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Isolation and FTIR spectroscopy characterization of chitin from local sources

K. Prabu* and E. Natarajan

Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, India

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ABSTRACT

Chitin the second most abundant after cellulose biopolymer found in nature after, is produced by many living organisms, and is present usually in a complex with other polysaccharides and proteins. Chitin was found as a major component in arthropods (insects, crustaceans, arachnids and myriapods), nematodes, algae and fungi. In this study Chitin was isolated from various species (crab, squilla, and shrimp) from the local sources and the functional properties of chitin were studied by Fourier transform infra red spectroscopy (FTIR) and Peak Fit version was used for data acquisition and handling

Keywords: Chitin, FTIR, biopolymer, Crustaceans, arthropod.

INTRODUCTION

Chitin is one of the most abundant biopolymers in nature and the most widespread amino-polysaccharide. It is widely distributed in nature in the exoskeleton of all animals with an outer skeleton such as insects and crustaceans. It is also found in microorganisms, e.g. in the cell walls and structural membranes of mycelia of fungi, yeast and green algae [1]. Chitin is a linear polymer of linked 2-acetamido-2-deoxy-D-glucopyranose Chitin has a resemblance to cellulose both in chemical structure and in biological function as a structural polysaccharide and may be regarded as a cellulose derivative with an acetamido group at carbon 2. Both polymers mainly serve as structural components supporting cell and body surfaces: cellulose strengthens the cell wall of plant cells whereas chitin contributes to the mechanical strength of fungal cell walls and exoskeletons of arthropods [2].

Chitin is mainly used as a raw material to produce chitin-derived products, such as chitosans, chitin/chitosan derivatives, oligosaccharides and glucosamine. An increasing number of useful products derived from chitin continue to attract commercial development. Chitin chains folded and aggregate and form microfibrils in living systems [3]. The chitin microfibrils combine with other sugars, proteins, glycoproteins and proteoglycans to form fungal septa and cell walls as well as arthropod cuticles and peritrophic matrices, notably in crustaceans and insects [4]. In animals chitin is associated with proteins, while in fungal cell wall it is associated with glucans, mannans or other polysaccharides. In fungal walls, it is found covalently bound to glucans, either directly or via peptide bridges [5]. In insects and other invertebrates, the chitin is always associated with specific proteins, with both covalent and noncovalent bonding, to produce the observed ordered structures. There are often also varying degrees of mineralization, in particular calcification, and sclerotisation involving interaction with phenolic and lipid molecules. In both fungi and invertebrates, varying degrees of deacetylation have been determined, giving a continuum of structure between chitin (fully acetylated) and chitosan (fully deacetylated) [6]. Chitin (β-(1-4)-2-acetamido-2-deoxy-D-glucopyrnaose) is second most abundant natural biopolymer generally obtained from exoskeletons of crustaceans such as crabs, shrimp, lobsters and krill. The most important derivative of chitin is chitosan ((1, 4)-2-

amino-2-deoxy- β -Dglucose) obtained by excessive deacetylation of chitin with alkali [7]. Chitosan is the most important derivative of chitin which is the structural element of the exoskeleton of crustaceans, [8]. Chitosan, the linear polymer of d glucosamine in β-(1-4) linkage has been recommended as a suitable functional material because of its biocompatibility, biodegradability, non-toxicity, adsorption properties and regulation cell activation, Pae *et al* [9] and Yoon *et al* [10]. Due to its antimicrobial activity chitosan have many pharmaceutical applications and generally used as natural preservative for the safety of food [11] and [12].

MATERIALS AND METHODS

Crustaceans (crab, squilla, and shrimp) with an average weight of 30-70 g were caught from southeast coast of India along the parangpettai. The crustaceans, off-loaded approximately 9h after catch, were placed in ice and transported to the laboratory, CAS in Marine Biology, Annamalai University, and Parangipettai. The crustaceans were immediately washed and manually excised the carapace. The exoskeletons were collected and used for chitin extraction.

