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# The preparation and characterization of chitin and chitosan under large-scale submerged fermentation level using shrimp by-products as substrate

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ABSTRACT

The crustacean shells of crabs and shrimps produces quantities of by-products, leading to seriously environmental pollution and human health problems during industrial processing, yet they turned into high-value useful products, such as chitin and chitosan. To prepare them under large-scale submerged fermentation level, shrimp shell powders (SSPs) was fermented by successive three-step fermentation of *Serratia marcescens* B742, *Lactobacillus plantarum* ATCC 8014 and *Rhizopus japonicus* M193 to extract chitin and chitosan based on previously optimal conditions. Moreover, the key parameters was investigated to monitor the changes of resulted products during fermentation process. The results showed that the yield of prepared chitin and chitosan reached 21.35 and 13.11% with the recovery rate of 74.67 and 63.42%, respectively. The degree of deacetylation (DDA) and molecular mass (MM) of produced chitosan were 81.23% and 512.06 kDa, respectively. The obtained chitin and chitosan was characterized using Fourier transform infrared spectrometer (FT-IR) and X-ray diffraction (XRD) analysis. The established microbial fermentation method can be applied for the industrial large-scale production of chitin and chitosan, while the use of chemical reagents was significantly reduced.



## 1. Introduction

Chitin and chitosan has widely been prepared from the exoskeletons of crustaxean shells such as shrimps and crabs shell, but their wastes including shells, heads and tails account for approximately 50% of raw materials produced. The abundant wastes often is processed to make cheap feed and most of useful substances are discarded [1]. These unused wastes could be converted into highly value-added products including protein, astaxanthin, polysaccharides and chitin [2,3]. Among them, chitin and its deacetylated form, chitosan, have been applied in multiple fields, including food, pharmaceutical, medicine, water treatment and agriculture due to their many unique properties such as biodegradability, biocompatibility, nontoxiocity and etc [4–6].

The three steps including deproteinization (DP), demineralization (DM) and removal of lipids and pigments were employed to prepare chitin from shrimp shell powders (SSPs). The SSPs of headless *Penaeus vannamei* used in this work contains almost 45% of protein, so DP is the main and critical step. Now, strong alkali and acids are employed for DP and DM of SSPs, respectively, which has brought out many drawbacks including hydrolysis of polymer, final inconsistent physiological properties of polymer, environmental pollution problems, and etc [7,8]. Compared to chemical method, alternative and eco-friendly treatment methods are the utilization of microbial fermentation and proteolytic enzymes [9]. The enzymatic methods include the use of trypsase, papain or pepsase, but the high cost of enzymes is the major pitfall of this method [10]. Hence, more eco-friendly microbial fermentation method has recently attracted great interests, the types of bacterial strains to produce protease for the DP of SSPs include *Bacillus subtilis* [11], *Pseudomonas aeruginosa* [12] and *Serratia marcescens* [13,14]. The *S. marcescens* strains has been employed for DP at shaking flask levels [15]. It is well-known that shrimp and crabs shell contains higher than 90% Ca among mineral substances, *Lactobacillus plantarum* is efficiently used to remove Ca from SSPs after DP based on previous report [15]. Thus, these two bacterial strains can be employed to prepare chitin in our previous study [15].

Now, the strong alkaline deacetylation of chitin was used for the conversion of chitin into chitosan, which has also resulted in

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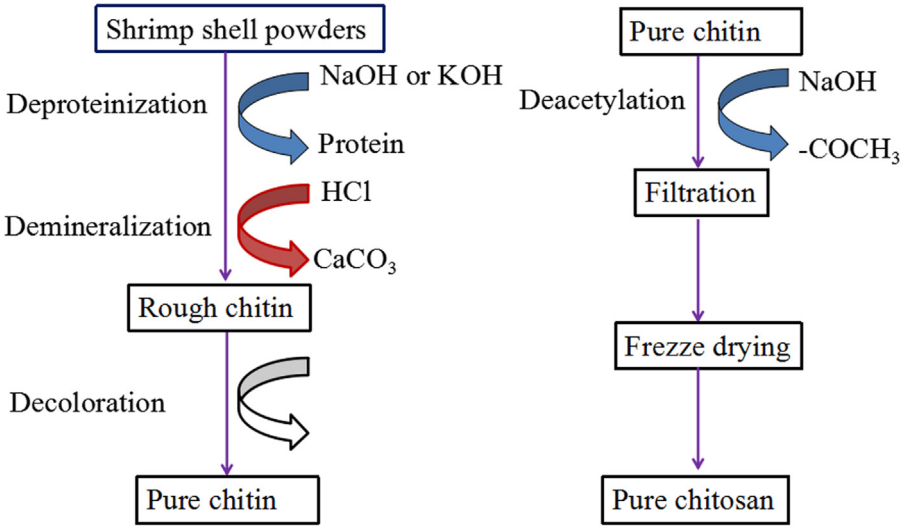



