Organic and mineral acid demineralizations: effects on crangon and Liocarcinus vernalis – sourced biopolymer yield and properties

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ABSTRACT
Chitin is chemically extracted from crustacean shells whose composition and structure differ from one another. It is reported that mineral acids distort chitin’s physiochemical properties compared to organic acids. Investigations on the effectiveness of these acids on chitin isolation from crab and shrimp exoskeletons are carried out. Shell particles were demineralized using acetic acid (CH$_3$COOH) and HCl while NaOH was used for deproteinization. Acetic acid possesses low potency for complete CaCO$_3$ removal from crab shell while it fully demineralizes shrimp shell. With the use of CH$_3$COOH, the biopolymer extracted shows characteristics of chitin but with lower content and physicochemical properties compared to chitin isolated from the shells using HCl. Mineral acid (HCl) will thus isolate chitin from exoskeletons of marine invertebrates irrespective of their shell nature while organic acid (CH$_3$COOH) will be effective for soft shells whose embedded chitin content is more than the mineral content such as shrimp shells.

1. Introduction
The need to reduce society’s dependence on fossil fuels has led to significant research efforts at the conversion of biomass. Remarkable progress is being made in the development and commercialization of usable products derived from waste materials which are abundant and cheap. Most living organisms are made of molecules that exhibit features and properties which constitute an inspiring reserve for the development of outstanding engineering products. Polysaccharides are biopolymers constituted by carbohydrate monomers (normally hexoses) linked by glycosidic bonds. The second most abundant structural polysaccharide after cellulose is chitin, which is composed of β(1 → 4) linked units of N-acetyl-2-amino-2-deoxy-D-glucose [1–2]. Chitin is present in insect’s body wall (cuticle), gut lining (peritrophic matrix, PM), salivary gland, trachea, eggshells and muscle attachment points. These structures are primarily composites of chitin fibres and proteins (some of the chitins are covalently linked to protein) with varying degrees of hydration and trace materials distributed along the structures [3–4]. The composition and biosynthesis of chitin have been studied in insects and terrestrial crustaceans such as beetle, cockroaches, honeybees, silkworms, Drosophila melanogaster (fruit fly) and land crabs [3–4,5]. In marine crustaceans, chitin has been extracted from sea crabs, shrimps, prawn, periwinkle and lobsters [6–8]. The composition and structure of their organic matrices, however, may be different from one another. Organic matrix of crustacean shell contains chitin–protein fibres that are associated with various amounts of calcium carbonate, CaCO$_3$ [9]. A traditional method for the commercial preparation of chitin from crustacean shell (chemical method) consists of two basic steps that include demineralization which entails CaCO$_3$ separation by acidic treatment and protein separation (deproteinization by alkali treatment), followed by a bleaching stage with chemical reagents to obtain a colourless product [6,10–12]. During chitin isolation, hydrochloric acid, HCl and sodium hydroxide NaOH seem to be the preferred reagents for CaCO$_3$ and protein removal as they are often been used in many studies. Chitin extracted from mussel shell has been characterized by Abdulkarim et al. [13] where 0.68 M HCl and 0.62 M NaOH solutions were used during the extraction process. The mussel shell was discovered to contain 51.62% CaCO$_3$ and 21.32% chitin. These acid and alkali solutions (though with varied concentrations) have also been used to isolate chitin from Gammarus crangon and Liocarcinus vernalis, adult beetle [12], silkworm [5], carp fish scales [11], bat guano [10], crab and shrimp shells [6]. Mineral acid such as HCl has been confirmed to cause harmful effluents and cause high cost of chitin purification [15]. This has culminated in the use of organic acids such as lactic (CH$_3$CH (OH) COOH) and acetic acid (CH$_3$COOH) for demineralization process because of the lesser harm they pose on the environment [16]. Greene et al. [17] reported that the use of CH$_3$CH (OH) COOH or CH$_3$COOH could be employed during demineralization process as HCl can result in significant modification of the physicochemical properties of chitin such as decrease in molecular...
and significant deacetylation (DA) of the chitin can also occur. This is justified from recent studies [18] where varying concentrations of HCl were used in removing CaCO3 from exoskeletons of crab and shrimp. Results showed that for each shell, the DA, thermal stability, crystallinity and hydrogen bond strength of extracted reduced as the concentration of HCl increased. Earlier studies [19] affirm that CaCO3 is more predominant and strongly bonded to embedded chitin in the crab shell than that of the shrimp. Ameh et al. [16] were able to successfully study shrimp shell demineralization kinetics with CH3CH(OH)COOH using the shrinking core model. Response surface methodology was developed in the prediction of shrimp shell demineralization with CH3COOH [20]. In their works, predicted values by the model and the experimental values of the demineralization agreed closely. Brummer et al. [21] reported an effective isolation of chitin from the skeleton of the marine demosponge Ianthella basta, a composite material containing organic constituents with CH3COOH. In this study, the potency of organic acid such as CH3COOH in the demineralization process during chitin extraction is investigated and evaluated on chitin’s physicochemical properties including realized content in comparison with properties obtained using a similar concentration of HCl.

