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Extraction of Chitin and Chitosan from Biowaste of Scampi *Macrobrichum rosenbergii* and Tiger Shrimp *Penaeus monodon*

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A B S T R A C T

Chitin and chitosan is an important natural resources and are estimated that almost as much as cellulose. Chitin is the fiber in shellfish such as crab, cobster, shrimp, and prawn. The shrimp industry generates a huge amount of shell waste per processing which usually cause environmental nuisance. This waste can be utilized as an economic source of chitin and its derivative chitosan. The chitin and chitosan are considered versatile and promising biomaterial. Keeping the significance of chitin and chitosan, the present investigation has taken up to evaluate the yield percentage and quality parameters between the fresh water prawn *Macrobrachium rosenberfii* and tiger shrimp *Penaeus monodon*. Compare with the between two species *M. rosebergii* was better chitosan producer.

Introduction

Nanoparticles are becoming increasingly important in many areas, including catalysis, biological applications and information storage. Their unique size-dependent properties make these materials superior Chitin and chitosan are the second most available biopolymer after cellulose. Chitosan extraction consists of four common steps such as demineralization, deproteinisation, decolourisation and N-deacetylation. Synthetic polymers have all along been the major materials involving in our daily life. They are seen in a wide range of applications from dietary to mechanical support. However, they have also created problems in disposal as they are considered not biodegradable and take up millions years to degrade back to the nature. In other words, they consume a large space and become a major issue as environmental pollution increasing the demands for biopolymers, which exhibit the characteristic of biocompatibility, biodegradable, and non-toxicity. Chitosan being as a biopolymer extracted from shrimp shells can be developed to act as a solution for environmental issue. In addition, chitosan also gains its fame in wastewater treatment and bio medical field due to its metal absorption and antibacterial properties respectively. Derivatives of chitin oligomers have also been implicated as morphogenic factors in the communication between

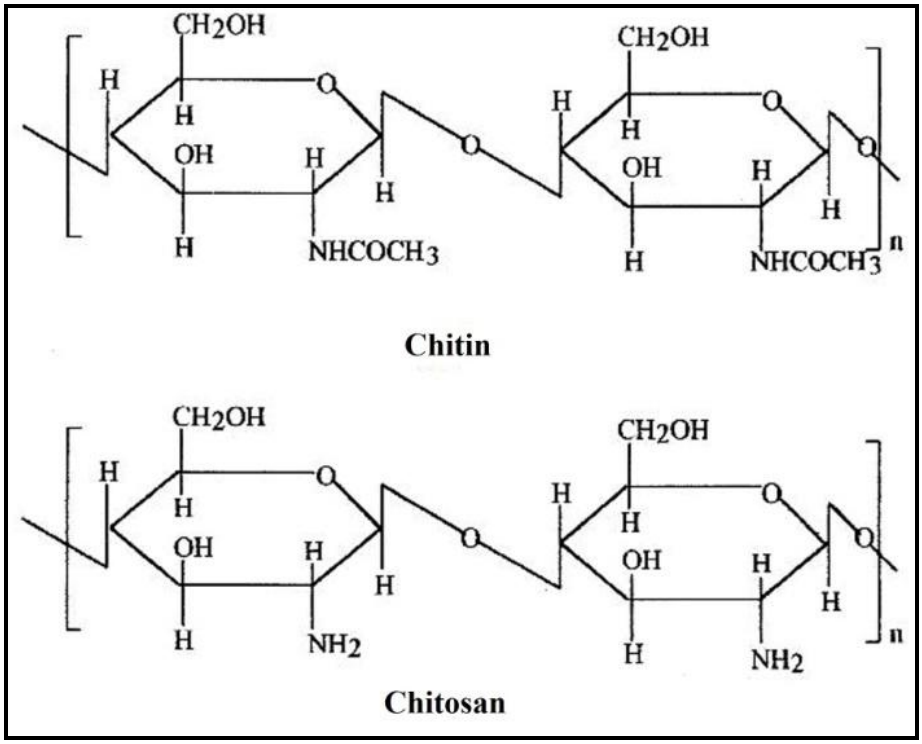
leguminous plants and *Rhizobium* and even in vertebrates, where they may be important during early stages of embryogenesis (Bakkers *et al.*, 1999). In India alone 60,000 to 80,000 tonnes of chitinous wastes are produced annually, from which a lot of chitin can be recovered from crustacean biowaste (Suresh and Chandrasekaran, 1998). At present only a small quantity of shell waste is utilized for animal feed or chitin isolation (Synowiecki and Al-Khateeb, 2003).

