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Extraction and Physico-Chemical Analysis of Chitosan from Shell of Marine Crab Scylla Serrata.

KEYWORDS: Scylla serrata crab, Chitosan, FTIR , SEM, EDAX.

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ABSTRACT *Chitosan is deacetylated form of chitin. It is obtained from various sources like fishes, molluscans, arthropods and fungi. Chitosan has diverse industrial applications. The present study was carried out for extracting Chitosan from carapace of locally available crab Scylla serrata. The study was conducted in five replicates. The mean of obtained Chitosan was 1.28gm Chitosan per 3 gram of carapace. Extracted Chitosan was subjected to FTIR, SEM and EDAX analysis. The spectra has characteristic band for Chitosan. The FTIR spectral analyses showing a peak at 3396.99 cm-1 indicate symmetric stretching vibration for OH group and amide group. Absorption peak at 2932.23cm-11 indicate presence of CH stretch , Absorption band at 1594.84cm-1 amide I and 1398.14 was due to C-H stretching and 872.631 cm-1- is a ring stretching a characteristic bond for β-1-4 glycosidic linkage. SEM Studies showed that Chitosan has smooth, nonporous, plane texture with particle size ranging from 3.05 µm to 10.82 µm.*

INTRODUCTION

Chitosan is a deacetylated derivative of chitin. It is extracted from the exoskeleton of arthropods, like crabs, lobster, shrimps cockroaches, scales of fishes and cell wall of fungi. Chemical composition of Chitin is β- (1-4) 2 acetamido-2-deoxy-β–D-glucose. (N-acetylglucosamine). Chitosan is linear polymer of α (1-4 linked-2- amino-2-deoxy- β-D-glucopyranose) [1]. It has three types of functional groups, an amino group at C-2and primary and secondary hydroxyl group at C-3 and C-6 positions. Aranaz *et al.* studied the physicochemical, behavioral and functional properties of chitin and Chitosan. They also reported about the studies on specific applications in drug delivery, tissue engineering, food preservative, biocatalyst, immobilization, waste water treatment, molecular imprinting and metal-nano-composites. [2]

Chitosan is semipermeable and has a film forming property. Chitosan films are tough, long lasting and flexible. They have moderate water permeability and are extremely good barriers for the permeability to oxygen. Film forming ability has been successfully used as food wraps, thus having the property of extending shelf life (Muzzarelli, 1986)[3]. The Chitin and Chitosan has been used as food additive due to their low toxicity, digestibility and ability of reducing cholesterol in human blood (Knorr,1983)[4]. Furthermore, Chitin and Chitosan, obtained from crustacean, can be used in pharmacy, agriculture and the industrial applications other than food (Tharanathan and Kittur, 2003)[5]. In food industry, it has been used as covering material, in applications of packing, gelling agent, additive, antimicrobial, and functional material of food (Shahidi *et al.,* 1999).[6]

It is an eco-friendly pesticide substance that boosts the innate ability of plants to defend themselves against fungal infections. EPA has been regulated the applications of Chitosan for outdoors and indoors plants and crops as well as the USDA National Organic Program regulates its use on organic certified farms and crops[7], [8],[9]. Chitosan elicits natural innate defense responses within plant to resist insects, pathogens, and soil-borne diseases when applied to foliage or the soil [10]. Chitosan enhances photosynthesis, promotes plant growth, stimulates nutrient uptake as well as increases germination rate and sprouting. [11][12].

Chitosan is an important additive in the filtration process. It helps in sedimentation of particles by binding it with phosphorous, heavy minerals and oils from the water. As an important additive in the filtration process, Chitosan can also be used in water processing engineering as a part of a filtration process. Sand filtration apparently can remove up to 50% of the turbidity alone, while the Chitosan act as clari flocculation with sand filtration resulting in 99% purification [13]. Chitosan has been used to precipitate caseins from bovine milk and cheese making. [14][15]. It combines with bentonite, gelatin, silica gel, isinglass, or other fining agents; it is used to clarify wine, mead, and beer. Added late in the brewing process, Chitosan improves flocculation, and removes yeast cells, fruit particles, and other detritus that cause hazy wine. Chitosan combined with colloidal silica which is becoming a popular fining agent for white wines, because Chitosan does not require acidic tannins [16]. [17], [18]. Fungal source of Chitosan has shown an increase in settling activity, reduction of oxidized polyphenolics in juice and wine, chelation and removal of copper (post-racking) and control of the spoilage yeast Brettanomyces. These products are used and approved for European use by the EU and OIV standards [19].

