International Journal of Biological Macromolecules 89 (2016) 118–126

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Short communication

High similarity in physicochemical properties of chitin and chitosan from nymphs and adults of a grasshopper

Sevil Erdogan[a,*], Murat Kaya[b]

[a] Fisheries Programme, Keşan Vocational College, Trakya University, 22800 Keşan, Edirne, Turkey
[b] Department of Biotechnology and Molecular Biology, Faculty of Science and Letters, Aksaray University, 68100 Aksaray, Turkey

ARTICLE INFO

Article history:
Received 17 February 2016
Received in revised form 13 April 2016
Accepted 20 April 2016
Available online 22 April 2016

Keywords:
Grasshopper
Nymph
Adult
Chitin
Chitosan
Characterization

ABSTRACT

This is the first study to explain the differences in the physicochemical properties of chitin and chitosan obtained from the nymphs and adults of *Dociostaurus maroccanus* using the same method. Fourier transform infrared spectroscopy, thermogravimetric analysis and x-ray diffraction analysis results demonstrated that the chitins from both the adults and nymphs were in the α-form. The chitin contents of the adults (14%) and nymphs (12%) were of the same order of magnitude. The crystalline index values of chitins from the adult and nymph grasshoppers were 71% and 74%, respectively. Thermal stabilities of the chitins and chitosans from adult and nymph grasshoppers were close to each other. Both the adult (7.2 kDa) and nymph (5.6 kDa) chitosans had low molar masses. Environmental scanning electron microscopy revealed that the surface morphologies of both chitins consisted of nanofibers and nanopores together, and they were very similar to each other. Consequently, it was determined that the physicochemical properties of the chitins and chitosans from adults and nymphs of *D. maroccanus* were not very different, so it can be hypothesized that the development of the chitin structure in the nymph has almost been completed and the nymph chitin has the same characteristics as the adult.

1. Introduction

Chitin has been isolated from many organisms, including some insect species as well as crab, shrimp and crayfish [1–6]. Previously, in a single study [7], chitin and chitosan were obtained from adults and larvae of potato beetle and compared with each other in terms of their physicochemical properties. However, there has been no study on how physicochemical properties of chitin and chitosan differ in the nymphs and adults of any hemimetabolous insect.

Chitin and its deacetylated form, chitosan, are commonly used in different fields, such as medicine, food technology, pharmacology, cosmetics, waste water treatment, agriculture, textile, bio/nano-technology and biomedical due to their biodegradable, biocompatible and non-toxic nature [8]. The physicochemical properties of chitin and chitosan biopolymers are the most important factors determining their use in various fields [9–11], and the physicochemical properties vary due to the source of the chitin and chitosan [10]. Hence, it is necessary to extract chitin from different sources and determine its physicochemical properties to expand the use of chitin and chitosan in different application areas.

Orthoptera is one of the most common insect groups (more than 20,000 species) in the world [12]. Populations of some species belonging to this order can reach extremely large numbers of individuals and can cause significant economic losses in cultivated areas [13,14]. *Dociostaurus maroccanus*, which is one of these species, is a species that can easily survive under extreme environmental conditions, can overgraze and has a very high reproductive capacity [14]. For these reasons, it is regarded as the most dangerous grasshopper species in the Mediterranean zone [15–17]. We selected this species because it over-reproduces and creates mass outbreaks. When they die, they accumulate in piles and are not used. Therefore, it will be beneficial to transform such an invasive organism into chitin and chitosan, which are biotechnologically important products.

This study aimed (1) to isolate chitin and chitosan from *D. maroccanus* for the first time, (2) to characterize the chitin and chitosan obtained with Fourier transform infrared spectroscopy, thermogravimetric analysis, x-ray diffraction analysis and Environmental scanning electron microscopy and (3) to reveal how the physicochemical properties of the chitin and chitosan differed between the adults and nymphs of *D. maroccanus*.

* Corresponding author.
E-mail addresses: serdogantrakya@gmail.com, sevilerdogan@trakya.edu.tr (S. Erdogan), muratkaya3806@gmail.com (M. Kaya).

http://dx.doi.org/10.1016/j.ijbiomac.2016.04.059
0141-8130/

2. Materials and methods

2.1. Material

D. maroccanus (Thunberg, 1815) is a grasshopper species belonging to the subfamily Oedipodinae of the family Acrididae from the order Orthoptera. It is also known as the Moroccan grasshopper. It is distributed in Europe, North and Northern East Africa, the Canary Islands, Caucasia, Kazakhstan, Cyprus, Central Asia, Sicily and Sardinia [18,19].

The grasshopper samples used for chitin and chitosan production were procured from Fethiye District of Muğla on 15.07.2014. Adult and nymph grasshoppers, which are dead, were collected and brought to the laboratory. Samples were dried at room temperature.