2. Material and methods

2.1. Chitin extraction

Shells of crab and shrimps were washed, dried and ground to powder prior to demineralization. During this process, powders were refluxed in 1M HCl and ground to powder prior to demineralization. During this operation, the temperature for the onset of thermal decomposition (T,onset) and the content of chitin were deduced from the thermograms. To further study the thermal stability of constituents, the activation energy (Ea) for their thermal degradation was calculated from the TGA curves using the Broido method [22] according to Equation (1):

\[ \ln(\frac{-\ln(1-X)}{T}) = -\frac{Ea}{RT} + \text{Const.} \]  

Ea is the activation energy of the degradation reaction (kJ/mol), R is the universal gas constant (8.314 J/mol K) and T is the absolute temperature (K). The degree of decomposition X is given by:

\[ X = \frac{(W_0-W_f)}{(W_0-W_i)} \]  

where \(W_0\) is the initial weight of the sample, \(W_i\) is the instantaneous weight of the sample at time \(t\) and \(W_f\) the final weight of the sample. The plot of \(\ln(-\ln(1-X))\) against \(1/T\) gives a straight line whose slope is \(-Ea/R\).

2.2. Fourier transform infrared spectroscopy (FTIR)

A Nicolet 6700 M spectrometer in transmission mode was used in carrying out FTIR spectra of samples. Ten milligrams of fine samples were dispersed in a matrix of KBr (500 mg), followed by compression at 22–30 MPa to form pellets. The transmittance measurements were carried out in the range 400–4000 cm\(^{-1}\) at a resolution of 4 cm\(^{-1}\).

2.3. Thermogravimetric analysis (TGA)

TGA was used to quantitatively determine the constituent of minerals and organic compound (including the biopolymer) in the samples. Analysis of samples was carried out on TGA Q500 instrument where 2 mg of samples was heated to 750°C at 10°C/min heating rate. During this operation, the temperature for the onset of thermal decomposition (T,onset) and the content of chitin were deduced from the thermograms. To further study the thermal stability of constituents, the activation energy (Ea) for their thermal degradation was calculated from the TGA curves using the Broido method [22] according to Equation (1):

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where \(W_0\) is the initial weight of the sample, \(W_i\) is the instantaneous weight of the sample at time \(t\) and \(W_f\) the final weight of the sample. The plot of \(\ln(-\ln(1-X))\) against \(1/T\) gives a straight line whose slope is \(-Ea/R\).

2.4. Scanning electron microscopy/electron dispersive spectroscopy (SEM/EDS) analysis

An ASPEX 3020 model variable pressure SEM operated with an electron intensity beam 15 kV and equipped with Noran-Voyager energy dispersive spectroscope was used to observe the morphological features of all samples. The samples to be observed under the SEM were mounted on a conductive carbon imprint left by the adhesive tape prepared by placing the samples on the circular holder and coated for 5 min to enable it conduct electricity. For EDS analysis, samples were analysed at an accelerating voltage of 15 kV.

