosan conversion using the microwave irradiation method resulting in high molecular weight chitosan from shrimp shells with 80% deacetylation.

Enzymatic methods for shellfish waste processing

Enzymes from various sources like plants, animals, prawn/shrimp, fishes, and microbes are used for the processing of shellfish waste.

Fish proteases for degradation of shellfish wastes

Fish proteinases are valuable for various industrial applications due to their high proteolytic activity at different pH and temperature ranges (Coppola et al. 2021). Table 1 discusses the deproteination efficiency of fish proteases on shellfish wastes. Various enzymes, namely proteases, chitinase, hyaluronidase, protease, etc., are reported to be isolated from shellfish and fish wastes (Caruso et al. 2020; Shahidi and Kamil 2001; Venugopal 1995). Alkaline proteases from the fish viscera of goby (*Zosterises sorophiocephalus*), thornback ray (*Raja clavata*) and scorpionfish (*Scorpaena scrofa*) were used in the deproteination of dry shrimp waste powder. The deproteination efficiency using E/S of 10 at 45 °C for 3 h resulted in a DP range of 76–80% (Nasri et al. 2011).

Proteases from Grey triggerfish (*Balistes capriscus*) at concentrations of 20 U/mg were used for the deproteination of *Metapenaeus monoceros* (shrimp) shells leading to deproteination efficiency of 78 ± 2%when incubated for 3 h at 45 °C (Younes et al. 2014). Other proteolytic enzymes from *Bacillus* species, *Vibrio metschnikovii* J1 and *Aspergillus clavatus* ES1, and fish alkaline proteases were also attempted (Younes et al. 2014). Trypsin purified from the pyloric ceca of bluefish *Pomatomus saltatrix* was used for the extraction of carotenoprotein from the *Penaeus monodon* (Klomklao et al. 2009).

Proteases from microbes

Biological methods for recovering chitin and other value-added products are preferred as an alternative compared to chemical processes. Therefore, biofermentation of these crustacean wastes, namely; crab waste (Jung et al. 2006; Oh et al. 2007; Jo et al. 2008), crayfish shell (Bautista et al. 2001; Cremades et al. 2003), prawn carapace (Fagbenro 1996) and shrimp waste (Xu et al. 2008; Younes et al. 2012; Cira et al. 2002) and Scampi wastes (Zakaria et al. 1998) are executed using proteolytic microbes.

Different deproteination efficiencies were observed using purified enzymes and proteolytic microorganisms (Table 2). Bustos and Michael (1994) did a comparative study of purified proteases from *P. maltophila* along with the whole microorganism. When *P. maltophila* was used directly, it resulted in 82% deproteination after 6 days incubation compared to purified proteases, resulting in only 64% DP efficiency. Bhaskar et al. (2007) biofermented shrimp wastes with *Pediococcus acidilactici* CFR2182 and optimized their fermentation conditions by response surface methodology to obtain chitin by demineralization and deproteination.

Alkaline proteases isolated from *Micromonosporachaiyaphumensis*S103 were used to recover chitin from *P. kerathurus* waste shells (Mhamdi et al. 2017). A 93% deproteination efficiency was achieved using an E/S ratio of 20 U/mg of shrimp waste for 3 h at 45 °C and pH 8.0, whereas in the absence of enzyme, the deproteination degree was 30% probably due to the breaking down of electrostatic or hydrogen bonds due to thermal treatment. Some proteins linked by covalent bonds require enzymatic and chemical approaches for separating the protein from chitin (Mhamdi et al. 2017).