**Scheme 1.** The diagram of preparing chitin and chitosan using chemical method.

numerous problems, including seriously environmental pollution, tedious to control, and inconsistent range of the resulted chitosan products [16]. Thus, selecting an effective and efficient strains is critical to catalyze the deacetylation of *N*-acetyl-D-glucosamine residues. *Rhizopus japonicus* M193, possessing a high intracellular and extracellular deacetylase activity, has been investigated to prepare the chitosan in our previous study [17]. Generally, fermentation processes at shaking flask levels cannot perform as well in large-scale reactors due to the difficulty to transfer optimal operation parameters from small to large volumes [18]. To investigate the industrial large-scale production of chitin and chitosan, the 10 Liters (L) of fermentation tank was used to prepare them using successive three-step submerged fermentation procedures in this work.

The objectives of this work are 1) to verify the previous fermentation conditions at shaking flask levels, 2) to prepare the chitin and chitosan under large-scale submerged fermentation level, 3) to calculate the yield and recovery of chitin and chitosan, respectively, 4) to compare the structural properties of chitin and chitosan obtained by successive three-step submerged fermentation and chemical treatment method using FT-IR and XRD analysis. No study has reported the preparation of chitin and chitosan using large-scale submerged fermentation, while use of chemical reagents was significantly reduced.

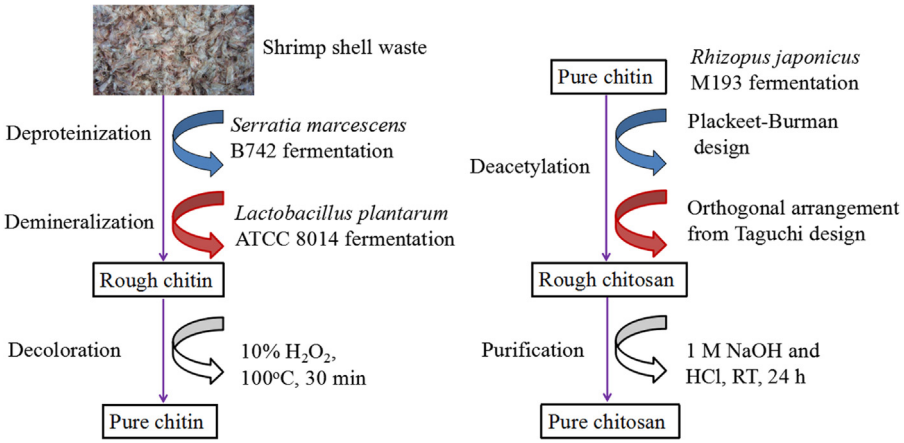
## 2. Materials and methods

### 2.1. Materials and reagents

Shrimp shells of headless *Penaeus vannamei* were purchased from Nantong Xingcheng Biological Products Factory (Nantong, China) and stored under dried conditions till further usage. The dried shrimp shell was pulverized with Waring blender (Shanghai Shibang Machinery Co., Ltd China) and passed through a 0.75 mm sieve to prepare SSPs. *L. plantarum* ATCC 8014 was obtained from American Type Culture Collection (ATCC, USA), and *S. marcescens* B742 and *R. japonicus* M193 from Shanghai Institute of Industrial Microbiology (Shanghai, China). The Mann-Rogosa Sharpe (MRS) broth and Luria Bertani (LB) broth were purchased from Shanghai Yayan Biotechnology Co., Ltd (Shanghai, China). $La_2O_3$ (99.9% purity), acetic acid, nitric acid, NaOH, HCl and other chemical reagents were all obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

### 2.2. Chemical extraction of chitin and chitosan

The diagram of preparing chitin and chitosan using chemical method is shown in Scheme 1. SSPs was treated with 40% NaOH (1:40) at 100 °C for 1 h to remove proteins, and then soaked in




**Scheme 2.** Experimental diagram of preparing chitin and chitosan using microbial fermentation method.