Chitin extration, Composition of Different Six Insect Species and Their Comparable Characteristics with That of the Shrimp

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Abstract: Chitin and chitosan attracting great interest due to their beneficial biological properties, their potential applications in various industrial fields and their notable bioactivity in biomedical fields. Traditionally, chitin is prepared mainly from crab and shrimp shells; recently, the production of chitin and chitosan from insect sources has drawn increased attention but until now, only limited numbers of insect species have been documented to be sources of chitin without an effective comparison studies; so in this work chitin was isolated from six different common insect species & compared with natural chitin of shrimp by means of FTIR infrared spectroscopy (IR), CHN elemental analysis and X-ray diffraction (XRD), their degree of acetylation was calculated. Chitins exhibited similar chemical structures, physiological properties and were suitable for chitosan production. The serial higher chitin yields were reported to all examined specimens. It was found that characters of chitin are more specific to each species, can be used as a diagnostic taxonomic character and to appear the relationships between species especially if it will be used to all species as possible and be added to data base bank.
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Key words: Chitin, chitosan, extraction, spectroscopy (IR), CHN elemental analysis, X-ray diffraction (XRD), grass hoppers, cockroaches, green bugs, vespid wasps & scarab beetles.

1. Introduction:

Chitin is one of the most abundant biopolymer with several applications [Shahidi & Abuzaytoun, 2005; Teli & Sheikh, 2012; Akila, 2014]. Its extraction could be from crustacean shells, fungi, insects and other biological materials, in addition to other commercial sources such as the shell waste of shrimps, krills, and crabs. Several millions tons of chitin are harvested annually in the world, making this biopolymer an inexpensive and readily available resource [Dash *et al*., 2011; Wan Ngah *et al*., 2011& Arabia *et al*., 2013].

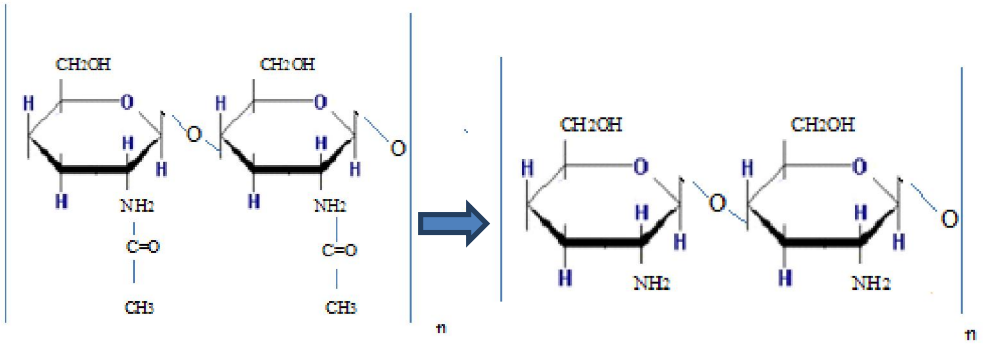
Chitin is classified into: α- chitin (anti-parallel chains), β-chitin (parallel chains), and γ-chitin (the combination of parallel and anti-parallel chains) according to the different orientations of its microfibrils [Khor& Lim, 2003]. It is insoluble in most solvents due to its compact structure.

Chitosan (soluble analogs) could be derived from chitin by partial *N*-deacetylation. Chitosan is a non-toxic, biocompatible, and biodegradable polymer that exhibits promise in a wide range of biomedical applications including wound dressings, tissue engineering, implant coatings and therapeutic agent delivery systems [Ong, *et al*., 2008 & Aranaz, *et al*., 2009].

The difference in chemical structure between chitin and chitosan is shown in Fig. 1. The numbers on the extreme left ring are conventionally assigned to the six carbons in the glucopyranose ring, from C-1 to C-6. Substitution at C-2 may be an acetamido or amino group. Chitosan contains more than 50% (commonly 70 to 90%) of acetamido residues on the C-2 of the structural unit, while amino groups predominate in chitin. The degree of deacetylation (DD) serves as a diagnostic to classify the biopolymer as chitin or chitosan [Rinaudo, 2006 & Dash *et al.,* 2011]. Notice that DD + DA =1.

