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Extraction and physicochemical characterization of chitin and chitosan isolated from house cricket

E B Ibityoe 1,2,*, I H Lokman 1, M N M Hezme 1, Y M Goh 1, A B Z Zuki 1 and A A Jimoh 2

1 Department of Veterinary Pre-Clinical Science Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang Selangor Darul Ehsan, Malaysia
2 Department of Theriogenology and Animal Production, Faculty of Veterinary Medicine Usman Danfodiyo University Sokoto, Nigeria

E-mail: lokidris@gmail.com

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Abstract

Chitin ranks next to cellulose as the most important bio-polysaccharide which can primarily be extracted from crustacean shells. However, the emergence of new areas of the application of chitin and its derivatives are on the increase and there is growing demand for new chitin sources. In this study, therefore, an attempt was made to extract chitin from the house cricket (Brachytrupes portentosus) by a chemical method. The physicochemical properties of chitin and chitosan extracted from crickets were compared with commercial chitin and chitosan extracted from shrimps, in terms of proximate analysis in particular, of their ash and moisture content. Also, infrared spectroscopy, x-ray diffraction (XRD), scanning electron microscopy and elemental analysis were conducted. The chitin and chitosan yield of the house cricket ranges over 4.3%–7.1% and 2.4%–5.8% respectively. Chitin and chitosan from crickets compares favourably with those extracted from shrimps, and were found to exhibit some similarities. The result shows that cricket and shrimp chitin and chitosan have the same degree of acetylation and degree of deacetylation of 108.1% and 80.5% respectively, following Fourier transform infrared spectroscopy. The characteristic XRD strong/sharp peaks of 9.4 and 19.4° for α-chitin are common for both cricket and shrimp chitin. The percentage ash content of chitin and chitosan extracted from B. portentosus is 1%, which is lower than that obtained from shrimp products. Therefore, cricket chitin and chitosan can be said to be of better quality and of purer form than commercially produced chitin and chitosan from shrimp. Based on the quality of the product, chitin and chitosan isolated from B. portentosus can replace commercial chitin and chitosan in terms of utilization and applications. Therefore, B. portentosus is a promising alternative source of chitin and chitosan.

Introduction/research background

Chitin is a nitrogen-modified polysaccharide made up of N-acetylglucosamine, bound together in β-(1-4)-N-acetyl-d-glucosamine bonds. Structurally, it is very similar to cellulose, but it has an additional amine group and a hydroxyl substituent on each monomer. It is the second most ubiquitous bio-polysaccharide after cellulose (Rudall and Kenchington 1973), and it is widely distributed in marine invertebrates, insects, fungi and yeast (Austin et al. 1981). Chitin is not soluble in most solvents due to its compact structure (Kurita 2001). This limits its utilization and has led to several chemical modifications in an attempt to produce a more soluble and utilizable derivative such as chitosan. Thus, chitosan ((β-(1-4)-linked D-glucosamine and N-acetyl-d-glucosamine) is a collective name for a group of fully or partially deacetylated chitin. Chitin and chitosan have recently become the focus of researchers due to their beneficial biological properties, such as biocompatibility, biodegradability, non-toxicity, non-antigenicity, adsorption, ability to form films and to chelate metal ions (Rout 2001, Shahidi and Abuzaytoun 2005). Therefore, they have been applied in different industrial fields (Shahidi et al. 1999, Ong et al. 2008), as well as in poultry health and production in Malaysia (Kaikabo et al. 2016a, 2016b).

Due to their natural origin and high level of chemical and physical variability, the properties of chitin and chitosan may have a direct impact on their utilization.
This variability not only relates to the sample origin but also to their method of isolation (Aranaz et al 2009). The success of the utilization of chitin and chitosan in various fields such as food, biomedicine and agriculture, among others, is directly related to their physicochemical properties. Commercially, chitosan is produced via the chemical deacetylation of crustacean chitin under treatment with strong alkali (Chatterjee et al 2005, Silva et al 2007). Crustacean chitin and chitosan is not the same in its physicochemical characteristics, probably due to the variability in raw materials, the destructive nature of the isolation and the conversion processes, the caustic effects of the chemicals used in the extraction process, and variability in the levels of deacetylation and protein contamination (Nwe et al 2002, Tajdinia et al 2010), and this may limit the utilization of these products. Also, commercial chitin and chitosan from shrimp, lobster and crabs may become unfeasible due to the continuous harvest of these animals without replacement, coupled with the fact that they are limited seasonally (Tajdinia et al 2010). In order to expand the chitin–chitosan base source and obtain chitin and chitosan of a better and more consistent quality, a few insect species have been researched to some extent as an alternative source (Zhang et al 2000, Ai et al 2008, Liu et al 2012). Most of these studies only compare insect chitin with commercial chitin, with a paucity of information comparing the chitosan counterpart. The aim of this study, therefore, is to isolate and study the chitin and chitosan structures of the house cricket (Brachytrupes portentosus) so as to reveal the differences between cricket chitin and chitosan and the commercial products by determining their physicochemical and nutritional properties.

