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Production of chitin from two marine stomatopods *Oratosquilla spp.* (Crustacea)

Thirunavukkarasu. N*, K. Dhinamala, R. Moses Inbaraj

Department of Marine Studies & Coastal Resource Management, Madras Christian College (Autonomous), Tambaram, Chennai, India

ABSTRACT

Many multinational pharmaceutical companies are producing chitin products in a commercial scale. The crustacean (crab and shrimp) shell wastes are also utilized for this production. Only limited works are available about the crustacean stomatopods pertaining to the chitin extraction. Due to the lacunae in this field the present study had been carried out to extract chitin from the marine stomatopods Orotosquilla quinquedentata and O.nepa. The yield of chitin was more in Oratosquilla nepa where 20g of the shell yielded 2.145g of chitin with percentage contribution of 10.725%. The FT-IR spectrum of chitin was also confirming the presence of chitin in the shell of stomatopods. The results of the present investigation paves way and provides concrete information for the utilization of chitin in the development of drugs, artificial bone and raw material for the food industries in the near future.

Keywords: stomatopods, shell, chitin, FT-IR

INTRODUCTION

Chitin is a poly - B - (1-4) - N - acetyl -D-glucosamine. It is a nitrogen containing polysaccharide, related chemically to the cellulose. Chitin is the second most abundant natural polymer, after cellulose. Like cellulose, it is a polysaccharide – a compound formed of many identical simple sugar molecules. Chitin was first found in mushrooms in 1811 by Professor Henri Braconnot, Director of the Botanical Gardens at the Academy of Sciences in Nancy, France.

The crustacean processing industries over the world turn out more than 60,000 tonnes of waste every year. The head and shell of crustaceans such as shrimp, lobster, crab, squilla, krill etc. form the waste. These biological wastes contain about 10% of chitin on dry weight basis. The market

price of the stomatapod waste too varies widely, from around US $ 5.00 per kg for the crude grades used in agriculture to around US $ 200 for ultra pure grade used in healthcare industry[1].

Various applications of chitin are of great industrial importance. The proper utilization of these shell wastes not only solves the problem of their disposal but also forms the basis for many potential products used in the fields such as textiles, photography, medicine, agriculture, food processing etc. The global attention on the importance of chitin has been demonstrated in three International conferences on chitin and held in the years 1977, 1982 and 1985[2].

The total annual world production of purified chitin is about 1600 tonnes, with Japan and USA being the main producers. Smaller amounts of these polymers are manufactured in India, Italy and Poland. In countries such as Brazil, Cuba, Ireland, Norway, Uruguay and Russia, production of these polymers is under consideration. Ministry of Health and Welfare, Japan has approved chitin and their derivatives as food additives. In India, chitin has been found to promote growth in broiler chicks. Incorporation of chitin in poultry feed at a level of 0.5 percent decreases the food consumption ratio and increases the body weight by 12 percent in comparison with chicks fed on a chitin-free diet[3]. The Central Institute of Fisheries Technology (CIFT), Cochin conducted experiments on chicken that was fed with chitin-incorporated diet. The results showed 10 - 12% increase in the chicken than fed with the normal feed[4].

Further the polymer chitin and the derivatives are used in the manufacture of non-allergic contact lenses. During 1998, the market value of the world demand for chitin was about 1000 tons. In Japan, the textile companies are successfully manufacturing a new type of artificial non-allergic skin for humans, using chitin derived from stomatopod (mantis shrimp) shell. The chitin with calcium combination decolorise waste products and hence the stomatopod shells were used as wastewater filters for textile industries in USA[5]. The chitin will trap pollutants such as insecticides DDT and PCBs. Chitin can also be used in printing and finishing preparation in the textile industry. Because of the accelerating significance of chitin and the present study has been aimed with the following objectives:

-To isolate the chitin from the exoskeleton of stomatopod (mantis shrimp)
-To quantify the yield of chitin and
-To check the chitin extracted from the stomatopod shell compared with standard chitin through FT-IR spectral analysis.