Table 1 Fish proteases in shellfish waste degradation

Crustacean shell wastes	Enzymes used	Incubation time and temperature	Enzyme/substrate ratio	DP (%)	References
Shrimp waste (*Penaeus longirostris*)	Purified trypsin from *Barbuscallensis*	1 h, 30°C	1.0 U/g shrimp shells	NA	Sila et al. (2012a; b)
Penaeus monodon	Trypsin from *Pomatomus saltatrix*	1 h at 25°C	1.2 U/g shrimp shells	NA	Klomklao et al. (2009)
Shrimp waste (*Metapenaeus monoceros*)	Crude alkaline protease from *Balistes capriscus*	3 h 45°C, pH 9.0	5 U/mg	78 ± 2	Jellouli et al. (2009), Younes et al. (2014)
Shrimp waste (*Metapenaeus monoceros*)	Crude alkaline protease from *Sardinella aurita*	3 h, 45°C, pH 8.0	20 U/mg	75 ± 2	Ben Khaled et al. (2011), Younes et al. (2014)
Shrimp waste (*Metapenaeus monoceros*)	Crude alkaline protease from *Z. ophiocephalus*	3 h, 45°C, pH 9.0	10 U/mg, 20 U/mg	76 ± 2	Nasri et al. (2011), Younes et al. (2014)
Shrimp waste	Crude alkaline protease from *Raja clavata*	3 h, 45°C, pH 8.0	10 U/mg	76	Nasri et al. (2011)
Shrimp waste	Crude alkaline protease from *Scorpaena scrofa*	3 h, 45°C, pH 10.0	10 U/mg	80	Nasri et al. (2011)

Table 2 Proteolytic microorganisms involved in shellfish deproteination

Proteolytic microorganism	Crustacean source	Deproteination (DP) in %	References
Aspergillus niger 0576	Shrimp shell	96.7 ± 0.3	Teng et al. (2001)
Aspergillus niger 0307	Shrimp shell	97.2 ± 0.5	Teng et al. (2001)
Aspergillus niger 0474	Shrimp shell	97.1 ± 0.3	Teng et al. (2001)
B. subtilis	Shrimp waste of *Metapenaeus dobsoni*	84	Sini et al. (2007)
Pseudomonas aeruginosa F722	Crab shell	63	Oh et al. (2007)
B. cereus 8-1	Shrimp shell	97.1	Sorokulova et al. (2009)
Exiguobacterium acetylicum	Shrimp shell	92.8	Sorokulova et al. (2009)
B. subtilis A26	Shrimp shells from *Metapenaeus monoceros*)	91.55	Ghorbel-Bellaaj et al. (2012b)
Bacillus mojavensis A21	Shrimp shells from *Metapenaeus monoceros*)	90.05	Ghorbel-Bellaaj et al. (2012b)
B. cereus SV1	Shrimp (*Metapenaeus monoceros*)	95.65	Ghorbel-Bellaaj et al. (2012b)
Bacillus licheniformis RP1	Shrimp shells from *Metapenaeus monoceros*)	94.4	Ghorbel-Bellaaj et al. 2012b
Bacillus pumilus A1	Shrimp shells from *Metapenaeus monoceros*)	94	Ghorbel-Bellaaj et al. (2013)
Bacillus amyloliquefaciens An6	Shrimp shells from *Metapenaeus monoceros*)	83.4	Ghorbel-Bellaaj et al. (2012b)
Pseudomonas aeruginosa A2	*Metapenaeus monoceros* (shrimp waste)	90	Ghorbel-Bellaaj et al. (2012a)
Paenibacillus woosongensis TKB2	Shell wastes of *Penaeus monodon*	80	Paul et al. (2015)

A maximum deproteination of 88% was obtained using 20 U/mg proteases of *B. cereus* SVI on shrimp wastes (Manni et al. 2010). Ghorbel-Bellaaj et al. (2012b) used six protease-producing *Bacillus* species (*B. licheniformis* RP1, *B. cereus* SV1, *B. subtilis* A26, *B. amyloliquefaciens*, *B. mojavencis*A21 and *B. pumilus*A1) for chitin extraction from shrimp shell wastes. The addition of 5% glucose to these strains promoted demineralization and the obtained protein hydrolysates showed high antioxidant activity. Response surface methodology was further employed to analyze the shrimp shell using *B. pumilus* A1 (Ghorbel-Bellaaj et al. 2013). The optimized conditions with shrimp shell concentration of 7%, glucose 5% with pH 5.0 at 35 °C for 6 days using *B. pumilus* A1 resulted in 88% demineralization and 94% deproteination. *B. licheniformis* strains deficient in chitinase were used for the deproteination of the shrimp shell wastes along with 0.9% lactic acid resulting in deproteination efficiency of 99% and demineralization of 98.8%, resulting in high-quality chitin (Waldeck et al. 2006). Protease from *Pseudomonas aeruginosa* K-187 was used for the deproteination of shrimp and crab shell powder (SCSP), shrimp wastes of *Penaeus japonicas* resulting in deproteination efficiency of 55, 48, and 61% after incubating for 7, 5 and 5 days, respectively (Wang and Chio 1998). The mineralized and demineralized lobster shell wastes were deproteinated with crude protease from *Erwinia chrysanthemi* resulting in 87.6% and 96% DP values (Giyose et al. 2010). The crude protease of *E. chrysanthemi* had 22.4 U/mL enzyme activity that was better than the protease activity recorded in *B. subtilis,* which was20.2 U/mL (Yang et al. 2000).

