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Extraction of chitin from six different insect species as alternative source for biological applications

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ABSTRACT

Chitosan, the most dominant and abundant polymer in scientific researches with its wide range biopharmaceutical and biomedical applications is now extracted from different interesting resources in large and valuable quantities.

Six different groups of insects were used in this study. These were Ground dark beetle, Solitary (Carpenter) bee, Damsel fly, Earwig, Black bug and Egyptian grasshopper. These insect species showed easy capturing and preparation through deproteinization, demineralization and dehydration. By using Infrared spectrophotometry technology and calculating the degree of deacetylation, the potential effect of these insects can be insured as alternative source for chitin extraction and chitosan industrial applications.

Key words: Extraction, chitin, insects, alternative source, biological applications, chitosan.

INTRODUCTION

Chitin, the second most abundant biopolymer after glucose (Park *et al*., 2009), is a natural aminopolysaccharide. It is found in algae, yeast, fungi, arachnids, myriapods, other biological materials and recently from fish scales (Kumari and Rath, 2014). Generally crab and shrimp shells are the main sources for chitin extraction (Islam *et al.*, 2011; Hossain and Iqbal, 2014). Recently the insect cuticles become the alternative sources and take great attention due to its lower inorganic materials compared with crustaceous shells and also due to their enormous biodiversity (Liu *et al*., 2012). However until now, only limited numbers of insect species have been documented to be sources of chitin (Rawda and Hadeer, 2015).

Chitin is insoluble in most solvents due to its compact structure, while chitosan, the most abundant renewable resource, is performed by thermochemical N- deacetylation of chitin in alkaline solution (Yaghobi and Mirzadeh, 2004). The process of deacetylation involves the removal of acetyl groups from the molecular chain of chitin, leaving behind a chitosan with free amino groups along its chain allow this macromolecule to dissolve in diluted aqueous acid solvent (Rinaudo, 2006; Kean and Thanou, 2010).

Detecting the degree of deacetylation of this molecule can be occured according to the number of its amino groups present. The high degree of deacetylation due to more protonation of this macromolecule, increasing the degree of its covalent crosslinking, increasing reactivity, consequently the biological properties and physiochemical characteristics of this chitosan (Croisier and Jerome, 2013; Besser and MacDonald, 2016).

So the aim of this work is isolating convenient cheap alternative sources of chitin and chitosan from six different insect species and comparing between the chemical composition and physiological properties of these extracted chitin and chitosan with those from commercial one. This will help in using waste -products of insects to produce

effective useful biopolymers for many biological and medical applications.

MATERIALS AND METHODS

A- Sample collection

Six different insect species were collected for chitin extraction. These insect species are: Solitary bee, Ground beetle, Damsel fly, Earwig, Black bug and Egyptian grasshopper. Samples were coded as shown in Table (1).

Table (1): Sample code of collected insect species.

Sample code	Species
A	Ground dark beetle
B	Solitary(carpenter) bee
C	Damsel fly
D	Earwig
E	Black bug
F	Egyptian grasshopper

The investigated insects belong to the Kingdom: Animalia, Phylum: Arthropoda, Class Insecta with the following characters:

- Body is divided into three parts, head, thorax and abdomen.
- With chitinous exoskeleton
- Without bones or endoskeleton.
- Head with a pair of compound eyes, usually three simple eyes and three modified appendages that form mouth parts.
- Thorax with three pairs of segmented legs
- Abdomen consists of eleven segments contains most internal system.

B- Preparation of chitosan:

Chitosan extracted from the exoskeleton of the previous insect according to the following steps:

I-Chitin extraction:

The collected samples of insects were killed by freezing at 3 °C, the internal organs were eliminated. Samples were dried at room temperature, grinding to powder then weighed (Wo).

II-Deacetylation process:

The dried crude chitin was converted into chitosan by the following steps:

1- Deproteination step:

The powder was treated with 10% KOH for 48h at room temperature. The residue was filtered off and washed repeatedly with distillate water. Finally the samples were dried at room temperature.

2-Demineralization step:

The prepared dried crude chitin from insect exoskeleton was treated with excess amount of 5% acetic acid for 12h at 40 ^{O}C to remove minerals. The residue was filtered off and washed repeatedly with distillate water. Finally the sample was dried at room temperature and weighed (W_f).