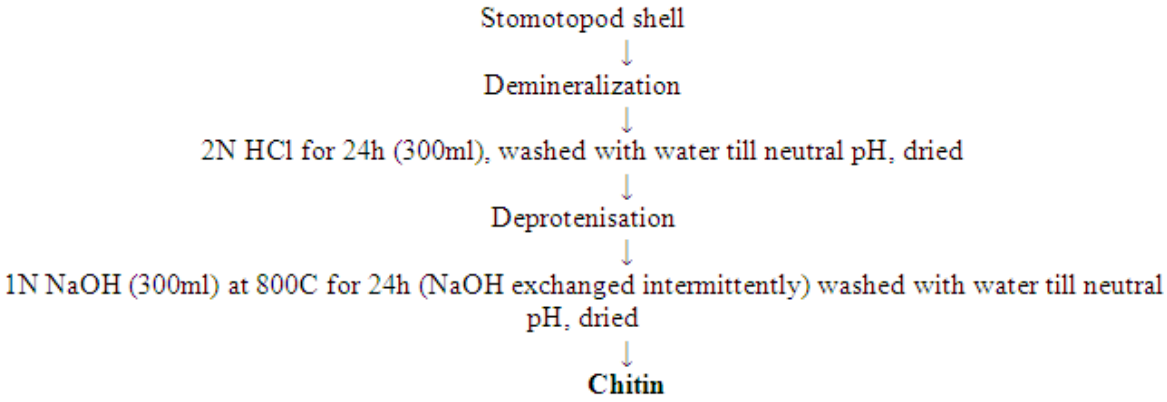
EXPERIMENTAL SECTION

The study animals chosen for the present study are *Orotosquilla quinquedentata* and *Orotosquilla nepa* (Fig 1&2).

Fig.1. *Oratosquilla quinquedentata*

Fig.2. *Oratosquilla nepa*

The chitin was extracted from the shells following the method of Takiguchi[6] using hydrochloric acid and sodium hydroxide.



Demineralization

In demineralization, type of acid Concentration, temperature and extent of reaction time are the important factors, which determine the quantity of chitin. Because of its low cost and ready availability, commercial Hydrochloric acid is commonly used for demineralization. Acid treatment carried out on chitin, leads to a partial or extended depolymerization. Powdered stomatopod shell of squilla was demineralised in HCl by dissolving the powder in HCl and leaving it overnight for demineralization.

Deprotenization

In deproteinisation, the dimineralised residue was filtered in a filter paper and the residue was removed of all its moisture content. The filtered residue was deproteinized in the presence of NaOH. A water bath were used to maintain the temperature at 80ºC and NaOH was changed over and over again for 24 hrs. The deproteinized material was dried and was subjected to FT-IR spectrum to make sure the end product is indeed chitin.

Fourier Transform – Infra Red (FT-IR) Spectrum

The quality parameters of chitin was analysed by Fourier Transform Infrared (FT-IR) Spectrometry (Schimadzu FTIR-4200, Reference: KBr, Apodization: Happ, Resolution: 4.0, Type: Hyper IR) (Shigemasa *et al.,* [1996])[14]. FT-IR spectroscopy of solid samples of chitin relied on a Bio-Rad FTIS-40 model, USA. Sample (10 µg) was mixed with 100 µg of dried Potassium Bromide (KBr) and compressed to prepare a salt disc (10mm diameter).

RESULTS AND DISCUSSION

The yield of chitin from the stomatopod shell is given in Table.1. In the present study, the yield of chitin was more in *Oratosquilla nepa* (2.145g) and the yield was 10.725% from the 20g of the shell sample. *O.quinquedentata* yielded 2.125g with 10.625% (Table 1).

