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Isolation of chitin from the oriental hornet *Vespa orientalis* (L) and its conversion into chitosan by a chemical method

Magda H. Rady[1], Eman Essa[1], Ma'moun Sh. A.M.[1], Mo`men, S.A.[1], M.S. Salama[1] , Barakat, E.M.S[1], and Wael S. I. Abou-Elmagd[2]
1- Entomology Department , Faculty of Science, Ain Shams University, Cairo, Egypt.
2- Chemistry Department , Faculty of Science ,Ain-Shams Universi ty, Cairo, Egypt.
* Corresponding author E-mail: drmagdaradi@yahoo.com.

ABSTRACT

Chitin extracted from the oriental hornet wasp *Vespa orientalis* (L),Vespinae, Hymenoptera, is a new source of insect chitin and converted into chitosan using chemical methods. In the present study the physical properties of the wasp chitosan was assessed using two approaches, Fourier transform infrared spectroscopy (FTIR) and Nuclear Magnetic Resonance spectroscopy (^{1}H-NMR). The results showed that it gives a higher purity as compared to crustacean chitosan. The degree of deacetylation (DDA) (92-93%) was also higher than that of the crustacean chitosan (83.8-85.8%). Therefore, the oriental hornet wasp could be a novel alternative source of chitosan.

Key words: *Vespa orientalis* – Chitosan -Deacetylation –Characterization.

INTRODUCTION

Chitin and chitosan are the most frequently cited polymers in the scientific research dealing with a wide range of biopharmaceutical and biomedical applications together with food science and technology. It has been strongly designated as a suitable functional material in view of its excellent biocompatibility, biodegradability, safety, and adsorption properties.Chitosan is often appealed to be GRAS (Generally Recognized as Safe) and bioabsorbable (Bellich *et al*., 2016). Chitosan is derived by the deacetylation of chitin (Qi Lifeng *et al*., 2004).The majority of studies of chitin and chitosan refer to the isolation and properties of these substances from the shells of commercially harvested crustaceans, because this is the most readily available material for large-scale manufacturing. However, recent works described the obtaining of chitin and chitosan from the cuticle of insects (Lamarque *et al.*, 2004 ; Nemtsev *et al.*, 2004).

MATERIALS AND METHODS

1. Materials

The local resource used to extract chitin was *Vespa orientalis* collected from the apiary yard of the Apiculture Research Department, Plant Protection Research Institute, Dokki, Egypt. Commercial chitosan (deacetylation degree is 85%, and viscosity is 100 cps) was purchased from Shanghai Bioscience and Technology Co., Ltd., China.

2. Chitin extraction

The chitin was extracted from *Vespa orientalis* following the standard procedure according to Majtán *et al*. (2007) and Wanule *et al*. (2014) with little modification,

3. Chitosan preparation

The extracted chitin from *Vespa orientalis* was treated with 50% NaOH (15 ml/g) at 100 °C for 2 h on a hot plate. The mixture was stirred after some times for homogenous reaction. Samples were then cooled for 30 min at room temperature, after cooling sample were washed continuously with 50 % of NaOH and filtered in order to retain the solid mater. This solid mater is further washed with deionized water. This solid mater was dried in oven at 120 C for 24hrs, from our trials if the produced chitosan is poorly soluble in 1% acetic acid repeat the deacetylation process for additional 2 hours (Majtán,*et al*., 2007 ; Wanule *et al*., 2014) .

4. Characterization

1. Determination of the degree of deacetylation of wasp chitosan.

The degree of deacetylation (DD) of chitosan was measured by titration method according to Abdou *et al*. (2008).

2.Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of chitosans obtained from wasp was recorded with FT-IR (4100Jasco-Japan) spectrophotometer. The spectral region between 4000 and 400 cm^{-1} was scanned. Specimens were prepared as KBr pellets. Dried wasp chitosan powder was mixed thoroughly with KBr and then pressed to form an ultimate thin homogenous disc with a thickness of 0.5 mm. The wasp chitosan concentration in the samples was 2% calculated with respect to KBr.