Chitosan is found naturally in certain fungi (Mucoraceae), is prepared mainly from shrimp, recently from few species of insects (Majtan *et al*., 2007 & Liu *et al*., 2012); but it is easily obtained by the thermochemical deacetylation of chitin in the presence of alkali. Several methods have been proposed, most of them involving the hydrolysis of the acetylated residue using sodium or potassium hydroxide solutions, as well as a mixture of anhydrous hydrazine and hydrazine sulfate. The conditions used for deacetylation determines the polymer molecular weight and the degree of deacetylation (DD) [Lavorgna *et al*., 2010 & Dash *et al*., 2011].

The DD is the key property that affects the physical and chemical properties of chitosan, such as solubility, chemical reactivity and biodegradability and, consequently their applications (Acharyulu *et al*., 2013 & Wanule *et al*., 2014).



Chitin **Chitosan**

Figure 1. Difference in chemical structure between chitin and chitosan

Both chitin and chitosan are biocompatible, biodegradable, and nontoxic, and act as anti-microbial and hydrating agents. From the medical view, chitin and chitosan are easily processed into gels [Nagahama *et al*., 2009], membranes [Jayakumar *et al*., 2009; Madhumathi *et al*., 2009a & Madhumathi *et al*., 2009b], nanofibers [Schiffman & Schauer, 2007a; Shalumon *et al*., 2009], beads [Jayakumar *et al*., 2006], microparticles [Prabaharan & Mano, 2005], nanoparticles [Anitha *et al*., 2009], scaffolds [Maeda *et al.,* 2008 & Madhumathi *et al.,*2009c], sponges [Portero *et al*., 2007] forms and nanoscale thin films and fibers of chitin/chitosan [Pillai *et al*., 2009].

The aim of the present work is to isolate the useful biopolymer chitin or chitosan from different six common insect species. The experimentally prepared chitin or chitosan were characterized and compared with that of the shrimp by means of FTIR spectroscopy, CHN elemental analysis and X-ray diffraction.

2. Material and Methods

1- Sample collection

Six insect species were chosen belonging to Class Insecta (Three pairs of legs, body have head, Thorax & abdomen) Subclass Pterygota (with Metamorphosis), they were:

A- Devision: Exopterygota (gradual Metamorphosis: Egg-nymph or naiad-Adult).

Order Orthoptera, Family: Acrididae (Dessert Short horned grass hoppers), *Shistocerca gregarea* Forsskal [Speciemen (A)].

Order Hemiptera, Family: Pentatomidae**,** *Nezara viridula* (L.) (Green bugs) [Speciemen (C)].

Order Blattodea (Cockroaches)Family Blattidae, *Periplaneta americana* (L.) (American cockroach) [Specimen (B)] and Family: Blattellidae, *Blattela germanica* (L.) (German cockroach) [Specimen (F)].

B: Devision: Endopterygota (Complete Metamorphosis: Egg-Larva-Pupa-Adult).

Order Hymenoptera, Family: Vespidae**,** *Vespa orientalis* L.(Vespid wasp, Yellow jacket wasp) [Specimen (D)].

Order Coleoptera (Scarab beetles), Family: Scarabaeidae**,** Subfamily**:** Dynastinae *Pentodon algerinum* (Fuessly) [Specimen (E)].

2- Sample preparation

The collected insects were killed by freezing, the internal organs were eliminated, deproteinization was performed using 10% KOH at 40 C˚ for 48 hours, then by distilled water, demineralization with 5% acetic acid at 55 C˚ for 2 hours, dehydration by series (30-100%) of ethyl alcohol, then the lightly brown dried chitin were milled to a powder (Excess acetylation was carried out by duplicated concentration and time may give positive results as shown on specimens A).