2.5. X-ray diffraction (XRD)

The XRD of each sample was conducted using PANalytical X’ Pert PRO MDX X-ray diffraction system PW3040/60 machine. Samples were exposed to a monochromatic Cu K\(\alpha\) radiation (\(k = 1.5406 \AA\)), operating at 40 kV and 40 mA. Crystallinity index (CrI) determination for chitin was done using Equation (3) [23].

\[ \text{CrI} = \left( \frac{l_c}{l_c + l_a} \right) \times 100 \]  

where \(l_c\) and \(l_a\) represent the intensities of the crystalline and amorphous regions, respectively.
3. Results and discussion

3.1. FTIR

Figure 1 shows the FTIR spectra of shells before and after treatment with HCl and CH₃COOH. The dominant mineral CaCO₃ exists at 1473 and 874 cm⁻¹ in the virgin crab shell (Figure 1(a) and (b)) and forms at 1443 and 874 cm⁻¹ on CH₃COOH crab chitin sample but with reduced intensities. This implies that the concentration of CH₃COOH is not sufficient to fully isolate chitin which is strongly bonded to CaCO₃. Demineralization with the concentration of HCl (Figures 1(a) and (b)) produces sample whose spectrum is devoid of CaCO₃ peaks. Its functional groups (as shown in Table 2) are typical of chitin peaks [24], expressing HCl’s potency of chitin isolation from CaCO₃. Using HCl and CH₃COOH on shrimp shell completely isolate chitin after deproteinization as CaCO₃ which is initially absorbed at 1456 and 844 cm⁻¹ (Figures 1(c) and (d)) is completely eliminated after treatment. Functional groups of HCID and CH₃COOHD shrimp chitin is shown in Table 2 while that of virgin crab and shrimp shells are shown in Table 1.

Using Equation (4), the amount of acetyl group removed during extraction as characterized by chitin’s DA during demineralization with HCl and HCOOH and deproteinization with NaOH was calculated:

\[
DA = \left( \frac{A_{1650}}{A_{3450}} \right) \times 100 / 1.33
\]  (4)

where \(A_{1650}\) is the absorbance of amide I vibration; \(A_{3450}\), absorbance of OH vibration; 1.33 is a factor that represents the ratio of \(A_{1650}/A_{3450}\) for fully N-acetylated chitin.

The DA of HCID and CH₃COOHD shrimp chitin samples are 95.9% and 77.9%, respectively. This implies that more of N glucosamine units are maintained when shrimp shell is demineralized with HCl other than CH₃COOHD prior to deproteinization with NaOH. Chitin extracted from crab shell with HCl possesses DA of 90.7%. Increasing amount of acetyl groups (function

![Figure 1. FTIR of crab samples (a) between 1800 and 1300 cm⁻¹ (b) 1100–700 cm⁻¹ (c) shrimp samples between 1800 and 1300 cm⁻¹ and (d) 1100–700 cm⁻¹.](image-url)
of increasing DA) imparts improved resistance to thermal, chemical [19] and mechanical degradation [25] on the biopolymer with shrimp chitin having the better property of the two shells. More detailed OH and NH (3600–3000 cm$^{-1}$) groups are exhibited by HCl crab chitin sample compared to that observed in the CH$_3$COOH crab chitin and the virgin sample (Figure 2(a)). This is typical of structural polysaccharides and attributed to the different packing arrangements of the macromolecules. The shoulder that appears in the HCl chitin spectrum at 3491 cm$^{-1}$ is attributed to the intramolecular hydrogen bond involving the OH(6) ... O = C which is missing in the virgin shell and CH$_3$COOH crab chitin. A band appearing at 3447 cm$^{-1}$ corresponds to the intramolecular hydrogen bond O(3)H ... O(5) from the ring. This vibration appears at 3446 and 3440 cm$^{-1}$ in the spectra of virgin shell and CH$_3$COOH chitin respectively. Vibrational modes of the intermolecular hydrogen bond C = O ... HN and OH ... OC are represented by 3267 and 3099 cm$^{-1}$ respectively. This NH amide vibration (C = O ... HN) appears at a lower frequencies of 3247 and 3261 cm$^{-1}$ in the virgin shell and CH$_3$COOH crab chitin. While OH ... OC bond shows a small peak at 3097 cm$^{-1}$ in CH$_3$COOH crab chitin and absent in the virgin sample. In addition, there exist symmetric CH$_3$ stretching and asymmetric CH stretching at 2948 and 2926 cm$^{-1}$ (Figure 2(a)) in HClD crab chitin spectra; vibration at 2865 cm$^{-1}$ shows aliphatic CH stretching, which also exists in virgin shell and CH$_3$COOH crab chitin spectra at 2886 cm$^{-1}$.

