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Biotechnological processes for chitin recovery out of crustacean waste: A mini-review

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Abstract

Background: Chitin is an important natural resource. The annual worldwide production is estimated in approximately 10^{10}-10^{12} ton. It is produced by arthropods (insects and crustaceans), molluscs and fungi. Its main biological function is structural. Crustacean shells are the most important chitin source for commercial use due to its high content and ready availability. Chitin and its derivatives have great economical value because of their numerous applications: food, cosmetics, pharmaceuticals, textile industries, waste water treatment and agriculture. In nature, chitin is closely associated with proteins, minerals, lipid and pigments, which have to be removed.
Results: Several techniques to extract chitin from different sources have been reported. The most common method for recovery of chitin from crustacean shells is the chemical procedure. It involves two mayor steps: elimination of inorganic matter (demineralization) and extraction of protein matter (deproteination) using strong acids and bases. However, these processes may cause depolymerization affecting the polymer properties such as molecular weight, viscosity and degree of acetylation. In addition, the chemical purification of chitin is hazardous, energy consuming and threatening to the environment. As an alternative to the chemical process, different biological processes have been investigated: microbiological fermentation and methodologies using enzymatic crude extracts or isolated enzymes.
Conclusions: The results reported are extremely variable; however, they offer new perspectives for the production of chitin with the concomitant reduction of the environmental impact.

Keywords: biological extraction, chitin production, crustacean waste.

INTRODUCTION

Chitin is a linear heterogeneous polysaccharide of *N*-acetyl-D-glucosamine (~50-100%) and D-glucosamine (~50-0%) linked by β(1→4) glycosidic bonds. Like cellulose, it functions as structural polysaccharide. Chitin occurs in a range of organisms but is particularly important as constituent of arthropods. Information on the physical, chemical and biological characteristics, properties, processing, economic aspects and applications of chitin and its derivatives is abundant in the recent literature (Ravi Kumar, 2000; Synowiecki and Al-Khateeb, 2003; Tharanathan and Kittur, 2003; Dutta et al. 2004; Kurita, 2006; Pillai et al. 2009; Domard, 2011).

Chitin is an abundant renewable resource. It was estimated that every year about 10^{11} tons of chitin are produced by crustaceans, mollusks, insects, and fungi. (Synowiecki and Al-Khateeb, 2003; Tharanathan and Kittur, 2003). The annual worldwide commercial production of crustaceans was 10,795,000 ton in 2008 (FAO, 2010). The significant increase in the amount of waste resulting from the industrial processing of seafood (shells of crab, shrimp, prawn, krill and lobster) has become a problem, both for the environment and for the processing plant. About 45% of processed seafood consists of shrimp, the waste of which is composed by the exoskeleton and the cephalothorax. This waste represents 50-70% of the weight of the raw material, and it contains valuable components such as chitin, protein, and pigments, their amounts depending on the processing conditions, the species, the body parts, the seasonal variations, etc. (Duarte de Holanda and Netto, 2006; Rodde et al. 2008; Xu et al. 2008; Al Sagheer et al. 2009; Palpandi et al. 2009; Wang et al. 2011). Crustacean shells are the most important chitin source for commercial use due to its high content and ready availability. Crustaceous which are considered trash or not edible can be used as sources of chitin, which adds more values to the bycatch and represents an economic asset for the fishermen (Das and Ganesh, 2010). Chitin and its deacetylated derivative chitosan have numerous applications in foods, pharmaceuticals and biotechnological products, cosmetics, textiles, in waste water treatment and in agriculture (Shahidi et al. 1999; Gryndler et al. 2003; Kato et al. 2003; Şenel and McClure, 2004; De Jin et al. 2005; Kim and Mendis, 2006; Gortari and Hours, 2008; Lárez-Velázquez, 2008; Bhatnagar and Sillanpää, 2009; El Hadrami, 2010; Park and Kim, 2010; Portes et al. 2009; Ramírez et al. 2010; Rangel-Mendez et al. 2010; Limam et al. 2011; Muzzarelli et al. 2012; Raja et al. 2012). Routinely applications require specific structures, and effectiveness of polymers has been shown to depend on the molecular weight and the degree of acetylation (Daum et al. 2007; Pillai et al. 2009; Kassai, 2010; Domard, 2011). The greatest obstacle for increasing the use of these biopolymers in various industries is the comparatively high cost of their manufacturing process (Sini et al. 2007).

Several techniques to extract the chitin from different sources have been reported. Most of them, including the currently used industrial method, rely on chemical processes for the hydrolysis of protein and removal of inorganic matter. Some include a pigment removal step to improve the color of the extracted chitin, using solvent extraction or chemical oxidation (Beaney et al. 2005). The traditional methods for commercial preparation of chitin from crustacean shell waste consist in mechanical grinding, demineralization (DM) with strong acids and protein removal (deproteination, DP) with alkali at 90-100ºC (Khor, 2001; Percot et al. 2003; Naznin, 2005; Palpandi et al. 2009; Thirunavukkarasu and Shanmugan, 2009; Thirunavukkarasu et al. 2011). However, the use of these chemicals causes depolymerization of the product and therefore affects properties such as molecular weight, viscosity, and degree of acetylation. Chemical chitin purification is extremely hazardous, energy consuming and threatening to the environment because of the high concentrations of mineral acid and caustic employed. Another disadvantage is that the valuable protein components can no longer be used as animal feed (Sini et al. 2007; Xu et al. 2008; Al Sagheer et al. 2009; Manni et al. 2010; Das and Ganesh, 2010; Pacheco et al. 2011). As regulations have become more stringent now, there is a need to treat and utilize the waste in a more efficient manner. Therefore, there is a significant interest regarding recycling of crustacean biowaste (Prameela et al. 2010).

To overcome the problems of chemical treatments, different biotechnological processes have been proposed. Microorganisms (microbiological fermentation) and proteolytic enzymes (enzymatic extracts or isolated enzymes) have been used to remove the proteins and mineral content (Sini et al. 2007; Jung et al. 2007; Oh et al. 2007; Xu et al. 2008; Sorokulova et al. 2009; Manni et al. 2010; Giyose et al. 2010) (Table 1).

