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Extraction of Chitin and Chitosan from Prawn Shell Waste

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ABSTRACT
Chitin is a natural polysaccharide made up of N-acetyl-D-glucosamine units linked through a β-(1, 4)-glycosidic bond and is produced by a variety of living organisms. Chitin is a major component present in the exoskeleton of arthropods cell wall of fungi and yeast. The most vital function of chitin is to provide strength and structure to the organism and protection. Chitin tries to exhibit more properties when it is converted into a chitosan. A chitosan is a linear polymer that consists of β-linked D-glucosamine and N-acetyl-D-glucosamine. These are non-toxic, biodegradable and biocompatible polymers. When chitins present in shells of crustaceans are treated with an alkaline substance like sodium hydroxide, chitosans can be obtained. Organisms like shrimp, crab, lobster, prawn, and squid also contain roughly about 14-35% of chitin. The present study was to extract chitin and chitosan from the shells of the prawn and convert these biological wastes into a useful product. Chemical methods like deproteinization, demineralization and decolorization for chitin, and further deacetylation for chitosan were used to extract the same from prawn shells. FTIR analysis of chitin and chitosan were also carried out to identify the functional groups present in it. India generates approximately 9.5 million tons of shell wastes per year. The whole idea is to recycle these wastes and make use of these as fibers, biofilms, affinity chromatography column matrix, plant disease resistance promoter, anti-cancer agent, wound healing promoting agent, antimicrobial agent, preservation of fruit, cosmetics and in pharmaceuticals, instead of dumping them and piling up the waste.
Keywords: Chitin, Chitosan, Exoskeleton, FTIR, Biocompatible polymers.

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INTRODUCTION

Chitin is typically a natural biopolymer predominantly found in the exoskeletal structures of a variety of crustaceans [1]. Chitin is a polymer of amino sugars and creates a very hard and tough outer shell in various organisms for protection [2]. Consumption of chitin externally through diet serves as a tremendous source of insoluble fibers by providing prebiotic properties to the flora present in the gut [3, 4]. The enzyme responsible for breaking down chitin is known as chitinase [5]. This enzyme is required by insects and other crustaceans when they undergo molting [6]. Humans express two kinds of chitinases called chitotriosidase 1 and acid mammalian chitinase [7, 8]. They play a role in the destruction of cell walls of pathogens entering our body. Chitosan is a derivative of chitin and tends to exhibit even greater properties than that of chitin [9]. Chitosan is made up of two monomers- glucosamine and N-acetyl-glucosamine [10]. Chitosans are soluble in water; hence they are biodegradable and biocompatible polymers [11]. They have a wide range of applications in the food and biomedical industry [12, 13]. Crustaceans and arthropods are the main and rich sources of chitin [14]. Tons of waste shells obtained from prawns get dumped. Chitin is acetyl glucosamine groups while chitosan is obtained by removing enough acetyl groups and they are highly soluble in diluted acids. Chitosan is a biodegradable polymer has wide application in pharmaceutical and biomedical industrial, wastewater treatment and food industries [15, 16]. Chitin exhibited anti-proliferative capacity against colon cancer cell HCT116 with its unique feature of degree of acetylation chitosan showed high anti-tumour activity [17]. Chitin polymer films have greater tensile strength compared to commercially available films [18]. The objective of the present study is to extract chitin and chitosan from the waste shells by chemical methods.

MATERIAL AND METHODS

Prawn shell was collected from a local fish market at Aminjikarai, Chennai, Tamil Nadu. The prawn shell was washed 2 to 3 times with tap water, further with distilled water, dried in the oven at 60°C for 24 hours and later they were powdered and stored in – 4°C until use.

Extraction of Chitin and Chitosan:

Powered prawn shell of 25g was deproteinized using 3.5% sodium hydroxide and kept in oven at 65°C for 2 hours. Then the samples were washed using distilled water and filtered. The filtrate was discarded and residue was further demineralized using 1N Hydrochloric acid. Later the samples were decolourized using 1% Potassium permanganate followed by addition of 1% Oxalic acid obtained chitin. Chitin extract was further deacetylated to obtain chitosan using 50% Sodium Hydroxide and content was incubated at 121°C for 30 minutes. This mixture is filtered and filtrate is discarded and the obtained residue was chitosan [19].

PHYSICO CHEMICAL ANALYSIS

Determination of ash content:

Chitin and chitosan of 2gm were taken in two different crucibles. The crucibles were weighed and placed in pre-heated oven at 100°C for 3 hours and transferred to a muffled furnace till the substances turned completely into ashes. Further, it was cooled and weighed and the ash content of samples was calculated using the standard formula [20].