The absorbance spectrum between 3800 and 2600 cm$^{-1}$ for virgin shrimp shell (Figure 2(b)) shows more pronounced peaks than the virgin crab shell and having almost similar peaks as HClD and CH$_3$COOH shrimp chitin samples (Figure 2(c)–(d)). Intra molecular hydrogen bonds OH(6) ... O = C and O(3)H ... O(5) with intermolecular hydrogen bonds C = O ... HN and OH ... OC exist on 3489, 3442, 3257 and 3007 cm$^{-1}$ respectively for HClD shrimp chitin (Figure 2(d)). In the same manner, these intra and inter molecular hydrogen bonds appear at 3468, 3449, 3264 and 3098 cm$^{-1}$ for CH$_3$COOHD shrimp chitin (Figure 2(c)). Comparing these bonds with that of virgin shrimp, OH(6) ... O = C bond does not exist while vibrations at 3425, 3264 and 3101 represent O(3)H ... O(5), C = O ... HN and OH ... OC (Figure 2(b)).

### 3.2. TGA

Thermal decomposition curves for virgin crab shell, HClD and CH$_3$COOHD crab and shrimp samples are presented in Figure 3. Presence of chitin (10.4%) embedded in the virgin crab shell is stable between 316°C and 397°C while 30.9% CaCO$_3$ is observed within the range of 682 and 729°C as it decomposes to CaO and CO$_2$ within the temperature. Chitin content is further increased to 16.1% with the use of CH$_3$COOH while CaCO$_3$ content is reduced to 19% (Figure 3a). Using this organic acid has also improved the thermal stability of CH$_3$COOHD crab chitin as the temperature at which the biopolymer decomposes ($T_{\text{onset}}$) is 320.7°C. The existence of CaCO$_3$ within 676–753°C however, still shows that demineralizing with CH$_3$COOH can increase the content of chitin and lower the amount of CaCO$_3$ from crab shell without full isolation of the biopolymer. The maximum content of chitin is obtained when treated with HCl as 70% of the biopolymer decomposes within 340°C and 436°C without evidence of CaCO$_3$ decomposition. Existing with the greatest magnitude of $T_{\text{onset}}$, the highest thermal energy will be required to separate CH$_2$, CH$_3$ functional groups (aliphatic compounds) from the structural ring of HClD than HCOOHD crab chitin. This will intern influence the necessity of a higher