Conventionally these wastes are disposed off either by burning or land filling better use methods are harmful to the environment, since burning releases carbon dioxide and carbon monoxide to the environment, which adds to global warming while land filling is harmful due to slow rate of degradation and concomitant release of a potent pollutant of ground water, namely, ammonia (Muzzarelli, 1997). The cost of transporting such as waste, environmental pollution concern and ethical questions as to the morality of ignoring 70-80% of the dry weight of the catch have highlighted the necessity of finding alternative method (Simpson and Haard, 1985; Vyas and Deshpande, 1991). Utilization of such chitinous wastes for the production of some useful products is being considered lately, and two different approaches are being investigated: The formation of a useful product such as chitin and chitosan through biological (Gagne and Simpson, 1993) and their uses in sewage treatment, animal feed, food preservation, and formulations of biofungicides (Muzzarelli, 1997; Gohel *et al.*, 2005), Using the waste as a carbon source in fermentation processes for the production of useful products such as chitinolytic enzymes by microorganisms.. Recently the commercial value of chitin has increased because of the beneficial properties of its soluble derivatives, which are suitable in chemistry, biotechnology, agriculture, food processing, cosmetics, veterinary, medicine, dentistry, environment protection and paper or textile production (Synowiecki and Al-Khateeb, 2003; Tharanathan and Kittur, 2003). Generation of this enormous amount of waste and more importantly the increasing commercial value of the soluble derivatives of chitin necessitates the development of a suitable process for solubilization of chitinous waste and its conversion into useful polymers. The chemical methods by which these polymers and their oligomers are produced commercially involve treatment with harsh chemicals like hydrochloric acid and sodium hydroxide. Besides being environmentally unsafe, the use of these chemicals leads to products that lack uniformity. The enzymatic methods, which employ enzymes such as chitinases, are mild and eco-friendly, and thus preferred over chemical methods.

Chitosan made from chitin is a white to light-red solid powder, insoluble in water, soluble in organic acids, but indigestible by human digestive enzymes. It does not dissolve in standard polar and non polar solvents. Chitosan is insoluble in most organic solvents and in water at neutral pH. However, it dissolves in acidic solutions. Chitin and its derivative chitosan are of commercial interest due to their excellent biocompatibility, biodegradability, non-toxicity, chelating and adsorption power. With these characteristics especially chitosan has many attractive applications in biotechnology, food and pharmaceutical industry, in cosmetics, environmental engineering, in agriculture and aquaculture (Muzzarelli *et al.*, 2012; Franco and Peter 2011; Ling *et al*. 2011). Quality of chitosan is determined from several parameters, the degree of deacetylation is a quality parameter that indicates an acetyl group which can be removed from yield of

chitosan. High deacetylation degree of chitosan means that the acetyl group contained in the chitosan is weak. Deacetylation degree of chitosan varies between 56-99% an average of 80% depending on the source and method of preparation (Hussain *et al.*, 2013). Other quality parameters of chotosan are the colour of a chitosan, wherein the application of chitin and chitosan also dependent on its colour. Chitosan with white colour or looks clean has better quality so commonly used in the field of health and food industries.

Keeping in view of significance and applications of chitosan, the present investigation has been taken up to evaluate the difference in yield % and in the quality parameters between the fresh water prawn *Macrobrachium rosenbergii* and marine shrimp *Penaeus monodon.*



Materials and Methods

One Kg of freshwater prawn *Macrobrachium rosenbergii* and marine shrimp *Penaeus monodon* collected from culture ponds at Bhimavaram. Bo-waste (carapace, exoskeleton, appendages, etc) are separated from the both samples, and wet weight of both samples was noted. Shell material now allowed to dry at 50^{o}C in oven for 24 h and homogenized in a laboratory mixer before shipping for further processing. The yield of both dried shell samples were determined by weighing after being dried. The obtained shell samples were stored at about 25^{0}C in the storage facility till needed. The most common procedure is chemically very simple, treatment of biowaste with 4% alkali to separate the protein and treatment with 4% acid to remove the calcium carbonate. The resulting chitin products can also be further deacetylated by concentrated 50% alkali to produce chitosan.

