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# Biological Method of Chitin Extraction from Shrimp Waste an Eco-friendly low Cost Technology and its Advanced Application

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**Abstract**
Biological method of chitin extraction from crustacean shells is an advanced and new technique which involves extraction of long chain carbohydrate polymer chitin by using lactic acid bacteria and it produces a good quality end product. This eco-friendly and low cost technique requires 24-48 hours old *Lactobacillus* inoculums for fermentation. The 10% culture inoculums is added with crustacean shells along with same quantity of carbon sources for 180 hrs incubation followed by filtering and the obtained solid cake i.e. chitin is dried in hot air oven. The additional advantage of this technique is production of liquid fraction which is rich in protein and can be used for human and animal feed. However, this method is still limited in laboratory condition as because demineralization and deproteinization have not yet reached up to the desired yields as compared to chemical method.

**Keywords:** Chitin, Biological methods, Processing industry, Low cast technology

## 1. Introduction

Chitin is a biopolymer and a major component of the supporting tissues of organisms such as crustaceans, fungi and insects. It is the most important organic constituent of invertebrates' exoskeleton. Chitin is the second most abundant natural biopolymer after cellulose. It is the single largest waste resource for the fish processing industry. Chemically chitin is the natural carbohydrate polymer. It is a long-chain polymer of N-acetyl D-glucosamine and derivative of glucose. Chitin is poly-β-(1-4)-N-acetyl-D-glucosamine; whereas chitosan is poly-β-(1-4)-D-glucosamine. Chitin is a stable compound and generally biodegradable by some organisms including humans. The average head and shell waste yield from the shrimp is around 60 % by weight of the whole shrimp. The shells contain 15–40% chitin and its amount in the whole marine environment has been estimated around 1560 million tons Kurita (2006) [3]. In India its availability is estimated to be 1, 00,000tons annually Gopakumar (2002) [2]. The shrimp processing industry has been rapidly growing with the significant increase in cultured shrimp production in the South-East Asian region. A huge amount of bio-waste such as carapace (head shells) and abdominal (tail) shells are produced from the industries because shrimps are normally sold as headless or peeled or both. Therefore, chitin producers are attracted to the shrimp wastes.
The main commercial process for chitin extraction from shrimp waste is based upon demineralization by acid treatment and deproteinization by alkali treatment. In recent years, several enzymatic deproteinization processes have been introduced as an alternative treatment for alkali digestion to reduce quality loss of chitin due to depolymerization of its chain and also to produce protein hydrolysate with a well-balanced amino acid composition Synowiecki, (2000) [9]. A new method for extraction of chitin by using proteolytic microorganisms or fungi or purified enzymes has been introduced which is a low cost technology and eco-friendly. It is an advanced application for extraction of bio-polymer from crustacean shell.

## 2. Properties of chitin and chitosan

Most of the naturally occurring polysaccharides viz. Cellulose, dextrin, pectin, alginicacid, agar, agarose and carrageenan are naturally acidic in nature, whereas chitin and chitosan are examples of highly basic polysaccharides. They have several properties such as solubility in various media, solution, viscosity, metal chelating, and structural characteristics. The chemical properties of chitosan are due to the presence of linear polyamine and reactive amino groups which can chelate many transitional metal ions. Chitin also has biological properties like biocompatibility, accelerate the formation of osteoblast which is responsible for bone formation, haemostatic, fungistatic, spermicidal, antitumor anticholesteremic, and accelerate bone formation.

## 3. Proximate composition of shellfish waste:

| Parameter | Prawn waste | Squilla | Crab shell |
|---|---|---|---|
| Moisture | 75-80 | 60-70 | 60-65 |
| Ash | 30-35 | 33-37 | 45-50 |
| Protein | 35-40 | 40-45 | 30-35 |
| Chitin | 15-20 | 12-16 | 13-15 |
| Fat | 3-5 | 2-3 | 1-1.5 |

Gopakumar (2000) [2]

## 4. Resource of chitin

Chitin is the main component of the invertebrates belongs to protostomia. The dry organic matter of their cuticle can contain up the 80% chitin. Chitin and chitiosan prepared from crab and shrimp shells are now available as commercial products throughout the world Muzzarelli, (1977) [5]. Typically the total wastes generated by shell fish processing varies between 65-85% of the total landing and also depends on the species processed by shellfish processing industry. The mollusks like clam and oyster shells which contain significant quantity of chitin are the main marine source of chitin. The total annual potential availability of chitin from the clam oyster shells might be 22,000 tons. The extracellular fibers of these organisms constitute the only recognized source of pure, unassociated chitin found in nature Letourneau *et al*., (1976) [4].