Weight loss of chitin due to deacetylation process (due to deproteination and demineralization) was calculated:

$$\text{Weight loss} = Wo - W_f$$

C- Characterization analysis of the extracted chitosan:

The structure of the extracted chitosan samples were confirmed by Fourier transform infrared (FT-IR) spectra that were recorded by Nicolet 6700 at 4 cm^{-1} resolution by Infrared spectro-photometry (Fig. 1) (Ain Shams Central Lab). The FTIR spectra were measured in the 4000-400 cm^{-1} region with samples dispersed in KBr pellets. This technique is utilized in this work to confirm the structure of chitin and chitosan.

Fig.(1): Infraed Spectrophotometry for infrared spectra analysis

The degree of deacetylation (DD) was calculated according to the following equation (Akila, 2014):

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$$DD=100-\left[\frac{\frac{A_{1655}}{A_{8450}}\times 100}{1.33}\right]$$

RESULTS AND DISCUSSION

A- Samples Taxonomy and Diagnostic characters:

Six different species were collected for chitin extraction as shown in Figure (2) belongs to the following:

Group (I): Subclass Endopterygota

A: Ground beetle

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Coleoptera
Family: Tenebrionidae
Blaps bifurcata Solier, 1898

Diagnostic characters:

Black, elongated, oval beetle with 2.5-3 cm, with chewing mouth parts, elongated filiform antennae. The 3rd antennal segment is twice the length of the 2nd one. Thorax with rounded sides, prothorax slightly wider than long; elytra forewing with fine terminal punctuation and the hind wing membranous. The last abdominal segment with hairy spot ventrally and prolonged and curved up cauda.

B: Solitary bee (Carpenter bee):

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Hymenoptera
Family: Apidae
Xylocopa pubescens
(Spinola, 1838)

Diagnostic characters:

Large, robust, hairy bee with dark blue color and yellow fur at thorax, with geniculate antennae and chewing lapping mouth parts. With membranous four wings, forewing larger than hind wing and with hooks (hamuli) wing coupling apparatus. Hind tibia with dense hairs for collecting pollen grains (pollen brush).

Group (II): Subclass Exopterygota

(C) Damselfly:

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Odonata
Sub order Zygoptera
Family: Coenagriionidae
Ischnura senegalensis
(Ramber, 1842)

Diagnostic characters

Damsel flies are quick agile flies have slender bodies, highly movable head and large compound eyes covering most of the head. Antennae very short and bristle like, mouth parts chewing with strong teeth on their jaws. Abdomen slender and elongated.

(D) Earwig:

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Dermaptera
Family: Forficulidae
Forficula aircularia
(Linnaeus, 1758)

Diagnostic characters:

Small sized (0.5-1.0 mm), brownish, slender elongated species with long filliform antennae and chewing mouthparts. Forewing short tegmina, hind wing membranous with radiating veins. Abdomen ended by horny defense strong elongated cerci.

(E) Black bug:

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Hemiptera
Family Cydnidae
Cydnus aterrimus
(Forster, 1771)

Diagnostic character:

Small dark bug with 5- segmented antennae, piercing sucking mouth parts. Wings without anal lobe and with limited

number of veins. Forewing hemelytra, scutellum large reaching base of membrane. Abdomen without cerci.

(F)Egyptian grasshopper:

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Orthoptra
Family: Acrididae
Anacridium aegyptium
(Linnaeus, 1764)

Diagnostic characters:

Stout insect with chewing mouth parts and elongated filiform antennae. Fore wing tegmina (with sound producing file on its lower surface), hind wing membranous & large fan-like. Hind leg enlarged and adapted for jumping with specialized auditory organs (Tympana).

