Optimization and Application of Chitosan as Natural Product from Locally Available Shrimp Shell in Bangladesh

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Authors’ contributions

This work has been carried out in collaboration among all authors. Authors IAJ, HH, NS, MI and MSI have made contributions to conception and design, performed the statistical analysis, have written the protocol and the first draft of the manuscript. Authors NS, KSA, MSI, SMMH and MSB have helped with the analysis and interpretation of data. Authors NS and HH have managed the literature searches. All authors have read and given final approval of the version to be published.

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ABSTRACT

The experiment was designed to investigate the optimization condition of chitosan and its application on fruit as natural preservative prepared from locally available shrimp shell in Bangladesh. Materials and methods. The preparation of chitosan was done by gravimetric method; characterization of chitosan was performed by solubility, pH, viscosity, FT-IR, SEM, XRD and DDA test. The prepared chitosan (0.5-0.25%) was completely soluble in 1% and 2% acetic acid solution. Based on these spectra, the major absorption band was observed at 3485.14 cm\textsuperscript{-1} which indicated

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1. INTRODUCTION

A common physical and chemical change of food is spoiling. Preservatives are mainly added to avoid this problem and enhance the self life. Food preservatives like as formaldehyde are often used which is very dangerous to health that's why currently it is very concerned about safety. Therefore, it is necessary to study a natural preservative to replace harmful chemical preservatives. This study aims to increase the shelf life of fruits, vegetables, meats and fish etc. Natural preservatives commonly used include plants extracts, chitosan and chitooligosaccharide, bacteriocins, bioactive peptides, and essential oils, among others [1]. Chitosan is a modified natural carbohydrate polymer derived from chitin which has been found in a wide range of natural sources such as crustaceans, fungi, insects and some algae [2]. Generally, the shell of selected crustaceans consists of 30-40% protein, 30-50% calcium carbonate and calcium phosphate and 20-30% chitin [3]. After cellulose, chitin is the second most abundant natural biopolymer found in nature [4]. Shrimp is one of the important fisheries products worldwide including Bangladesh. This product is mostly exported in the frozen condition that has undergone a process of separation of the head and skin [5]. The crude shrimp head and skin materials have only a low economic value and are treated as bio-waste or sold to animal feed manufacturers [6]. Shrimp industries generate large amounts of shrimp bio-waste during processing, approximately 45-55% of the weight of raw shrimp [7]. However, this bio-waste can be used to produce value-added products such as chitosan. Due to its excellent properties, this has made chitosan attained increasing commercial interest worldwide such as agriculture, biochemistry, pharmaceuticals, biotechnology, biomedical, cosmetics, food, and paper industry [7]. Hence, in this experiment, we attempted to investigate the optimization condition and application of chitosan on fruit as natural preservative prepared from locally available shrimp shell in Bangladesh.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The raw shrimp shell waste was collected from Sathkhira, Khulna at December, 2018 from one of the factories which produced large quantities of shrimp shell waste. The sample was collected in fresh conditions where it was collected during the peeled of the process of the shrimp shell. The samples were washed with tap water to remove any insoluble material on the shell then dried under the sun for 10 hrs and was stored in a closed container prior to use. The shrimp shell was grinded with the help of a grinding machine. The powder was treated with 12 balls in “Ball Mill” for six hours. Fine powder (ASTM 200 mesh size) was separated by using a sieve shaker machine.

2.2 Chitin Extraction

This process contains two steps

(a) **Demineralization**: 100 g shrimp shell powder was treated for 24 hrs in 25°C with 7% HCl.

(b) **Deprotination**: After neutralizing with DI water it was treated with 10% NaOH at 60°C for 24 hrs. This process was repeated for two times. Then neutralized the powder with DI water, finally washed with rectified spirit and EtOH and dried in an oven at 50°C.
(c) Yield of Chitin was 22.48% from dried shrimp shell.

2.3 Chitosan Preparation

(a) 20 g chitin was treated with 50% NaOH for 8 hrs at 60°C. Then washed with DI water at 60°C for three times and dried at 50°C for 7-8 hrs. Yield of Chitosan was 11.02% from dried shrimp shell.

(b) Water Soluble Chitosan Preparation: 5 g of crude chitosan was treated with 20 mL of 2% acetic acid solution and stirred with a magnetic stirrer at 50°C. After the reaction, 10% NaOH was used for neutralization and two fold volumes of EtOH was added to the filtrate for crystallization of water-soluble chitosan.

2.4 Characterization of Chitosan

The characterization of the extracted chitosan was carried out in term of the solubility, pH, Viscosity, FT-IR, SEM, XRD and degree of deacetylation (DDA).

2.4.1 Solubility in acid solution

1.0 g of chitosan was dissolved in 100 mL of 1% and 2% acetic acid solution and stirred by a magnetic stirrer until a homogeneous solution was obtained. The chitosan in acidic solution was then filtered using a vacuum pump. The procedure was repeated three times. The percentage of the solubility was calculated according to relations (1) and (2) [8]:

\[ \text{Insoluble (g)} = \frac{\text{final weight of filter paper (g) - initial weight of filter paper (g)}}{\text{initial weight of filter paper (g)}} \]  \hspace{1cm} (1)

\[ \text{Solubility (%) = 100 - % insoluble} \] \hspace{1cm} (2)

2.4.2 pH

pH of 0.25% chitosan solution was measured in 1% & 2% acetic acid at 27°C by using digital pH meter (HACH, USA).