Crushed biowaste samples were placed in 1000 ml beakers and soaked in boiling

sodium hydroxide (2 and 4% w/v) for one hour at 70-120^{0}C in order to dissolve the proteins and sugars thus isolating crude chitin. 4% NaOH is used for chitin preparation, concentration used by the scientists at the Sonat corporation. After the samples are boiled in the sodium hydroxide, the beakers containing the shell samples are removed from the hot pate, and allowed to cool for 30 minutes at room temperature. The exoskeletons are then further crushed to pieces of 0.5-5.0 mm using a meat tenderizer. The grounded exoskeleton samples are de-mineralized using 1% HCl with four times its quantity. The samples are allowed to soak for 24 h. to remove the mineral (mainly calcium carbonate). The de-mineralized samples were then treated for one hour with 50 ml of a 2% NaOH solution to decompose the albumen into water soluble amino-acids. The remaining chitin is washed with deionized water, which is then drained off. The chitin was further, converted in to chitosan by the process of deacetylation

The deacetylation process is carried out by adding 50% NaOH and then boiled at 100^{0}C for 2 h on a hot plate. The samples are placed under the hood and cooled for 30 min. at room temperature. After wards the samples are washed continuously with the 50% NaOH and filtered in order to retain the solid matter, which is the chitosan. The samples were then left uncovered and oven dried at 110^{0}C for 6 h. the chitosan obtained will be in a creamy white form (Muzzarelli and Rochetti, 1985).

Characterization of Prepared Chitosan

Degree of Deacetylation

Degree of deacetylation refers to the removal of acetyl group from the chain, this is determined by potentiometric titration. Chitosan homogenous solution is prepared using dil. HCl containing 0.010 mol/L which is titrated against 0.1 M NaOH. The end point is detected by the inflection of the pH values. Two inflections were mainly noted out of which first one corresponds to neutralization of HCl and second one to the neutralization of ammonium ions for chitosan chain. The difference between two points give the amount of the amino group in the chtosan chain (degree of deacetylation) (Zhanga *et al.*, 2010).

Ash Value

To determine the ash value of chitosan, 2.0g of chitosan sample is placed into previously ignited, cooled, and tared crucible. The samples are heated in a muffle furnace preheated to 6500C for 4 h. the crucibles are allowed to cool in the furnace to less than 2000C and then placed inti desiccators with a vented top. Percentage of ash value is calculated using the following.

$$\% \text{ Ash } = \frac{\text{Weight of Residue (g)}}{\text{Sample Weight (g)}} \text{x } 100$$

Moisture Content

Crude chitin sample was placed in a pre-weighted aluminium dish. The dish and contents were then placed in an oven at 1050C for 24h. The aluminum dish along with the dried sample was first placed in a desiccator to cool down and then weighted. The moisture content was determined as follows (Mahmoud, 2007)

$$\text{Moisture Content } = \frac{\text{Wws} - \text{Wds}}{\text{Wws}} \text{ X } 100$$

MC = Moisture content (%)
Wws = Weight of the wet sample (g)
Wds = Weight of the dry sample (g)

Proteins

Protein was estimated according to the method described by Lowery *et al.*, (1951). One gram of cultured copepod, *P.parvus* was homogenized with double distilled water and the extract was centrifuged at 4000 rpm for 10 minutes. To 1ml of the supernatant, 4ml of Biuret reagent was added and incubated for 20 minutes. The optical density (OD) of the color developed was read at 540nm using spectrophotometer and the protein was calculated by referring to the standard graph of Bovine serum Albumin. The result was expressed in percentage.

Fats

For the estimation of fats, chloroform: methanol method was followed (Folch *et al.*, 1957). Sample (400 mg) was homogenized with 5ml of chloroform: methanol mixture (2:1) and filtered by a fat filtering unit. The filtered solution was poured into a previously weighed 10 ml beaker and kept in an oven at 70ºC for 24 hrs. The difference in weight between the empty beaker and the beaker containing fat was expressed as the amount of fat in the sample analyzed.