Most investigations dealing with biotechnological processes have been focused on the preparation of chitin and chitosan; however, in some cases they also have included the production of high value commercial by-products such as chitooligosaccharides, protein hydrolyzates, chitinases, carotenoids (astaxanthin), lactic acid, antioxidants, etc. (Wang et al. 2006; Auerswald and Gäde, 2008; Bueno-Solano et al. 2009; Wang et al. 2009; Pacheco et al. 2011; Wang et al. 2011; Cahú et al. 2012; Flores-Albino et al. 2012; Jeyandra Saravanan et al. 2012; Khorrami et al. 2012; Sharaf et al. 2012). In this review we compile, compare and discuss the most significant and updated information published on biotechnological processes for chitin recovery from crustacean waste. Some additional information on closely related by-products is also included.

Microbial fermentation of crustacean biowaste

Lactic fermentation bioprocess

Fermentation of crustacean shell biowaste using a selected *Lactobacillus* sp. strain as inoculum resulted in medium conditioning, supposedly by production of lactic acid and proteases. Lactic acid is produced by breakdown of glucose, creating the low pH condition of silage that suppresses the growth of spoilage microorganisms. Lactic acid reacts with the calcium carbonate present in the chitin fraction, leading to the formation of calcium lactate, which precipitates and can be removed by washing. DP of the biowaste and simultaneous liquefaction of the shrimp proteins occurs by the action of proteolytic enzymes produced by the added strains, by gut bacteria present in the intestinal system of the shrimp, or by proteases present in the biowaste. The efficiency of fermentation using lactic acid bacteria depends on factors such as the quantity of inoculum, glucose (or carbon source) concentration, initial pH and pH evolution during fermentation, and fermentation time (Rao et al. 2000; Oh et al. 2007; Prameela et al. 2010).

Rao et al. (2000) studied the effect of different fermentation parameters (initial pH adjustment with different acids, initial glucose concentration and inoculation with different quantities of *Lactobacillus*) individually and in combination, on DP and DM, and the prevention of spoilage during fermentation of shrimp biowaste. Combined treatment with *Lactobacillus* and reduction of initial waste pH by addition of acetic acid produced lower DP and higher DM than did the treatment with *Lactobacillus* or acid individually. In addition, inoculation with *Lactobacillus* resulted in a high-quality protein liquor output, whereas autofermented waste (due to the presence of shrimp microflora) imparted a strong and pungent acidic smell to the protein fraction. Therefore, fermentation using only endogenous microorganisms does not seem to be a good option. In the fermentation with lactic acid bacteria, the DM efficiency and the quality of the derived product are high, and the addition of commercial proteases may increase DP. They concluded that combined *Lactobacillus* and acetic acid treatment remains a cost-effective and environmentally friendly procedure for the fermentation of shrimp waste.

The *in situ* production of lactic acid from whey for the DM of crayfish exoskeleton or chitinous fraction was reported by Bautista et al. (2001). In this study, lactic acid was produced from whey lactose in a fed-batch fermentation process, using immobilized cells of *Lactobacillus pentosus*. While protein and mineral contents were significantly reduced (81.5 and 90.1%, respectively), chitin content was increased 3.4 fold. Their results show that complete DM was not achieved by *in situ* production of lactic acid and a final treatment with 0.5 M HCl was necessary. Nevertheless, the process proposed for crayfish chitin purification was less polluting than the traditional HCl-NaOH procedure.

The effect of the carbohydrate source (sugar cane, lactose and whey powder) and inoculum level (0.5 and 10% of *Lactobacillus plantarum*) in the solid-state lactic acid fermentation of prawn wastes (*Penaeus sp.*) on the removal of proteins and minerals for chitin recovery was studied by Cira et al. (2002). Under the selected conditions (10% sugar cane and 5% inoculum level) and using a solid-state column reactor, at the sixth day of fermentation they obtained a low cost chitin with adequate DP and DM levels (89.4 and 82.5%, respectively). Also, Cira et al. (2002) evaluated lactic fermentation of shrimp on solid substrates as a means of preservation for chitin recovery. They tested several levels of inoculation of *Lactobacillus* spp. strain B2 (isolated from shellfish waste) as well as different types and levels of carbohydrates. DM and DP degree obtained was 85 and 87.6% respectively. The chitin fraction, after 6 days of fermentation, treated with HCl (0.5 M) and NaOH (0.4 M) showed the greatest decrease in mineral and protein content.

Beaney et al. (2005) compared the physicochemical properties and some quality aspects of chitin (presence of spoilage bacteria, enzymes and heavy metals) produced by a chemical technique with chitin extracted by a biological method using a lactic acid bacterial fermentation (with a commercial inoculant: SIL-ALL 4 x 4™ silage additive). At the end of fermentation, the product obtained by the biological method retained around half of the original protein content of the shell and DM was 68.29%. They concluded that the chemical process may be the most effective way of obtaining chitin of the highest purity. However, biotechnological processes can be considered as a viable alternative.

One-step extraction of crude chitin from red crab shell waste by single-strain or co-fermentation using two distinct strains: *Lactobacillus paracasei* subsp. *tolerans* (KCTC 3074), a lactic-acid-producing bacterium, and *Serratia marcescens* (FS-3), a protease-producing bacterium was conducted by Jung

et al. (2006). DM level of KCTC 3074 + FS-3 (1:1) co-fermentation increased up 97.2% after 7 days of fermentation, suggesting that co-fermentation was highly effective for removal of ash from the shells. In this process, FS-3 was less contributing to DP. This was probably due to the lower pH than the optimum neutral pH for the proteolytic activity in the culture supernatant because of the organic acids produced by the co-cultured KCTC 3074. They suggested that fermentation process by KCTC 3074 and FS-3 is efficient and applicable for one-step extraction of crude chitin from red crab shells waste by simultaneously removing ash and protein in a fermenter, but needs improvement in DP. These results show the necessity of developing other protocols in order to improve the removing of protein in crab shells.

Jung et al. (2007) studied a two-step fermentation with the same two bacteria (KCTC-3074 and FS-3). The successive fermentation with the combination of KCTC-3074 and FS-3 gave the best results in the co-removal of $CaCO_3$ and proteins from crab shells. Employing this combination, the levels of DM and DP at the end of fermentation were 94.3% and 68.9%, respectively. Their results suggest that bio-deproteination is less efficient than bio-demineralization in every treatment and that the sequential order of processing is important.

The best lactic acid bacteria among five strains tested, which significantly acidified the shrimp biowaste on fermentation, and the effect of lactic acid fermentation on carotenoid recovery, DP and DM of shrimp biowaste for chitin preparation were determined by Bhaskar et al. (2007). The strain *Pediococcus acidilactici* CFR2182 was found to be efficient in fermenting the biowaste besides resulting in a product with the highest DP and considerably high DM.

