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Role of $CaCO_3$ in the physicochemical properties of crustacean-sourced structural polysaccharides

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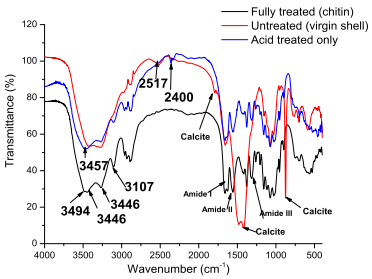
HIGHLIGHTS

- Thermal stability of crab chitin increase by 244% and shrimp chitin, by112.5%.
- Shrimp shell contains more of chitin than $CaCO_3$ while crab shell has the opposite.
- Shrimp chitin has higher hydrogen bond occupancy.

GRAPHICAL ABSTRACT



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ABSTRACT

Crustacean shells are rigid and comprise mainly calcium carbonate ($CaCO_3$), chitin and protein. These principal minerals vary with specie and full elimination could influence the physicochemical properties of chitin. In this study α-chitin sourced from crab and shrimp shells was extracted via chemical means using 0.4 M hydrochloric acid (HCl) and sodium hydroxide (NaOH) for demineralization and deproteinization processes respectively. Results from Fourier Transform Infrared Spectroscopy (FTIR) and thermogravimetric analysis (TGA) showed that $CaCO_3$ is more predominant in the crab shell than that of the shrimp. Average hydrogen bond energy (E_H) in shrimp chitin was calculated to be 5.25 kCal while that obtained from crab measured 4.71 kCal. Presence of $CaCO_3$ in each exoskeleton is responsible for low thermal stability of embedded chitin, which improves after treatment with both chemicals.

1. 1Introduction

Chitin, a linear structural polysaccharide and second most common biopolymer in the biosphere (after cellulose), has a highly ordered crystalline structure. It is found in three polymorphic forms namely: α, β, and γ −chitin, which differ in their unit cell size, [1,2]. The α-form has been mostly obtained from crab and shrimp shells [3] and is confirmed to have best crystalline structure and abundant of the three. The anti-parallel arrangement in this polymorph (Fig. 1) as disclosed by X-ray diffraction studies, gives rise to extensive hydrogen bonding that culminates in its high structural flexibility [4].

The chains of the β-form are arranged parallel (Fig. 1) and it is being obtained from squids, snails, spider and silkworms, [5]. Thus parallel arrangement yields weak intermolecular forces [6]. As for γ −chitin found in fungi and yeast [5], two out of three chains are parallel with the third oriented in the opposite direction (Fig. 1) i.e., it is a mixture of the intermediate form of α and β-chitins [7].

The FTIR analysis has been one of the major techniques used in

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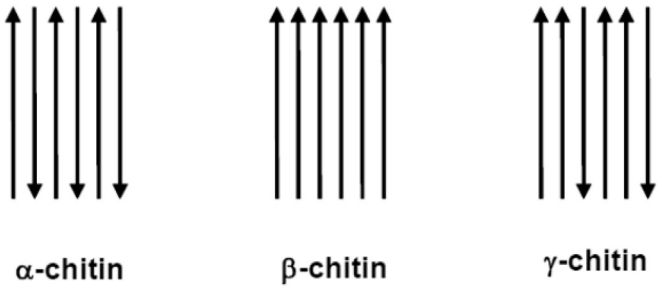

Fig. 1. Polymorphic representations of α, β, and γ chitin [1].

distinguishing [8] between the two major polymorphs of chitin- (α and β forms). Cardenas, Cabrera, Taboada and Miranda [9], affirmed that the frequency of vibration modes of amide I in the region 1660–1620 cm^{-1} is the distinguishing factor. Two absorptions were observed at 1660 and 1627 cm^{-1} for α-chitin obtained from shrimps, lobsters and prawns while only one band at 1630 cm^{-1} was observed for β −chitin sourced from squid. Brunner et al. [10], study on *Ianthella basta* (elephant ear sponge), reported that for α-chitin, the amide I band is broken into two components at 1660 and 1630 cm^{-1} respectively. However, 1646 and 1666 cm-^{1} bands observed for amide I confirmed the α-chitin structure in black coral [11]. The formation of two amide I bands has been attributed to the occurrence of the intermolecular hydrogen bond C═O···H N and the intra molecular hydrogen bond C═O···$HOCH_2$ existing in α-chitin while for β-chitin, only the intermolecular hydrogen bonds exist [12]. Chitin chains generally are organized in sheets and tightly held by a network of hydrogen bonds, which is linked by the strong C═O and H-N-groups. The band between 3600 and 3000 cm^{-1} is due to the OH and NH stretching vibration, which gives considerable information concerning the hydrogen bonds. The first intra molecular hydrogen bond occurs when a carbonyl group on C-2 bonds to the hydroxyl group on C-6 i.e. O H (6)···O═C while the second hydrogen bond exists between the OH group on C-3 and the ring oxygen (OH (3)···O-5). The bands related to NH of the amide group are assigned to the vibrational modes involved in strong intermolecular hydrogen bond networks of C═O···HN and OH···O═C respectively [13]. It is known and accepted that these hydrogen bonds play important roles in determining the conformational and mechanical properties of structural polysaccharides [14]. Recently, Cui, Yu, and Lau [15] used a molecular dynamics approach to decipher the relationship between hydrogen bonding and mechanical properties of chitin utilizing steered molecular dynamics simulation (SMD). It was observed that chitin with high hydrogen bond occupancy will possess a greater resistance to fracture. However, quantum mechanical calculations on α − chitin have also shown that response of this biomolecule to stresses is influenced by strong covalent bonds which dominates along its axis [4,16].