| Vibration modes | HClD crab chitin | CH$_3$COOHD crab chitin | HClD shrimp chitin | CH$_3$COOHD shrimp chitin |
|----------------|-----------------|-------------------------|-------------------|--------------------------|
| OH stretching  | 3491,3447       | 3446                    | 3489,3442         | 3468,3449                |
| NH stretching  | 3267            | 3261                    | 3257              | 3264                     |
| NH stretching  | 3099            | 3097                    | 3097              | 3098                     |
| Symmetric CH$_3$ stretching and asymmetric CH$_2$ stretching | 2948,2926 | –                      | 2961,2931          | 2959,2923                |
| CH stretching  | 2865            | 2886                    | 2885              | 2891                     |
| C = O secondary amide stretch (amide I) | 1662          | 1660                    | 1661              | 1660                     |
| C = O secondary amide stretch (amide I) | 1628          | 1630                    | 1626              | 1633                     |
| NH bend, CN stretch (amide II) | 1559          | 1550                    | 1559              | 1559                     |
| CH$_2$ bending and CH$_3$ deformation | 1417          | –                      | 1416              | 1418                     |
| CH bending and CH$_3$ symmetric deformation | 1379          | –                      | 1379              | 1379                     |
| CH$_2$ wagging (amide III) | 1314          | 1317                    | 1312              | 1315                     |
| Asymmetric bridge oxygen stretching | 1157          | 1157                    | 1157              | 1157                     |
| Asymmetric in-phase ring stretching mode | 1114          | 1111                    | 1117              | 1111                     |
| C–O–C asymmetric stretch in-phase ring | 1074          | 1072                    | 1074              | 1072                     |
| CO stretching  | 1028            | 1030                    | 1028              | 1071                     |
| CH$_3$ wagging along chain | 953           | 953                     | 953               | 953                      |
| CH stretching (saccharide rings) | 893            | –                      | 897               | 897                      |
| NH out-plane bending | 750           | 713                     | 750               | –                        |
| OH out-of-plane bending | 694           | 606                     | 694               | 693                      |
| Calcium carbonate | –             | 1443,874                | –                 | –                        |
Figure 2. FTIR of samples (a) virgin crab shell, CH₃COOH and HCl crab chitin (b) Virgin shrimp shell (c) CH₃COOH shrimp chitin (d) HCl shrimp chitin samples between 3600 and 3000 cm⁻¹.

Figure 3. TGA curves of virgin shell and extracted chitin using HCl and CH₃COOH on (a) crab and (b) shrimp shells.

energy to distort C = O, N–H (amides I and II groups) and C–O–C (saccharide structure) as proposed by Juarez et al. [23].

More of chitin is embedded in the shrimp shell than the crab shell matrix (Figure 3(b)). Witan 321 and 398°C, the existence of chitin in the untreated shell is revealed as 33.7% of chitin decomposes. A greater quantity (57.1%) is observed for CH₃COOH shrimp chitin between 342°C and 417°C with no evidence of CaCO₃ existence. This shows chitin – CaCO₃ bond is weaker in shrimp shell compared to that in crab shell [19] hence, CH₃COOH will completely isolate chitin from shrimp shell. In addition to this, a much reduced CaCO₃ content of 18.9% observed between 665°C and 713°C may have further enhanced its easy removal by this organic acid. Harder shells such as periwinkle and snail shells have been investigated with results confirming that high contents of CaCO₃ could have culminated in much reduced chitin yield [26–27]. Comparing CH₃COOH shrimp chitin to its
Table 3. Activation energies of samples.

| Samples                  | CaCO₃  | Chitin |
|--------------------------|--------|--------|
| Virging crab shell       | 221.6  | 32.7   |
| Virgin shrimp shell      | 199.8  | 56.3   |
| CH₃COOH D crab chitin    | 138.7  | 53.4   |
| HCl D crab chitin        | –      | 100.64 |
| CH₃COOH D shrimp chitin  | –      | 97.89  |
| HCl D shrimp chitin      | –      | 116.32 |

HCl D equivalent, it is noticed that 80% chitin can be obtained with improved thermal stability where its $T_{\text{onset}}$ is measured to be 346°C.

The Ea values shown in Table 3 presents a more stable CaCO₃ in virgin crab shell as 221.6 kJ/mol energy is required to decompose the mineral. This is greater than that present in the virgin shrimp shell which requires 199.8 kJ/mol. The lower thermal stability of CaCO₃ in the shrimp shell is responsible a greater embedded chitin Ea (56.3 kJ/mol) compared to that embedded in the crab shell (32.7 kJ/mol) where CaCO₃ has a higher Ea value. Gradual removal of CaCO₃ from shells will further lower its Ea and enhance the thermal stability of embedded chitin as observed in CH₃COOH D crab chitin sample (Table 3). Complete removal of the mineral increases Ea for the decomposition of the embedded biopolymer in virgin shrimp and crab shells by 208, 107 and 74% in HCl D crab chitin, HCl D and HCOOH D shrimp chitin, respectively.