Chitosan has blood clotting property and has recently gained approval in the United States and Europe for using in bandages and other hemostatic agents. Chitosan hemostatic products have been shown in testing by the U.S. Marine Corps to quickly stop bleeding and to reduce

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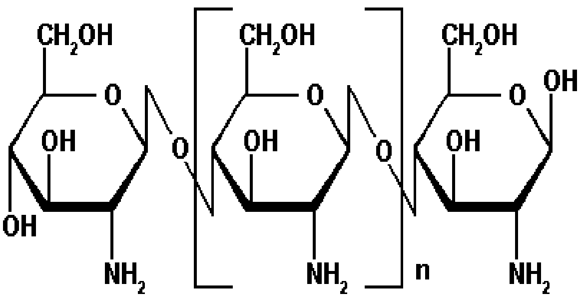
blood loss, and result in 100% survival of otherwise lethal arterial wounds in swine [20]. Homeostasis products of Chitosan reduces blood loss more as compare to gauze dressings and increase patients survival[21]. US and UK have already used the bandages on the battlefields of Iraq and Afghanistan [22]. As a hypoallergenic and having natural antibacterial properties, Chitosan further support its use in field bandages [23]. These hemostatic agents are often Chitosan salts made from mixing Chitosan with an organic acid (such as succinic or lactic acid)[24]. The properties like muco-adhesiveness and positive charge on it, in acidic condition allow Chitosan to used in dermal drug delivery system. Also it can be used to transport a drug to an acidic environment, where the Chitosan packaging will then degrade and releasing the drug to the desired environment for example the transport of insulin [25] [26].

A manufacturing largescale Chitosan from natural sources like nacre (Shellfish), shrimp carapace or insect cuticles,[27] [28][29] has led to development of several Chitosan based industries [30][31] [32]



CHITOSAN

Scylla serrata is widely distributed in western coast of Indian ocean and abundantly available in local market. Scylla sp. is a genus of swimming crabs, composing four species,[3] of which *S. serrata* is the most widespread. They are found across the Indo West Pacific [4].

The main objective of the present research is extraction and chemical characterization of Chitosan from *Scyalla serrata* keeping in view its economic and industrial importance.

MATERIALS AND METHODS

Five *Scylla serrata* were procured from local market of kalyan City, Maharashtra, India. They were sacrificed and carapaces were dried in oven at 60 $^{\circ}$ C for 48hrs. Carapaces were crushed to pieces of 0.5-5.0mm separately and extracted for Chitosan as per the standard method reported by Felicity *et al* [33]. 5gm of carapace powder was boiled into 4% of 100ml NaOH for 1hr on hot plate. Sample was allowed to cool at room temperature. Sample was demineralized with 100ml of 1% HCl. The sample was allowed to soak for 24 hr. to remove the minerals (mainly Calcium Carbonate). The demineralized sample was treated with 50 ml of a 2% NaOH for 1 hr., yield chitin. The chitin was washed with deionized water, thrice and boiled with 100 ml of 50% NaOH at 100^{0} C for 2 hrs. for deacylation. After 2 hrs sample was cooled at room temperature and washed thrice with the 50% NaOH. The sample filtered to obtained solid mass and dried at 120 $^{\circ}$ C in oven for 24 hrs. Chitin to Chitosan synthesis was revealed by adding 5ml of I2 / KI and concentrated Sulphuric acid to the sample. Colour changes from yellow/ brown to dark purple indicate presence of Chitosan [34] (Kumar and Verma). Physical and Chemical characterization of Chitosan was done using FTIR, EDX and SEM.

RESULTS AND DISCUSSION

Results of Chitosan extraction of *Scylla serrata*, is shown in Table 1. The results showed that Chitosan can be successfully obtained from crab. An average of 1.28 gm of Chitosan was obtained from / 3gm carapace. Formation of Purple colour in test solutions confirmed conversion of chitin to Chitosan.

FTIR Spectra of Chitosan was showed in Fig 2. The spectra has characteristic band for Chitosan. The spectra showing a peak at 3396.99 cm-1 indicate symmetric stretching vibration of OH group and amide, Absorption peak at 2932.23cm-11 indicate presence of CH_2 stretch , Absorption band at 1594.84cm-1 amide I and 1398.14 was due to C-H stretching and 872.631 cm-1- is a ring stretching a characteristic bond for β-1-4 glycosidic linkage.

Wanule et.al reported that FTIR analysis of Chitosan extracted from cockroach having characteristic peak of OH and NH symmetric stretch at 3400 CM-1 , 2923 CH stretch , 1095. 49 CM-1 peak for CO group and 872 CM-1 ring stretching characteristic bond for β glycosidic linkage. Those are peaks were similar to Peaks of Chitosan extracted from crab Scylla.

In the present study peak at 3396.99 CM-1 is found very broad indicating that proper deacetylation of chitin. Also characteristic peak present in chitin between 1600 CM-1 to 1700 CM-1 was missing and indicating absence of CO group proving proper deacetylation of Chitosan.

Zakaria et.al reported that peak became wider between 3100 CM-1 to 3400CM -1 with increase in degree of deacetylaion [35] . They also reported that presence of peak at 1639 CM-1 for CO group id indication of incomplete deacetylation of chitin to Chitosan. Shanmugam and Arabi reported the characteristic FTIR peak for Chitosan at 1558 CM-1 for amide group I [36].