## 5. Extraction methods

Chitin is mainly extracted from shrimp and crab shells. The major shell components are chitin, proteins, lipids, pigments and trace elements. Chitin, calcium carbonate and proteins account about 90% of the dry shell weight Ferrer *et al*., (1996) [1]. In order to obtain purified chitin, it must be separated from the proteins, minerals and other components. This separation is achieved mainly by three steps:

1) Demineralization to eliminate the calcium carbonate,

2) Deproteinization,

3) Elimination of lipids and decolourisation.

The chitin extraction steps can be achieved mainly by two methods, either chemically or biologically. For the biological extraction two methods are available that is fermentation and enzyme assisted extraction. The use of purified enzymes is more expensive, but can result in high yield and selectivity. The major concern is the quality of the final product, which is a function of the molecular mass (average and polydispersity) and the degree of acetylation (DA).

### 5.1 Chemical method

In chemical method of chitin extraction, the shell is deproteinized by alkali treatment with NaOH and demineralization by inorganic acid (HCl) treatment. Organic solvent treatment is done for the elimination of lipids and colour. This method predominantly leads to the formation of monomers to trimers, but not tetramers to heptanes, which are more desirable from the biological activity point of view Shahidi *et al*., (1999) [7]. Another problem is the possible denaturation of the chitin. Moreover, the use of organic solvents which can be toxic, corrosive, degradative or mutagenic makes the resulting chitin unsuitable for medicinal use and also the cost of processing food waste will be more.

### 5.2 Biological methods of chitin extraction

This is an advanced and a new technique for chitin extraction. It can be done using proteolytic microorganisms or fungi or purified enzymes. It results in the production of oligomers with an optimum degree of polymerization for different applications and does not denature the chitin. Enzymatic extraction can be highly specific and yield chitin with higher molecular weights. Besides environmental advantages as compared to the chemical method, the use of enzymes also eliminates the hazards associated with the reactive reagents. Even the extracting cost of chitin by biological method can be optimized by reducing the cost of carbon sources (glucose, dextrose, lactose, sucrose). There are following steps involved in chitin extraction:

#### 5.2.1 Preparation of inoculums

Take the lactobacillus cell and transfer in 5 ml MRS broth and incubate at 30 ºC for 24 hrs. Then take 2 ml of starter culture and transfer in 100 ml sterile MRS broth and incubate at 30 °C for a further 24 hrs. Now, these inoculums are ready for fermentation.

#### 5.2.2 Fermentation

Take the shell fish waste and grind them properly. Then add 10% of any carbon sources followed by 10% culture inoculums. Then incubate it for 180hrs followed by filtering and the obtained solid cake is dried in hot air oven.

#### 5.2.3 Protein separation

Separation of protein is carried out by proteases secreted into the fermentation medium. In addition deproteinization can be made by adding exo-proteases or by proteolytic bacteria.

#### 5.2.4 Calcium carbonate separation

It is carried out by lactic acid producing bacteria through the conversion of an added carbon source.

**5.2.5 Bacteria used for deproteinization and demineralization:**

| Organic acid producing bacteria | Protease producing bacteria |
|---|---|
| *Lactobacillus plantarum* | *Pseudomonas aeruginosa* |
| *L. salivarius* | *P. acidilactici* |
| *L. paracasei* | *Bacillus subtilis* |
| *Serratia marcescens* | *B. firmus* |