RESULTS AND DISCUSSION

1. Chitosan production and preliminary identification

The chitosan was extracted from the oriental hornet. The recovery rate of chitosan from the oriental hornet was 12.2%; whereas that from the black tiger shrimp head is 14.6% as reported by Hongpattarakere and Riyaphan (2008), however yield was moderately low as compared to crabs and other crustaceans. This may be due to less amount of chitin in exoskeleton ofthe oriental hornet. By comparing the reported insect species, the oriental hornet gives much higher yield comparing to *Periplaneta americana* which gives 2.0% and lower yield comparing to the blowfly 26.2% (Song *et al*., 2013; Wanule *et al*., 2014) but of all it's the most pure one.

2. Degree of deacetylation of chitosan

The degree of deacetylation (DD) of chitin/chitosan is the most important parameter that influences their various properties including biological, physicochemical and mechanical properties and it depends on the method of isolation and the reaction conditions that should be taken into consideration prior to the use of chitosan as drug delivery system (Khan *et al.*, 2002). It was obvious that the DD of the resulted chitosan isolated from wasp (WCS) exhibits the highest (92-93%), while chitosan isolated from shrimp (SCS) has lower DD (83.8-85.8%).

3.Fourier transform infrared spectroscopy(FTIR)

FTIR spectra of chitosans obtained from wasp and crustacean chitosan were recorded with a Nicolet FT-IR (4100Jasco-Japan). The chemical structure of the chitosans derived from the *Vespa orientalis* was confirmed by FTIR analysis. FTIR Spectrum of chitosan showed characteristic peaks at 3500 , 3300 and (br) 3448 Cm^{-1} and indicated symmetric stretching vibration of OH and amine Absorption peak, at 2918 and 2884Cm^{-1}indicate presence of CH stretch (Brugnerotto *et al*., 2010 ; Anicuta *et al*., 2010 ;Ramya *et al*., 2012).

5.^{1}H-NMR

^{1}H-NMR spectrum showed singlet peaks at 1.8 and at 4.6 ppm corresponding to CH_3 of acetyl group and H-1(Ac) of acetylated chitosan, respectively, beside the characteristic peaks of deacetylated chitosan at 2.9, 3.4-3.9 and 4.7 ppm.

Further support for the structure and ratio of acetylated and deacetylated chitosan were gained from ^{1}H-NMR. The spectra revealed that the percentage of methyl group of acetamido group of acetylated chitosan at δ=1.8 ppm decreases and the degree of deacetylation increases as the time of deacetylation increase.

Conclusions.

To the best of our knowledge, this is the first report on the sequential extraction of chitosan from *Vespa orientalis*, a new source, using modified process of previous studies. The characteristics of produced wasp chitosan were in accordance with the commercial standard. The investigation revealed that wasp has lower yield of chitin of 12.2% but of higher purity compared to that of the shrimp. The degree of deacetylation (DDA) was determined. It was found that the DD of chitosans derived from wasp cuticles was found to be (92-93%) which was higher than that of shrimp chitosan (75%). Chitosan with such properties has several commercial applications and greater scope of industrial applications and this results encourage us to continue the study of this kind of chitosan. Further studies in this area are in progress and will be reported in due courses.

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عزل الكيتين من الدبابير الشرقية *Vespa orientalis* (L) وتحويلها الى الشيتوزان بطريقة كيميائية

ماجدة راضي [1] ، إيمان عيسى ، مأمون ش. م[1]، محمد سلامة[1] ، عماد بركات[1] ، وائل أبو المجد [2]
1- قسم الحشرات ، كلية العلوم ، جامعة عين شمس ، القاهرة ، مصر.
2- قسم الكيمياء ، كلية العلوم ، جامعة عين شمس ، القاهرة ، مصر

المستخلص

يعتبر الكيتين المستخرج من دبور الدبور الشرقية *Vespa orientalis* (L)، مصدرًا جديدًا للكيتين الحشري ويتحول إلى شيتوزان باستخدام طرق كيميائية. في هذه الدراسة ، تم تقييم الخواص الفيزيائية لخيط الدبابير باستخدام طريقتين ، تحويل فورييه الطيفي للأشعة تحت الحمراء (FTIR) وطيف الرنين المغناطيسي النووي (H-NMR1). أظهرت النتائج أنه يعطي درجة نقاوة أعلى مقارنة بالشيتوزان القشري. كانت درجة نزع النسيج (92-93٪) (DDA) أعلى أيضًا من درجة الشيتوزان في القشريات (83.8-85.8٪). لذلك ، يمكن أن يكون الدبور الشرقي مصدرًا بديلاً جديدًا للشيتوزان.