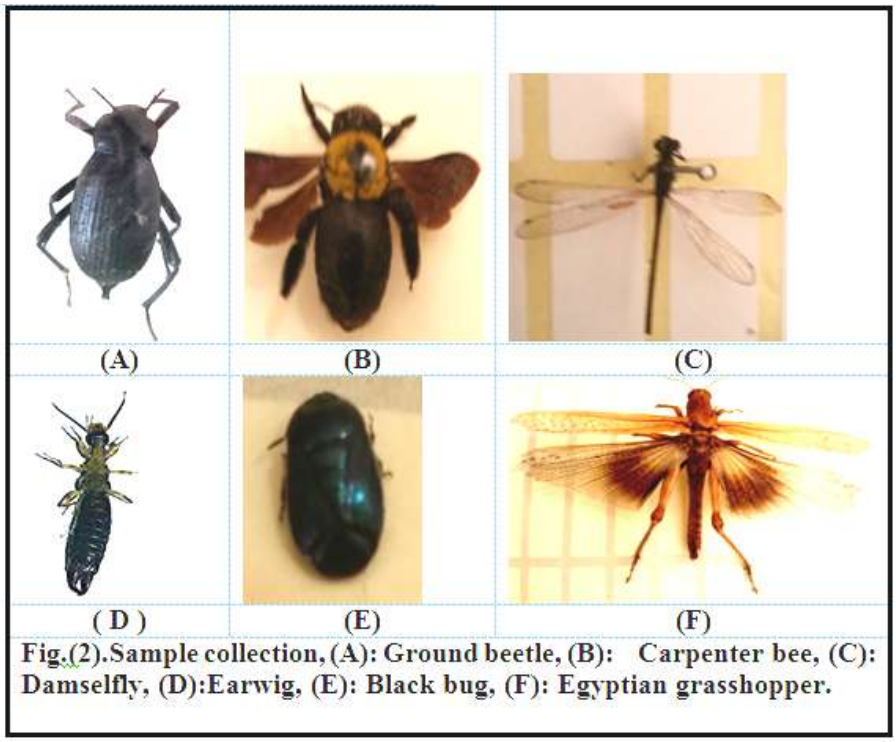

Fig.(2).Sample collection, (A): Ground beetle, (B): Carpenter bee, (C): Damselfly, (D):Earwig, (E): Black bug, (F): Egyptian grasshopper.

B- Structural analysis:

FT-IR results

FTIR spectra of all groups characterized by absorption band within the 1422-603 cm^{-1} region confirmed the presence of CH_3, CH_2 and CH as well as primary and secondary OH groups attached to the pyranose ring, and the oxygen atoms in either groups (Zvezdova, 2010).

Table (2): Clarify characteristic peaks for chitin and chitosan

Functional Group	Wave number (cm^{-1})	Chitin	Chitosan
(O-H)	3424-3440	•	•
N-H_2 stretching	3438	-----	•
(N-H)	3262	•	-----
$COCH_3$	2934	•	----
(C-H)	2884	•	----
(C-O)	1655	•	----
C=O of N-acetyl group	1620-1640	•	•
amide band –CONH- or (N-H of N-acetyl group)	1561	•	•
-CH_3 symmetrical deformation	1348	-----	•
(O-H) or C-O-C bands	1255	-----	•
-CH vibration	1152	-----	•
C-O stretching	1090-1020	-----	•

Group (I)

Figure (3) shows IR spectra of the extracted chitosan from insects of group (I) Carpenter bee and beetle compared with control (commercial chitosan). Table (2) clarifies characteristic peaks for chitin and chitosan.

FT-IR spectra clarify the presence of specific bands at 1665 cm^{-1} indicated the presence of carbonyl group and another band at 3450 cm^{-1} indicated the presence of amide group in all samples. These bands were the baseline bands used in calculating the degree of deacetylation. Figure (3) shows slight differences in the intensity of these bands as deacetylation progress.

Both bands were decreased in control sample [commercial chitosan] with respect to the other samples. As shown in Table (**3)** the lower absorbance intensity of C=O and the broadening of the peak in the range 3450 to 3100 cm^{-1} indicate the higher DD of chitosan (Aranaz *et al.*, 2009)

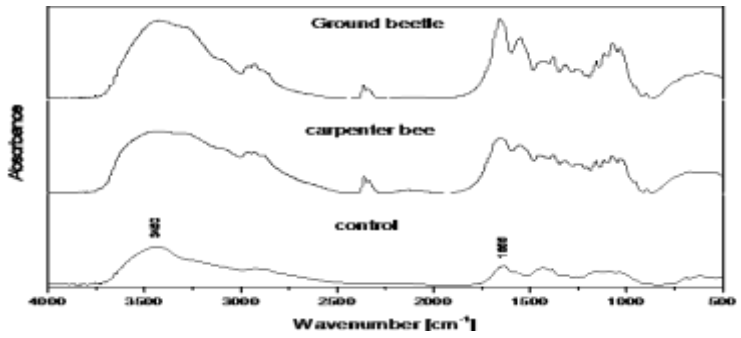

Fig. (3): FTIR spectra of insects of group (I)