Bioconversion of squid pens, shrimp, and crab shells with *B. cereus* TKU006 produced protein hydrolysates containing proteases and chitinases (Wang et al. 2009). Hence, shellfish wastes can be utilized as a cheap alternative for carbon and nitrogen source in the generation of industrial enzymes (Doan et al. 2019). Maruthiah et al. (2015) used *Bacillus* sp. APCMST-RS3 proteases for the deproteination of crustacean shell wastes from shrimp, crab lobster shells yielding a DP efficiency of 84.35% after 7 days fermentation that was tolerant to organic solvent and salts. Jo et al. (2008) deproteinated snow crab wastes with proteolytic bacteria *Serratia marcescens* FS-3 resulting in 84% DP efficiency after 7 days of fermentation. The deproteination efficiencies using various commercial enzymes, namely Delvolase®, Cytolase PCL5®, EconaseCEPi®, Econase MP 1000®, Maxazme™ NNP®, and Cellupulin MG® was also analyzed, of which Delvolase showed better deproteination efficiency of the crab shells. When 1% of commercial enzyme Delvolase® was blended with 10% inoculum of *S. marcescens* FS-3, it resulted in 85% of deproteination.

Valdez-Peña et al. (2010) screened several commercial enzymes for chitin recovery from shrimp heads. The commercial enzymes likeAlcalase® 2.4 L FG (Novozymes, the source is *B. licheniformis*), papain, Trypsin Vl, Flavorzyme® 500 MG (Novozymes, the source from *Aspergillus oryzae*) were incubated with shrimp heads for 6 h at 37 °C at 40 rpm. From their study, high-quality chitin was obtained by demineralization using microwave-assisted technology along with enzymatic deproteination. In similar research, Baron et al. (2017) screened 11 commercial proteases that could function at lower pH ranging from 3.5 to 4.0 to carry out a single biorefinery process for chitin extraction and deproteination of shrimp shell wastes to obtain above 95% deproteination. These commercial proteases belonged to fungal sources (*T.*

reesei, A. oryzae and *A. niger*), aspartate protease and pepsin (gastric mucosa). The important parameters for enzymatic hydrolysis of crustacean wastes are temperature, pH, duration of hydrolysis, enzyme/substrate (E/S ratio) and pH (Diniz and Martin 1997; Deng et al. 2002).

Commercial enzymes

Some commercial enzymes were traditionally exploited by the seafood processing industries for applications like shrimp deveining, descaling, etc. The enzymes that have been utilized in shellfish degradation are mentioned in Table 3.

Papain

The plant-based enzyme, papain derived from *Carica papaya* was reported to be used in the extraction of chitin (Jasmine et al. 2006). Broussignac (1968) observed that commercial enzymes like papain, pepsin, and trypsin produced good quality chitin with little deacetylation. Papain facilitated the production of high-grade chitosan from shellfish wastes (Gopalakannan et al. 2000). The shrimp shell wastes of *Penaeus indicus* were demineralized with 1.75 N glacial acetic acid followed by deproteination with papain to yield values of 73.1% compared to the chemical process having a DP value of 98%. The degree of deacetylation (DD) using enzymatic approaches gave good quality chitosan of 19.4% than chemical approaches having DD of 17.2%. Muzzarelli et al. (1994) used covalently immobilized papain to depolymerise lobster chitosan in its lactate salt under acidic conditions and other modified chitosans. The usage of this plant-based low-cost commercial enzyme was widely accepted for the production of chitosan hydrolysates in comparison to using lysozyme and other chitinases for various applications.