3- Sample characterization

Infrared Spectra (IR) Analysis

Chitin and chitosan samples were characterized from 4,000 to 500 cm^{-1} by infrared spectrophotometry (Ain Shams Central lab) with KBr pellets. Commercial chitin (Qualikens Laboratory Chitin) exported from New Delhi was used as standard. The DD of chitin samples were determined by comparing the absorbance of the measured peak to that of the reference peak. The DA was calculated from the absorbance (A) ratios [Akila, 2014]:

$$DD = 100 - [\frac{\frac{A1655}{A3450}x100}{1.33}]$$

CHN analysis

The C-H-N analyzer (Elementar, Vario EL III) is used for elemental analysis of collected samples to calculate the degree of deacetylation according to [Jiao*et.al,* 2011].

$$DD = \left(1 - \frac{{}^{C}/_{N} - 5.145}{6.862 - 5.145}\right)x100$$

X-ray Chitin Powder Diffraction

XRD analysis was used to detect the crystallinity of chitins prepared, and their patterns were recorded

using a D/Max-rAdiffractometer (Rigaku, Tokyo, Japan) with Cu radiation.

Data were collected at a scan rate of 1°/min with the scan angle from 5° to 40°. The crystalline index (CrI) was determined according to [Jiao *et al*., 2011]

$$CrI\ 110 = \frac{(I110 - Iam)}{I110} x100$$

where I110 is the maximum intensity at 2θ ≅ 20° and Iam is the intensity of amorphous diffraction at 2θ ≅ 16°.

3. Results and Discussions

Characterization of Chitin

1. Morphological characterization

Devision: **Exopterygota**:

Shistocerca gregarea (Desert grass hopper) (Fig.2a & d), stout larg brownish insect with narrow elongate leathery (tegmina) fore wing, membranous hind wing; mouth parts chewing, antennae many segmented filiform, hind leg enlarged for jumping; with sound produsing organ (Stridulatory file) on lower surface of the fore wing, hearing organ (Tympanum) on the sides of the first abdominal segment. While treatment of *Nezara viridula* (Green bug) (Fig.2b & e)small green pentagonal bug, fore wing with basal leathery & membranous tip; mouthparts are piercing sucking in the form of a slender segmented beek a rising from the front of the head, usually extends back along the ventral side of the body; with distinct large triangular scutellum**.**Large American cockroach *Periplaneta americana* (Fig.2f & g), Small German cockroach *Blattela germanica* (Fig. 2c), with 2 blackish lines on pronotum; they havebrownish dorsoventrally flattened body with long setaceous antennae, chewing mouth parts; fore wing tegmina, hind larg membranous; legs walking.

Devision Endopterygota: *Vespa orientalis* (Vespid wasp, Yellow jacket wasp) (Fig. 2h), large brownish wasp with yellow markings on their head, thorax& yellow bands on the abdomen; brownish membranous two pairs of wings with few veins, pigmented pterostigma, elongated marginal cell & a minute row of hooks (Hamuli) for coupling the two wings. Head hard & mobile, mouth parts chewing lapping with well developed glossa (tongue like sucking structure) and *Pentodon algerinum* (Scarab beetles), **(**Fig.2i) showed blackish beetles, with horny (Elytra) fore wing, membranous hind wing; mouth parts chewing (mandibulate); with hard teeth on the head & legs.