Materials and methods/research methodology

Proximate analysis of house cricket (B. portentosus)
Mixed sex house crickets, B. portentosus, about eight weeks old, were obtained from an insect breeding farm in Kuala Selangor, Malaysia. They were starved for 48 h to eliminate gut contents, weighed, sacrificed by freezing at −20 °C for 24 h, washed with water, then oven dried at 60 °C for 48 h. The moisture and ash content of the processed crickets were then determined (AOAC 2016).

Extraction of chitin and chitosan from B. portentosus
Chitin was isolated by modifying the methods of (Song et al 2013, Jarolimkova 2015). For deproteinization, 20 g of the dried cricket was treated with 200 ml (i.e. a sample:NaOH ratio of 1:20) of 1 M solution of NaOH at 95 °C for 6 h. It was then filtered through a 100-mesh sieve and washed with distilled water until the neutral pH of the distilled water was reached. For demineralization, the deproteinized sample was then treated using the same ratio with 200 ml 1 g 100 ml⁻¹ oxalic acid for 3 h at room temperature with moderate stirring, then filtered with a 100-mesh sieve and washed with distilled water until the neutral pH of the distilled water was reached. The sample was then mixed with 200 ml 1% sodium hypochlorite solution (1%, w/v), at room temperature for 3 h with moderate stirring to decolourize it, filtered using a 100-mesh sieve and washed with distilled water until the neutral pH of the distilled water was reached. The whole sequence was repeated once more. The sample was dried overnight at 60 °C and the dry weight was recorded. Chitosan was then purified by deacetylation of the chitin sample according to the method of Ploydee and Chaiyanan (2014). The percentage yield ((weight of chitin or chitosan/weight of dried sample) × 100%) of the chitin and chitosan was calculated, labeled and stored in airtight containers until required. For the purpose of comparison, commercial shrimp chitin and chitosan was obtained from Bita Lifescience Sdn. Bhd.

Elemental analysis
This was conducted by an Elemental Analyzer Flash 2000, to ascertain the % C, N and H content of the chitin and chitosan from both the house cricket and shrimp.

Physicochemical characterization of cricket chitin and chitosan
Fourier transform infrared spectroscopy (FT-IR) was used to determine the presence of the characteristic IR bands, which are the characteristics of chitin and chitosan. The FT-IR spectra were taken on an IRTracer-100 spectrometer with a universal Zn-Se ATR (attenuated total reflection) accessory in the 600–4000 cm⁻¹ region. The degree of acetylation (DA) and the degree of deacetylation (DD) of the house cricket and shrimp chitin and chitosan samples, respectively, were determined by comparing the absorbance of the measured peak to that of the reference peak at 1655/A3450. Therefore, for chitin the DA was calculated from the absorbance ratio A1655/A3450.

X-ray diffraction (XRD) analysis was done to evaluate the crystallinity of the cricket and the shrimp chitin and chitosan using a D/Max–Rα diffractometer. Data was collected at a scan rate of 2° min⁻¹ with the scan angle from 5° to 40°. The crystallinity index (CrI) was determined using the following equation (Liu et al 2012):

\[
CrI_{10} = \left( \frac{I_{10} - I_{am}}{I_{am}} \right) \times 100.
\]

\(I_{am}\) is the maximum intensity at 2 θ ≈ 19°, while \(I_{10}\) is the intensity of amorphous diffraction at 2θ ≈ 15°.
Statistics were used for the moisture and ash content. Presented in tables and pictures, while descriptive statistical analysis and presentation of results.

Ter coater and chitosan samples were coated with gold by a sputter coater (Cressington Auto 108).

**Statistical analysis and presentation of results**

The results of the physicochemical properties are presented in tables and pictures, while descriptive statistics were used for the moisture and ash content.

**Results**

This study presents the nutritional value and first attempt to investigate the physico-chemical characterization of house cricket (*B. portentosus*) chitin and chitosan. This study reveals that the chitin and chitosan yield of the house cricket are 4.3%–7.1% and 2.4%–5.8% respectively on a dry matter basis. Table 1, shows that the moisture and ash content of the house cricket on a dry matter basis consist of 5.45% moisture, which decreases to 4.0% and 3.33% in the chitin and chitosan of *B. portentosus* respectively. The moisture content of cricket and shrimp chitin was similar, while the chitosan from shrimp had a higher moisture content than cricket chitosan. The *B. portentosus* contained 6.77% ash, while it dropped to 1.0% both in the chitin and chitosan extracted from this species, which was lower than that obtained from the commercial chitin and chitosan.