Isolation of Chitin

Chitin was prepared from a crab, shrimp and squilla exoskeletons. The material was purified with aqueous 1M HCl for 2h at 25^0 C and thus refluxed n 2M Na OH for 48h at 25^0 C. The resulting chitin was washed in deionised water by several centrifugations until neutrality was reached. The whole procedure was repeated twice. The resultant chitin was for Fourier transforms infra red (FTIR) and absorption spectra studies.

FTIR Method

Characterization of the chemical structure of a polymer is one by using Fourier transform infra red (FTIR) technique. The specific chemistries and orientation of the structure will be known from the IR spectrum. The chitin infra red spectra were measured using of the Perkin Elmer FTIR model 2000 spectrophotometer. The absorption spectrum was recorded in the wave number range from 4000 cm^{-1} to 400cm^{-1}. FTIR spectra were recorded with a Perkin Elmer–Spectrum RxI Spectrometer equipped with a mullard I–alanine doped triglycine sulfate (DTGS) detector installed at Centralized Instrumentation and Services Laboratory (CISL), Annamalai University. Pellets were scanned at room temperature (25 ± 1 ºC) in the 4000–400 cm–1 spectral range. To improve the signal to noise ratio for each spectrum, 100 interferograms with a spectral resolution of ±4 cm^{-1} were averaged. Background spectra, which were collected under identical conditions, were subtracted from the sample spectra automatically.

Peak Fit (version 4.121) was used for data acquisition and handling. Direct comparisons of spectral changes were done on vector normalized second derivatives of average spectra of the discussed spectral classes, because most IR absorption bands are broad and composed of overlapping components. Second derivative spectra were vector normalized at 3500–3000 cm^{-1}, at 1500–1800 cm^{-1}, 1200 to1600 cm^{-1} and at 800–1200 cm^{-1}. Fourier self-deconvolution and second derivative resolution enhancement were used to narrow the width of infrared bands and increase the separation of the overlapping components with software Peak Fit Version 4.12. The resolution enhancement resulting from self-deconvolution and the second derivative was such that the number and the position of the bands to be fitted were readily determined. Curve fitting was accomplished during a curve-fitting process with Peak Fit software (version 4.121) for the amide I, II, and III band region. The program iterated the curve-fitting process by adjusting the peak high and width to achieve the best Gaussian-shaped curves that fit the original spectrum. The results were presented as ‘‘mean ± standard deviation’’, and the differences between Crab, shrimp and squilla chitin.

RESULTS AND DISCUSSION

FTIR spectra (Fig 1-4) revealed extensive homology between the samples. Replicate spectra of individual carapace sample demonstrated that differences in absorption were well within the resolution employed. Common to all spectra were vibrations due to the carbohydrate backbone. Strong complex absorptions centered between 1021 and 1081 for the three carapace polymers are ascribed to complex vibrations of ring structures (including bending, stretching and coupling between these modes; [13]). Bonds between 1200 and 1100 $cm^{-1}$are assigned to vC-C and vC-O modes including a shoulder due to the v_{as} C-O-C of the glycosidic linkage (1135 cm^{-1}) whilst the main absorption is due to vC-O weakly coupled to δC-O-H [14]. Bands at 1717 to 1775 are due to vC=O associated with O-acetyl ester bonds. The observed spectra for the OH bending modes and sugar OH stretching vibrations in the region 3268-3432 cm^{-1} together are results of the shrimp, squilla and crab carapace polymers (Fig 4a-c).

Table 1. Band assignments of major absorptions in IR spectra of Crustaceans (Shrimp, Squilla and Crab) in 3500-800 cm^{-1}