Xu et al. (2008) purified chitin from shrimp shells (*Penaeus monodon* and *Crangon crangon*) using a two-stage fermentation process with anaerobic DP followed by decalcification through homofermentative lactic acid fermentation. Mixed cultures from different sources were tested for DP: cultures M1 and M2 (from autochthonic flora of Indonesian shrimp shells), cultures SM (from *C. crangon* shrimp), S (from soil), SS (from sewage sludge), SK (from sauerkraut) and GM (from ground beef meat). Cultures SS and GM were selected for the treatment of either dry or wet shrimp shells, yielding high DP values (from 83 to 98% for *P. monodon* and ~98% for *C. crangon*). For DM, a pure culture of *Lactobacillus casei* MRS1 was used for lactic acid production from glucose, obtaining DM from 58 to 92% for *P. monodon* and above 99% for *C. crangon*. This process for DP and decalcification is not commonly used, but offers a good possibility to biologically produce highly pure chitin.

The effect of three strains of lactic acid bacteria on the fermentation efficiency of shrimp biowaste for chitin recovery was studied and compared with the chemical extraction method by Khanafari et al. (2008). Their results showed that the microbial method is more effective, especially for the recovery of chitin, when compared with the chemical method. No significant differences were observed in chitin yields obtained by microbial extraction by *Lactobacillus plantarum* PTTC 1058, *L. acidophilus* PTTC 1643 and *L. plantarum* PTTC 1637. The percentage of DM, DP and degree of acetylation (DA) was not reported, but the results suggested that *L. plantarum* (PTTC 1058) is an attractive source for the recovery of chitin and chitosan.

A response surface methodology for the determination of DM in fermented shrimp shells was used by Choorit et al. (2008). DM was determined with the selected strains of lactic acid bacteria at various sucrose concentrations, initial pH values and soaking times. The highest DM (83.47%) was observed with 50 g/L sucrose, initial pH 7.0 and soaking time of 36 hrs.

Aytekin and Elibol (2010) investigated the characteristics of chitin from prawn waste using a bacterial system (individual strains and also their co-culture) in the presence of various concentrations of glucose. *Lactococcus lactis*, a protease producer and a marine bacterium *Teredinobacter turnerae*were evaluated in comparison with chemical treatment. *L. lactis* was more effective in mineral removal compared to *T. turnerae*, while the reverse was true for protein removal. A comparison of process yields in these treatments showed that *L. lactis* and *T. turnerae* used individually are not a good choice for commercial chitin extraction. On the contrary, in all co-culture processes, the values of DM were higher than those of the mono-cultivation at the same glucose level. The highest process yield (95.5%) was obtained in the co-cultivation of two bacteria, *T. turinae-L. lactis,* in a medium containing 5% glucose. The extraction of chitin was not complete as in the chemical method. However, the prawn shells were demineralized and deproteinized to a considerable extent. These results suggest that biological treatment could be an alternative to the chemical method.

Das and Ganesh (2010) evaluated the effectiveness of lactic acid produced by *L. plantarum* and the proteolytic activity of *Aspergillus niger* for the extraction of chitin from trash crabs. The study showed that the use of an organic acid for DM is a promising option and concluded that the combination of lactic acid and *A. niger* is a cost-effective and eco-friendly process for the production of chitin. López-Cervantes et al. (2010) used immobilized cells of a commercial probiotic strain (*Lactobacillus* sp). The objective of this study was to utilize the lactic acid fermentation of shrimp waste to facilitate the separation of products such as raw chitin, protein rich liquid hydrolyzates and lipidic paste. The raw chitin obtained showed a partial DM and DP with an average purity of 59.1%. This investigation suggests that lactic fermentation facilitates the separation and partial purification of the main components of shrimp waste. Also, Prameela et al. (2010) used a natural probiotic for chitin and carotenoid production. Under optimal conditions of fermentation, the DM and DP of shrimp biowaste were 69 and 89% respectively. The study showed that the probiotic strain was efficient in fermenting the shrimp biowaste.

Lactic acid fermentation as a biological method for chitin and astaxanthin recoveries was evaluated by Pacheco et al. (2011). The biologically obtained chitin (BIO-C) refers to the product obtained by fermentation and mild treatment with HCl and NaOH to eliminate the remaining minerals and proteins. In addition, the physicochemical characteristics of the materials obtained after prolonged fermentation time were compared with those of commercial chitin and chitin recovered by chemical methods. These authors concluded that lactic acid fermentation is an effective biological method for chitin recovery. The characteristic of BIO-C makes it a promising starting material for chitosan production.

Wahyuntari et al. (2011) compared the order of microbiological DP and DM in chitin extraction of shrimp shell waste. The first experiment considered DP (*Bacillus licheniformis*) followed by DM (*Lactobacillus acidophilus* FNCC116) process (DP-DM) and the second considered DM followed by DP (DM-DP). DM-DP of shrimp waste produced higher chitin extraction efficiency than DP-DM.

Prameela et al. (2011) reported on two bacterial cultures isolated from shrimp gut indentified as lactic acid bacteria. Their use resulted in DP and DM efficiencies of 87 ± 0.2% and 72.2 ± 0.1% respectively.

Duan et al. (2012) reported an improvement in the removal of minerals (in comparison with previous researches) using the epiphytic *Lactobacillus acidophilus* SW01, isolated from shrimp waste (SW). *L. acidophilus* SW1 was compared with other lactic acid bacteria (LABs) in terms of acid production and DP in shrimp waste fermentation. The results showed that *L. acidophilus* SW1 produced much more acid than other LABs and had a much higher proteolytic activity. With more protein removed, more minerals are exposed to an acidic environment, which has led to the improvement in the removal of minerals. In laboratory-scale fermentations, the mineral and protein content was 0.5 and 5.4% at 168 hrs (the end of fermentation). This level of mineral content has reached China's standard for chitin (the ash and protein content is usually bellow 1% for high-quality products). Authors concluded that the material obtained at the end of the process can be transformed into chitin by a simple bleaching procedure. Furthermore, in pilot-scale fermentation, the mineral content decreased to 0.72% (after 120 hrs) and protein was removed to 7.01% (120 hrs). These results illustrate that *L. acidophilus* SW01 is suitable for SW treatment and industrial applications.