Result from researchers on chitin investigations have proven that results obtained vary with extraction methods [12] and sources [13]. Regardless of the chosen treatment, the isolation of chitin begins with the shell selection. Chitin, however, is a constituent of a complex network with proteins onto which $CaCO_3$ deposits to form the rigid shell [17]. There is therefore need to investigate the role of shells' principal biomineral, $CaCO_3$ on hydrogen bond strength and thermal stability of the two major sources of α-chitin.

Researchers have employed the use of scanning electron microscopy (SEM), optical microscopy and X-ray micro diffraction to decipher the functional role of $CaCO_3$ in American lobster cuticle [18–20]. In the works of Al-Sawalmih et al. [18], the chitin − protein fibers in the cuticle of this crustacean is made up of two components. The first fiber component maintains a twisted ply wood structure within the cuticle plane while the second is roughly oriented perpendicular to the cuticle surface. They suggested that the crystalline $CaCO_3$ (calcite) portion in the organic matrix is associated with the fibers oriented perpendicular to the surface. Chitin − protein fiber supports the orientated growth of crystalline $CaCO_3$ by either acting as a template with specific crystal growth occurring on active sites on the surface or within the organic fibers. The abundant crystalline $CaCO_3$ in the outermost layer of American lobster exocuticle offers a mechanical function such as impact and wear resistance. Generally, crustaceans reinforce the load bearing part of their cuticle with $CaCO_3$ and sometimes, the $CaCO_3$ may contain magnesium atom, Mg which must be of low concentration to be thermodynamically stable in it [21]. The Mg improves stiffness of $CaCO_3$ crystals. Results affirm that in several crustaceans, the mineral content varies in different parts of their exoskeletons with the carapace having the least [22]. This work employs isolation of α-chitin by full elimination of $CaCO_3$ from crab and shrimp shells from African origin via chemical treatment; hence, the exoskeleton part with the lowest mineral content (carapace) was considered for this investigation.

2. Material and methods

2.1. Chitin extraction

Shells of crab and shrimps (the carapace) were washed, dried and ground to powder prior to demineralization, which occurred by refluxing the powders in 0.4 M HCl at 32 °C. This was repeated several times until gas evolution stopped. Demineralized samples were washed with distilled water to neutral pH7.0 and dried in an oven at 70 °C for 4 h to constant weights. Deproteinization was carried out by heating the mineral free samples in 0.4 M NaOH solutions in a beaker at 100 °C for 1 h. At the end of this period, samples were filtered and soaked in a fresh set of 0.4 M NaOH for 18 h at 32 °C for effective protein removal. Samples were washed with distilled water to pH 7.0, filtered and oven dried at 70 °C. Depigmentization (decoloration or bleaching to remove carotenoids) was later carried out by soaking extracted chitin in 1 M H_2O_2 for 24 h at 32 °C. Chitin obtained was washed in distilled water and dried for 4 h in an oven at 70 °C for characterizations.

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 milligram 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. Hydrogen bond

Broad and overlapped FTIR absorption spectra existing between 3600 and 3000 cm^{-1} were resolved and improved by their deconvolution from a background scattering using a Gaussian function curve-fitting analysis with an $r^2 > 0.99$. The energy of the hydrogen bond E_H ($kCal^{-1}$) was calculated using the Equation (1) as adopted by Ciolacu et al., [14].

$$EH = [1/kx(Vo - V)/Vo] \quad (1)$$

where: Vo is the standard frequency corresponding to free OH groups (3600 cm^{-1}); V is the frequency of the bonded OH groups and $k = 1.68 \times 10^{-2}$ $kcal^{-1}$.

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