3.3. SEM with EDS

The morphologies of raw crab and shrimp shells (Figure 4(a, b)) show particles with strong calcium and oxygen as revealed by results from EDS which identifies elements in each phase (Table 4). The existence of these two elements at maximum quantities justifies the dominance of CaCO₃ in each exoskeleton with...
crab shell having a greater amount of this mineral with \( \approx 60\% \) more of calcium. There still exist traces of CaCO\(_3\) in the morphology of CH\(_3\)COOH \( D \) crab chitin samples (Figure 4(c)) where 21.5\% reduction of calcium (see Table 4) is observed. The CaCO\(_3\) particles present agglomerate to form lumps. Comparing this with HCl \( D \) crab chitin morphology, there is a transformation from particles to fibrilar structures which forms agglomerations (Figure 4(d)). This gives the evidence that the CaCO\(_3\) is heavily removed with 94\% calcium reduction. Surface morphologies of CH\(_3\)COOH \( D \) and HC\(_3\)D shrimp chitins show sheet and plate-like fibrils (Figure 4(e, f)), implying that both CH\(_3\)COOH and HCl will isolate chitin from shrimp shells. Reduction of CaCO\(_3\) by 95\% is achieved using HCl to demineralize shrimp shell while CH\(_3\)COOH reduces the mineral by 86\%. For each treated sample, there is an increase in carbon content compared to their raw sources (Table 4). This shows that there is an increase in the formation of chitin chains when the amount of CaCO\(_3\) is reduced.

### 3.4. XRD

The XRD result shown in Figure 5(a) informs that CaCO\(_3\) is a principal mineral present in crab’s exoskeleton whose strongest diffraction intensity lies at \( 2\theta = 29.7^\circ \) and corresponding to (104). Other CaCO\(_3\) peaks are diffracted on \( 2\theta = 39.6, 48.9, \) and \( 47.4^\circ \) which are

### Table 4. Elemental constituents of samples obtained from EDS.

| Elements (wt.\%) | C  | O  | N  | Ca | Mg | Si |
|-----------------|----|----|----|----|----|----|
| Virgin crab shell | 3.9 | 61.5 | 7.8 | 24.7 | 0.8 | 1.3 |
| Virgin shrimp shell | 4.2 | 72.6 | 7.9 | 15.4 | -   | 0.4 |
| HCl crab chitin  | 13.5 | 71.6 | 7.4 | 1.5  | -   | -  |
| CH\(_3\)COOHD crab chitin | 15.1 | 57.9 | 7.6 | 19.4 | -   | -  |
| HC\(_3\)D shrimp chitin | 20.2 | 71.5 | 5.8 | 0.8  | -   | -  |
| CH\(_3\)COOHD shrimp chitin | 8.9  | 74.2 | 7.0 | 2.1  | -   | -  |

![Figure 5. XRD of samples (a) virgin crab shell (b) CH\(_3\)COOH \( D \) crab chitin (c) HC\(_3\)D crab chitin (d) virgin shrimp shell (e) HC\(_3\)D and CH\(_3\)COOH \( D \) shrimp chitins.](image-url)
indexed as (113), (116) and (018), respectively. The diffractogram of the virgin crab shell also reveals the existence of embedded chitin in the matrix which prevails on $2\theta = 26.7, 23.2, 36.1, 43.8$ and $57.2^\circ$ ((013), (012), (110), (020) and (122)). Partial isolation of biopolymer is noticed in CH$_3$COOH D crab chitin samples as CaCO$_3$ still exists in its spectrum but with reduced intensities (Figure 5(b)). Full conventional chitin peaks are observed on HCID crab chitin samples with Crl 66% which implies that complete chitin extraction from crab shell is achieved using HCl as demineralizing reagent (Figure 5(c)). The peaks include 9.5, 12.9, 19.5, 21.1, 23.7 and 26.9° ((020), (021), (110), (120), (130) and (013)). Unlike the virgin crab shell, embedded chitin in the shrimp shell matrix has its strongest intensity at $2\theta = 34.5^\circ$ (110) (Figure 5(d)). Comparing these chitin peaks (embedded) with only $27.7^\circ$, $40.5^\circ$ and $48.3^\circ$ of CaCO$_3$ peaks present, stronger peaks are observed in the former. This explains why XRD of CH$_3$COOH D shrimp chitin has complete chitin peaks devoid of CaCO$_3$ as a result of weaker CaCO$_3$ – chitin bond in the exoskeleton of shrimp. With the same diffraction angles, HCID shrimp chitin exhibits similar diffraction pattern but with more intensified peaks which shows more of chitin yield. Calculated Crl of HCID and CH$_3$COOH D shrimp chitin samples in this study are 68.2% and 64.6%, respectively.