Table 1.The yield of chitin from 20g sample of different species

S. No	Species	Sample used(g)	Chitin Yield(g)	Yield (%)
1	*Oratosquilla quinquedentata*	20	2.125	10.625
2	*Oratosquilla nepa*	20	2.145	10.725

Table 2. Showing the FT-IR spectral values (cm^{-1}) of standard chitin *O.quinquedentata* and *O.nepa* from stomatopod shell samples

S.No	Standard chitin (cm^{-1})	*Oratosquilla quinquedentata* (cm^{-1})	*Oratosquilla nepa* (cm^{-1})
1	530.11	569.00	529.15
2	690.99	-	695.23
3	896.28	-	897.35
4	1026.33	-	1028.95
5	1073.93	1070.77	-
6	1203.4	-	1200.00
7	1315.40	-	1324.28
8	1377.38	1370.41	1371.11
9	1563.42	1555.02	-
10	1661.50	1656.82	1634.55

Figure.3. FT-IR Spectrum of standard chitin

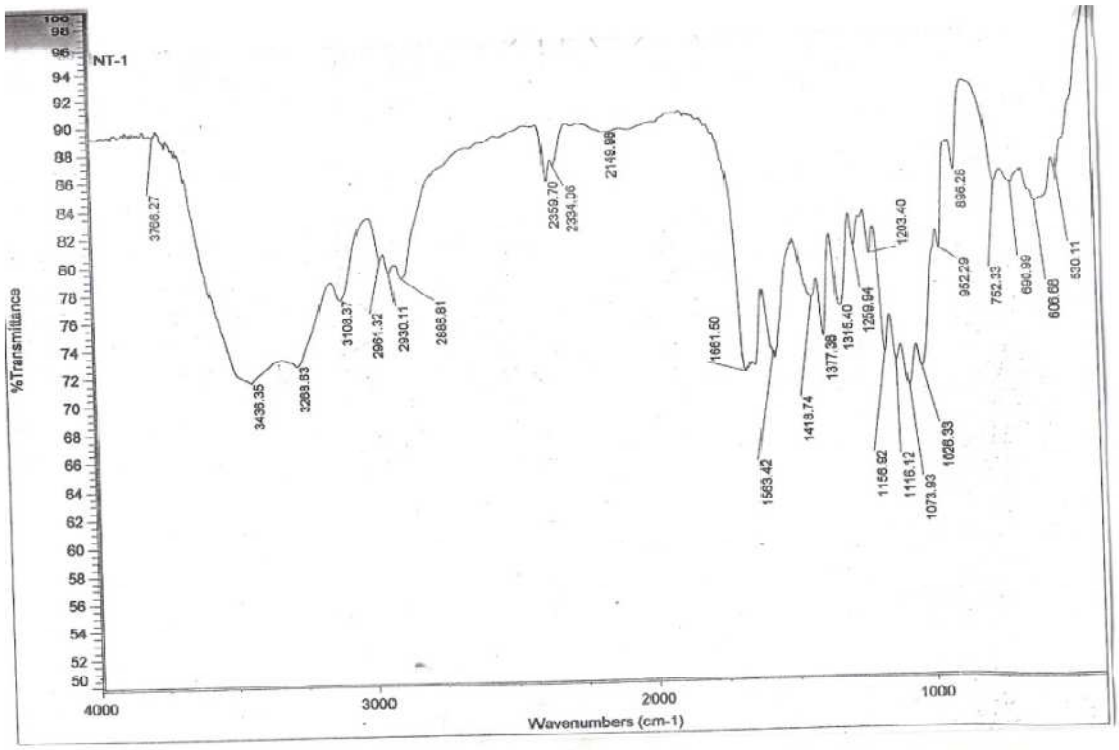


Figure.4. FT-IR spectrum of chitin from *Oratosquilla quinquedentata*

The FT-IR spectrum of the chitin isolated from the carapace of *Oratosquilla nepa* and *Oratosquilla quinquedentata* were compared with the standard chitin (Fig.3). The IR spectrum of the standard chitin contains 10 majorpeaks (Table 2) whereas the IR spectrum of the sample of stomatopod shell recorded five peaks (Fig.4) in *O.quinquedentata*