Chitin and chitosan obtained from the house cricket, as well as that commercially obtained from shrimp, were subjected to elemental analysis. From this study, and as shown in table 2, the percentage of N present in cricket chitin (6.022%) is higher than that of commercial shrimp (4.794%), and both were observed to be lower than 6.89%, which is the reference N content for fully acetylated chitin (Liu *et al* 2012). Conversely, commercial chitosan had a higher percentage of N (6.182%) than house cricket chitosan (5.932%), while the carbon content of cricket chitin and chitosan were lower than that of shrimp.

Table 3 and figure 1 present the FT-IR bands for chitin extracted from the house crickets and that commercially extracted from shrimp. The chitin structure examination using FT-IR spectroscopy reveals the different characteristic bands for α-chitin in different studies (Zhang *et al* 2000, Jang *et al* 2004, Sajomsang and Gonil 2010). Also, table 4 and figure 2 present the recorded FT-IR spectra of house cricket and commercial shrimp chitosan.

One of the most important chemical properties that can determine the performance of chitosan and chitin utilization is the degree of *N*-acetylation (Pillai *et al* 2005, Rinaudo 2006). Many various analysis techniques have been developed for the determination of DA (Kasaii 2009). This study employed FT-IR for the determination of the DA and DD of house cricket and commercial chitin and chitosan. The DA of house cricket and commercial shrimp chitin by FT-IR is 108.1%, while the DD of their chitosan is 80.5%.

This study also revealed that the chitin extracted from cricket in this study is of the α-form and is very similar to that of commercial shrimp chitin. The results of the XRD of chitin extracted from the house cricket (B. portentosus) scanned at 2θ, and between 5 and 40°, shows a total of ten peaks at 9.4, 12.8, 17.1, 19.4, 21.1, 23.2, 26.3, 28.5, 35.0 and 39.0°, while that of commercial shrimp chitin shows a total of nine peaks at 9.4, 12.8, 17.4, 19.4, 21.1, 23.2, 26.2, 28.2 and 39.1°. Both cricket and commercial shrimp chitin are similar in that they both have three strong peaks, namely 9.4, 19.4 and 21.1°, with 9.4 and 19.4° being the sharpest peaks for both. Also, in the XRD of the house cricket and commercial shrimp chitosan, a total of nine peaks for both. Also, in the XRD of the house cricket and commercial shrimp chitosan, a total of nine peaks were observed for the former, with three strong peaks at 9.6, 19.6 and 21.2°, and six weak ones at 12.4, 23.0, 26.2, 28.5, 35.0 and 39.0°. Commercial shrimp chitosan showed a total of 15 different peaks, with three strong peaks at 10.1, 20.2 and 22.3° with others as weak peaks. It has been revealed in previous studies

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**Table 1. Moisture and ash content of chitin and chitosan from house crickets and shrimps.**

<table>
<thead>
<tr>
<th>Parameters (%)</th>
<th>Meal</th>
<th>Chitin</th>
<th>Chitosan</th>
<th>Meal</th>
<th>Chitin</th>
<th>Chitosan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>5.45</td>
<td>4.00</td>
<td>3.33</td>
<td>3.98</td>
<td>7.62</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>6.77</td>
<td>1.00</td>
<td>1.00</td>
<td>4.48</td>
<td>11.77</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Elemental analysis of chitin and chitosan from house cricket and shrimp.**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Carbon</th>
<th>Nitrogen</th>
<th>C/N</th>
<th>Carbon</th>
<th>Nitrogen</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitin</td>
<td>41.30</td>
<td>6.022</td>
<td>6.858</td>
<td>43.61</td>
<td>4.794</td>
<td>9.10</td>
</tr>
<tr>
<td>Chitosan</td>
<td>38.98</td>
<td>5.932</td>
<td>6.571</td>
<td>40.97</td>
<td>6.182</td>
<td>6.63</td>
</tr>
</tbody>
</table>
that the CrI values of chitin vary between 47% and 91%, depending on the species, the method of isolation and calculation (Fan et al 2008, Fan and Saito 2009, Ifuku et al 2011, Liu et al 2012). In this study, the chitin from house crickets shows a CrI value of 88.02%, while house cricket chitosan has a CrI of 86.64%. These are comparable to the CrI of commercial shrimp chitin and chitosan at 90.43% and 94.42%, respectively.