4. Conclusion

Chemical isolation of chitin from two marine sources has been studied. Properties and yield of the biopolymer depend on the reagent type used which is also a function of the nature of (make up) of the exoskeleton. The dominance of 30.9% CaCO$_3$ (with higher absorbance peaks and 221.6 kJ/mol Ea)) in crab shell matrix is more prevalent than that in shrimp shell (18.9% and 199.8 kJ/mol Ea with lower absorbance peaks) such that demineralizing with CH$_3$COOH still leaves presence of CaCO$_3$ of reduced amount with lesser thermal stability in the former. While CH$_3$COOH has been discovered to possess low potency for complete CaCO$_3$ removal and chitin isolation from crab shell, the acid fully demineralizes shrimp shell and after deproteinization, the biopolymer extracted shows characteristics of chitin but with lower content (57.1%), Ea (97.89 kJ/mol), DA (77%) Crl (64%) and lower inter and intra molecular hydrogen bond occupancy compared to HCID shrimp chitin with 80% yield and possesses 116.32 kJ/mol, 95% and 68.2% Ea, DA and Crl. HCID crab chitin samples possess comparable properties as HCID shrimp chitin with lesser features but higher than CH$_3$COOH D shrimp chitin. It can be concluded that HCl will isolate chitin from exoskeletons of marine invertebrates irrespective of their shell nature while CH$_3$COOH will be effective for soft shells whose embedded chitin content is more than the mineral content such as shrimp shells.

Acknowledgements

The authors thankfully acknowledge the University of Lagos – Nigeria, Redeemer’s University – Nigeria, Covenant University – Nigeria and Soochow University – China for making their facilities available for this work.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Zhao Y, Ju WT, Jo GH, et al. Perspectives of chitin deacetylation research. Biootechnol Polym. 2011;131–144.

[2] Kim S. Chitin, chitosan, oligosaccharides and their derivatives; biological and their applications. CRC Press, Taylor & Francis Group; 2011. p. 3–633.

[3] Nemtsev SV, Zueva OY, Khismatullin MR, et al. Isolation of chitin and chitosan from honeybees. Appl Biochem Microbiol. 2004;40:39–43.

[4] Arakane Y, Specht CA, Kramer KJ, et al. Chitin synthases are required for survival, fecundity and egg hatch in the red flour beetle, Tribolium castaneum. Insect Biochem Mol Biol. 2008;38:959–962.

[5] Paulino AT, Simionato JI, Garcia JC, et al. Characterization of chitosan and chitin produced from silkworm chrysalides. Carbohydr Polym. 2006;64:948–103.

[6] Isa MA, Ameh AO, Gabriel JO, et al. Extraction and characterization of chitin from Nigerian sources. Leonardo Electron J Pract Technol. 2012;21:75–81.

[7] Nikolov S, Fabritius H, Petrov M, et al. Robustness and optimal use of design principles of arthropod exoskeletons studied by ab initio based multiscale simulations. J Mech Behav Biomed Mater. 2011;4:129–145.

[8] Nam YS, Park WH, Ihm D, et al. Effect of the degree of deacetylation on the thermal decomposition of chitin and chitosan nanofibers. Carbohydr Polym. 2010;80:291–295.