Chymotrypsin

Chymotrypsin can be isolated from the shrimp (*Fenneropenaeus chinensis*) (Shi et al. 2008) and fish viscera of sardine (*Sardinops sagax caerulea*) (Castillo-Yañez et al. 2009). Chymotrypsin type II (EC 3.4.21.1) and papain were employed for the deproteination of demineralized shrimp shell wastes by Response surface methodology (Gagné and Simpson 1993). Optimum conditions for chymotrypsin were 40 °C at pH 8.0 with an E/S ratio of 7:1000 (w/w) for deproteination of shrimp wastes, whereas the conditions for papain enzymes were 38 °C, pH of 8.7 and E/W ratio of 10:1000 (w:w).

Alcalase

Alcalase is a commercially available protease used for the treatment of crustacean wastes to produce hydrolysates rich in bioactives (Gildberg and Stenberg 2001). Alcalase 2.4 L belongs to bacterial serine endopeptidases produced by *Bacillus licheniformis* (Dey and Dora 2014). Shrimp wastes from *Pandalus borealis* were treated with Alcalase enzyme (2.4 L FG) to obtain protein hydrolysates containing amino acids, nitrogen, and carotenoid astaxanthin. Australian rock lobster (*Jasus edwardsii*) shells were treated with Alcalase 2.4 L FG (Novozymes) followed by microwave intensified enzymatic deproteination that enhanced the deproteination efficiency from 58% to 85.8%. This method released higher yields of bioactive compounds with a reduction in chitin recovery time with minimum solvent usage (Xiao et al. 2008). The cooked and minced lobster shells were treated with Alcalase (1:1) at 55 °C using a microwave (input energy 40 W, stirring 95%) for deproteination (Nguyen et al. 2016).

Table 3 Commercial enzymes in shellfish waste degradation

Commercial enzymes	Crustacean source	Deproteination (%)	References
Alcalase 2.4 L	Demineralised shrimp shells (*Crangon crangon*)	95.5 ± 0.53	Synowiecki and Al-Khateeb (2000)
Alcalase 2.4 L	Shrimp (*Xiphopenaeus kroyeri*)	93.41	De Holanda and Netto (2006)
Alcalase®	Shrimp head	NA	Valdez-Peña et al. (2010)
Alcalase 2.4 L added along with sodium sulphite and NaOH	Shrimp (*P. semisulcatus*)	99.13	Mizani and Aminlari (2007)
Swine pancreatin	Shrimp (*Xiphopenaeus kroyeri*)	92.23	De Holanda and Netto (2006)
Pepsin	White shrimp shell	92	Duong and Nghia (2014)
Protease	*Litopenaeus vannamei*	91.1	Hongkulsup et al. (2016)
Bluefish trypsin	*Penaeus monodon*	NA	Klomklao et al. (2009)
Delvolase	Snow crabs (*Chionoecetes opilio)*	90 ± 2.9	Jo et al. (2008)
Crude protease from *Bacillus cereus* SV1	Shrimp shell from *Metapenaeus monoceros*)	88.8 ± 0.4	Manni et al. (2010)

Significant by-products like chitin, protein, and astaxanthin from shrimp shell wastes of *Xiphopenaeus kroyeri* were obtained using two different commercial enzymes, namely Alcalase and swine pancreatin. Alcalase gave better recovery of protein hydrolysates (65%) with the reduction in bitter taste and improvement in functional characteristics (De Holanda and Netto 2006). In a similar study, the shrimp wastes of *P. monodon* were treated with four microbial food-grade proteases namely Alcalase, Neutrase, Protamex (*Bacillus* protease complex) and Flavourzyme 500 MG (fungal protease derived from *A. oryzae*) which are functional at alkaline pH (Dey and Dora 2014; Aunstrup 1980). Optimization by central composite design showed that the Alcalase enzyme showed deproteination compared to other enzymes (Dey and Dora 2014). Alcalase treated shrimp shell wastes showed better protein hydrolysis than others, releasing more peptides and amino acids than other enzymes (Valdez-Peña et al. 2010; Synowiecki and Al-Khateeb 2000). Mizani et al. (2005) used a successful method to develop protein powder rich in amino acids from the shrimp head of *P. semisulcatus* using Alcalase along with Triton X-100 and sodium sulphite. The addition of mild acids like lactic acid and gluconic acid for demineralization of shellfish wastes followed by enzymatic conversion with Alcalase can release by-products like calcium lactate and calcium gluconate used as fortificant in soymilk (Dechapinan et al. 2017).