Determination of moisture content:

Chitin and chitosan of 1 gm each was taken in a crucible and placed in oven at 100°C for 3 hours. It was then cooled and weighed. The process was repeated for about 2 to 3 times till a constant weight was obtained. The moisture content of sample was calculated using the standard formula [19].

Estimation of carbohydrate by Anthrone method:

100 mg of Chitin and chitosan was added to 5ml of 2.5 N Hydrochloric acids and heated for 30 minutes at 65▪ C, the samples were cooled and neutralized with sodium bicarbonate the volume was made upto 100ml with distilled water, centrifuged and supernatant were used for the estimation of carbohydrate by Anthrone method [21].

Estimation of protein by Lowry's method:

1g of chitin and chitosan samples was taken and 5ml of 10% TCA was added to it. The solution was kept in ice for 30 minutes. The Homogenate was centrifuged at 1000rpm for 20 minutes and the supernatant was discarded. The pellet obtained was dissolved in 5ml of 0.1N sodium hydroxide. This extract was used for estimation of protein by Lowry's method [22].

Estimation of calcium:

Ash of each 5 gm of chitin and chitosan was dissolved in a few drops of 0.1N Hydrochloric acid and the solution was made up to 100ml in a standard flask. 20ml of this solution was taken and made up to 40ml by adding 20ml of 4% ammonium oxalate and kept overnight to allow precipitation of calcium oxalate. It was then filtered and then washed with ammonium hydroxide (2-3 times). After washing the precipitate was dissolved in 100ml of 2N sulphuric acid and heated to bearable warmth (60°C). The liberated Oxalic acid was then titrated against potassium permanganate. The titration value was noted and the amount of calcium present in a given food sample was calculated [23].

Anti-microbial activity:

The antimicrobial properties of sample chitin and chitosan were tested against gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli, Klebsiella aureus, Pseudomonas*) by Agar well diffusion method. 50µl of extracts in different concentrations was added to respective wells. The plates were placed in the refrigerator for 30mins to let the extracts diffuse well into the agar. Then, the plates were incubated at 37°c for 24hrs under aerobic conditions. After incubation, confluent bacterial growth was observed [24].

FTIR analysis:

Identification of chemical constituents and elucidation of structures of compounds was carried out with Fourier transform infrared (FTIR) spectrometry.

RESULT

The percentage yield of wet prawn shell was determined. 25g of powdered prawn shell was deproteinized, deminerialized and decolourized to obtain chitin and deacetylated to chitosan (Fig 1). The moisture, total ash, carbohydrate, protein and calcium content in chitin and chitosan are discussed in **Table 1.** The amount of carbohydrate in chitin and chitosan was found to be 4.1mg and 1.2 mg in 100ml of sample respectively. The protein content in chitin and chitosan was found to be 5.7 mg and 0.98 mg in 100ml of the sample respectively. The Calcium present in chitin and chitosan extracted prawn shell was found to be 140 mg and 183 mg respectively. The antimicrobial activity of chitin and chitosan at different

concentrations has a potent antibacterial effect against *E. coli, Klebsiella, Staphylococcus aureus and Pseudomonas* organisms (**Table 2)**. Various types of chemical bonds present in molecules can be identified using FTIR Spectroscopy by producing an IR absorption spectrum; molecular finger (**Fig 2, 3**). The frequency 1458 – 1591 is phenol ring, 600- 700 C-S linkage, 550-690 is halogen compound. The functional group detected using FTIR is presented in **Table 3.**