[9] Hild S, Marti O, Ziegler A. Spatial distribution of calcite and amorphous calcium carbonate in the cuticle of the terrestrial crustaceans Porcellio scaber and Armadillidium vulgare. J Struct Biol. 2008;163:100–108.

[10] Kaya M, Baran T, Mentes A, et al. Extraction and characterization of α-chitin and chitosan from six different aquatic invertebrates. Food Biophys. 2014;9:145–157.

[11] Zaku SG, Emmanuel SA, Aguzue OC, et al. Extraction and characterization of chitin; a functional biopolymer obtained from scales of common carp fish (Cyprinus carpio L.) a lesser known source. Afr J Food Sci. 2011;5(8):478–483.

[12] Liu S, Yu J, Bi C, et al. Extraction and characterization of chitin from the beetle holotrichia parallela motschuskyi. Molecules. 2012;17:4604–4611.

[13] T. G. Odulakar A, Isa MT, Abdullahi SA, et al. Extraction and characterization of chitin and chitosan from mussel shell. Civil Environ Res. 2013;13(2):108–114.

[14] Kaya M, Lelesius E, Nagrockait R, et al. Differentiations of chitin content and surface morphologies of chitins extracted from male and female grasshopper species. PLOS ONE. 2015;1–14.
[15] Mahmoud NS, Ghaly AE, Arab F. Unconventional approach for demineralization of deproteinized crustacean shells for chitin production. Am J Biochem Biotechnol. 2007;3(1):1–9.

[16] Ameh AO, Isa M.T, Abutu D, et al. Kinetics of demineralization of shrimp. Leonardo Electron J Pract Technol. 2014;24:13–22.

[17] Greene HBC, Robertson KN, Young COC, et al. Lactic acid demineralization of green crab (Carcinus maenas) shells: effect of reaction conditions and isolation of an unusual calcium complex. Green Chem Lett Rev. 2016;9(1):1–11.

[18] Gbenebor OP, Adeosun SO, Lawal GI, et al. Acetylation, crystalline and morphological properties of structural polysaccharide from shrimp exoskeleton. Eng Sci Technol Int J. 2017;20:1155–1165.

[19] Gbenebor OP, Adeosun SO, Lawal GI, et al. Role of CaCO₃ in the physicochemical properties of crustacean-sourced structural polysaccharides. Mater Chem Phys. 2016;184:203–209.

[20] Galadima MS, Ameh AO, Agbane MO. Empirical modeling of acetic acid demineralization of shrimp shell using response surface methodology. Proceedings, International Engineering Conference FUTMINNA; 2015. p. 388–392.

[21] Brunner E, Ehrlich A, Schupp P, et al. Chitin-based scaffolds are an integral part of the skeleton of the marine demosponge Ianthella basta. J Struct Biol, NIH Public Access. 2010;1–21.

[22] Broido AA. A simple, sensitive graphical method of treating thermogravimetric analysis data. J Polym Sci Part A: Polym Phys. 1969;7:1761–1773.

[23] Juarez-de la Rosa BA, Quintana P, Ardisson PL, et al. Effects of thermal treatments on the structure of two black coral species chitinous exoskeleton. J Mater Sci. 2012;47:990–998.

[24] Daraghmeh NH, Chowdhry BZ, Leharne SA, et al. Chitin. In: Brittain HG, editor. Profiles of drug substances, excipients and related methodology. Elsevier Inc. Academic Press. 2011;36:35–102.

[25] Cui J, Yu Z, Lau D. Effect of acetyl group on mechanical properties of chitin/chitosan nanocrystal: a molecular dynamics study. Int J Mol Sci. 2016;7(61):1–13.

[26] Gbenebor OP, Akpan EI, Adeosun SO. Thermal, structural and acetylation behavior of snail and periwinkle shells chitin. Prog Biomater. 2017;6:97–111.

[27] Akpan EI, Gbenebor OP, Adeosun, SO. Synthesis and characterization of chitin from periwinkle (Tympanotus fusatus (L.)) and snail (Lissachatina fulica (Bowdich)) shells. Int J Biol Macromol. 2018;106:1080–1088.