Trypsin

Trypsin is a member of serine proteases that hydrolyze the protein at the carboxyl side of arginine and lysine residues (Sriket 2014; Klomklao et al. 2006, 2009). Carotenoproteins are extracted from the shellfish wastes using trypsin from fish viscera and Bovine (Klomklao et al. 2009). Although chemical applications using solvents and oil helped in an efficient carotenoid recovery, it affected the stability of the pigment because of oxidation (Mezzomo and Ferreira 2016). In the crustacean shell wastes, a third of the constituents belonged to proteins, mainly carotenoids. These carotenoproteins are extracted from shellfish wastes like crab, shrimp and lobster using trypsin along with the extraction buffer (Klomklao et al. 2009).

Different astaxanthin and protein removal efficiencies were observed using shrimp discards treated with bovine trypsin and Atlantic cod trypsin. Better carotenoprotein retrieval of 64% was observed using Atlantic cod trypsin than bovine trypsin with 49% (Cano-Lopez et al. 1987). Application of commercial enzymes like trypsin for astaxanthin recovery for food-related applications due to their stability and GRAS status (Lee et al. 1999). Trypsin was added along with EDTA for extracting carotenoids from shrimp wastes (Sowmya et al. 2014). Lee et al. (1999) used an effective method for the extraction of carotenoid pigments from cooked shrimp wastes of *Pandalus borealis* using proteolytic enzymes (concentrates) derived from various organisms, namely *Aspergillus melleus*, *A. oryzae* and *Bacillus licheniformis*. Proteolytic enzymes from *B. licheniformis* showed maximum astaxanthin recovery of 91.9% with EDTA. Snow crab waste treated with trypsin removed the carotenoids. Simpson et al. (1992) recovered carotenoproteins from lobster (*Homarus americanus*) shell wastes using trypsin from Atlantic cod offals and bovine pancreas.

The shrimp shell wastes of *Metapenaeus monoceros* were extracted for carotenoprotein using three commercial enzymes namely trypsin, papain, and pepsin. Trypsin showed the highest carotenoid recovery of 55% after 4 h at 28 °C using citrate phosphate buffer at pH 7.6. Under similar temperature and incubation conditions, 50% of the protein was obtained using papain with citrate buffer (pH 6.2) and 50% protein using pepsin with pH 4.6 (Chakrabarti 2002). The shrimp shells were ground and mixed in the ratio 3:7 with citrate phosphate buffer (corresponding pH 5.0), subsequently adding enzyme in the ratio of 1:3000 based on shell waste protein.

Pepsin

Duong and Nghia (2014) utilized pepsin enzymes for the deproteination of demineralized white shrimp shell wastes for chitin recovery. Pepsincould carry out deproteination at a lower pH of 2.0 at optimized conditions of factors like temperature, time, and enzyme concentration. This resulted in a 0.92 degree of deproteination when the enzyme concentration was 20 U/g protein incubated at 40 °C for 16 h. This process was beneficial as it reduced the utilization of chemicals in chitin recovery.

Proteinase K

Crab shell wastes were enzymatically degraded using proteinase K, leading to amino acid production which can be used as a nutrient supplement in seed cultivation and microbial growth (Padmalochana and Prema 2016). In addition, an efficient method was devised for the removal of minerals and proteins from cray shell waste powder using enzymatic action using proteinase K and fermentation with *B. coagulans* LA204 resulting in DP value of 93%, DM of 91% and chitin recovery of 94% (Dun et al. 2019).

Pectinase

Commercial pectinase enzyme, Pectinex™ produced by *Aspergillus niger* was reported to hydrolyze insoluble chitin (Roy et al. 2003). The chitinolytic activity of Pectinex™ on chitin was comparatively higher than lysozyme or even chitinase enzyme from *Serratia marcescens* (Roy et al. 2003).