Khorrami et al. (2012) used *Lactobacillus plantarum* for biological extraction of chitin from shrimp shell. DM and DP obtained were 45 and 54% respectively. In both studies, the product fermented was transformed into chitin by a mild chemical post-treatment. Flores-Albino et al. (2012) reported the simultaneous production of chitin and L(+)-lactic acid from crab (*Callinectes bellicosus*) wastes by submerged fermentation of *Lactobacillus* sp. B2 (isolated from shrimp wastes) using sugar-cane molasses as carbon source. DM and DP achieved were 88% and 56%, respectively. Their results demonstrate that the production of added-value products can be achieved under mild conditions and in an environmentally friendly way.

A summary on the above mentioned 21 reports published during the last decade is shown in Table 2. In all cases, lactic acid fermentation was used for the bioprocessing of crustacean wastes. Different strains of *L. plantarum* were used in 7 cases for the treatment of shrimp (6 cases) or crab (1 case) wastes. Other reports dealt with the use of single (*L. pentosus*, *L. salivarius*, *E. faecium*, *P. acidilactici*, *L. paracasei*, *L. casei*, *L. rhamnosus*, *L. lactis*, *Pediococcus*) or mixed *(L. plantarum* and *A. niger*; *L. paracasei* and *S. marcescens*; *L. acidophilus* and *B. licheniformis*, *L. lactis* and *T. turnerae*) microbial cultures on wastes from different origin: crayfish, prawn shell, red crab shell and trash crab. Various

fermentation procedures included auto fermentation, fermentation in a single-step, successive fermentations, co-fermentation, and different process conditions were evaluated.

Production and characterization of chitin, including the efficiency of the DM process, were the main targets in all these reports, although in some cases the simultaneous recovery of carotenoids, chitosan and other by-products was considered. The reported results highlight DP and DM as the main quality parameters of the obtained chitin. Reported DP values varied between 45 and 99%, whereas DM values varied between 18.5 and 99%. It is remarkable that only three papers reported on the DA of the obtained chitin. This is an important chemical characteristic that affects the properties and potential use of chitin. Results of the amount of chitin produced were expressed in many different ways: *i.e.* as percentage of original waste processed, as percentage of the original amount of chitin contained in the waste, as component percentage of the fermented raw material, etc.

Miscellaneous fermentations

The concurrent production of chitin from shrimp shells and fungi by placing shrimp shells in direct contact with the fermentation of filamentous fungi was analyzed by Teng et al. (2001). Proteolytic enzymes released from the fungi were anticipated to deproteinize and demineralize the shrimp shells releasing amino acids that would act as a nitrogen source for fungal growth. The results of the experiments showed that the percentage of residual protein in the chitin isolates from shrimp shells was below 5% and the DA was between 60 ± 3 and 72 ± 1. The protein content in the fungal chitin isolate was higher (10-15%) and DA was between 75 ± 4 and 82 ± 3. When fungi and shrimp shell powder supplemented with glucose were combined in a single reactor, the release of protease by the fungi enhanced the DP of shrimp-shell powder and the release of hydrolyzed proteins. The hydrolyzed proteins in turn were utilized as a nitrogen source for fungal growth, leading to a lowering of the pH of the fermentation medium, thereby further enhancing the DM of the shrimp-shell powder. They concluded that concurrent DP of shrimp shells by three proteolytic *A. niger* (strains 0576, 0307 and 0474) was demonstrated and that DM occurred under these conditions. Therefore, proteolytic fungal fermentation of shrimp shells is a simple, effective and inexpensive process to concurrently recover relatively good quality chitin from shrimp shells and fungal mycelia.

Sini et al. (2007) used *Bacillus subtilis* for the production of chitin and chitosan. About 84% of the protein and 72% of the minerals were removed from the shrimp shell after fermentation. They concluded that in order to produce chitin of standard quality, residual protein and ash content of the fermented residue have to be removed by mild acid and alkali treatments.

The efficiency of DM and DP using *Pseudomona aeruginosa* F 722 was evaluated by Oh et al. (2007) with various concentrations of glucose as carbon source and two particle sizes of crab shell waste. The strain produced a high protease activity in the culture medium in addition to organic acids, mainly lactic, succinic, and citric acid. At the optimal incubation temperature, the efficiency of DM was dependent on the glucose concentration and the decrease of pH in the medium. Regression analysis was undertaken to determine the correlation of DM and DP with the measured variables: glucose concentration, pH, total titratable acidity and protein in the medium. The highest correlations were found between DM and glucose amount, and DP and glucose amount (r^2 = 0.821 and 0.787, respectively). Shell particle size had a small effect on DM and DP.

The application of *Serratia marcescens* FS-3 for DP of crab shell waste was studied by Jo et al. (2008). They compared four treatments on the crab shells: FS-3 inoculum, Delvolase (commercial enzyme), FS-3 culture supernatant and FS-3 supernatant plus Delvolase. Also, they compared the DP efficiency of commercial enzymes such as Delvolase, Cytolase PCL5, Econase CEPi, Econase MP 1000, Maxazme NNP and Cellupulin MG. After biological treatment, the DP was in the 81-90% range (Delvolase has the highest DP activity) and DM was in the 0.01-47% range (FS-3 showed the highest DM activity). The protein removal from crab shell wastes with 10% FS-3 inoculum was 84% after 7 days of fermentation, highlighting the efficiency of the bio-deproteination process. About 50% of DM occurred in accordance with DP during microbial fermentation. Authors postulated that organic acids (acetic acid) produced by the bacteria dissolved the calcium carbonate from the crab shell waste. However, little DM occurred in the treatment with enzymes alone, such as Delvolase, and FS-3 supernatant plus Delvolase. These results demonstrated that the protease produced by *S. marcescens* can be effectively used for DP of crab shell wastes.

Sorokulova et al. (2009) isolated and characterized two bacterial strains for their proteolytic activity which were identified as *Bacillus cereus* and *Exiguobacterium acetylicum*. They obtained a high level of DP (97.1 and 92.8%) and DM (95.0 and 92.0%) of shrimp waste with *B. cereus* and *E. acetylicum*, respectively, at laboratory scale. High efficiency of strain *B. cereus* 8-1 was demonstrated in the large-scale fermentation of waste (78.6 ± 2.6% DP and 73.0 ± 1.5% DM). They concluded that such high percentages of DP and DM, together with the use of a shell waste without any additional procedures or supplements, have never been reported and highlight the potential of these bacteria for the development of environmentally friendly processes for chitin extraction from chitin-rich wastes.