**Scanning electron microscopy (SEM)**
The morphology of house cricket chitin and chitosan was studied by SEM and FESEM and the micrographs at different magnifications and different areas of chitin and chitosan are shown in figure 3. From this study, the surface area morphology of house cricket chitin and chitosan is different from those of commercial (shrimp) chitin and chitosan. The morphology of chitin and chitosan isolated from *B. portentous* is similar to what was reported by (Kucukgulmez et al 2011). Generally, at lower magnification (×500) house cricket chitin shows a combination of rough and smooth layers of flakes; also big pores can be seen in some areas, while fibrillar structures can easily be distinguished in other parts (figure 3(a)). At different magnification (×1000), house cricket chitin has big

### Table 3. The FT-IR bands (cm$^{-1}$) of chitin isolated from a house cricket, *B. portentosus.*

<table>
<thead>
<tr>
<th>Functional group and vibration modes</th>
<th>Classification</th>
<th>House cricket chitin</th>
<th>Commercial shrimp chitin</th>
<th>Other commercial chitin</th>
</tr>
</thead>
<tbody>
<tr>
<td>O–H stretching</td>
<td></td>
<td>3433</td>
<td>3431</td>
<td>3437</td>
</tr>
<tr>
<td>N–H stretching</td>
<td></td>
<td>3103–3257</td>
<td>3105–3259</td>
<td>3101–3259</td>
</tr>
<tr>
<td>CH$_3$ symmetrical stretch and CH$_2$ asymmetric stretch</td>
<td>Aliphatic compounds</td>
<td>2881</td>
<td>2889</td>
<td>2937</td>
</tr>
<tr>
<td>CH$_3$ symmetrical stretch</td>
<td></td>
<td></td>
<td></td>
<td>2867</td>
</tr>
<tr>
<td>C–O secondary amide stretch</td>
<td>Amide I</td>
<td>1653</td>
<td>1653</td>
<td>1654</td>
</tr>
<tr>
<td>C–O secondary amide stretch</td>
<td>Amide I</td>
<td>1622</td>
<td>1622</td>
<td>1620</td>
</tr>
<tr>
<td>N–H bend, C–N stretch</td>
<td>Amide II</td>
<td>1554</td>
<td>1554</td>
<td>1553</td>
</tr>
<tr>
<td>CH$_2$ ending and CH$_2$ deformation</td>
<td></td>
<td>1423</td>
<td>1415</td>
<td>1430</td>
</tr>
<tr>
<td>CH bends CH$_3$ symmetrical deformation</td>
<td></td>
<td>1375</td>
<td>1375</td>
<td>1376</td>
</tr>
<tr>
<td>CH$_2$ wagging</td>
<td>Amide III, components of protein</td>
<td>1311</td>
<td>1307</td>
<td>1318</td>
</tr>
<tr>
<td>Asymmetric bridge oxygen stretching</td>
<td></td>
<td>1153</td>
<td>1153</td>
<td>1155</td>
</tr>
<tr>
<td>Asymmetric in-phase ring stretch- ing mode</td>
<td></td>
<td>1112</td>
<td>1112</td>
<td>1114</td>
</tr>
<tr>
<td>C–O–C asymmetric stretch in phase ring</td>
<td>Saccharide rings</td>
<td>1066</td>
<td>1068</td>
<td>1068</td>
</tr>
<tr>
<td>C–O asymmetric stretch in phase ring</td>
<td></td>
<td>1014</td>
<td>1010</td>
<td>1024</td>
</tr>
<tr>
<td>CH$_3$ wagging</td>
<td>Along chain</td>
<td>952</td>
<td>952</td>
<td>952</td>
</tr>
<tr>
<td>CH ring stretching</td>
<td>Saccharide rings</td>
<td>896</td>
<td>894</td>
<td>896</td>
</tr>
</tbody>
</table>

References

Current study

Current study

(Kaya et al 2015b)
pores that are surrounded by numerous nanopores on a smooth surface devoid of fibres (figure 3(b)). Both nanopores and ordinary pores are found in house cricket chitin with a range of 67.7 nm to 0.27 μm, and most of the big pores are oval in shape. Also, at a magnification of ×1500, a large thread-like fibrous structure ranging 0.30 to 0.89 μm was observed in some other parts of the house cricket chitin surface (figure 3(c)). For the commercial shrimp chitin, on the other hand, at higher magnification (×20 k) there were only fibres on its cracked surface structure (figure 3(d)). The general surface morphology of house cricket chitosan at a lower magnification (×400) presents a smooth surface, with big pores and fibres (figure 3(e)). When evaluated at a different magnification (×2000 and ×8000), the pore arrangement is like that found in the chitin counterpart, but the pores in the house cricket chitosan are less numerous when compared to the cricket chitin, and they are found in combination with nanofibres (figures 3(f) and (g)) unlike in the house cricket chitin. The pore size in the house cricket chitosan ranges from 72.1 nm to 0.12 μm, in contrast to the commercially obtained chitosan from a shrimp, which has a cracked-like surface morphology only (figure 3(h)).