Frequency, cm^{-1}	Assignments
890	Glycopyrenose,
952	Amide III
1040-1070	Carbohydrate back bone (Schrader, 1995)
1152-1156	Glycosidic linkage, C-H stretch, saccharide structure (Lu et al., 2006)
1261	Amide III, acetyl group (Sherbrock-Cox et al., 1984)
1340	Methyl C-H stretch, Amide III (Castello et al., 2003; Acosta et al., 1993)
1432	Amide III (Castello et al., 2003; Acosta et al., 1993)
1495	Amide III (Castello et al., 2003; Acosta et al., 1993)
1560	N-acetyl ester bonds, Amide II (Castello et al., 2003; Acosta et al., 1993)
1627	Amide I (Castello et al., 2003; Acosta et al., 1993)
1672	Amide I (Castello et al., 2003; Acosta et al., 1993)
1720	Acetyl ester bonds (Beech et al., 1999)
1760	Acetyl ester bonds (Beech et al., 1999)
3102	Amno peak, alpha-Chitin (Castello et al., 2003; Acosta et al., 1993)
3265	Amno peak, alpha-Chitin (Castello et al., 2003; Acosta et al., 1993)
3420	Amno peak, alpha-Chitin (Castello et al., 2003; Acosta et al., 1993)
3450	Amno peak, alpha-Chitin (Castello et al., 2003; Acosta et al., 1993)

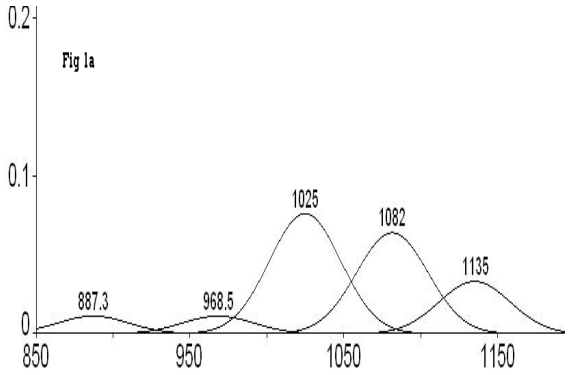


Figure: 1a

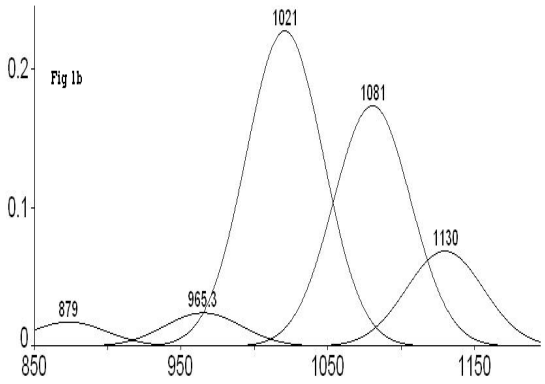


Figure: 1b

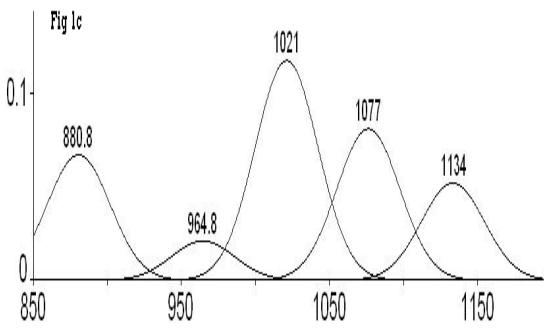


Figure: 1c

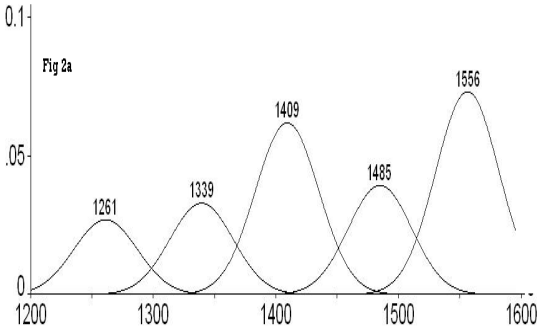
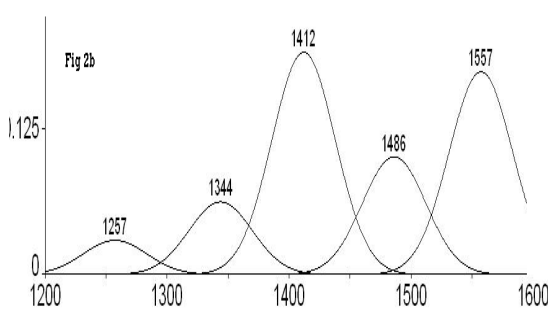