Endogenous enzymes

A cost-effective technology was employed by using autochthonous microorganisms from the shellfish wastes for the breakdown of the shellfish wastes. Endogenous enzymes from the shrimp head of *Pandalus borealis* along with fermentation with *Bacillus licheniformis* was used for retrieving protein hydrolysates and chitin (Guo et al. 2019). Mixed cultures from various sources like endogenous microflora from Indonesian shrimp shells and cultures from *C. crangon* shrimps, from the soil, sewage sludge (SS), ground beef meat (GM), and sauerkraut. The cultures from SS and GM were added to wet and dry shrimp shells resulting in high deproteination values ranging from 83 to 98% in *P. monodon* and approximately 98% for *C. crangon* (Xu et al. 2008). High-quality chitin was derived in this process. Chitin was extracted by the autofermentation of *Penaeus vannamei* shells (Sjaifullah and Santoso 2016). Hamdi et al. (2017) used alkaline protease from the digestive prawn viscera of *P. segnis* for the deproteination of shellfish wastes from *Portunus segnis* (Blue crab) and *P. kerathurus* (shrimp). The deproteination efficiency was 84.69% for blue crab and 91% for *P. kerathurus* by incubating 5 U/mg protein for 3 h at 50 °C.

Chemical demineralization followed by deproteination with enzymes

Minerals like calcium carbonate are dislodged from the chitin-protein complex before enzymatic treatments. As mentioned earlier, mild concentrations of organic or inorganic acids facilitate demineralization, which is succeeded by the addition of enzymes. Hamdi et al. (2017) demineralized blue crab shell wastes with 1:10 w/v of 0.55 M HCl followed by the addition of crude protease from the prawn waste of *P. segnis* incubated at pH 8.0 at 50 °C for 3 h resulting in a deproteination value of 85%. Shrimp shell powder was demineralized with 1.5 NHCl, 1:2 w/v for 2 h incubation at room temperature followed by deproteination with crude proteases of *Erwinia chrysanthemi* incubated at 37 °C for 16 h resulting in a DP of 95% (Sami 2010). Shrimp wastes demineralized with 1:10 (w/v) of 1.5 M HCl incubated for 6 h, 25 °C followed by deproteination with proteases (1:2) from *B. cereus* SV1 resulted in 89% deproteination (Manni et al. 2010). Younes et al. (2016) demineralized shrimp shells 1:10 (w/v), 0.5 M HCl followed by deproteination with crude proteases from *Bacillus mojavensis* A2 (7.75 U/mg) and crude protease from *Scorpaena scrofa* (10 U/mg) resulting in 96% deproteination.

Conversion of chitin to chitosan using enzymes

Enzyme-based conversion of chitin and chitosan is widely preferred and is gaining interest. Hydrolysis of chitin and chitosan is attained with enzymes like chitinases, chitosanases, chitin deacetylases, and lytic polysaccharide monooxygenases (LPMO) (Kaczmarek et al. 2019). Chitinases (EC 3.2.1.14) are enzymes that break down the β-1, 4 linkages of the chitin polymer in crustacean shells to form *N*-acetylglucosamine units. Degradation of chitin occurs in two parts (1) breakdown of chitin to chitooligosaccharides (2) breakdown of chitooligosaccharides to form *N*-acetyl glucosamine units (Chavan and Deshpande 2013; Adrangi and Faramarzi 2013). Details are depicted in Fig. 1. *N*-acetyl glucosamine (GlcNAc) is produced from chitin-containing materials by chitinolytic enzymes like β-*N*-acetyl-D-hexosaminidase (Slamova et al. 2010; Yang et al. 2008) and Lytic polysaccharide monooxygenases (LPMO) (Vaaje-Kolstad et al. 2010). Chitin deacetylases convert chitin to chitosan, a polymer preferred over chitin due to their soluble nature, high molecular weight, and degree of deacetylation (DD) (Tsigos et al. 2000). Ideally, microbes secreting chitinolytic enzymes are preferred for the bioconversion of chitin and chitosan to their respective oligosaccharides. Ilyina et al. (2000) immobilized chitinolytic enzyme from *Streptomyces kurssanovii* for the preparation of water-soluble chitosan (devoid of acid) from crab with a molecular weight of 2–9 kDa. Chitinase from *Aeromonas hydrophila* H-2330 degraded α chitin resulting in 77% of *N*-acetyl glucosamine (Sashiwa et al. 2002).