Surface morphology, porosity, particle size and texture of Chitosan was studied using Scanning Electron Microscopy (SEM). Morphology of Chitosan was shown in fig 3. It has crystalline, uneven surface, non porous and plane texture of Chitosan. Magnified micrograph (Fig.4) of Chitosan showed Chitosan particle of size ranging from 3.05 μm to 10.82 μm and even larger sized particles. Bhuveneshwari *et.al* reported that pure Chitosan film is nonporous, smooth and uneven [37].EDAX analysis reveals presence of Carbon, Calcium , Oxygen and Magnesium.

This study revealed that chitin and Chitosan can be extracted from *Scylla Serrata.* Crab chitin and Chitosan may be supplementary to other sources of chitin and Chitosan extracted from other animals and may be applicable in many areas like agriculture primarily as natural seed treatment and plant growth enhancer, and as an ecologically friendly bio-pesticide substance that boosts the innate ability of plants to defend themselves against fungal infections. It can be used as a chelation of heavy metal ions, fining agent in food industries, for wound healing and other medical uses, in shampoos and hair conditioners, in water processing engineering as a filter, as fining agent for wine, etc.

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Fig1. Scylla serrata

Table : 1

sample	Weight of entire carapace (in gm)	Weight crushed carapace taken (in gm)	Final product (in gm)
1	5.14	3	1.214
2	5.62	3	1.395
3	4.59	3	1.339
4	3.45	3	1.250
5	3.34	3	1.190
Mean of product = Σ 1.28gm			

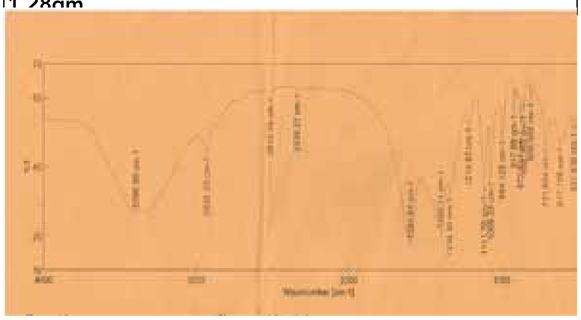

Fig. 2 FTIR spectroscopic analysis of Chitosan extracted from crab Scylla serrata

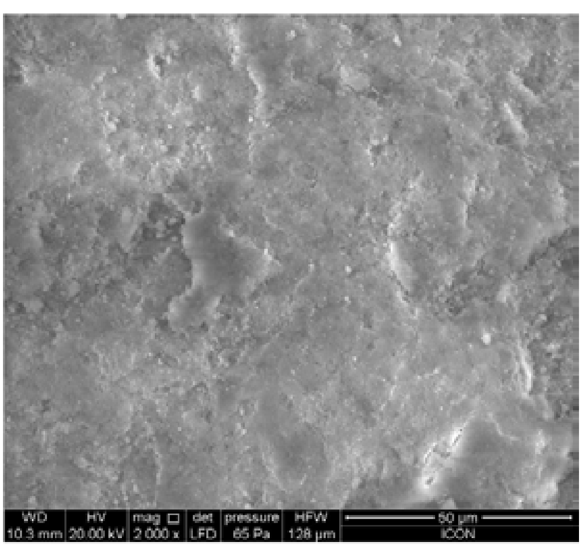

Fig 3 SEM micrograph of Chitosan

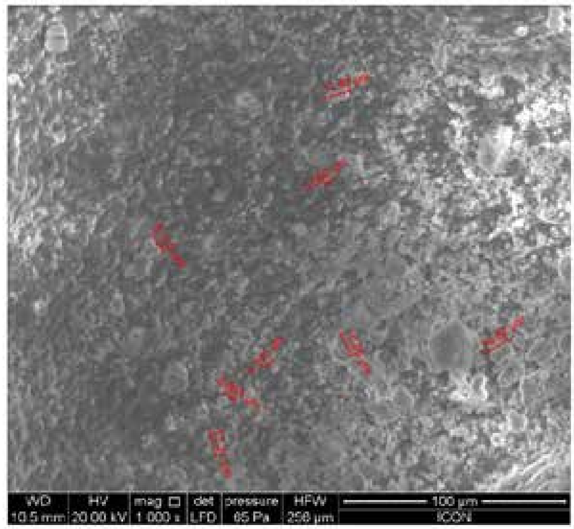

Fig 4 SEM Micrograph Magnified view of Chitosan

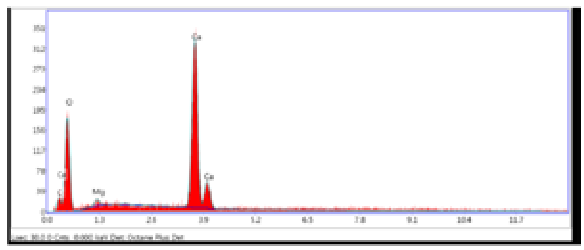

Fig 5. EDAX Analysis of Chitosan .

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