DP of shrimp shell waste is a crucial step in chitin production. Shrimp waste DP with different microorganisms, such as *Pseudomonas aeruginosa*, *Enterococcus faecium, Bacillus subtilis* and *Candida parapsilosis,* has been repeatedly attempted (Waldeck et al. 2006). However, the degree of DP was insufficient or the process was too time-consuming and/or costly sterilization processes had to be included. To obtain high-quality chitin, two parameters are of the utmost importance: high proteolytic activity and no chitinolytic activity during the process. In this sense, Hoffmann et al. (2010) studied the genetic improvement of *Bacillus licheniformis* strains for efficient DP of crustacean shells and production of high quality chitin. *B. licheniformis* F11 (wild type) produced large amounts of proteolytic enzymes and had low chitinase activity. Several genetically modified strains of *B. licheniformis* were constructed and used in that study: *B. licheniformis* F11.1, *B. licheniformis* F11.2, *B. licheniformis* F11.3 and *B*. *licheniformis* F11.4. The applying of variants of *B. licheniformis* F11 resulted in the production of high-molecular-mass chitin from shrimp shells. The best result was achieved with the double mutant F11.4, demonstrating that genetic manipulation is a powerful tool for the production of long-chain chitin and chitosan (chitinase activity was completely abolished).

Ghorbel-Bellaaj et al. (2012) evaluated six proteolytic strains on the fermentation (without and with glucose addition) of crustacean biowaste for chitin recovery under several conditions of fermentation, being: *Bacillus pumilus* A1, *B. mojavensis* A21, *B. licheniformis* RP1, *B. cereus* SV1, *B. amyloliquefaciens* An6 and *B. subtilis* A26. All the strains were able to deproteinize shrimp waste. However, the highest DP was obtained with *B. cereus* SV1. They concluded that values of DP were related not only to the level of protease activity produced, but also to the particular properties of the enzyme that affect its accessibility to the substrate. The addition of glucose to shrimp waste medium had no significant effect on DP but was critical for the regulation of medium pH, so it affected DM.

A summary of the above mentioned 6 reports published during the last decade on the fermentation of crustacean wastes with microorganisms different than lactic acid bacteria is shown in Table 3. Among them, three research groups worked with different *Bacillus* sp, in one case in comparison with *E. acetylicum*, whereas the other 3 research groups worked with *P. aeruginosa*, *S. marcescens* and *A. niger*. The main objectives of these six reports were similar to those discussed above using lactic acid bacteria, *i.e.*: chitin and/or chitosan production and DP and DM of crustacean wastes. Results obtained for DP ranged between 63 and 97% and for DM between 37 and 95%. Only two studies reported values of chitin produced and DA.

Enzymatic hydrolysis of crustacean biowaste

For DP of the shells during chitin isolation, a simple base extraction is usually employed. However, this process produces a liquid waste containing residual base, protein and protein degradation products. The enzymatic digestion and separation of the shell waste proteins should allow the recovery of the protein hydrolyzate with a well balanced amino acid composition. Synowiecki and Al-Khateeb (2000) employed an enzymatic DP of previously demineralized (10% HCl) shrimp shells for the production of chitin and a nutritionally valuable protein hydrolyzate. They used Alcalase 2.4L (Novo Nordisk A/S), which is a serine endopeptidase obtained from *Bacillus licheniformis*. The choice of this enzyme was made based on its specificity for terminal hydrophobic amino acids, which generally leads to the production of non-bitter hydrolyzate and allows an easy control of the degree of hydrolysis. The hydrolyzate obtained by Alcalase digestion is a good source of essential amino acids in foods products. However, the effectiveness of DP was limited by the presence of residual small peptides and amino acids directly attached to chitin molecules and resistant to enzymatic hydrolysis. The enzymatic DP of the shrimp shells using Alcalase 2.4L is a suitable method for the production of protein hydrolyzates with a good value of essential amino acid index. It also allows the isolation of chitin containing only about 4% of protein impurities and 0.31 to 1.56% of ash. Such purity is sufficient for many non-medical applications of chitin.

The digestive system of some marine invertebrates contains a variety of highly active proteases that can be used as a source of enzyme preparations. Mukhin and Novikov (2001) studied the possibility of using crustacean processing waste (CPW) for producing enzymatic protein hydrolyzates, which can be used as components of microbiological nutrient media. They investigated the dependence of the degree of protein hydrolysis on enzyme concentration, duration of hydrolysis, and pH of the reaction mixture. In this research, CPW was utilized both as substrate and as source of proteases. The CPW proteins were degraded with a proteinase cocktail isolated from the hepatopancreas of king crab. This way of utilization of the processing waste of crustaceans would provide material for nutrient media and solve the environmental problems associate with crustacean processing. In addition, the residual non hydrolyzed material is a good source for chitin recovery.

Although various enzymatic methods have been described for the recovery of crustacean waste components, little work has focused on processes that lead, in a single operation, to the recovery of the three main components: chitin, protein and carotenoid pigments. The objective of the study of Duarte de Holanda and Netto (2006) was to optimize the recovery of the main components from *Xiphopenaeus kroyeri* shrimp waste (one of the most important commercial species found in Brazil) using an enzymatic hydrolysis process. Two enzymes were used: Alcalase 2.4L and swine pancreatin, a mixture of endo- and exo-peptidases (trypsin, chymotrypsin, and carboxypeptidase) obtained from pig pancreas. Both enzymes are already being used in the processing of marine products, giving hydrolyzates with a good balance of essential amino acids. Various authors reported that Alcalase resulted in higher protein recovery (PR), in addition to providing hydrolyzates with good functional properties and a mild bitter taste. An increase in the degree of hydrolysis (DH) from 6 to 12% was concomitantly obtained with an increase in PR from 26 to 28%. Although there is a relationship between PR and DH, a previous work showed that PR did not improve significantly at DH values higher than 12%. The alkaline method (treatment with NaOH or KOH solutions) used for PR was more efficient (88.39% against 59.50% for enzymatic hydrolysis). However, it must be pointed out that alkaline hydrolysis is not indicated for PR since some amino acids are lost, reducing the nutritional and technological value. DP with Alcalase was more efficient than with pancreatin. The higher nitrogen contents found in chitin after the enzymatic treatment were possibly due to incomplete DP. These results were confirmed by the presence of 9.31% residual protein. After enzymatic hydrolysis, the chitin ash content varied from 2.1 to 2.9%. Enzymatic hydrolysis of industrial waste from *X. kroyeri* shrimp gave a PR of 59.5%, in addition to providing adequate conditions for astaxanthin and chitin recovery.