(569.00cm^{-1},1070.77cm^{-1},1370.41cm^{-1},1555.02cm^{-1} & 1656.82cm^{-1}) and in *O.nepa* eight peaks (Fig.5) were recorded (529.15cm^{-1}, 695.23cm^{-1}, 897.35cm^{-1},1028.95cm^{-1}, 1200cm^{-1}, 1324.28cm^{-1}, 1371.11cm^{-1} & 1634.55cm^{-1}) compared with the standard chitin.

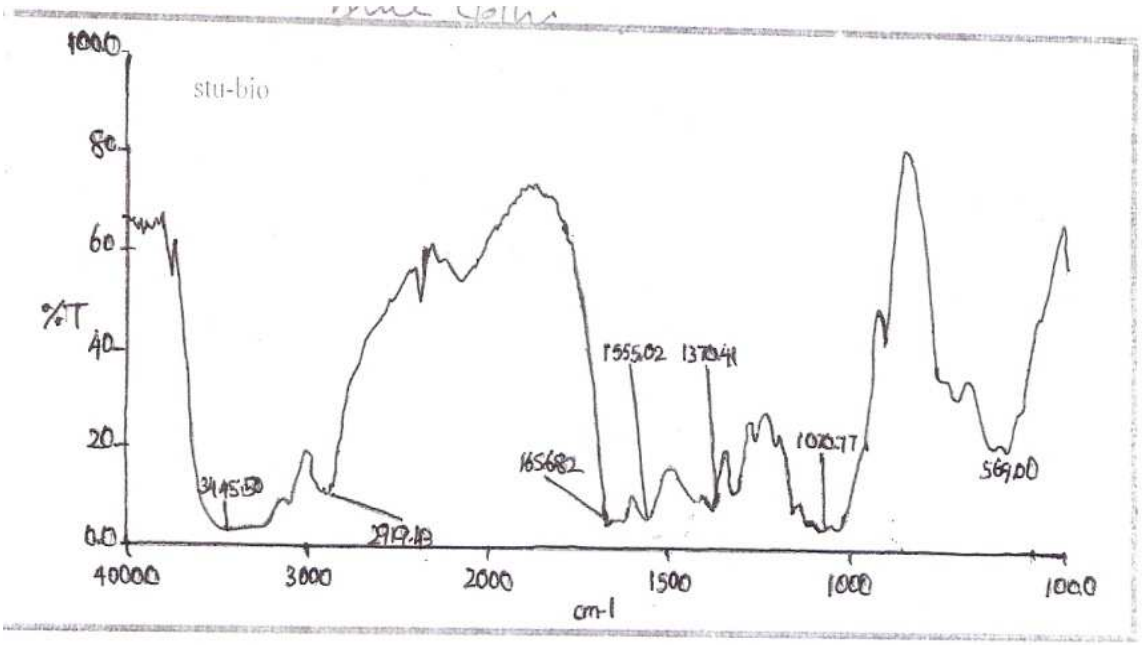
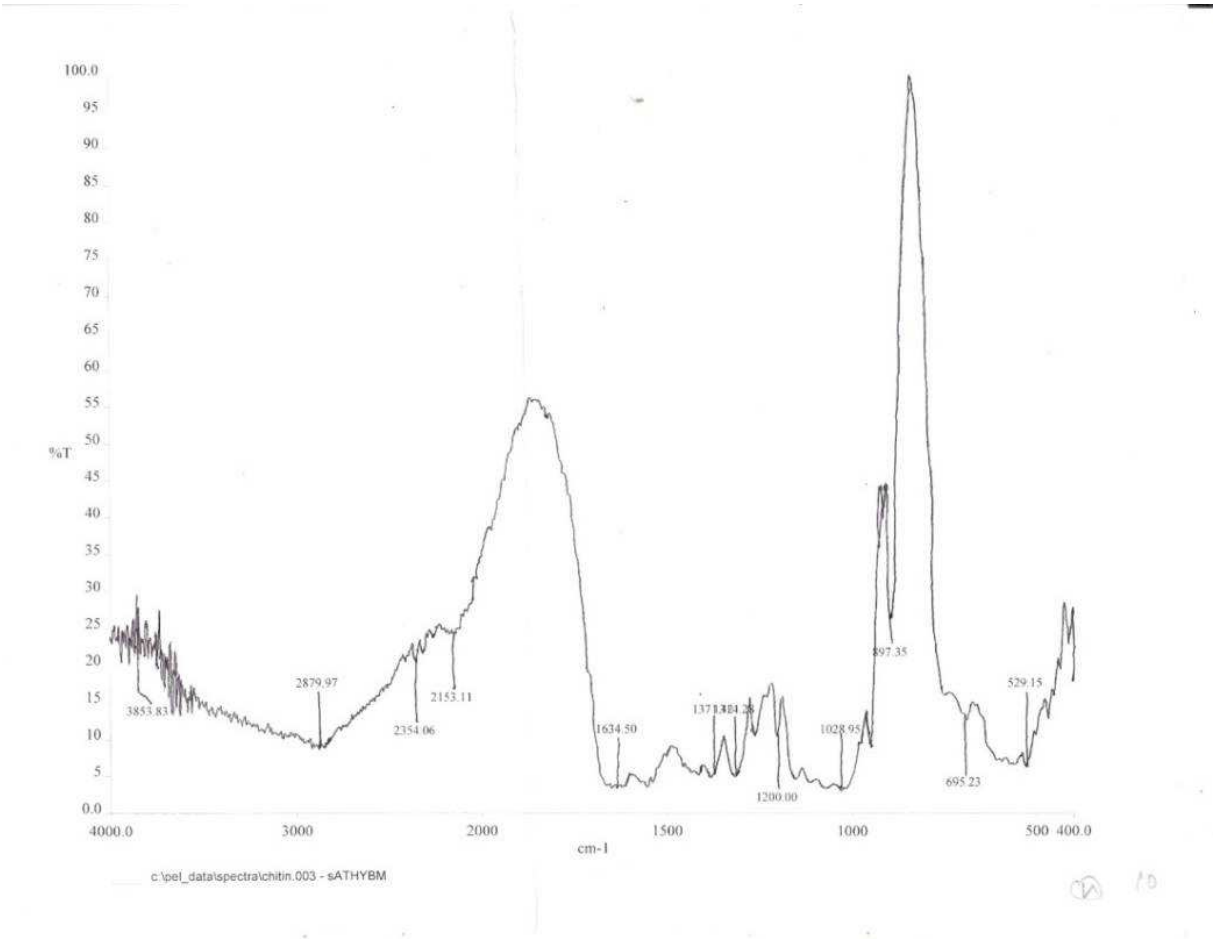