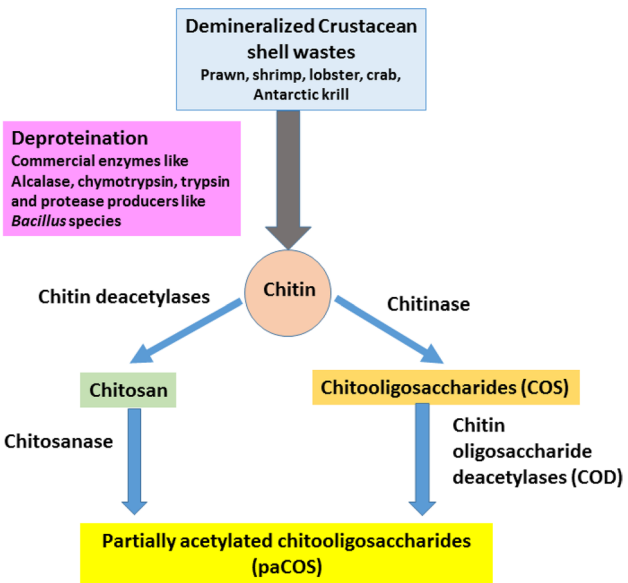

Fig. 1 Enzymatic conversion of shellfish wastes to partially acetylated chitooligosaccharides (paCOS)

Other enzymes like cellulases and lysozymes were exploited for the digestibility of chitin. Commercial cellulase from *T. reesei* and *Acremonium cellulolyticus* was beneficial for the production of *N*-acetyl glucosamine from the chitin of powdered squid pen and crab shells. The yield of *N*-acetyl glucosamine enhanced the mixing of *T. reesei* cellulase and *A. cellulolyticus* cellulase (Sashiwa et al. 2003). Chitinases from *Bacillus* sp. PI-7S showed higher digestibility of chitin from squid pen and shrimp shells and deacetylated chitins in comparison to using commercial lysozyme from Hen egg white (Shigemasa et al. 1994).

Sabry (1992) analyzed the degradation of shrimp shell wastes using microbes secreting chitinase enzyme. Lobster shells were degraded using chitinases from *Streptomyces* species, which exhibited high deproteination, demineralization, and chitinolytic efficiency. These digested shell extracts were reported to control the infection caused by *Pseudomonas syringae* and *Botrytis cinerea* on *Arabidopsis* plants (Ilangumaran et al. 2017). Shrimp wastes were completely degraded by chitinase enzymes from marine isolates of *Paenibacillus*AD for the production of chitinases and chitooligosaccharides (Kumar et al. 2018). The enzymatic conversion of chitin from crab shells to *N*,*N* -diacetylchitobiose $(GlcNAc)_2$ with the pretreatment of sub and supercritical water was achieved using chitinase from *Streptomyces griseus* (Osada et al. 2012, 2015) in another method.

Chitin-active LPMOs are copper-based enzymes that belong to auxiliary activity enzyme families of groups 10, 11, and 15. These enzymes may aid in the hydrolysis of chitin by oxidation, leading to the easy accessibility of chitin by chitinases. Unlike the chitinases and chitosanases, which are glycosyl hydrolases; LPMOs can directly attack the glycoside linkage in highly crystalline chitin and require the presence of external reducing agents like H_2O_2 (Mutahir et al. 2018; Arnold et al. 2020). Vaaje-Kolstad et al. (2010) studied the role of chitin-specific LPMO for the Chitin binding protein (CBP21) from *Serratia marcescens* AA10. Chitinases were produced during the bioconversion of shellfish wastes like crab and shrimp using *B. subtilis* W-118 (Wang et al. 2006). The derived chitinase hydrolysates contained chitooligosaccharides with inhibitory activity against human leukemia cell lines and *Fusarium oxysporum* (Wang et al. 2006). Wang et al. (2018) used a potent recombinant chitinase from *Bacillus subtilis* expressed in *E. coli* for the degradation of crab shells. Thus, recombinant chitinases are used in the degradation of shellfish wastes.