2.2. Chitin extraction

Dried nymph and adult grasshoppers were ground by crashing in a mortar, and 10 g of each sample was used for chitin extraction. Samples were treated with 100 mL of 2 M HCl for removal of the minerals in the structure. The mixture was heated at 55 °C with stirring at 1000 rpm for an hour. After that, it was filtered through filter paper with a pore size of 0.4 μm. Filtration was enabled by washing the sample with distilled water and continued until reaching a neutral pH. In the next step, the sample was refluxed in 50 mL of 2 M NaOH to remove the residual proteins in the sample. Deproteinization was performed at 50 °C with stirring at 1000 rpm for 18 h. Later, the samples were washed with distilled water again until the pH was neutral. Filtered samples were taken into a glass container and treated with 50 mL of a solution including methanol, chloroform and distilled water (in the ratio of 2:1:4). In that step, samples were stirred at 1000 rpm without heating to whiten the sample and to remove the lipids, pigments and other residues in the sample. The mixtures were washed for the last time and filtered. Finally, the chitin was isolated. The obtained materials were dried in an oven at 50 °C for 6 h. The dried chitins were weighted and the dry weight chitin contents were calculated.

2.3. Deacetylation

One gram of the chitins isolated from adult and nymph grasshoppers were transformed into chitosan. For this, the chitins were treated with 60% NaOH. The samples were stirred at 150 °C and 600 rpm for 4 h. Later, the mixture was filtered and washed with distilled water until a neutral pH was reached. Then, the chitosans derived from the chitins of the adult and nymph grasshoppers were dried in a drying oven at 60 °C for 24 h.

2.4. Fourier transform infrared spectroscopy (FTIR)

The infrared spectra of the chitins and chitosans obtained from adults and nymphs of *D. maroccanus* were recorded with a PerkinElmer FTIR spectrometer according to the attenuated total reflection (ATR) technique. Absorbance values were evaluated between 4000 and 625 cm^{-1}.

2.5. Thermogravimetric analysis (TGA)

To determine the thermal degradation behavior of chitins and chitosans from adults and nymphs of *D. maroccanus*, the chitin and chitosan samples were heated from 25 °C to 650 °C at a heating rate of 10 °C under inert conditions using a thermogravimetric analyzer (EXSTAR S11 7400 system).

2.6. X-ray diffraction (XRD)

X-ray diffraction patterns of the chitins and chitosans obtained from adults and nymphs of *D. maroccanus* were determined by a Rigaku D Max 2000 system X-ray diffraction analyzer. Data were obtained at 40 kV, 30 mA and 2θ with a scan angle from 5° to 45°. Crystalline index values (CrI) of chitins from adult and nymph grasshoppers were determined according to the following formula [2]. In this formula, I_{110} corresponds to the maximum intensity at $2\theta \cong 20^\circ$ and I_{am} corresponds to the intensity of amorphous diffraction at $2\theta \cong 13^\circ$.

$$CrI_{110} = \left[\left(\frac{I_{110} - I_{am}}{I_{110}}\right)\right] \times 100.$$

2.7. Environmental scanning electron microscopy (ESEM)

The surface morphologies of the chitins of adult and nymph grasshoppers and the chitosans derived from these chitins were examined with a Quanta 200 FEG ESEM. Samples were coated with gold using a Gatan precision etching coating system (PECS) and microstructures on the surface were displayed at magnifications of 25,000× and 50,000×.

2.8. Viscosimetric determination of molar mass

To determine the molar mass of the chitosans obtained from the adult and nymph grasshoppers, a solvent system including 0.1 M of acetic acid and 0.2 M of NaCl (1:1, v/v) was used. The solvent system was prepared at five different concentrations. Measurements were performed at room temperature using an Ubbelohde Dilution Viscometer. Measurement results were entered into the equation by Mark–Houwink [20] and the molar masses of the chitosans were calculated. The symbol [η] represents the intrinsic viscosity, Mv represents the viscosity–average molar mass of the polymer and k and α represent the Mark–Houwink–Sakurada constants. k = 1.81 × 10 − 3 and α = 0.93.

$$[\eta] = kM_v\alpha$$

2.9. Elemental analysis

Nitrogen, Carbon and Hydrogen contents of the chitins and chitosans from the adults and nymphs of *D. maroccanus* were determined with FLASH-2000CHNS-O Elemental Analyser. Degree of acetylation (DA) of the chitins and degree of deacetylation of the chitosans (DD) were calculated according to the given formula. C/N; represents the ratio of Carbon to Nitrogen.

$$DA = \left[\left(\frac{C/N - 5.14}{1.72}\right)\right] \times 100$$

Ref. [21].

$$DD = \frac{6.89 - (C/N)}{1.72} \times 100$$

Ref. [22].

3. Results and discussion

3.1. Molar mass

Molar masses of chitosan samples obtained from *D. maroccanus* were found to be 7.2 kDa for the adults and 5.6 kDa for the nymphs. The molar mass of the chitosan from the adult was a little higher than that of the nymphs. According to the literature [23], chitosans