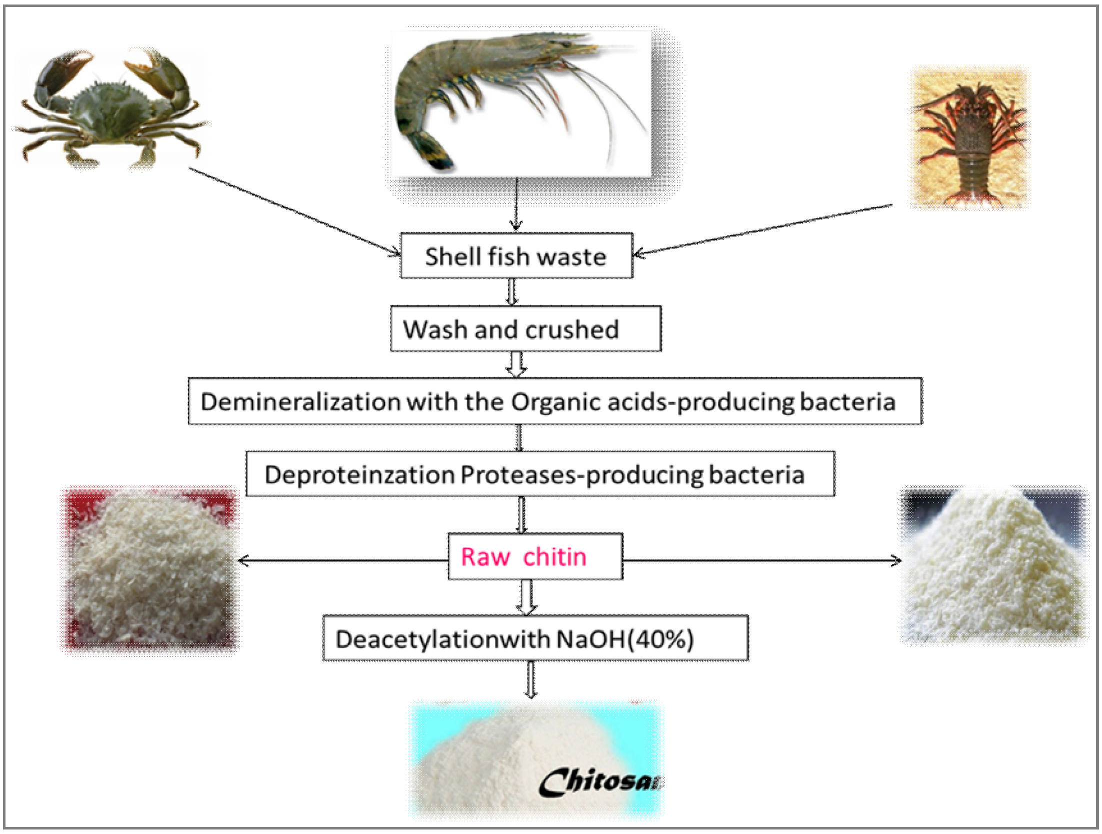


**Fig 1:** A Flow diagram showing biological method of chitin production

## 6. Application of chitin and chitosan:

Chitin is mainly used as the main raw material to produce chitin derived products such as chitosans, oligosaccharides, and glucosamine hydrochloride. The worldwide industrial production of these derivatives in the year 2000 was estimated to be above 10,000 tons Kurita. (2006) [3]. Chitosan have the important food related applications which include purification of drinking water, recovering protein from fish wash water and meat processing plants, using in animal feeds, clarifying wine etc. Chitosan is used in the thin layer chromatography for the separation of nucleic acid Takeda, (1961) [10]. Chitosan fulfill all the requirements of an ideal material applicable in paper industry Muzzarelli, (1977) [5]. It was also reported that cartons made of duplex boards coated with chitosan were excellent in packing of frozen fish in place of wax coated one. Animal nutrition has been shown that the utilization of whey has been improved by the addition of small amount chitin in the diet as it change the intestinal flora of the animal Spreen *et al.,*(1980) [8]. It has diverse applications in agricultural field. It has been found that chitosan in solution acts as the bacteriostatic agent and haemostatic agents Radhakrishnan *et al*., (1991) [6]. Chitosan derivatives serve as emulsifiers, moisturizers, antistatic agents and emollients, nail polishes and tooth pastes.

## 7. Conclusion

Chitin is one of the most abundant biopolymers in nature and is a major component in the supporting tissues of organisms such as crustaceans, fungi, and insects. It has wide application in various fields. Biological and chemical methods are mainly employed for the production of chitin and chitosan. The quality of chitosan produce by chemical methods is not homogeneous as compared to biological method. In biological method, chitin is produced by using lactic acid bacteria and produces a good quality product. It also leads to a protein rich liquid fraction which can be used as human and animal feed. But the use of this method is still limited to laboratory scale because demineralization and deproteinization have not yet reached up to the desired yields as compared to chemical method. So, the optimization and standardization of the extraction process is necessary to produce high yield with minimum cost

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