Figure.5. FT-IR spectrum of chitin from *Oratosquilla nepa*



Chitin are used in the preparation of the materials like wound dressing, antibacterial, antifungal agents, dialysis membrane, biomedical beads, fabrics and gauzes[1] in the biomedical field. It is a promising product in the R&D field for production of important medical products.

In the present study, the yield of chitin was more in *Oratosquilla nepa*. 20g of the shell yielded 2.145g (10.725%) in *Oratosquilla quinquedentata*, the chitin yield was 2.125g representing 10.625%. In the past investigation chitin isolated from the carapace and the shell of the claw and legs of *Scylla tranquebarica* was found to be 10.74%, 7.91% and 14.62% respectively. The content of chitin in crab shell varies depending on species as well as the body part within the same species[7]. In supporting this Nair and Madhavan reported 20.5%, 10.0% and 14.0% chitin in body shell, claw shell and legs shell of *Scylla serrata* in which the chitin content of the leg shell[8]. Subasinghe also noticed more chitin yield in snow crab legs (32%) than claws (24%)[1]. Further Das *et al*. also reported the same trend in the other portunids such as in *S. serrata* and *Portunus pelagicus*[9]. They reported that the yield of chitin was more in the shell of legs (16.07% and 20.19%) than in body (11.67% and 13.51%) and claw (10.42% and 11.66%) representing in *S. serrata* and *P. pelagicus*. Cuttle bone of *Salvia officinalis* was found to contain 24% of chitin[10], whereas in general the squid/octopus reported 3-20% of chitin[4].