Opportunities and challenges associated with shellfish wastes processing

Enzyme technology is gaining attention in recent years, mainly in the seafood-processing sector. These enzymes are mainly categorized under proteases named ficin, papain, subtilisin, bacillolysin (Neutrase®), trypsin, and Protamex® (a combination of bacillolysin and subtilisin) that function endogenously or exogenously and aid in shrimp peeling, deskinning and descaling of fishes (Fernandes, 2016). Combining endoproteases and exoproteases namely, Endocut-03L and 0.25% Exocut-A0 resulted in 100% peeling of *Pandalus borealis* shrimps (Dang et al. 2018). These commercial enzymes are generally expensive than microbial enzymes that are easy to produce on a large scale (Younes et al. 2014). The gut of invertebrates is also a reservoir of active proteases that can be used for enzyme preparation. These non-commercial enzymes (microbial and fish proteases) were studied to carry out the deproteination of shellfish wastes (Younes et al. 2014). Deproteination of about $77 \pm 3\%$ and $78 \pm 2\%$ was recorded using *Bacillus mojavensis* A21 and *Balistes capriscus* proteases, respectively, after 3 h of hydrolysis at 45 °C using an enzyme/substrate ratio of 20 U/mg.

Enzymes are beneficial as they minimize or generate near-zero wastes by converting the shellfish wastes from crustaceans to value-added products like chitin and protein hydrolysates. This method is effective in reducing wastes dumped into the environment. Enzymes are preferred over chemicals in the conversion of shellfish wastes as the chemical effluents released are either highly acidic or basic, which need to be neutralized before disposal. Usage of chemicals in chitin recovery is expensive, as they require high temperatures and several steps of washing. Furthermore, alkali deproteinized shellfish waste hydrolysates cannot be used as animal feed (Gortari and Hours 2013; Zargar et al. 2015). In the case of enzymes, the hydrolysates obtained can be concentrated and used as feed applications in animals, fishes, and shrimps and used as soup concentrates (Mathew et al. 2020; Das et al. 2013). The derived hydrolysates act as bioactives, displaying antioxidant activity.

The production of the enzymes can be enhanced through the heterologous expression of enzymes in a cost-effective manner. Harnessing enzymes produced by marine microflora isolated from crustacean shells can reduce the optimization conditions through computational prediction models. In addition, these enzymes are effective in tolerating harsh processing conditions like high pH, temperature, etc. Thus, using enzymes for degrading shellfish wastes is a promising greener alternative to chemicals.

The demerits of using enzymes in shellfish deproteination are that the costs of commercial enzymes are

comparatively expensive. In addition, the usage of enzymes retains 5–10% of proteins attached to the chitin, compared to the complete removal of proteins using chemicals in a shorter duration (Younes and Rinaudo 2015). The enzyme-based application studies are still at a laboratory scale and require pilot-scale bioprocessing for promoting this technology in shell waste degradation on a wider platform industrially.

Conclusion and future prospects

Greener technologies are accepted globally to eliminate toxic and hazardous effluents from the environment. The usage of enzymes in shellfish waste management is a cleaner way to protect the ecosystem over chemicals that affects the ecosystem. As enzyme technology is yet to be implemented on an industrial scale for shellfish waste degradation, there is a need to prioritize and develop cheaper and environmentally friendly ways over chemical approaches. This can be achieved by combining several cheaper commercial enzymes or using microbial enzymes for the effective degradation of shellfish wastes. The application of physical methods like microwave-assisted treatment or ultrasound extraction of shellfish wastes followed by enzymatic treatment is another economical and greener way of reducing the enzyme load and reducing the cost in the future. Furthermore, the valorized products obtained by enzymatic methods can be exploited to develop eco-friendly biopolymers like chitin. The protein hydrolysates from the enzymatic treatment are rich in oligopeptides and can also be used in the development of animal, fish, and poultry feed on a large scale. These enzyme-based methods are environmentally friendly and sustainable compared to chemical methods and generate near-zero wastes.

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Declarations

Conflict of interest The authors have declared that there is no conflict of interest.

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