By comparing the spectra of untreated and treated chitin, it was found that, the N -deacetylation was associated with a decrease of the intensity band at 1665 cm^{-1}, 3265 and 3100 cm-1(Seoudi and Nada, 2007).

It was found a peak at 2360 cm^{-1} and abroad one in the range 3000–2800 cm^{-1} in case of lower DD (as in carpenter

bee and ground beetle), however it was disappeared in commercial chitosan (60%). These bands indicate the presence of CH and (νCH) group of chitin, show low degree of deacetylation in this group.

A sharp band appeared at 1560 cm^{-1} region that is assigned the N-H groups of N-acetyl group of chitin showing that the degree of deacetylation was decreased (Yaghoubi and Mirzadeh, 2004).

Table (3): Degree of deacetylation of samples of group I

sample notation	A_{1655}	A_{3450}	DDA (%)
(A) Ground beetle	0.212	0.210	24.35
(B) Carpentr bee	0.148	0.164	32.0
Commercial chitosan	0.060	0.112	60

Group II:

Figure (4) illustrates the comparison between IR spectra of the extracted chitosan from insects of group II (dragonfly, earwig, bug and grasshopper with control (commercial chitosan)). Figure (4) shows the characteristic bands of chitin such as N-H, $COCH_3$, C-H, C-O and C=O of N-acetyl group as shown in Table (2). Nearly similar spectra for all samples were observed and these spectra were confirmed in Table (4) which shows nearly similar DD. It was observed that when chitin deacetylation occurs, the band observed at 1570 cm^{-1}occurs indicating prevalence at NH_2 group (Zvezdova, 2010).

CH and CH_3 bands at 2365 &1350 cm^{-1} was observed in all spectra except commercial chitosan. It was observed that the lower intensity of absorbance at CH and CH_3 group related to increasing degree of deacetylation as shown in Figure (13), and Table (4). The lowest intensity of both bands found in commercial chitosan with highest degree of deacetylation.

Table (4): Degree of deacetylation of samples of Insects of group II

sample notation	A1655	A3450	DD%
(C)Damselfly	0.187	0.204	30.8
(D)Earwig	0.262	0.136	26.7
(E) Black bug	0.1373	0.1481	25
(F)Egyptian grasshopper	0.196	0.187	24.8
Commercial chitosan	0.060	0.112	60

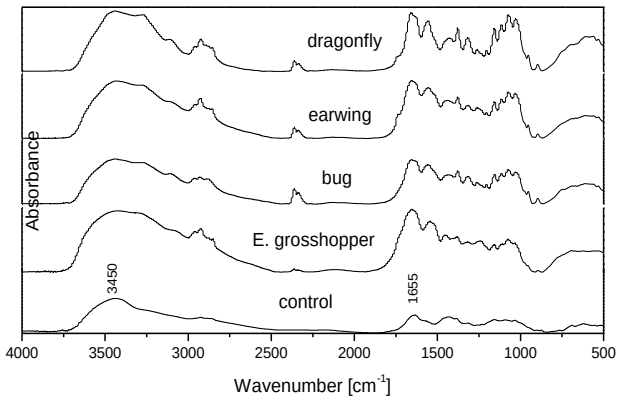

Fig. (4): FTIR spectra of insect of group (II)

Group III: Duplication the time of degree of deacetylation

By increasing the time of deacetylation for dark ground beetle and control the degree of deacetylation increased in beetles only. The lower intensity at 1655cm^{-1} was observed in Figure (7) related to increasing degree of deacetylation as in chitosan which extracted from beetle with the highest degree of deacetylation (Table 6) as reported in previous work (Arabia *et al*., 2013).