**Discussions**

As shown in table 1, the chitin content of the house cricket in this study favourably compares and competes with those of microcrustaceans and some insects;
for example 3%–7% in Daphnia (Cauchie et al 1995), 5.3%–8.9% in grasshoppers (Kaya et al 2015b), beetles (5%) (Marei et al 2016) and from spider species Geolycosa vultuosa (8%–8.5%) and Hogna radiate (6.5%–7%) (Kaya et al 2014b). It is, however, lower when compared to those of some commercial organisms (crab: 13%–26%, shrimp: 14%–42% and krill: 34%–49%) (Synowiecki and Al-Khateeb 2003). The chitin yield in this study is also lower compared to the results from other insects, like 8.5% in field crickets (Wang et al 2004) 18%–21% in the Daphnia magna resting egg (Kaya et al 2013a) and 15% in Holotrichia parallela (Liu et al 2012). This may be due to the difference in the species of crickets and insects used, as it has been reported that different species affect the yield of chitin in insects (Kaya et al 2015b). Moreover, it could also be the result of the small amount of wings possessed by the house cricket and the difference in the extraction methods used. It has been reported that the wings from a butterfly species (Argynnis pandora) yielded a higher amount of chitin (22%) than the body parts (8%) (Kaya et al 2015a). Also, sex can dictate chitin yield in insects, as a Decticus verrucivorus male yields (11.84%) more chitin than the Melanogryllus desertus female (4.71%) (Kaya et al 2015c), whereas the crickets used in this study were mixed sex. Hence, the house cricket stands as a promising alternative chitin source among other insects. Furthermore, the sequence of extraction (deproteinization then demineralization and decolourization) has been reported to lead to a decrease in chitin yield. Deproteinization before demineralization erodes the protein layer that protects the material matrix, so the chitin becomes unprotected and completely exposed to acidic treatment, leading to the extensive removal of inorganic materials, with significant hydrolysis and loss of solid material in the chitin fraction, leading to the low yield of chitin (Lertsutthiwong et al 2002). The chitosan yield of this stands at 2.4%–5.8% on a dry matter basis. This is higher in comparison to the yield of 0.24% from the American cockroach (Periplaneta americana).
americanawere) (Wanule et al 2014). This may be the result of different species of organism being used.

It has been suggested as a reference that the chitin of shrimp should possess about 0.21% moisture (Ambarish and Sridha 2015), but according to the Korea Food Additive Code (KFDA) (1995), the moisture content of chitosan powder should be lower than 10%, indicating that the chitosan from a house cricket is of acceptable quality. The moisture content of the chitin from a house cricket is higher than previous reports from some other insects (Ambarish and Sridha 2015), and this may be due to the high moisture content of fresh house crickets (73.7%) as determined by this study. This has been supported by a study conducted by (Woodring et al 1977) who reported 68.4% moisture. This may be explained by the fact that at 2–3 days to the end of the 7th–8th instars, food consumption initially stops but the cricket continues to consume water, and therefore there is no loss of either dry or wet weight (Woodring et al 1977, Roe et al 1980). On the other hand, the chitosan isolated in this study had a lower moisture content when compared with a previous report (Lertsutthiwong et al 2002). This may be attributed to the difference in the animal species and the methods used. The ash content of chitin is an indication of the effectiveness of the demineralization process. This study indicates a reduction of 85.2% in ash content, and it implies that the demineralization of the house cricket with 0.079 M oxalic acid for 3 h at room temperature was effective in removing enough organic salt from it. A high-quality grade of chitin and chitosan should have less than 1% ash content (Nessa et al 2010), and although the ash content of chitin and chitosan in this study was 1.0%, it is still said to be of high quality. Also, in terms of ash content, the chitosan in this study is far better than that isolated from honey bees (9.2%), shrimps (9.0%), beetles (2.0%) and locusts (1.6%) (Marei et al 2016). This may be attributed to the difference in species studied and methods of extraction. The ash levels for both the chitin and chitosan in this study are higher than those obtained isolated from fresh and frozen shrimp shells (<1%)
(Toan 2009). This may be due to the higher concentration of hydrochloric acid and longer period of time of 6 h used for demineralization in the study of (Toan 2009), as against 0.079 M oxalic acid and 3 h used for this study.