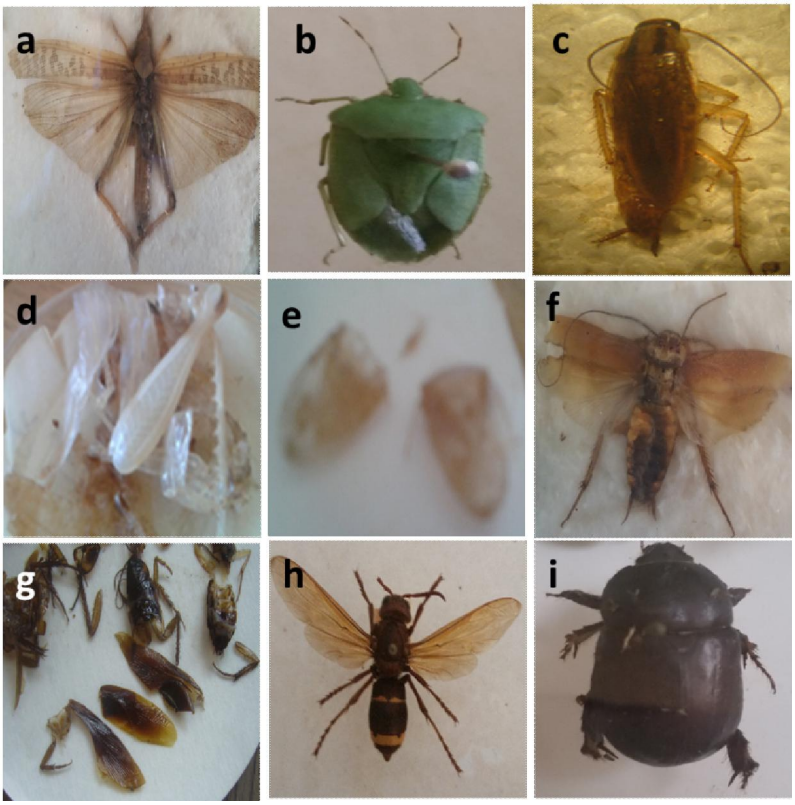


Figure 2. (a & d): Desert grass hopper, *Schistocerca gregaria,*Forsskal (before& after treatment); (b & e) Green bug, *Nezara viridula,* (L.) (Before& after treatment); (c)*:* German cockroache, *Blatella germanica* (L.); (f & g) American cock roach, *Periplanetta americana* (L.); (h): Vespid Wasp, *Vespa orientalis* L.*;* (i): Scarab beetle, *Pentodon algerinus* (Fuessly).

2. Structural characterization

IR Analysis

The degrees of deacetylation (DD) of chitin from all selected samples are calculated using FT-IR analysis and shown in table 1. From the calculated results, it is clear that the degree of de-acetylation (indication to chitosan contents) of seven samples (Six insect species & Natural shrimp shell).

Figure 3 shows that IR spectra of extracted chitin and chitosan from Division Exopterygota, (Desert grasshopper) [Specimen (A)]; (American cockroach) [Specimen (B)] and (Green bugs) [Specimen (C)] are quite similar, and comparable to those of α-chitin from other sources in previous literature [Kumirska, *et al*., 2010; Liu, *et al*., 2012]. There are three significant amide bands characterize the spectra at 1654, 1560 and 1310 cm^{-1}, which correspond to the amide I stretching in C=O, the amide II in N-H and amide III in C-N, respectively. Also the stretching vibrations of C=O and NH in ($NHCOCH_3$) are displayed as a small shoulder at 3085 cm^{-1} and 3265 cm^{-1} correspondingly [Teli & Sheikh, 2012]. As for chitosan, absorption band is observed at 3440 cm^{-1} due to OH group and the bands at 2930 cm^{-1}, 2858 cm^{-1} and 1375.2 cm^{-1} may be due to stretching vibration and bending vibration of C-H respectively. The recorded band at 1021 $cm^{-1}$was characterized to C-O-C stretching vibrations [Acharyulu, *et al*., 2013]. From this result, it is demonstrated that all samples contain chitins are in α form mixed with chitosan.

Table 1: The degree of acetylation (DD) of all samples

Sample notation	A_{1655}	A_{3450}	DD
(A) Desert grasshopper	0.1511	0.1817	37.5
after excess acetylation	0.126	0.151	37.3
(B)American cockroach	0.116	0.143	30.0
(C)Green bug	0.2	0.21	28.4
(D) Vespid wasp	0.086	0.109	40.7
(E) Scarab beetle	0.153	0.162	29.0
(F)German cockroach	0.154	0.184	37.1
Shrimp shell	0.033	0.02	0.0

