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Extraction and physicochemical characterization of chitin and chitosan from *Zophobas morio* larvae in varying sodium hydroxide concentration

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ABSTRACT

Large amount of sodium hydroxide (NaOH) is consumed to remove the protein content in chitin biomass during deproteinization. However, excessive NaOH concentration used might lead to the reduction of cost effectiveness during chitin extraction. Hence, the present study aimed to extract and evaluate the physicochemical properties of chitin and chitosan isolated from superworm (*Zophobas morio*) larvae using 0.5 M–2.0 M of NaOH. The extracted chitin and chitosan were subjected to Fourier Transform Infrared Spectroscopy (FT-IR), elemental analysis, Scanning Electron Microscope (SEM), Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC) and X-ray Diffraction (XRD). The 0.5 M NaOH treatment resulted in the highest yield of chitin (5.43%), but produced the lowest yield (65.84%) of chitosan. The extracted chitin samples had relatively high degree of acetylation (DA) (82.39%-101.39%). Both chitin and chitosan showed smooth surface with tiny pores. The extracted chitin samples were confirmed as α-chitin based on the FT-IR and TGA. The chitin samples were amorphous with low degree of crystallinity. From TGA, the Chitosan 3 extracted was partially deacetylated. Both DPPH radical scavenging and ferric-chelating assay showed positive correlation with DD of chitosan isolates. However, the chitosan isolates were not fully dissolved, resulting in lower radical scavenging and ferric-chelating ability compared to commercial chitosan.

1. Introduction

Chitin is a linear biopolymer with monomer β-(1 → 4)-N-acetyl-D-glucosamine unit. It is biocompatible, biodegradable, non-toxic and highly insoluble in most of the polar and non-polar solvent [1]. Due to these preferable properties, chitin has been extensively used in the food, cosmetics, composite material, wastewater treatment, and biomedical industry [2–5]. The chitin can be further treated with concentrated NaOH in extreme conditions to produce chitosan via deacetylation [6–8]. Chitosan is a linear co-polymer obtained by partial deacetylation of chitin derivative which consists of N-acetyl glucosamine units and D-glucosamine unit blocks. It is less amphiphilic and soluble in mild acidic solvent [9,10]. Chitosan can be used as the filler in packaging material development due to its antimicrobial properties to broad spectrum of microorganisms [11]. The chitosan with high degree of deacetylation (DD) was crucial as biomaterial in tissue engineering due to its polycationic nature that able to interact with negative-charged cell membrane [12]. Besides, the chitosan is the raw material to synthesis the *O*-carboxymethyl chitosan Schiff bases supported metal ion complex heterogeneous catalyst, which exhibits excellent catalytic activity and is reusable [13–15].

Recently, there is an interest in discovery of potential chitin and chitosan source from insects. Chitin fibre can be found abundantly in the cuticle of insects and is covered with protein matrix that contains catechol and minerals [16]. The current source for massive production of chitin is from marine crustaceans, such as

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shrimp, lobster, and crab [7,8,17–20]. Other than insect and marine source, chitin can also be found in fungus [19,21,22]. Nevertheless, the extraction of chitin from all the biosources mentioned utilizes the same facile chemical treatment procedure, namely demineralization, deproteinization and decolourization. In this study, superworm (*Zophobas morio*) was studied for the extraction of the chitin and chitosan.

Superworm is found abundantly in tropical countries and is widely used as aquaculture feed. A normal superworm has approximately 57% of water content. The dried superworm contains low amount of ash content (about 3.5%), closed to 6.9% of total nitrogen and relatively high amount of crude fat (around 40.8%) [23]. Interestingly, superworm was found to be able to produce antimicrobial peptides (AMPs) which showed relatively good antimicrobial activity against bacteria [24]. Considering its low land and space utilization per culture breeding, low requirement of culture maintenance, high adaptation to tropical climates, and large number of offspring per reproduction, *Z. morio* could have the potential as an alternative source of chitin and chitosan production [25,26].

The chitin biosources have the significant amount of protein content as mentioned. Therefore, deproteinization using alkaline treatment should be carried out in order to ensure the high purity of the chitin. The deproteinization parameters includes the concentration of NaOH, temperature and time of reaction [6,27–32]. From the study of the chitin extraction from silkworm chrysalides, it was concluded that the yield of the chitin decreased as the time of basic reaction or the temperature of reaction increased [6]. However, the relationship of the yield of chitin and its corresponding physicochemical properties was not yet revealed. Nonetheless, at least to the authors' knowledge, there was no research done in the investigation of the NaOH concentration during deproteinization to the physicochemical properties of chitin from insect source. Hence, the inspiration to conduct the present research was obtained from the finding of different researchers that utilized varying deproteinization conditions to purify chitin.