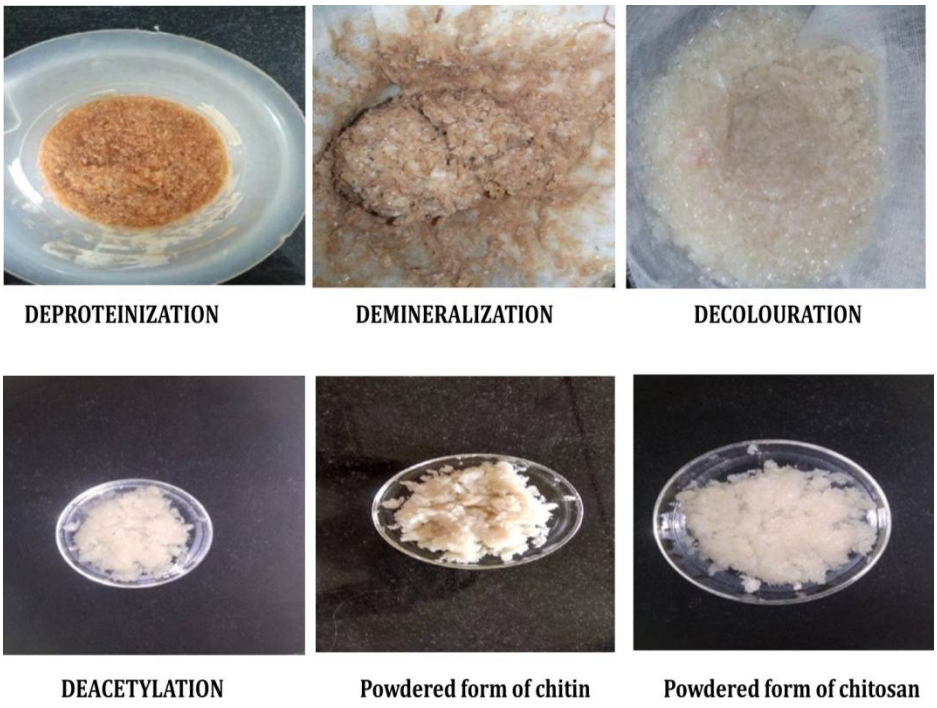

Fig1. Extraction of Chitin and Chitosan.