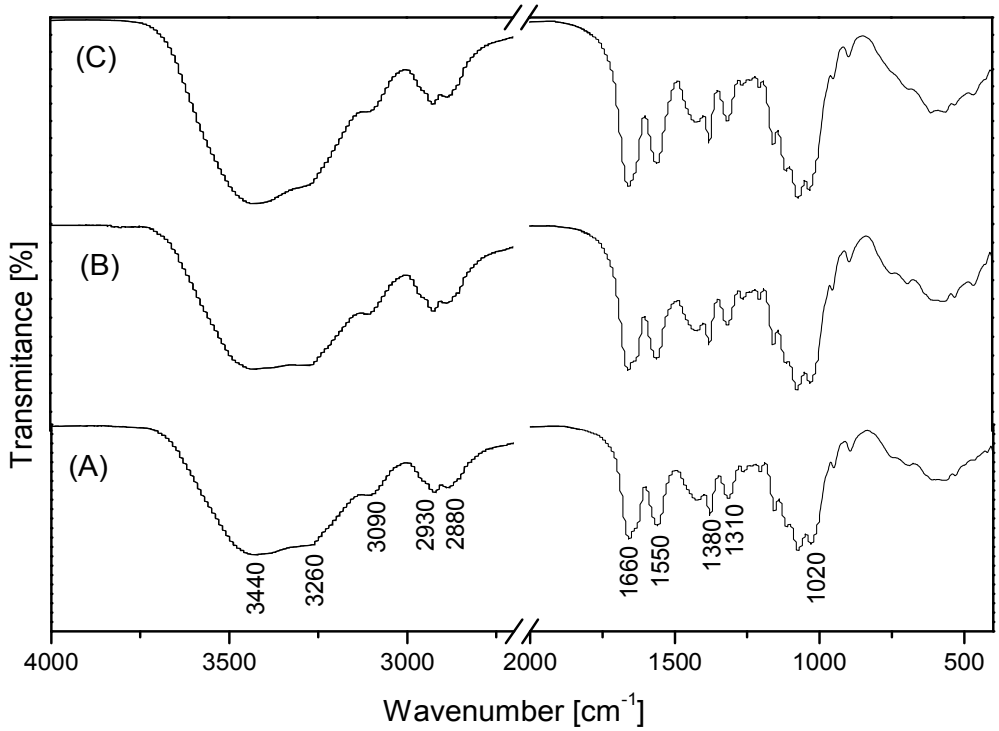


Figure 3: IR spectra of α-chitin and chitosan structure from Grasshopper (A), Cockroach (B) and Green bug (C).

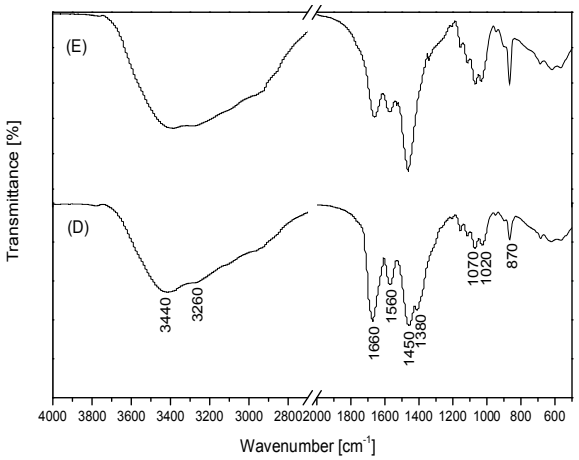


Figure 4: IR spectra of α-chitin and chitosan structure from Vespid wasp (D), and Scarab beetle(E).

Figure 4 shows that both chitin and chitosan structures of Devision Endopterygota, Vespid wasp [Specimen (D)], under the same extraction condition of Scarab beetle [Specimen (E)] are quite similar, and comparable to those of α-chitin in the other Class & Subclass [Fig.3]. Also, the band of amide III disappeared and that means chitin is transformed to chitosan through n-deacetylation process which reduces the amide content especially that observed as the reduction of band at 1655 cm^{-1} as it transformed from chitin to chitosan [Yaghobi, & Hormozi, 2010].

Chitosan peaks of Devision Endopterygota are observed at 3454cm^{-1} and 1021 cm^{-1}as discussed above in the Division Exopterygota, in addition to the presence of absorption band at 1450 cm^{-1} and 1380 cm^{-1}that are characteristic to the bending vibration of C-H of chitosan.

Figure 5 shows a comparison in structure between American cockroach (B) and German cockroach (F). The bands characteristic to α-chitin as shown in Fig.3 were displayed in sample F, but with reduction in the amide content. Also, Figure 5 shows the presence of chitosan absorption band of the stretching and bending vibration of C-H of chitosan and the stretching vibrations of C-O-C especially in case of German Cockroach (F).