In the present study, the chitin and chitosan samples were extracted from *Z. morio* larvae using different NaOH concentrations during deproteinization. The physicochemical properties of the chitin and chitosan samples were evaluated and the effects of the different basicity treatment were studied. The findings of this present study could provide a new insight of the physicochemical changes of chitin and chitosan at different extraction conditions and hence, promote the reduction of NaOH chemical concentration used during chitin extraction. Besides, it also served as an exploration to the potential of *Z. morio* as an alternative raw material for chitin and chitosan production, in par with the increasing demand worldwide.

2. Materials and methods

2.1. Materials

Processed (dried) *Z. morio* larvae was purchased from Macau Pet Trading Sdn. Bhd., Selangor, Malaysia. The chemicals used for chitin extraction were hydrochloric acid (HCl), sodium hydroxide (NaOH), and acetone. Acetic acid was used to dissolve the chitosan. All reagents used were of analytical grade. Commercial chitin (CAS: 1398-61-4) and chitosan (CAS: 9012-76-4) were purchased from Sigma Aldrich.

2.2. Chitin extraction from *Z. morio*

The processed larvae was washed with running tap water to remove impurities such as dirt sand and organic residues. Then, they were dried in convection oven at 70 °C overnight. The dried larvae was then blended in low speed and kept in air-tight plastic seal.

The extraction of chitin was modified in reference to Kaya, Akyuz, et al. (2016). The chitin extraction process consisted of stages as summarized below:

i Demineralization: 4 g of blended worms were mixed with 1.0 M of HCl solution (1:20 ratio of solid to solution) in 35 °C water bath at 100 rpm rotation for 30 min to remove mineral content and catechols. The samples were filtered out and washed with distilled water until neutral pH was achieved in the washing solution.

ii Deproteinization: The demineralized samples were mixed with three different concentration of NaOH solution (0.5 M, 1.0 M and 2.0 M) (1:20 ratio of solid to solution), which were referred to as Chitin 1, 2 and 3, respectively. Deproteinization was carried out in 80 °C water bath at 100 rpm rotation to promote the reaction for 20 h. The samples were filtered out and washed extensively with distilled water until neutral pH.

iii Decolorization: The partially pure chitin samples were soaked in glacial acetone (1:10 ratio of solid to solvent) for 30 min to remove organic pigment. The chitin was filtered out from the excess acetone and dried further in fume hood for 30 min for volatile acetone removal. Then, the chitin isolates were dried completely in convection oven at 70 °C overnight.

2.3. Chitosan preparation

The chitin isolates were deacetylated with 50 wt% NaOH in 90 °C water bath for 30 h and 100 rpm rotation. The samples were filtered and washed with distilled water until neutral pH. Chitosan samples were then dried in convection oven at 70 °C overnight. The Chitosan 1, 2 and 3 were the products of deacetylation from Chitin 1, 2 and 3, respectively.

2.4. Characterization tests

2.4.1. Elemental analysis

Elemental analysis was performed using (LECO CHNS-932, USA). The degree of acetylation (DA) of the chitin and degree of deacetylation (DD) of chitosan was determined using percentage composition of carbon (C) and nitrogen (N) as shown in Eq. (1) and Eq. (2), respectively [33].

$$DA\,(\%) = \left[\left(\mathrm{C/N} - 5.14\right)/1.72\right] \times 100 \tag{1}$$

$$DD\,(\%) = 100 - \left[\left(6.857 - C/N\right)/1.7143\right] \times 100 \tag{2}$$

2.4.2. FT-IR analysis

Both chitin isolates and chitosan samples were characterized using FT-IR (Thermo Scientific Nicolet iS5, USA) with the attenuated total reflectance (ATR) technique to determine the chemical compositions. The FT-IR spectrum was recorded in the range of wavelength 400 cm^{-1}–4000 cm^{-1} with 4 cm^{-1} spectral resolution. A total of 64 scans were conducted for each sample.

2.4.3. Scanning electron microscope (SEM)

A scanning electron microscope (JSM-IT100 SEM, Japan) was employed to observe the microstructure and morphology of all samples. The samples were sputter-coated with a thin gold layer in liquid nitrogen. The acceleration voltage was fixed at the range of 15–20 kV to ensure high resolution image.

2.4.4. Thermogravimetric analysis (TGA)

The thermal degradation properties of the *Z. morio* chitin and chitosan isolates were evaluated using thermogravimetric analyzer