Table (6): Degree of deacetylation of samples of beetle (group III) and control.

sample notation	A1655	A 3450	DD%
Beetle chitosan	0.0829	0.1125	50
Commercial chitosan	0.0600	0.1117	60

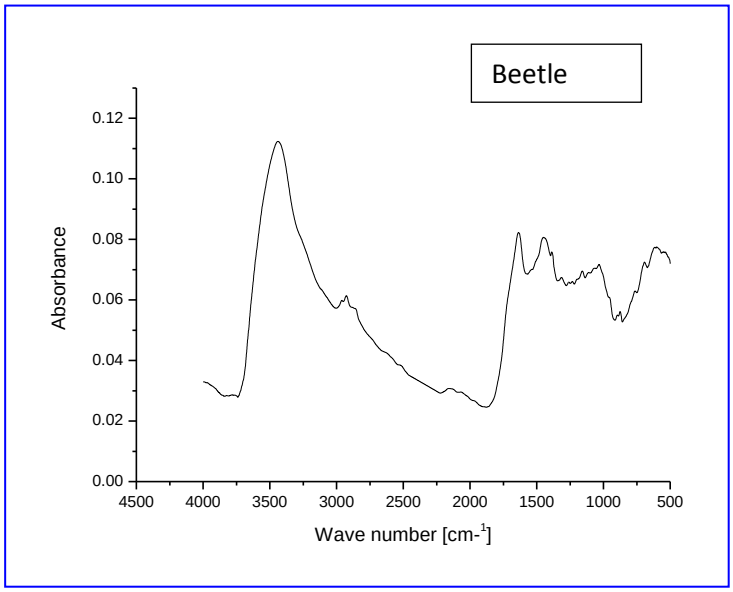

Fig. (7). FTIR spectra of beetle (group III)

From the previous results it was obvious that the molecule of chitosan is a copolymer consists of N acetyl- d-glucosamine and d-glucosamine units in different levels depending upon the degree of acetylation and the number of free amino acids groups in different chitosan polysaccharides (Li *et al*.,1992; Hoppe-Seiler, 1994), that can be used to differentiate between not only chitin and chitosan, but also physical, chemical and biological properties of chitosan which are influenced by the degree of deacetylation (removing of the acetyl group from chitin molecular chain leaving complete amino groups NH_2 leads to positive charge of chitosan) (Hussain *et al*., 2013; Hattori & Ishihara, 2015).

Conclusion:

The results of the present study indicated that the selected six insect species can be used for chitin and chitosan extraction and this will lead to save many tons of insect waste products and confirm that these species can be used as new sources for biological and pharmaceutical applications.

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Extraction of chitin from six different insect species as alternative source for biological applications

استخلاص الكيتين من ستة أنواع حشرية لأستخدامها كمصادر بديلة فى التطبيقات البيولوجية

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المستخلص

الكيتوزان هو بوليمر حيوى طبيعى واسع الأنتشار وله العديد من التطبيقات التكنولوجية فى مختلف المجالات الطبية والغذائية والصناعية. ويمكن تحضيره بنزع مجموعة الأستيل من مركب الكيتين خلال عمليتى نزع البروتين وازالة المعادن باستخدام حمض الخليك المخفف ومحلول البوتاسا الكاويا مما يجعله يمتاز بالشحنه الموجبة التى تساعده على الذوبان فى المحاليل المختلفة وتحسن من خصائصه الكيميائية والتطبيقية وخاصة كمضاد للميكروبات وكتركيبات حيوية وفى تأثيره على خلايا الدم.

يستخلص الكيتين تجاريا من قشرة الجمبرى وحديثا تم استخلاصه معمليا من بعض أنواع من الحشرات. لذلك استهدف البحث اختيار ستة أنواع جديدة من الحشرات المنتشرة فى البيئة المصريةلاستخلاص الكيتين وتحويله الى كيتوزان وحساب درجة نزع مجموعة الأستيل وعمل تحليل وصفى باستخدام الأشعة تحت الحمراء .

وأكدت النتائج أن الكيتين الحشرى يمتاز عن غيره بسهولة تحضيره وقلة المواد غير الغضوية بجامع كفاءته وبذلك ممكن الأستفادة من هذه الموارد الطبيعية وتحويلها الى منتجات طبية وصناعية ذات قيمة عالية .