From elemental analyses, this study reveals a percentage nitrogen (N) of 5.72% for cricket chitin and 5.98% for cricket chitosan. This is in agreement with the report of (Fadel El-Seed et al 2003) that commercial chitin and chitosan contain 58.8 mg g\(^{-1}\) and 67 mg g\(^{-1}\) N\(^{-1}\) respectively. The N content of chitin in this study is lower compared to that of earlier reports in other insects: 6.42% for Geolycosa vultuosa, 6.41% for Hognia radiata (Kaya et al 2014), 5.92% for bumblebee chitin (Majtán et al 2007) and 6.3% for Holotrichia parallela (Liu et al 2012). The usual value (6.89%) obtained for fully acetylated chitin in previous studies (Liu et al 2012, Kaya et al 2014) was higher than the values of 6.02% and 4.79% obtained for cricket and shrimp chitin respectively in the present study. A lower amount of nitrogen signifies the minimum residual protein remaining in chitin (Majtán et al 2007, Iwshina et al 2009); this is also a pointer to effective deproteinization. Also, the percentage N of chitin and chitosan is extremely important as it indicates the level of purity of the product. The N content of completely acetylated (pure) chitin is known to be 6.89% (Majtán et al 2007, Sajomsang and Gonil 2010, Liu et al 2012). At N levels above 6.89%, this suggests that there are protein remnants in the chitin sample, while below 6.89% this indicates the presence of inorganic materials (Sajomsang and Gonil 2010). It can therefore be said that the chitin extracted from house crickets is not completely in a pure form, but that it is purer than commercial chitin, as it may contain some inorganic materials, as suggested by the lower percentage N of 4.79% (Kaya et al 2014). However, house cricket chitin contains a higher N level than fungi (2.96%). This is because fungi possesses chitin-glucan residues (Iifu et al 2011) which cannot be removed completely by chemical processes, increasing the N content of the fungi chitin.

Considering the results of FT-IR, these studies explain that the 3442, 3267–3105, 1660, 1550 and 1310 cm\(^{-1}\) characteristic bands for chitin are attributed to O-H stretching, N-H stretching and amides I, II and III, respectively. In this study, all these characteristics bands are present and similar in the chitin extracted from the house cricket and those obtained commercially from shrimp. Chitin isolated from the B. portentosus (table 3 and figure 1) possesses peaks at 3433, 3257–3103, 1653, 1622, 1554 and 1311 cm\(^{-1}\) and is similar to that of other insects (Zhang et al 2000, Sajomsang and Gonil 2010, Kaya et al 2013a, Kaya et al 2015b, Kaya et al 2015d), as well as to that obtained from the shrimps evaluated in this study (3431, 3239–3105, 163, 1622, 1554 and 1307 cm\(^{-1}\)). Also, in agreement with some previous reports (Cardenas et al 2004, Jang et al 2004), the amide I band of B. portentosus and commercial shrimp chitin splits into two bands appearing at 1653 and 1622 cm\(^{-1}\) (figure 1), indicating that the chitin isolated from the house crickets in this study and that commercially obtained from shrimp is in the α-crystal form state (Cardenas et al 2004, Kaya et al 2013a, Kaya et al 2014a, Kaya et al 2014c, Kaya et al 2014e, Kaya et al 2014f, Kaya et al 2015b, Kaya et al 2015d, Marei et al 2016). For house cricket and shrimp chitin, some other strong and broad bands were also respectively observed at 3433 and 3431 cm\(^{-1}\) (O-H stretching), 3105–3257 and 3105–3259 cm\(^{-1}\) (N-H stretching), 2881 and 2889 cm\(^{-1}\) (aliphatic compounds), 1622–1650 and 1622–1653 cm\(^{-1}\) (amide I), 1554 and 1554 cm\(^{-1}\) (amide II), 1423 and 1415 cm\(^{-1}\) (CH\(_2\) bending and CH\(_3\) deformation), 1375 and 1375 cm\(^{-1}\) (CH bend, CH\(_3\) symmetrical deformation), 1311 and 1307 cm\(^{-1}\) (CH\(_2\) wagging), 1153 and 1153 cm\(^{-1}\) (asymmetric bridge oxygen stretching), 1112 and 1112 cm\(^{-1}\) (asymmetric in-phase ring stretching mode), 1066 and 1068 cm\(^{-1}\) (saccharide rings), 1014 and 1010 cm\(^{-1}\) (C-O asymmetrical stretching in-phase ring), 952 and 952 cm\(^{-1}\) (along chain) and 896 and 894 cm\(^{-1}\) (saccharide rings), and they agreed with other reports (Iifu et al 2009, Kaya et al 2014b, Kaya et al 2014e, Kaya et al 2015b). It was also observed that the 1540 cm\(^{-1}\) absorption band, which is attributed to protein, was totally absent in both cricket and shrimp chitin, hence confirming the effectiveness of deproteinization (Majtán et al 2007). From the FT-IR results, we can conclude that there is a very close similarity between the chemical composition and bonding types of chitin from the house cricket and shrimp chitin obtained commercially as well as for other insects and commercial chitin. Hence, house cricket chitin can be used in place of commercial shrimp chitin.