The content of chitin in stomatopod shell (mantis shrimp) varies depending on species within the same species. The FT-IR peaks at 569.00cm^{-1}, 1070.77cm^{-1}, 1370.41cm^{-1}, 1555.02cm^{-1}, and 1656.82cm^{-1} in chitin from the shell of *O.quinquedentata*, which resembling the peaks of standard chitin values, Whereas in the chitin sample of *t*he *O.nepa* peaks at 529.15cm^{-1}, 695.23cm^{-1}, 897.35cm^{-1}, 1028.95cm^{-1}, 1200.00cm^{-1}, 1324.28cm^{-1} and 1371.11cm^{-1},1634.55cm^{-1} also coincide with the standard value.

Saraswathy *et al.*, has observed the major absorption band between 1220 cm^{-1} and 1020 cm^{-1} represents the free primary amino group (-NH_2) at C_2 position of glucosamine, a major group present in chitin[11]. Further the sample showed the absorption based for the free amino group in, 1028.95 cm^{-1} and 1200.00cm^{-1} from *O.nepa* and 1070.77 cm^{-1} from *O.quinquidentata* which are very closely similar to the standard. When the peak is at 1384cm^{-1}, it represents the -C-O stretching of primary alcoholic group (-CH_2-OH). The primary alcoholic group is represented by a band in 1370.41 cm^{-1}*O.quinqudentata*, 1371.11cm^{-}1, 1324.28 cm^{-1} respectively from *O.nepa.* The values obtained through FT-IR spectrum co-related with the values obtained by Saraswathy *et al* (2001) and hence it can be confirmed that the compound passed through FT-IR spectrum is indeed chitin[11].

From *O. quinquedentata* and *O.nepa* we can infer the presence of free amino group and an alcoholic group. The presence of these functional groups has been confirmed after comparison with the standard FT-IR analysis. Parasakthi (2004) observed the FT-IR peaks at 534.61cm^{-1}, 1024.16cm^{-1}, 1321.88cm^{-1}, 1380.81cm^{-1}, and 1640.40cm^{-1} in chitin from the shell of *Sepia aculeata*. Whereas in the chitin sample of *Loligo duvauceli* the peaks at 533.44cm^{-1}, 896.73cm^{-1}, 1032.11cm^{-1}, 1315.60cm^{-1}, 1377.88cm^{-1}, 1561.36cm^{-1} and 1657.29cm^{-1} were also coincide with the same stomatopod shell samples[12]. Cuttle bone of *Salvia officinalis* was found to contain 24% of chitin[10], whereas in general the squid/octopus reported 3-20% of chitin[4].

Chitin is a wound-healing accelerator and its effectiveness in protecting wound from bacterial invasion by suppressing bacterial proliferation and it may act effectively against typhoid producing micro organisms[13]. It is interesting to note that inspite of the considerable progress in chitin research and the large number of potential application of chitin and its derivatives, their commercial applications are somewhat limited. Hence, it could be safely assumed that at the

present levels of industrial usage of chitin, the shrimp and crab processing industries world over would be capable of satisfying the raw material requirements for decades to come.

The slow growth of the industry has been attributed by some to the negative role of patents, which have tended to slow down market development. It is also believed that instead of examining ways of using chitin as a substitute for ingredients already at use in the agriculture and industry, more research on identifying specific uses and the advantages would help expand the spectrum.

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