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Preparation of chitosan from prawn shells

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Abstract
In the present study an attempt was made to synthesize chitosan from prawn shells. The prawn shells (*Penaeus indicus*) were collected from the local fish market of Bhandup, Mumbai. Chitosan was prepared from prawn shells waste by chemical method i.e. demineralization, deproteinization and deacetylation by using HCl and NaOH respectively. The procured chitosan yield was 12.25 % and the degree of deacetylation was 74.25%. Chitosan was found to be 78 %soluble in 1% acetic acid solution.

Keywords: Chitosan, Solubility, residue

Introduction

The shellfish business creates a lot of shell waste which causes environmental pollution. This waste can be utilized as an economic source of chitin and its derivative chitosan (Rashmi *et al*., 2016) [9]. Chitosan is polysaccharide and is characterized by its biocompatibility, biodegradability and non-toxicity (De Queiroz *et al*., 2017) [3]. Chitosan has application in various fields such as in agriculture, pharmaceuticals, cosmetics, biomedicals, paper industry, food and textile and in water purification (Muzzarelli, 1985 and Al-Manhel *et al*., 2018) [8, 1]. The objective of the present study was to extract chitosan from prawn shell and determine its degree of deacetylation.

Materials and Methods

- **Sample collection & Preparation:** The shells were obtained from local market of Bhandup, Mumbai. The samples were washed with tap water and sun dried for 2 days followed by oven drying for 3 hrs at 60^0C. After drying, the shells were grounded into powder and passed through a sieve of 250 µm.

- **Demineralization:** Prawn shell powder was treated with 5 % HCL solution for 24 hrs at ambient temperature as mentioned by Arafat *et al*., 2015 [2]. After 24 hrs the residue was washed and soaked in water to remove acid until the pH was neutral. The residue obtained was then oven dried.

- **Deproteinization:** Demineralized shell was treated with 5% NaOH solution for 48 hrs at ambient temperature as described by Arafat *et al*., 2015 [2]. After processing, the residue was washed and soaked in water until neutral pH. Then the residue was dried. The product obtained is called as chitin.

- **Deacetylation:** In this step, removal of acetyl group from chitin was experimented by using 60% NaOH solution at 65^0C temperature for 3 hours. The residue was washed with water until neutral pH. The resulting Chitosan was then dried.

- **Characterization of chitosan**

Determination of yield of Chitosan

The yield of the chitosan was obtained by following formula (Mohanasrinivasan *et al*. 2013).

$$\textbf{Yield (\%)} = \frac{(\textit{weight of chitosan obtained})}{(\textit{weight of the raw material})} \textbf{ x 100}$$

Determination of degree of deacetylation:

The degree of deacetylation (DD) was assessed by the acid-base titration method (Hossain and Iqbal 2014) [5]. The DD was calculated by the formula:

$$\text{DD (\%)} = \frac{(C1V1-C2V2)}{(Mx0.0994)} \text{ x } 0.016$$

Where, C1 = concentration of standard HCl aqueous solution (mol•dm-3), C2 = standard NaOH solution (mol•dm-3), V1= volume of HCl aqueous solution used to dissolve chitosan (ml), V2= volume of NaOH solution consumed during titration (ml), and M= weight of chitosan (g). The number 0.016 (g) is the equivalent weight of NH2 group in 1 ml of standard 1 mol/l HCl aqueous solution and 0.0994 is the proportion of NH2 group by weight in chitosan.

Solubility: Solubility of chitosan was checked in 1 % acetic acid solution. 0.1g of chitosan was placed into a pre-weighed centrifuge tube with 10 ml of acetic acid and was vigorously shaken with the interval of 5 minutes for 30 min at room temperature and centrifuged at 10,000 rpm for 20 min. The supernatant was removed and undissolved pellets were dried at 60^0C for 24hr. finally, the dried pellet was weighed and percentage solubility was determined by using the following formula. (Fernandez, 2004) [4]

$$\textbf{\% solubility} = \frac{((\textit{Initial weight of tube + chitosan}) - (\textit{Final weight of tube + chitosan}))}{((\textit{Initial weight of tube + chitosan}) - (\textit{Initial weight of tube}))} \textbf{X 100}$$

Table 1: Attributes of Chitosan

Attributes	Chitosan
Yield	12.25%
pH	7.2
Colour	Off white
Degree of deacetylation	74.25%
Solubility	78.06±0.9%

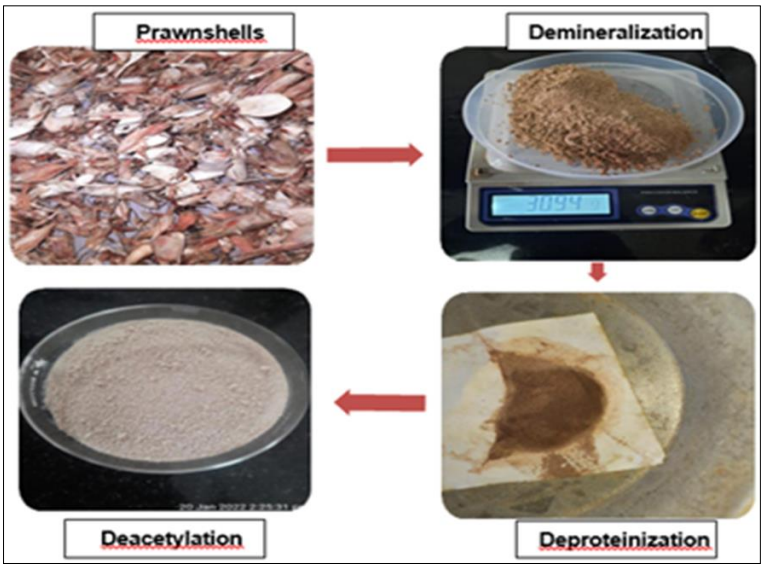

Fig 1: Preparation of Chitosan

Result & Discussion

Chitosan was extracted from the prawn shell waste (*Penaeus indicus*) by deminerlaization, deprotenization and deacetylation (Fig.1). The attributes of chitosan are shown in Table. 1. Chitosan obtained from 200 g of dried prawn shell was 24.50 g and the yield percentage was calculated as 12.25%. The yield obtained was found almost similar with Islam *et al*., 2011 (15.21%), But from 200 g of dried shrimp shell (*Penaeus monodon*) 36.24 g Chitosan was extracted and the yield percentage was 18.12 % (Singh, N., & Chahar, S.2021). Solubility is one of the parameter to determine the quality of procured chitosan. In the present study solubility of chitosan was found to be 78.06±0.9% in acetic acid. Degree of deacetylation is the proportion between glucosamine and N-acetyl glucosamine, which is predominantly influenced by NaOH concentration and temperature. The process of converting chitin into chitosan was determined by the formation of glucosamine. (Hossain and Iqbal 2014) [5]. In the present experiment, Degree of Deacetylation of the chitosan using acid-base titration method was 74.25%. As per previous studies, it is unusual to achieve the 100% Degree of deacetylation (Vijaykumar.R. *et al*., 2019) [11].

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