The spectra of house cricket chitosan were similar to those of commercial shrimp, and others extracted from insect and shrimp chitosan (Song et al 2013, Marei et al 2016). Chitosan from the house cricket exhibited a broad band at 3263 and 3421 cm\(^{-1}\) (figure 2) and 3358 cm\(^{-1}\) in shrimp chitosan, respectively corresponding to the stretching vibration of N-H and O-H, the extension vibration of N-H and the intermolecular hydrogen bonds of polysaccharide. The absorption bands at 2920 and 2881 cm\(^{-1}\) for cricket chitosan and 2900 and 2877 cm\(^{-1}\) for shrimp chitosan were assigned to the asymmetric and symmetric C-H stretching vibration, respectively. As expected, an evident absence of the split amide I band was observed in both cricket and commercial shrimp chitosan (figure 2) with a prominent characteristic band at 1556 cm\(^{-1}\) revealing the success of N-deacetylation in both types of chitosan. The absorption at 1425 and 1375 cm\(^{-1}\) and 1419 and 1377 cm\(^{-1}\) for cricket and commercial shrimp, respectively, was due to the C-N stretching vibrations. The band at 1303 (chitosan) and 1317 cm\(^{-1}\) (shrimp chitosan) were attributed to the O-H bending vibration (Crews
et al 1998). The C-O stretching vibration in the secondary alcohol was respectively evaluated for cricket and shrimp chitosan at a wave number of 1153 and 1151 cm⁻¹. Nevertheless, a C-O stretching vibration in the alcohol was seen at 1016 (chitin chitosan) and 1026 cm⁻¹ (shrimp chitosan). Also, absorption at 896 and 894 cm⁻¹ was ascribed to the C-H out-of-plane vibration of the ring of monosaccharides. From the FT-IR results we can suggest that the similarity between the chemical composition and bonding types of chitosan in the house cricket and commercial shrimp are very close, and can therefore be substituted for each other during utilization.

The same DA value of 108.1% was recorded for chitin extracted from B. portentosus in this study and that commercially obtained from shrimp. However, it is slightly higher than that previously reported for other shrimp (94.3%) and Holotrichia parallela (93.1%), determined using the same formula (Liu et al 2012), as well as that from bumblebees (87.3%) and shrimp α-chitin (99.0%) calculated using a different formula (Majtán et al 2007). Also, our result is slightly higher when compared with other insects such as locusts (98%), honey bees (96%) and beetles (95%) (Marei et al 2016). These differences may be attributed to different methods of chitin isolation as well as differences in the sources. A DA greater than 100% is an indication that some inorganic materials are left in the polymer structure (Sajomsang and Gonil 2010). In this case, our results speculate that some inorganic materials may still be present in the chitin samples extracted from B. portentosus and those from commercial chitin, and this is further corroborated by the slightly lower percentage of N in these products. Nevertheless, the extracted chitin from crickets in this study is still of acceptable purity, and in some instances is purer when compared to that obtained in other studies. For instance, the DA is 101.02% in shrimps (Liu et al 2012), 132.5% in bumblebees (Majtán et al 2007), 151.7% in crude crabs (Yen et al 2009), 239.76% in Lentinula edodes, 377.9% in Grifola frondosa and 560.9% in Hypsizygus marmoreus (Kaya et al 2014d).

It has been reported that the physicochemical and functional properties of chitosan are considerably affected by DD (Teng et al 2001, Chang et al 2003, Tsaih and Chen 2003). The DD of house cricket chitosan in this study is lower than that in previous ones (Marei et al 2016), and this could be due to the different concentration of alkali, temperature and duration used (Chang et al 2003, Tsaih and Chen 2003, Chen et al 2004, Yen et al 2009). It is, however, the same as that of commercially extracted chitosan from shrimp and as evaluated in this study, as well as from other studies (Hossain and Iqbal 2014). This may be due to the fact that the same concentration (50%) of NaOH was used in our study and that of (Hossain and Iqbal 2014). According to No and Meyers (No and Meyers 1997), the DD of chitosan ranges from 56% to 99%, therefore the chitosan produced from house crickets is of acceptable quality.