Figure: 2a



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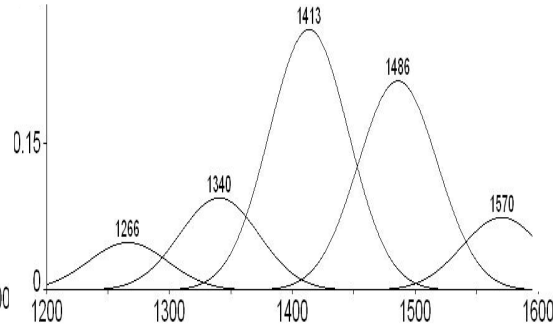

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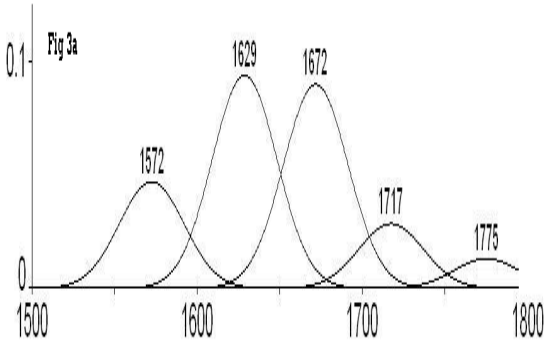


Figure: 3a

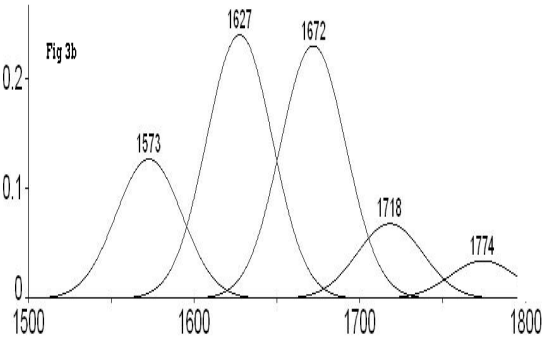
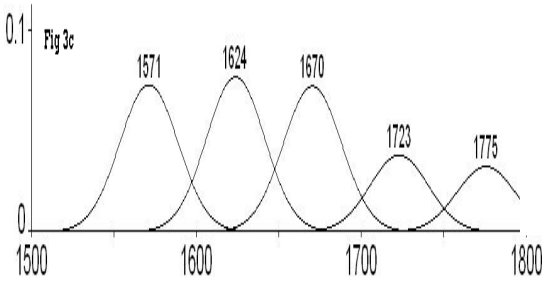


Figure: 3b



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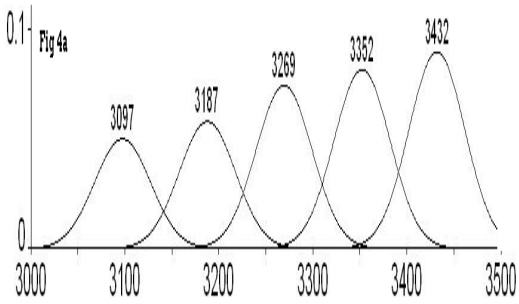