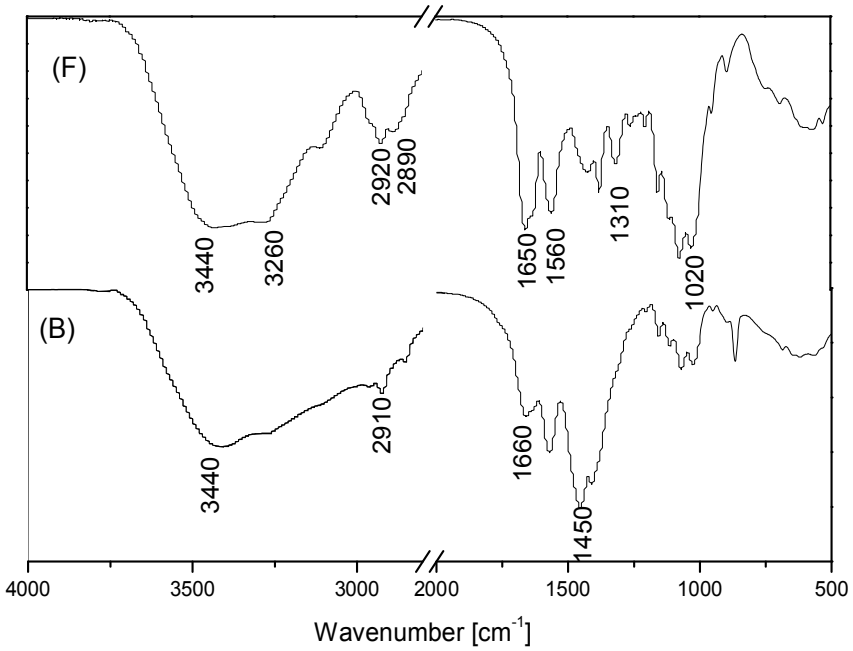


Figure 5. IR spectra of α-chitin and chitosan structure from *Periplaneta americana* (American cockroach) (B), and *Blattela germanica* (German cockroach) (F).

CHN analysis

Since the nitrogen content is less than 7% for chitin and more than 7% for chitosan [Rinaudo, 2006 & Dash *et al.,* 2011]. Figure 6 indicates that as C/N ratio increase the degree of deacetylation decrease especially for Green bug and Scarab beetle and this result confirmed IR result in table 1.

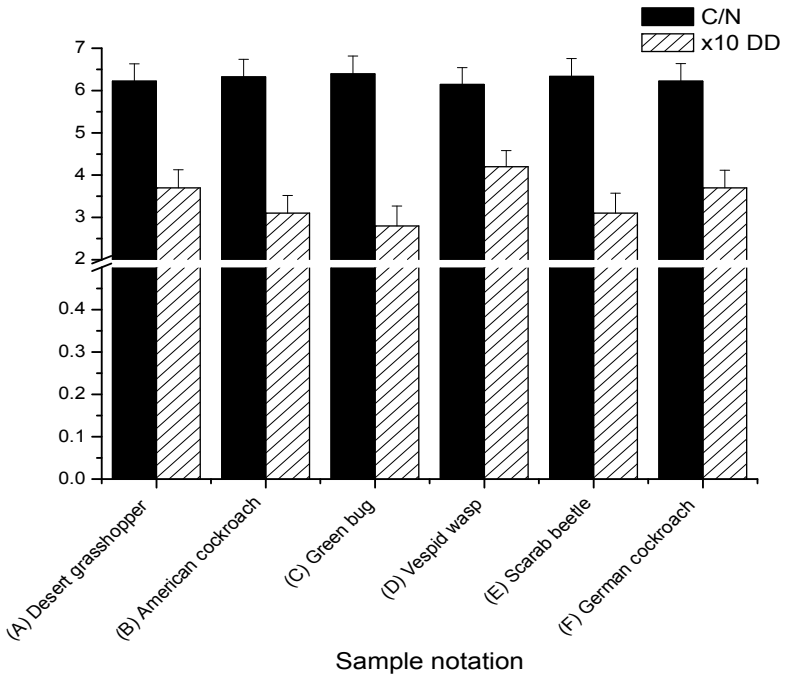


Figure 6. The C/N ratio of chitin from all samples and corresponding degree of deacetylation (DD)

Figure 7 represents the XRD pattern for α-chitin, with strong reflections at 9.2 and 19.1° and minor reflections at 12.6, 22.9 and 26.2°[Liu *et al.* 2012]. The results of other samples showed similar XRD patterns with appearance of additional peak at 21.9°, this peak was confirmed the presence of chitosan fragments as recorded by Abdel-Fattah *et al.*, 2007 (Figure 7(A) Grasshopper; (D) Vespid wasp; (F) German cockroach). The crystallinity index of 020 and 110 diffraction angle is calculated and shown in table 2. It is clarify that CrI_{020} and CrI_{110} is related to DD of chitosan where both CrI show decrease with increase of DD as reported in the previous literature Zhang *et al.* (2000). However, CrI_{020} displays a more significant relationship in DDA.

