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QUANTITATIVE AND QUALITATIVE ANALYSIS OF CHITIN AND CHITOSAN FROM THE SHELL OF THE MUD CRAB, *SCYLLA SERRATA* (FORSKAL, 1775)

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ABSTRACT

Chitin and Chitosan are polysaccharides found abundantly in nature. The presence of these polysaccharides is found largely in marine ecosystem next only to the plant source is noteworthy. Especially the production and consumption of crustaceans like crabs and prawns have increased in the recent years, thereby generating a large amount of solid shell waste. In the present study the crab, *Scylla serrata* having great importance in mangroves and a sought after species of crustaceans in the coastal region of the country is considered. The shell waste of the crab, *Scylla serrata* is taken for the extraction of Chitin and Chitosan by traditional method of demineralisation, deproteinisation and deacetylation process. Chitin and Chitosan extracted from natural source like crab is non-toxic and biodegradable biopolymer used in medicine, pharmacy and agriculture. By modifying some of the properties of these polysaccharides, they can act as bio adhesives, nanocomposites, antimicrobial agents and for other biocompatible applications. The Chitin and Chitosan obtained in the present study were analysed quantitatively and qualitatively. Thus ensuring the extracted Chitin and Chitosan could be considered for further applications.

Keywords: Chitin, Chitosan, *Scylla serrate*, Polysaccharides.

INTRODUCTION

Chitin is the most widespread biopolymer in nature. Chitin and its derivatives have great economic value because of their biological activities and their industrial and biomedical applications (Wassila Arbia *et al.*, 2011) Chitin, a homopolymer of N-acetyl-D-glucosamine (Glc- NAc) residues linked by β-1-4 bonds (Fig:1), is the most widespread renewable natural resources (Rinaudo, 2006). Chitin is the major component in the shell of the shrimps, crabs, cartilage of the squids, and outer cover of insects, it also occurs as ordered crystalline microfibrils forming structural components in the exoskeleton of arthropods or in the cell walls of fungi and yeast. (Abdulwadud *et al.*, 2013).

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Chitosan is chemically defined as a copolymer of α-(1,4) glucosamine $(C_6H_{11}O_4N)n$, with a varying content of N-acetyl groups. It is produced by deacetylation of Chitin. Figure 2 indicates the chemical structure of glucosamine, which is the building unit of Chitosan.

Chitin and Chitosan is extracted from various plant and animal sources. One of the major sources is the marine ecosystem. Chitin and Chitosan was extracted from sources like mussel shell (Abdulwadud *et al.*, 2013) shrimp type of *Penaeus monodon* (Sewvandi *et al.*, 2012) and Trash Crabs (*Podophthalmus vigil*) (Sunita Das and Anand Ganesh, 2010). Chitin has been extracted from two Tunisian crustacean species (Zouhour *et al.*, 2011). A critical evaluation of potential sources of Chitin and Chitosan concluded that shrimp, prawn and crab wastes are the principle source of Chitin and Chitosan (Wassila Arbia *et al.*, 2011). There are certain reports that Chitin and Chitosan extracted from the naturally occurring sources can be utilized as growth regulator and supplements which shows no toxicity (Junaid *et al.*, 2013).

In the recent years Chitin and Chitosan proved to be a versatile and promising biopolymer. The use of these biopolymers is in various fields. They have an important role as natural alternatives having some biological properties and some specific applications like drug delivery, tissue engineering, functional food, food preservative, biocatalyst immobilization, wastewater treatment, molecular imprinting and metal nanocomposites. The molecular mechanism of the biological properties such as biocompatibility, mucoadhesion, permeation enhancing effect, anticholesterolemic, and antimicrobial has been an area of interest for many researchers (Inmaculada *et al.*, 2009). Shellfish including Crab, lobster and crayfish continue to predominate due to at least two factors. The first is the growth of aquaculture, and the second is the large increase in consumption of crustaceans. The present study focuses on the extraction of Chitin and Chitosan derived from the crab, *Scylla serrata.* Chitin and Chitosan derived from this crab is also subjected to qualitative and quantitative analysis. The naturally derived Chitin and Chitosan (Yadav *et al.,* 2011) which is soluble in acidic aqueous media, can be used in many applications (food,cosmetics, biomedical and pharmaceutical applications). The emphasis on the high value-added applications of these materials in medicine and cosmetics is a promising field in the recent years (Rinaudo, 2006).The objective of the study is to utilize the shell waste of the commercially important crab, *Scylla serrata* to produce an important biopolymer which is subjected to analysis to ensure the quality of the soluble Chitin and Chitosan.

MATERIALS AND METHODS

The live mud crab, *Scylla serrata* was bought from the local markets in and around Chennai.

Extraction of Chitin and Chitosan

Method – I

The crab shells were washed thoroughly in running tap water to remove debris and tissue attached to them. The sun dried crab shells were crushed and soaked in 9% HCl in the ratio 1:14 for 40 hours at room temperature. Then the shells were treated with 5% NaOH. The neutralised shells were sun dried for 2 days and then grinded. The powdered form of Chitin obtained was further subjected to qualitative and quantitative analysis .The deacetylation was done with 70% NaoH solution in a ratio of 1:15 (w/v) for 72 hours at room temperature. The mixture was filtered and dried at 80^{o}C in the oven to obtain powdered Chitosan. (Abdulwadud *et al.,* 2013)

Method – II

The crab shells were washed and cleaned thoroughly to remove foreign materials. The shells were grinded to fine particles. Chitin and Chitosan was extracted using the traditional process involving the following steps. (Abdulwadud *et al.,* 2013). The demineralization process was done by using hydrochloric acid. The filtrate was soaked in 5% NaOH (w/v) in the ratio 1:10 and kept for 24 hrs at room temperature for deproteinization. The powdered form of Chitin obtained from the crab shell was deacetylated with 42.3% NaOH and the filtrate was dried to obtain Chitosan.