Figure: 4a

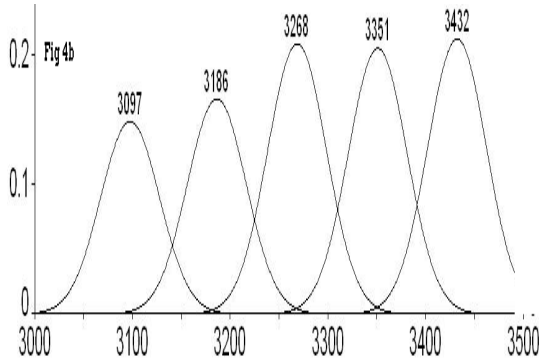

Figure: 4b

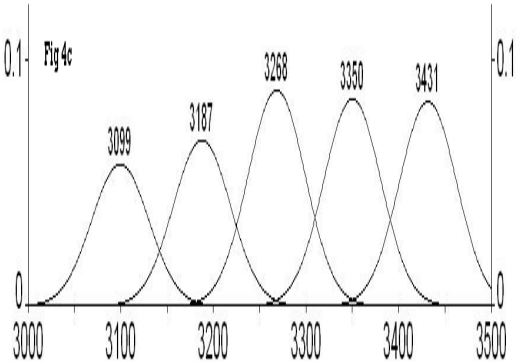

Figure: 4c

Protein related bands, the vC=O of the amide I and δN-H/vC=O combination of the amide II bands, were also observable for all three carapace samples. The principal amide I absorption between 1624 to 1672 $cm^{-1}$for the all

three carapace polymers. However, the presence of amino sugars, which is manifested by an absorbance band in the same region due to N-acetyl or even glucournamide (-$CONH_2$) groups, cannot be discounted. Fig 3a-c shows the FTIR spectrum of the carapace polymer together with the deconvoluted derivative spectra. Band positions obtained from the derivative spectrum were similar for the three carapace polymers. However, as will be shown later, the relative 'band intensities' were different for the three carapace polymers. A band at 1717-1723 cm^{-1} is assigned to the vC=O of O-acetyl ester bonds and is accompanied by a doublet at 1266-1257 cm^{-1} (Fig 2a-c) including vC-O absorption at 1257 cm^{-1} [15]. However, the doublet may arise from the amide III (vC-N and δipC-N-H) of proteins. N-acetyl ester bonds were also present; the unusually high amide III absorption at 1571-1573 has been variantly ascribed to either N-acetyl [16] or N-methyl groups [17]. Protein secondary structure was determined by fitting curves to peaks identified in the amide I region [18] and revealed distinct differences between the three carapace-polymers (Fig 3a-c and Table 1). The principal structure in all three sample was the β-sheet/N-acetyl or (-$CONH_2$).peak representing 69.31% of the amide I region of the shrimp, 69.60% of the squilla but only 52.96% in the crab carapace polymers. Whilst the shrimp and crab (1135) carapace polymers contained substantial amounts of polysaccharide structure (26.30% and 21.19%, respectively), the squilla carapace polymer contained considerably less (14.08%). In contrast, glycopyrenose (879-965 cm^{-1}) 17.99% in the crab carapace polymer, respectively.

The relative carbohydrate content was estimated as a proportion of the total acetyl groups content of the carapace polymer. This was achieved by calculating the ratios of integrated peak areas A, from the spectra of peaks at 1717 to 1775 cm^{-1} (vC=O, O-acetyl ester bonds, AI), 1572 cm^{-1} (N-acetyl ester bonds, A2), with the peak centered between 1025 cm^{-1} and 1082 cm^{-1} due to the carbohydrate back bone (A3). The shrimp and squilla carapace polymer contained roughly equivalent levels of acetyl ester bond residues. The greatest degree of carbohydrate content was found in the shrimp carapace polymer with A3:A1 and A3:A2 ratio of 4.8 and 2.4, respectively, compared to 5.4 and 2.6, respectively, for the squilla carapace polymer, and 2.4 and 1.9, respectively, for the crab carapace polymer.

CONCLUSION

The chitin obtained from the exoskeleton of crab, squilla, and shrimp, could be used in a variety of applications especially when transformed into the more useful compound chitosan. The increased use of biopolymer, due to their intrinsically beneficial properties for a broad spectrum of applications, have been gaining importance as raw materials in many industrial sectors such as food, textile, packaging, medicine and pharmacy. Biodegradable and biocompatible biomaterials that do not cause immune responses in organisms and/or are able to integrate with a particular cell type/tissue are required for medical and pharmaceutical uses. Biopolymers nowadays play crucial roles in applications where the materials are in direct contact with body tissue.

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