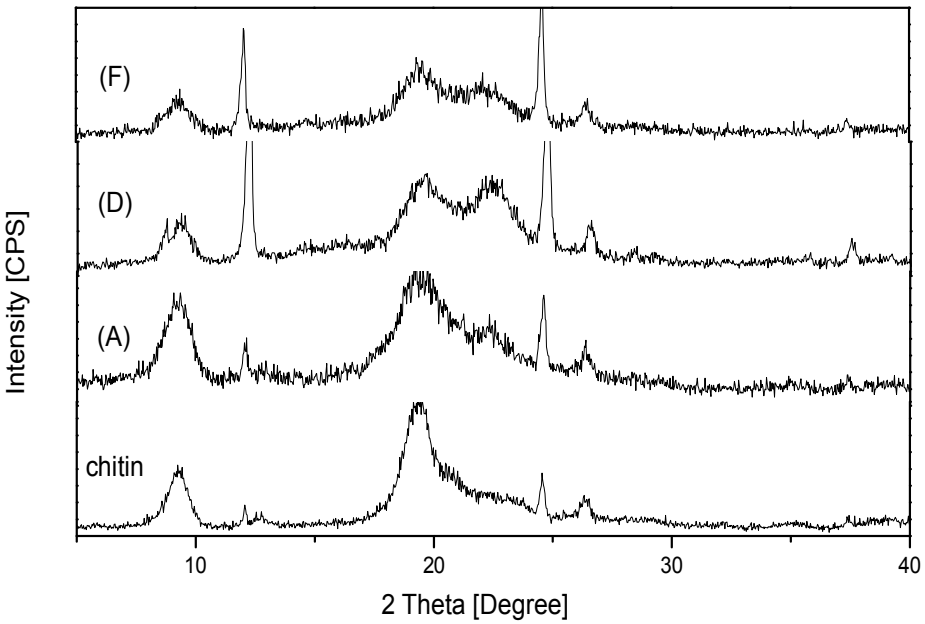


Figure 7. XRD pattarns of chitin and chitosan structure from Grasshopper (A), Vespid wasp (D), and German cockroach (F).

Table 2: Relation between the degrees of deacetylation (DD), $CrI_{110}$and CrI_{020}

Sample notation	DD	CrI_{110}	CrI_{020}
(A) Grasshopper	37.5	71.2	71.4
(D)Vespid wasp	40.7	63.9	39.4
(F) German cockroach	37.1	72.2	44.2

Conclusion

The experimentally prepared chitin, chitosan of six insect species and that prepared from shrimp shells were characterized by FTIR spectroscopy, CHN analysis, calculation of the degree of deacetylation and X-ray diffraction. It was found that: Insect Chitin, the primary component of the cuticle is an effective alternative source; especially insect cuticles have lower levels of inorganic material compared to crustacean shells, which makes their demineralization treatment more convenient and easier to be deacetayled in comparison with shrimp shell. By use these common insects, several millions tons of chitin are harvested annually in the world, making this useful biopolymer an inexpensive and readily available resource.

These results indicated that: All chitins exhibited similar chemical structures, physiological properties and were suitable for chitosan production. The higher DD (indicated chitosan yield) was reported dissentingly from Vespid wasp *Vespa orientalis* L, followed by grass hoppers *Chistocerca gregaria*, Forsskal; German cockroach *Blattela germanica* (L.); American cockroach *Periplaneta americana* (L.); scarab beetle, *Pentodon algerinum* (Fuessly) and then green bugs *Nezara veridula* (L.).

From all previous results, it is clear that: Characters of chitin are more specific to each species and can be easily used as a diagnostic taxonomic character as a finger print to each species and can appear the relationships between different species. So it will be more useful to do this taxonomic application in a wide range to all species as possible and is added to data base bank.

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