Qualitative estimation of Chitin and Chitosan

Chitin to be tested was taken with separated solution of KOH and heated at 160^{o}C for 15-20 min (Richards 1951). The persistence material was rinsed and divided for the following tests. Test – I: A few drops of Lugol's iodine were added to the precipitate. The precipitate changed to brown colour. The excess iodine was removed and 1% H_2So_4 was added. The precipitate was viewed under the microscope. Further addition of 75% of sulphuric acid resulted in an immediate colour change. Test – II: The precipitate was taken in a test tube and 3% of acetic acid was added. And then few drops of 1% H_2So_4 were added. A small amount of the powder was taken and a few drops of 1% acetic acid were added. The mixture was vigorously shaken to check the solubility of Chitin and Chitosan (Abhrajyoti and Gargi 2013).

Quantitative analysis of Chitin and Chitosan

The quantitative analysis of derived Chitin and Chitosan was done by estimating the total moisture, ash and protein content by standard methods (Reaff, 2009)

RESULTS AND DISCUSSION

Qualitative Analysis of Chitin and Chitosan

Test- I

The polysaccharide test performed for Chitin extracted by both the methods showed the presence of reddish purple precipitate. The colour of the precipitate changed to black indicating the presence of Chitin. The precipitate was viewed under the microscope and true colour appeared to be reddish purple. After the addition of 75% of sulphuric acid the colour changed to brown and eventually disappearance off.

Test-II

The precipitate taken with 3% of acetic acid and then addition of few drops of 1% H_2So_4 showed the appearance of white precipitate indicating the presence of Chitosan.

Quantitative analysis of Chitin and Chitosan:

Different workers have reported that Chitin and Chitosan yields differ between species. It is generally accepted that 20-30% of crustacean waste is Chitin (Johnston and Peniston, 1982). These proportions vary with species and season of harvest (Green and Kramer, 1984). Anderson *et al*., (1978) reported 60% yield from crab Chitin**.** In the present study (Table: 1) the total amount of Chitin obtained from method I is 70.17% and method II is

Table 1. Quantitative analysis of Chitin and Chitosan extracted from the shell of the mud crab, *Scylla serrata.*

S. No	Parameters(%)	Chitin extracted by		Chitosan extracted by	
		Method I	Method I	Method II	Method II
1	Yield	70.17	78.0	48.66	43.75
2	Protein	8.3	9.45	17.28	13.6
3	Moisture	4.1	2.68	4.81	6.14
4	Ash content	77	77.86	49.31	62.16
5.	Solubility	Insoluble	Insoluble	Soluble in 1% CH_3COOH	Soluble in 1% CH_3COOH

Figure 1. Chemical structure of Chitin (Lertsutthiwong *et al*., 2002)

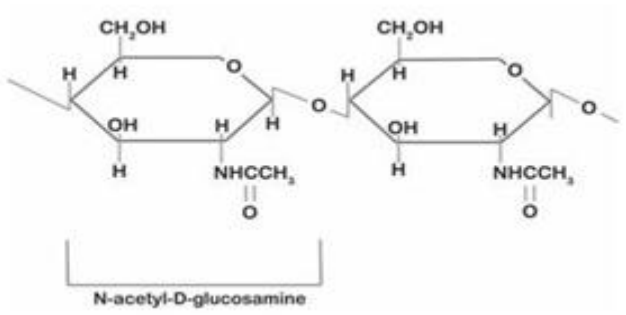
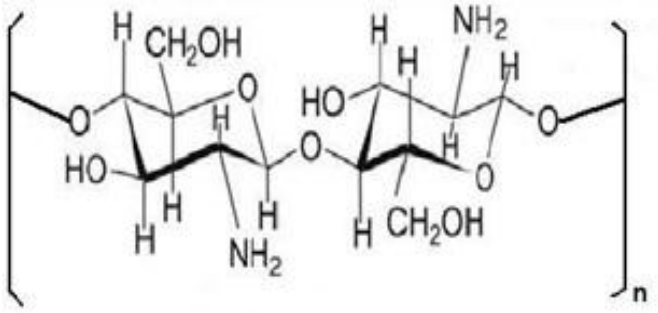


Figure 2. Chemical structure of Chitosan (Lertsutthiwong *et al*., 2002)



43.75%. The yield percentage of Chitosan obtained from both the methods is 78 % and 48.66 % respectively. The amount of protein present in Chitin and Chitosan are 8.3%, 13.6%, 9.45% and 17.28% respectively.

The deproteinisation was more effective in method II where high percentage of protein is obtained. Protein content of the Chitosan sample was considered high after deprotienization of the Chitin and this could be attributed to the low degree of deacetylation of the Chitin (Abdulwadud *et al.,* 2013). In both the methods of extraction the moisture content was below 10% (4.1, 2.68, 2.68% and 4.81% respectively) which indicates the good quality of Chitin and Chitosan. This was expected since the water in Chitin was dried to constant weight before the deacetylation process. The ash content of Chitosan was lower than that of Chitin. This could be attributed because of the presence of acetyl group in Chitin sample as also reported by Isa *et al* 2012. In the present study, the ash content was found to be more in method I than method II. Crab and lobster shells require stronger HCl concentration for demineralisation to reduce the ash in the final Chitosan product (Peter *et al.,* 2005). The solubility of Chitin was not defined when 1% of acetic acid was added but the powdered Chitosan was soluble. Thus in this study the yield of Chitin and Chitosan from the mud crab, *Scylla serrata* by chemical method fall within the ranges reported earlier.

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