Consistent with this study, previous studies reported that α-chitin has two characteristic sharp peaks at 9° and 19° (Jang et al 2004), while four other weak peaks were observed at 12°, 21°, 25° and 26° (Yen et al 2009, Sajomsang and Gonil 2010, Juárez-de la Rosa et al 2012, Liu et al 2012). The peaks observed in this study are suggestive of α-chitin and are similar to those XRD results of α-chitin produced by crab, shrimp, krill and insects (Yen et al 2009, Sajomsang and Gonil 2010, Liu et al 2012, Wang et al 2013, Kaya et al 2014f, Kaya et al 2015b). The peaks at 9.4°–39.1° observed for cricket chitin extracted in this study and commercial shrimp chitin were similar to the report of Kaya et al 2014b. Two sharp peaks characteristic of chitosan at approximately 9°–11° and 19°–21° are consistent with this current study—both the extracted chitin from cricket and commercial shrimp chitin—and this is in agreement with the reports of Kucukgulmez et al 2011, Wang et al 2013, Kaya et al 2014b. This does, however, differ from the work of Zaku et al 2011, who showed that the XRD of chitosan from fish had crystalline reflections at 14.4°, 20.0°, 26.7°, 37.3° and 54.3°. The results of this study suggested that N-deacetylation and purification were not able to alter the natural crystallinity of house cricket chitin and chitosan. Many other previous studies report that chitosan usually presents two sharp XRD peaks (Liu et al 2012, Kaya et al 2016). In this study, however, there is a third sharp peak at around 20.0° (cricket chitosan) and 21.1° (shrimp chitosan), just as reported in beetles and locust chitosan (Marei et al 2016). Also, there is an indication, as suggested by the presence of an extra XRD peak at 26.30°, that some inorganic material may be left in the chitin extracted from house crickets in this study (Kaya et al 2014d).

The CrI values of chitin and its derivatives play a major role in the determination and effectiveness of their application (Aranaz et al 2009). In this study, the CrI (88.02%) of chitin obtained from house crickets is favourably compared to that of commercial chitin from shrimp (90.43%). This is also consistent with other insects: Holotrichia parallela (89.05%) and cicada sloughs (89.7%) (Liu et al 2012). This might be due to the similar method of extraction and calculation used. However, house cricket chitin is much more crystalline than the 54% and 58% that was respectively obtained in chitin from larva cuticles and silkworm pupa exuviae (Bombymx mori) (Zhang et al 2000). This may be as a result of the difference in species, the method of chitin extraction and the purity of the chitin used. It has been speculated that the high molecular weight compound catechol that is left in the cricket chitin may be responsible for the high crystallinity (Liu et al 2012).

The chitin surface morphology of house crickets reported at higher magnification (×8000) in this study is similar to that of spiders, but the chitin isolated here
has fewer nanopores when compared to those of spiders (Kaya et al 2014f). The pores observed in the chitin and chitosan from this study are much larger than previously reported from other insects and commercial chitin (Ifuku et al 2011, Kaya et al 2014f). This may be due to the fact that chitin and chitosan isolated from different parts of an insect’s body and from different sexes has differing surface morphology, and this has been previously stated (Kaya and Baran 2015, Kaya et al 2015b, Kaya et al 2016). The presence of pores and fibres combined in this study is in agreement with previous studies on chitin and chitosan from crustaceans such as pink shrimp, krill and Gamma marus argeus as well as from other insects (Kucukgulmez et al 2011, Kaya et al 2013a, Wang et al 2013, Kaya et al 2016). Five different surface structures have been reported until now for chitin, and can also be used to define chitosan. They include (1) a hard and rough surface morphology with neither pores nor fibres, (2) surfaces with pores only, (3) a combination of fibres and pores, (4) surfaces with two types of pores with fibres, (5) and only fibres (Kaya et al 2014f, Kaya and Baran 2015). However, these different reported morphologies depend on the magnification of the area being viewed. Therefore, in the present study as a general observation, i.e. at lower magnification, we observed a mixed type of surface morphology for chitin and chitosan extracted from the house crickets, which has not been previously reported — i.e. a combination of smooth, rough, flaky, porous and fibrous chitin surface morphology (figures 3(a) and (g)). On the other hand, at higher magnifications in this study, the surface morphology of chitin resembles that of (2) and (5) above, i.e. only having pores (figure 3(b)) and fibres (figure 3(c)), while chitosan resembles that of (3) above, a combination of fibres and pores (figure 3(g)). The standard surface morphology of α-chitin and chitosan can be said not to have been established yet. This study reported that chitin and chitosan extracted from the same species of organism (house crickets) had different surface morphologies. Therefore, it is possible to vary the utilization of such chitin and chitosan isolated from the same species of organism, but having different surface morphologies.

Conclusion

This study has investigated the extraction of chitin from the house cricket (B. portentosus) using a chemical method. The low amount of ash and nitrogen content indicates the effectiveness of the chitin extraction method. The chitin extracted from B. portentosus is found suitable for the production of chitosan. The characteristics of the chitin and chitosan from B. portentosus were similar to those of commercial chitin extracted from shrimp, as evaluated by infrared spectroscopy, x-ray diffraction, scanning electron microscopy and elemental analysis. Therefore, B. portentosus is a suitable alternative source of chitin. In addition, chitin and chitosan extracted from B. portentosus can generally be said to be of better quality and purity than commercially produced chitin and chitosan from shrimp, making it a better alternative in terms of utilization and applications. Also, the utilizations of chitin and chitosan from B. portentosus in various fields as well as their effects on animal health and production should be evaluated.

ORCID iDs

E B Ibiteye @ https://orcid.org/0000-0003-3456-0009

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