Mizani and Aminlari (2007) propose the use of chemical agents, such as sodium sulphite and Triton X-100, along with proteolytic enzymes for improving PR efficiency from shrimp head waste and investigated the characteristic of the chitin produced. Eight different DP processes were compared. The highest PR was obtained by application of both sodium sulphite and Alcalase. The characteristics of the chitin produced by the two-stage process (considering an additional mild alkali digestion step) as compared with the commercial food grade product, confirmed its good quality. This research showed that by using chemical/enzymatic procedures, it is possible to produce standard quality chitin as a valuable by-product from shrimp head waste processing.

Manni et al. (2010) described the isolation and characterization of chitin from shrimp waste by treatment with a crude enzyme preparation from *Bacillus cereus* SV1 and its deacetylation into chitosan. In the recovery of chitin from shrimp waste, associated minerals were removed by mild acid treatment (dilute HCl solution). The protein content was significantly higher in the chitin isolated with enzymatic DP than with alkali treatment, but removal of the residual protein associated with the chitin was not complete. Although such DP percentage is lower than that of chemical treatment, enzymatic DP helps avoiding many drawbacks of chemical treatment such as heavy metals residues, over-hydrolysis, breakdown of chitin, etc. The treatments employed to extract chitin from shrimp wastes allowed the recovery of 16.5% of its initial mass as a water insoluble white fibrous material, indicating that a good yield for the chitin extraction was attained. The crude protease from *B. cereus* SV 1 could be used effectively in the DP of shrimp wastes to produce chitin and protein hydrolyzates.

Giyose et al. (2010) evaluated the activity of proteases (commercial and crude) produced by *Erwinia chrysanthemi* in a chitin production process. The calcium present in the crustacean waste was removed using acid-based DM. Protein removal efficiency (95%) was higher for the crude protease from both the mineralized and demineralized wastes. The proteases produced by *E. chrysanthemi* may find applications in the biotransformation of waste from sea-food processing industries into products of commercial value.

Chitin recovery using an enzymatic DP step combined with microwave-assisted DM was reported by Valdez-Peña et al. (2010). Five different food-grade commercial proteolytic enzymes were compared for the DP of shrimp heads and combined with the DM process mentioned for chitin recovery in order to achieve short reaction times. Protein hydrolysis occurred during the enzymatic treatment and the hydrolyzed protein was released into the liquid medium. Alcalase 2.4L FG treatment produced the lowest protein content in the insoluble solids, which indicates its high efficiency for DP of shrimp head waste. Protein removal was not complete with any of the enzyme preparations. The ash content after enzyme treatment was between 17.85 and 26.58%. These results suggest that the enzymatic treatments were not capable of releasing minerals from the protein-chitin complex. In addition, the use of microwave-assisted technology for DM of deproteinized shrimp heads with lactic acid in short reaction times promoted the elimination of the residual protein present in enzymatically deproteinized shrimp heads. Enzymatic DP followed by microwave-assisted DM allowed the recovery of high quality chitin under environmental friendly conditions.

Younes et al. (2012) investigated the influence of several operating parameters, such as enzyme/substrate ratio, temperature and incubation time, on the DP degree of shrimp shells by non-commercial crude enzyme preparations. They assayed microbial proteases from *Bacillus mojavensis* A21, *B. subtilis* A26, *B. licheniformis* NH1, *B. licheniformis* MP1, *Vibrio metschnikovii* J1 and *Aspergillus clavatus* ES1. The DP degrees obtained with the crude enzymes were 76 ± 4% for A21, A26, J1, and MP1, 65 ± 3% and 59 ± 3% for NH1 and ES1, respectively. The highest DP degree was obtained with the protease from *B. mojavensis* A21, being 88.5% under optimal conditions. The shrimp waste deproteinized by enzymatic treatment was subjected to mild acid treatment in order to remove minerals. These sequential treatments of chitin extraction allowed the recovery of 18.5 ± 2.3% of its initial dry mass as water insoluble white fibrous material.

A summary of the above mentioned 9 investigations about the application of proteolytic enzymes for the treatment of chitinous wastes (shrimp, prawn and crab) is shown in Table 4. The main purpose of these treatments was the elimination of the protein present in the waste with the concomitant production of protein hydrolyzate (with potential use as food ingredient) and raw chitin. The commercial enzymes used were: Alcalase 2.4L, Pancreatin, Delvolase, Cytolase PCL5, Econase CEPi and MP 100, Maxazime NNP and Cellupulin MG, as well as non-commercial protease complexes and crude proteases. Results obtained for DP varied between 54 and 97% and between 0 and 99.8% for DM. Only three reports mentioned the percentage of chitin produced (16.5, 18.5 ± 2.3 and 22%, respectively) whereas the DA reported was between 77.77 and 89%.

FINAL REMARKS

The above discussed investigations on the biological processing of crustacean wastes are difficult to compare. They differ in the objective of the study, origin of the waste to be treated, type of treatment (fermentation or enzymatic hydrolysis, in combination or not with chemical treatment), and fermentation conditions (pretreatment of the waste, additional starters, initial pH, temperature, type and concentration of supplements, type and concentration of C- and N-sources, inoculum size, etc.). Different parameters were evaluated and some important characteristics such as yield, quality and physicochemical properties of the chitin obtained were not always reported. In all cases, authors agreed with respect to the economic and environmental advantages demonstrated for the biological processing of crustacean wastes and concluded that less expensive and more eco-friendly methods for large scale extraction of chitin need to be developed.

It should be remarked that the microbiological contamination of the products obtained was not an aspect particularly considered in those reports, even though being a very important aspect to be evaluated. Only one paper mentioned the microbiological quality of the raw chitin obtained (López-Cervantes et al. 2010), where a study was done according to AOAC (1995) methods 46.030, 46.062, 46.115, 46011 and 4.030 for coliforms and *E. coli*, *Staphylococcus aureus*, *Salmonella*, fungi and *Pseudomona,* respectively. In addition, the total mesophilic count was registered. The microbiological quality of the product is linked to the level of hygiene in the production process and is a parameter that restricts the use of the by-products for human or animal consumption (standard regulations for the consumption of fresh shrimp). Pacheco et al. (2011) determined the growth of coliforms by colony counting. Some authors applied a pre-treatment with chlorine solution, removal of flesh and protein, or boiling distilled water for removing endogenous microbes (Choorit et al. 2008; Xu et al. 2008; Praamela

et al. 2010). In fermentation, lactic acid produced by the breakdown of glucose creates low pH condition of ensilation that suppresses the growth of the spoilage microorganisms (Prameela et al. 2010; Pacheco et al. 2011), pH being an important factor in the suppression of undesirable bacteria. This is due to the production of antimicrobial metabolites, as hydrogen peroxide, acetone and bacteriocins, by lactic acid bacteria (Beaney et al. 2005; López Cervantes et al. 2010).