Fig 2: FTIR Analysis of chitin

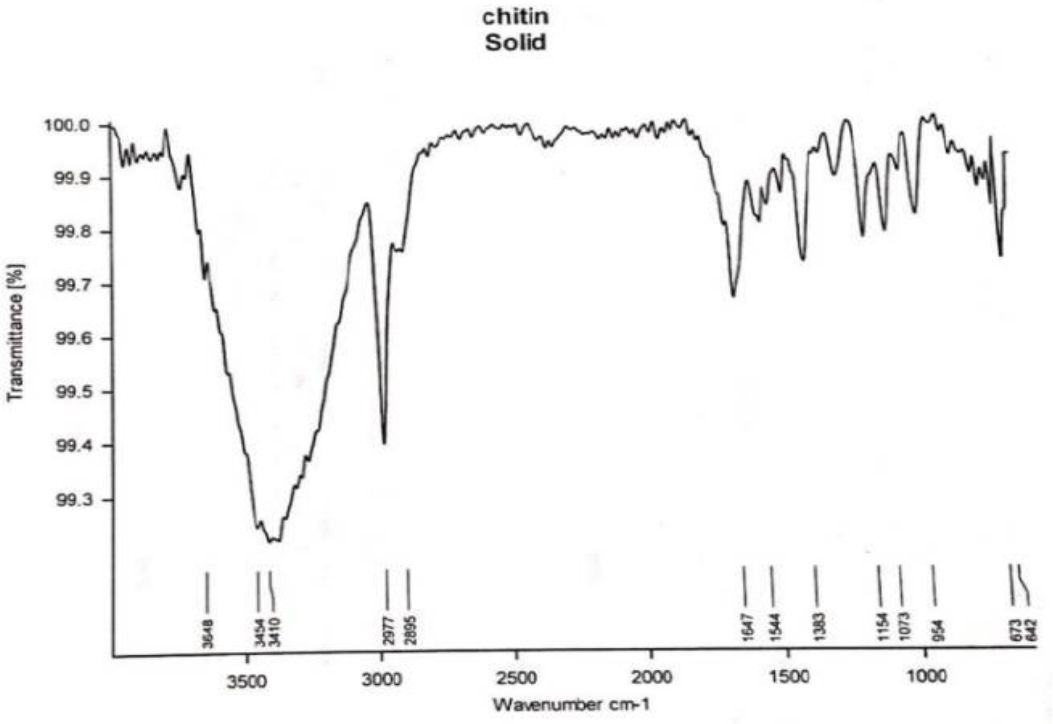

Fig3: FTIR Analysis of chitosan

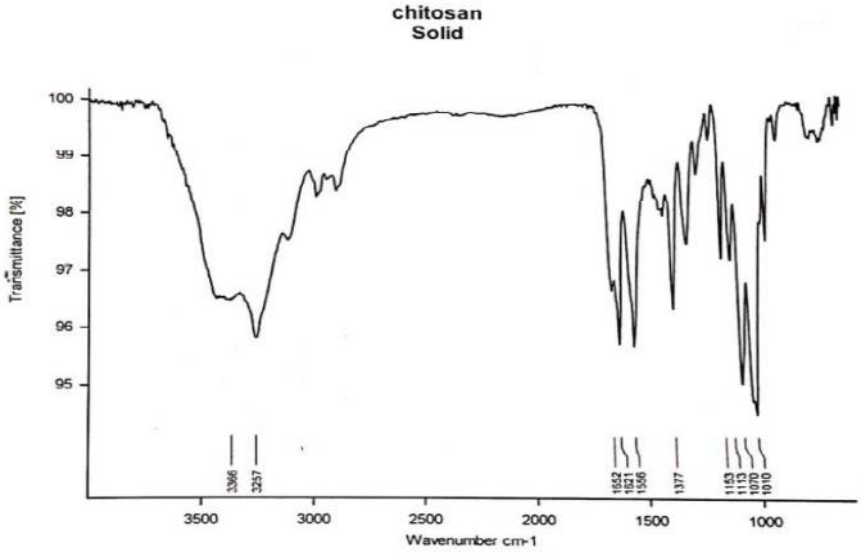

TABLE 1. YIELD PERCENTAGE, PHYSICO CHEMICAL ANALYSIS AND PROXIMATE ANALYSIS

SAMPLE	YIELD (%)	MOISTURE (%)	ASH (%)	Carbohydrates (mg)	Protein (mg)	Calcium (mg)
Chitin	62.92	2	105	4.26 ± 0.20	5.56 ± 0.15	140
Chitosan	68.4	1	113	1.03 ± 0.15	0.97 ± 0.025	183

Values were expressed as mean ± S.D for 3 different preparations.

TABLE 2. ANTIMICROBIAL ACTIVITY OF CHITIN AND CHITOSAN

Pathogens	Concentrations (µl)	Zones of inhibition (mm)	Zones of inhibition (mm)
		Chitin	Chitosan
Escherichia coli	**10**	**5**	**10**
	20	**10**	**14**
	30	**12**	**19**
	40	**15**	**23**
	50	**17**	**31**
Klebsiella	**10**	**2**	**7**
	20	**3**	**12**
	30	**8**	**20**
	40	**12**	**25**
	50	**15**	**32**
Staphylococcus aureus	**10**	**12**	**15**
	20	**13**	**20**
	30	**15**	**24**
	40	**20**	**27**
	50	**24**	**35**
Pseudomonas	**10**	**7**	**12**
	20	**9**	**16**
	30	**12**	**22**
	40	**17**	**25**
	50	**20**	**32**

TABLE 3. FUNCTIONAL GROUPS DETECTED USING FT-IR IN CHITIN AND CHITOSAN OF PRAWN SHELL

BOND	TYPE	FREQUENCY RANGE cm^{-1}	NATURE
O-H	Hydrogen bonding Alcohol's, Phenols	3200-3600	Variable, sometimes broad
C=C	Alkenes	1610-1680	Variable
C-H	Alkanes	1340-1470	Strong
C-O	Alcohols, ethers, carboxylic acids, esters	1050-1300	Strong
C-H	Alkenes	675-995	Strong
-C≡C-H	Alkynes	2100-2270	Variable
C-H	Alkanes	2850-3000	Stretching
N-O	Nitro compound	1556	Stretching
O-H	Alcohol	3584-3700	Stretching
C-O-C	Alkenes	871.82-671.233	Weak

DISCUSSION

The purpose of this study is to find solution for the dumping the waste into a renewable resource. Chitin and chitosan extracted from lobsters' shell source has shown the yield percentage of 35% and 41%, moisture and ash has showed 7.4, 0.73, 1.2 and 0.79%. Chitosan has antioxidant property and it can be used in pharmaceutical industry and tissue engineering**.** The proximate analysis of *Actinidia deliciosa* has higher fat and protein content than Persea americana fruit [25]. Chitin and chitosan extracted from marine organisms has antibacterial, antifungal, antitumor and antioxidant properties. Chitin is a natural polymer next to cellulose has more applications than the transformed to chitosan [26]**.** Different stretching vibration bands were observed in the range 3425-3422 cm-1 related to ν (N-H), ν (N-H), ν (O-H) and ν (NH2) [27]. Chitosan can be used as water treatment, as thickener in food industries, Column matrix, plant disease resistance promoter, anti-cancer agent, wound healing promoting agent, antimicrobial agent, preservation of fruit, cosmetics, artificial organs and pharmaceuticals [28].

CONCLUSION

India generates approximately 9.5 million tons of shell wastes per year. Our project was carried out on a smaller scale. However, if extraction can be carried out on a larger scale, it could be an alternative to control environmental pollution by avoiding dumping of shell waste in our surroundings. Chitin and chitosan can be extracted from various kinds of crustaceans and arthropods and used as fibers, biofilms, insecticides, plant disease resistance promoters, wound healing promoters, antimicrobial and anti-cancer agents. Further, they can be utilized in various analytical techniques such as, in affinity chromatography matrix, gas-selective membranes and bioplastic.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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