2.4.3 Viscosity

Viscosity of 0.25% chitosan solution was measured in 1% acetic acid at 27°C using Oswald viscometer.

2.4.4 FT-IR

FT-IR spectra of chitosan powder were recorded using Fourier Transform Infrared (FT-IR) spectrophotometer (IR Tracer-100, Shimadzu, Japan) in the range of 480 to 4800 cm\(^{-1}\).

The sample of chitosan was characterized by SEM using a Scanning Electron Microscope type Hitachi S-3400N and by X-ray diffraction using XRD type EMMA, GBC, Australia.

2.4.5 DDA

The DDA of chitosan sample was determined according to the method used by [9]. The A1320 was the peak area of the band 1320 cm\(^{-1}\), the A1420 was the peak area of 1420 cm\(^{-1}\) which representing the peak for amide group and amine group, respectively. The DDA calculation was carried out according to relations (3) and (4):

\[ \% \text{DA} = \frac{1}{0.382} \left( \frac{\text{A1320}}{\text{A1420}} \right) \] \hspace{1cm} (3)

\[ \% \text{DDA} = 100 - % \text{DA} \] \hspace{1cm} (4)

Where:

\[ \text{DDA} = \text{degree of deacetylation} \% \]

\[ \text{DA} = \text{degree of acetylation} \% \]

2.4.6 Application of Chitosan

Chitosan at a concentration of 0.25% solution in 1% acetic acid at 25-27°C was applied on red and green apple. After that, we made an observation regarding the changing of physical properties in comparison with control samples (untreated fruits) for 21 days.

3. RESULTS AND DISCUSSION

The physical appearance of the Chitosan was white powder. It was also odorless and in the form of crystalline powder. The characteristic of the chitosan produced from this study was similar to the chitosan standard and this indicates a good quality of chitosan was produced [10,11].

3.1 Solubility in Acid Solution

The prepared chitosan was completely soluble in 1% and 2% acetic acid solution. Among several characteristics, the solubility of chitosan is one of the most essential parameters for quality of chitosan, because a higher solubility implies high quality of chitosan. Chitosan is a compound which is very trouble to dissolve in water, alkaline solutions or most common organic solvents but it is soluble to some level in dilute acidic solutions [12].
3.2 pH
(a) 0.25% chitosan solution in 1% acetic acid at 27°C was 3.11
(b) 0.25% chitosan solution in 2% acetic acid at 27°C) was 2.78.

3.3 The Viscosity
The Viscosity of 0.25% chitosan solution measured in 1% acetic acid at 27°C was 137 cps.

3.4 FT-IR
FT-IR spectra of chitosan powder (shown in Fig. 1) reveal the following:

a) The major absorption band is observed at 3485.14 cm\(^{-1}\) due the stretching vibration of OH and NH groups.
b) The band from 1045.42 cm\(^{-1}\) represents the free amino group (-NH\(_2\)) at C2 position of glucosamine indicates that this group is present in chitosan. FT-IR spectra obtained for the prepared chitosan showed the absorption bands for the free amino group (the peaks from 1040 and 1254 cm\(^{-1}\)) and also some bands which is also reported by [11] where exist some strong peaks at 3485.14, 1566 and 1345.02 cm\(^{-1}\). Some of the bands we were also observed by Puvvada et al. [8], 2985 cm\(^{-1}\) (symmetric CH\(_3\) and asymmetric CH\(_2\) stretching) and 1428 cm\(^{-1}\) (-CN secondary amide).

3.5 Scanning Electron Microscope
The SEM analysis presented in Figs. 2-4, shown non-homogenous and non-smooth surface.

3.6 XRD
The XRD analysis (XRD pattern presented in Fig. 5) illustrates one sharp and strong characteristic broad diffraction peak at 2θ = 20°.

3.7 DDA
Degree of deacetylation (DDA) of chitosan is one the most essential parameter because DDA could be used to determine the quality of chitosan where this parameter reflects the physical, chemical, and biological properties such as covalent linking, adsorption and encapsulation [12]. The DDA value was determined by FTIR as ratio of the bands at 1320 and 1420 cm\(^{-1}\). Fig. 1 shows the FT-IR spectra for chitosan which produced in this study. Degree of deacetylation (DDA) of the prepared chitosan
was 84.0%. Nouri et al. [10] found DDA value ranged from 71.02-82.20% for deacetylation using traditional method, while 79.01-88.60% for using microwave method. Besides, Alishahi et al. [13] also performed deacetylation by using the microwave and obtained chitosan with DDA value ranged from 87.5 - 93%.

3.8 Application of Chitosan

At a concentration of 0.25% solution in 1% acetic acid at 25-27°C was applied on red and green apple is representing in the Figs. 6-13.
Fig. 4. Figure showing scanning electron microscope image at 5.00 K X

Fig. 5. X-Ray diffraction results of Chitosan

Fig. 6. First day

Fig. 7. 5th day (Control started to form wrinkle and test sample had no change)
4. CONCLUSION

Based on the results obtained from this study, this method of extracting chitosan from shrimp shell available in Bangladesh can produce highly soluble chitosan with DDA value 84.0% (this sentence must be rewritten). The characterization of prepared chitosan from different methods such as FT-IR, SEM, XRD reveal a high quality of chitosan produced from this method. Results obtained by application of chitosan solution on red and green apple reflects that the chitosan solution (0.25%) could be natural preservative for fruits (apple) preservation. However, a more extensive study is necessary to determine shelf-life of chitosan solution as well as antibacterial and antioxidant agent.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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