CONCLUDING REMARKS

Commercial chitin is extracted from crustacean wastes provided by the seafood processing industry. There are some reports of chitin extraction form insects and fungi, most of them at laboratory scale. The amount of utilizable chitin is much less than the billions of ton estimated to be available in the biosphere.

Crustacean shells are constituted mainly of a matrix made of chitin tightly associated with protein, minerals, lipids and pigments. The amount of each component can vary widely among species and also in an intra-specific way as a function of season, age, sex, the body part, geographic origin, and other environmental conditions.

The chitin produced depends to a large extent on the quality of the raw material.

The degree of acetylation is one of the most important characteristics of chitin, and its value depends on the raw material and the processes used for deproteination and demineralization. Observed differences in the degree of acetylation values were caused by deacetylation of polysaccharides occurring in the basic conditions during the deproteination process.

If pure chitin is required, for instance to make chitosan for medical applications, the fermented waste can be further subjected to a mild chemical treatment to remove the residual protein and minerals.

The efficiency of fermentation using microorganisms depends on the quantity of inoculum, the carbon source concentration, the initial pH and pH evolution during the culture, and fermentation time.

A cost-effective, fast, and easily controllable industrial process for producing chitin of high molecular mass and degree of acetylation still remains to be developed. Therefore, a great interest exists for the optimization of the extraction process to minimize the degradation of chitin, while, at the same time, bringing the impurity levels down to a satisfactory level for specific applications.

Further studies on the physico-chemical quality characteristic (chemical composition, FTIR analysis, NMR analysis, XRD analysis, degree of N-acetylation, TGA, SEM) of chitin prepared using biological processes is needed to ascertain its superiority over chitin prepared by conventional chemical methods.

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Tables

Table 1. Biotechnological processes proposed for chitin recovery from biowastes.

Biotechnological process	Biocatalyst	Type of bioprocess
Microbial fermentation	Selected strains of microorganism	Single-stage fermentation Two-stage fermentation Co-fermentation Successive fermentations
	Endogenous microorganism	Auto fermentation
Enzymatic hydrolysis	Alcalase Pancreatin Delvolase Enzymatic extract	

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able 2. Lactic acid fermentations used for the bioprocessing of crustacean wastes.

Microorganism	Waste source	DP (%)	DM (%)	DA (%)	Chitin (%)	Reference
Lactobacillus plantarum 541 Autofermentation (intestinal microflora)	Shrimp waste	61.61-87.97 74.87-93.48	64.07-90.76 44.14-82.35	ND ND	ND ND	Rao et al. 2000
Lactobacillus pentosus 4023 *Lactobacillus pentosus* 4023 + treatment with 0.5 M HCl and 0.25 M NaOH	Crayfish (*Procambarus clarkii*) CF (chitinous fraction)	81.5 99.4 ± 0.2	90.1 98.2 ± 0.4	ND 81.7	88.2 ± 0.8[a] 95.8 ± 0.5[a]	Bautista et al. 2001
Lactobacillus plantarum	Shrimp (*Penaeus* sp.)	89.4	82.5	ND	ND	Cira et al. 2002a
Lactobacillus spp. B2	Shrimp (*Penaeus* spp.)	87.6	85	ND	ND	Cira et al. 2002b
(*Lactobacillus salivarius* + *Enterococcus faecium* + *Pediococcus acidilactici*)	Prawn shell (*Nephrops norvegicus*)	49.37 [b]	68.29 [b]	67.0	67.9 [b]	Beaney et al. 2005
Co-fermentation (*Lactobacillus paracasei* subsp. *tolerans* KCTC-3074 + *Serratia marcescens* FS-3)	Red crab shell (*Chionoecetes japonicus*)	52.6	97.2	ND	ND	Jung et al. 2006
Successive fermentations (*Lactobacillus paracasei* KCTC-3074 + *Serratia marcescens* FS-3)	Red crab shell (*Chionoecetes japonicus*)	68.9	94.3	ND	33.50 ± 0.90	Jung et al. 2007
Pediococcus acidilactici CFR2182	Shrimp (*Penaeus monodon*)	97.9 ± 0.3	72.5 ± 1.5	ND	ND	Bhaskar et al. 2007
Mixed culture isolated from ground beef meat (GM) + *Lactobacillus casei* MRS1 Mixed culture isolated from sewage sludge (SS) + *Lactobacillus casei* MRS1 GM + *Lactobacillus casei* MRS1	Shrimp shells (Penaeus monodon) Shrimp shells (Penaeus monodon) Shrimp shells (Crangon crangon)	98[c] 83-98[c] >98[c]	78 - >92[d] 58 - >92[d] >99		37 ND 30	Xu et al. 2008
Lactobacillus plantarum (PTTC 1058) *Lactobacillus acidophilus* (PTTC 1643) *Lactobacillus rhamnosus* (PTCC 1637)	Shrimp waste (Penaeus semisulcatus)	ND ND ND	ND ND ND	ND ND ND	700[e] 700[d] 700[d]	Khanafari et al. 2008
Pediococcus sp. L1/2	Shrimp shells (*Litopenaus vannamei*)	ND	83.47	ND	ND	Choorit et al. 2008
Lactococcus lactis spp. Lactis NRRL-B-1821 *Teredinobacter turnirae* Co-cultivation *T. turnirae→L. lactis*	Prawn shell waste	66.5-69.4 63.2-77.8 ND ND	47.2-78.8 18.5-37.3 ND ND	ND ND ND ND	22.4-49.4 40.1-49.8 64.5 42.9	Aytekin and Elibol, 2010