Percentage of Yield

Percentage of yield for chitosan was calculated from the weight of chitosan produced as a percentage of starting dry raw material (Zaku *et al.*, 2011).

$$\% \text{ Chitin or Chitosan} = \frac{\text{Weight of Residue (g)}}{\text{Weight of initial dry raw materia (g)}} \text{ X } 100$$

Results and Discussion

The results of the present study given in Table No.1 and 2. Chitin and chitosan are attracting a great deal because of their distinctive biological and physico-chemical characteristics. Chitin and chitosan have been used in various industries ranging from waste management to aqua food processing, medicine and Biotechnology. To date a lot of research been done to produce chitin and chitosan from various sources like shrimp (Laila *et al.*, 2010; Kucukgulmez *et al.*, 2012; Tajik *et al.*, 2008); Crab (Felecity *et al.*, 2007; Matheis *et al.*, 2012) and other sources like insects and moluscus.

Table.1 Yield of bio-waste from two experimental organisms

Experimental Species	Total sample weight (g)	Wet Bio-waste (g)	Dry weight of crude (g)	Chitosan yield per Kg of sample (g)
Macrobrachium rosenbergii	1000	580	124.8	32.44
Penaeus monodon	1000	455	109.2	26.64

Table.2 Proximate analysis of exoskeleton *Macrobrachium rosenbergii* and *Penaeus monodon*

Parameter	*Macrobrachium rosenbergii*	*Penaeus monodon*
Moisture (%)	75.2 ± 5.6	76.2 ± 5.7
Ash Content (%)	33.25 ± 3.38	34.9 ± 3.87
Proteins (%)	31.25 ± 2.38	28.89 ± 2.17
Fats (%)	4.77 ± 0.15	6.77±0.95

The fresh water prawn *Macrobrachium rosenbergii* and shrimp *Peneaus monodon* are one of the potential species for chitosan production. In the present study biowaste of 580mg *M.rosenbergii* goes to 32.44gms chitosan, *P.monodon* produced 455 gms biowaste and this biowaste produced 26.64gms of chitosin.

Extraction of chitosan from freshwater prawn *Macrobrachium rosenbergii* and tiger shrimp *Penaeus monodon* exoskeleton requires chemical treatment the shell even though contains majority of chitin, also has proteins and minerals. The chitin and chitosan are used in the preparation of materials like wound dressing, antiviral and antifungal agents, dialysis membranes Biomedical beads, Fabrics and gauzes (Subashinghe, 1999). Chitosan is a wound healing accelerator, and its effectiveness in protecting wound from bacterial invasion by suppressing bacterial proliferation. It may cat as effectively against typhoid producing microorganism (Yadav and Bhine, 2004). The result of present study one kg of prawn *Macrobrachium rosenbergii* sample produced 580gms of biowaste and *Penaeus monodon* produced 455gms of biowaste. In case of yield goes to 124.8 gms (*M. rosenbergii)* and 109.2 gms (*P. monodon*) in the sample (Table. 1).

It has been observed that the percentage of chitosan yield from shrimp waste collected from *Penaeus semisulcatus* is found to be 32.25% (Khanafari *et al.,* 2008), chitosan yield from biowaste of *Penaeus carinatus* and *Penaeus monodon* was found to be 34% (Yateendra *et al.,* 2012),it is reported as 18.6% (Alimuniar *et al.,* 1992), 30% (Sibi *et al.,* 2013), 17% (Mohanasrinivasan, 2014), chitosan yield from biowaste of *Penaeus monodon*, chitosan yield from the biowaste of *Penaeus monodon* was found to be 67.47% and 46% (Anshar patria, 2013; Divya *et al.,* 2014). It is obvious that the amount of chitosan yield is proportional to the amount chitin obtained from the bio-waste of shellfish, the amount of chitin yield intern depend on the amount of biowaste obtained from shellfish. Partially acetylated chitosan polymers exhibit a number of biological activities, including antimicrobial activities, elicitor activities inducing disease resistance in plants, and diverse stimulating or inhibiting activities towards a number of normal or transformed human cell types. Purified and well characterized chitosan showed biological activities correlated with physico-chemical properties of the polymers used. The source made from waste (chitosan) shows an excellent antimicrobial activity against human pathogens. Thus, it can be used as good potent source against the infectious pathogens.

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