L. lactis→ T. turnirae						
(*Lactobacillus plantarum* + *Aspergillus niger*)	Trash crab (*Podophthalmus vigil*)	ND	ND	ND	20.15-22.6	Das and Ganesh, 2010
Lactobacillus sp. probiotic	Shrimp waste	81.88 ± 0.15	95.64 ± 0.26	ND	10	López-Cervantes et al. 2010
Natural probiotic (milk curd)	Shrimp waste (*Penaeus monodon*)	89	69		5.65	Prameela et al. 2010
Lactobacillus plantarun	Shrimp waste (*Litopenaeus vannamei*)	94	92	94	45	Pacheco et al. 2011
DM followed by DP DP followed by DM DP: Bacillus licheniformis F11-1 DM: Lactobacillus acidophilus	Shrimp shells (*Penaeus vannamei*)	79.61 47.37	88.65 50.23	ND ND	92.84 78.45	Wahyuntari et al. 2011
Lactobacillus plantarum	Shrimp waste (*Penaeus monodon)*	87 ± 0.2	72.2 ± 0.1	ND	ND	Prameela et al. 2011
Lactobacillus acidophilus SW1 Laboratory-scale fermentation Pilot-scale fermentation	Shrimp waste (*Penaeus vannamei*)	92.2 91.56	99.27 99.02	ND ND	ND ND	Duan et al. 2012
Lactobacillus plantarum 1058	Shrimp shell	45	54	ND	ND	Khorrami et al. 2012
Lactobacillus sp. B2	Crab waste (*Callinectes bellicosus*)	56	88	95	34	Flores-Albino et al. 2012

DP (deproteination), DM (demineralization), DA (degree of acetylation), ND (Not determined).

[a]Increment in the chitin content (%); [b]Calculated from reported data (mg/g); [c]Total Kjeldahl nitrogen; [d]As $CaCO_3$; [e]Chitin yield (mg/g).

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Table 3. Miscellaneous fermentations used for the bioprocessing of crustacean.

Microorganism	Waste source	DP (%)	DM (%)	DA (%)	Chitin (%)	Reference
Aspergillus niger 0576	Shrimp shells	96.7 ± 0.3[a]	ND	72 ± 1	48.32	Teng et al. 2001
Aspergillus niger 0307		97.2 ± 0.5[a]		60 ± 3	48.53	
Aspergillus niger 0474		97.1 ± 0.3[a]		62 ± 3	48.15	
Aspergillus niger 0576	Fungal mycelia	86.2 ± 0.5[a]	ND	76 ± 6	22 ± 2	
Aspergillus niger 0307		84.9 ± 0.6[a]		75 ± 4	27 ± 3	
Aspergillus niger 0474		88.9 ± 0.4[a]		82 ± 3	17 ± 3	
Bacillus subtilis	Shrimp shells (*Metapenaeopsis dobsoni*)	84	72	84.4 ± 5.3	93.2 ± 0.6	Sini et al. 2007
Pseudomona aeruginosa F722	Crab shell	63	92	ND	ND	Oh et al. 2007
Serratia marcescens FS-3	Snow crabs (*Chionoecetes opilio*)	84 ± 1.6	47 ± 1.2	ND	ND	Jo et al. 2008
Bacillus cereus 8-1	Shrimp shell	97.1	95	ND	ND	Sorokulova et al. 2009
Exiguobacterium acetylicum		92.8	92			
Bacillus mojavensis A21	Shrimp shell waste	90.05[b] - 88[c]	38.1[b] - 78.7[c]	ND	ND	Ghorbel-Bellaaj et al. 2012
Bacillus pumilus A1		88.25[b] - 91.2[c]	37.3[b] - 75.3[c]	ND	ND	
Bacillus licheniformis RP1		94.4[b] - 90.8[c]	59.4[b] - 55.5[c]	ND	ND	
Bacillus amyloliquefaciens An6		83.4[b] - 90.8[c]	41.7[b] - 66.0[c]	ND	ND	
B. cereus SV1		95.65[b] - 88.6[c]	67.1[b] - 77.3[c]	ND	ND	
B. subtilis A26		91.5[b] - 91.2 [c]	37[b] - 79.9[c]	ND	ND	

DP (deproteination), DM (demineralization), DA (degree of acetylation), ND (Not determined).

[a] (100 - residual protein); [b] Shrimp shell waste medium; [s] Shrimp shell waste medium supplemented with glucose.

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Table 4. Enzymatic hydrolysis for bioprocessing of crustacean wastes.

Enzymes	Waste source	DP (%)	DM (%)	DA (%)	Chitin (%)	Reference
Alcalase 2.4L	Demineralized shrimp shells (*Crangon crangon*)	95.5 ± 0.53	98.4 ± 0.48	88.9 ± 0.25	ND	Synowiecki et al. 2000
Protease cocktail (hepatopancreas of king crab)	Deep-water prawn (*Pandalus borealis)* King crab (*Paralithodes camtschaticus*) Crab species (*Hyas araneus; Lithodes maja*)	ND	ND	ND	ND	Mukhin et al. 2001
Alcalase 2.4L Swine Pancreatin	Shrimp (*Xiphopenaeus kroyeri*)	93.41 [a] 92.23 [a]	97.5 [b] 97.9 [b]	ND ND	ND ND	Duarte de Holanda and Netto, 2006
Alcalase 2.4L Alcalase 2.4L + Na sulphite + NaOH (Two-Stage Method)	Shrimp (*P. semisulcatus*)	54.3 99.13 [c]	ND 98.96	ND 77.67	ND ND	Mizani and Aminlari, 2007
FS-3 inoculum (*Serratia marcescens*) Delvolase Culture supernatant FS-3 Culture supernatant FS-3 + Delvolase	Snow Crabs (*Chionoecetes opilio*)	84 ± 1.6 90 ± 2.9 81 ± 2.9 85 ± 3.2	47 ± 1.2 0.01 ± 0.01 12.0 ± 0.05 0.03 ± 0.01	ND	ND	Jo et al. 2008
Crude protease (*Bacillus cereus* SV1)	Shrimp shell (*Metapenaeus monoceros*)	88.8 ± 0.4	99.56[a]	89.5	16.55 ± 1.5	Manni et al. 2010
Protease from *Erwinia chrysanthemi*	Demineralized lobster waste (*Palinurus* sp.)	95	99	ND	ND	Giyose et al. 2010
Alcalase 2.4L FG Alcalase 2.4L FG + microwave-assisted demineralization	Shrimp head (*Litopenaeus vannamei*)	ND ND	74.42 - 82.15 99.8	ND ND	ND 22	Valdez-Peña et al. 2010
B. mojavensis A21 (under optimal experimental conditions)	Shrimp waste (*Metapenaeus monoceros*)	88 ±5	98.1	ND	18.5 ± 2.3	Younes et al. 2012

DP (deproteination), DM (demineralization), DA (degree of acetylation), ND (Not determined).

[a]100 - residual nitrogen content (%); [b]100 - residual ash content (%); [c]100 - residual protein (%).