



Comparative study on Extraction, Physico Chemical Properties and Antibacterial activity of Chitosan from different crustacean shell wastes

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Abstract:

In India export of processed and frozen shell products is that the backbone of sea food export industry. The waste generated from the worldwide production and process of shellfish could be a significant issue of growing magnitude and could be a threat to the surroundings. Particularly the assembly and consumption of crustaceans like crabs, mussels and prawns have enhanced within the recent years, thereby generating an outsized quantity of solid shell waste. It is essential to convert shell waste into helpful products like chitin, chitosan etc., by recycle and reducing the waste additionally as contribute towards profitable work and economic advantages. Chitosan is created from chitin by a chemical change involving demineralization (DM), deproteinization (DP), and deacetylation (DA). This research aims to comparative study was undertaken to extract chitosan from some crustacean shells (*Scylla serrate* (mud crab), *litopenaeus setiferus* (white shrimp) and *modiolus modiolus* (horse mussel), wastes) and were subjected to physicochemical properties are moisture, ash, yields, degree of deacetylation, water, and fat binding capability were additionally determined. The active biomolecules like chitin and its derivatives undergoing a major and really quick development in the food application space. Therefore the current investigation is geared toward usage different shell waste by using it for work the bactericide activity against medically necessary pathogens. Finally, the tested materials were determined through disc diffusion technique against 3 strains of Gram-negative microorganism (*Escherichia coli*; *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and 2 Gram-positive bacteria (*Bacillus subtilis*; *staphylococcus aureus*). This study suggests that *Scylla serrate* may well be used as a supply of high amount of chitosan than *litopenaeus setiferus* and *modiolus modiolus* and it is an advantage for elaborated studies. Hence, this treatment is effective for realizing recent bioactive compounds from natural sources which can enhance the invention and development of recent drugs. Our study conjointly pointed to the chance of using of chitosan as a natural supply of bactericide compounds.

Keywords: chitin, chitosan, shrimp, and crab.

Introduction:

Chitosan is one among the foremost studied polysaccharides these days. Chitin and Chitosan are extracted from numerous plant and animal sources. One of the key sources is that the marine system. Polysaccharide and Chitosan were extracted from sources like mussel shell (Abdulwadud et al., 2013) [1] shrimp form of *Penaeus* genus *Monodon* (Sewvandi et al., 2012) [28], and Trash Crabs (*Podophthalmus vigil*) (Sunita Das and Anand Ganesh, 2010) [31]. Polysaccharide has been extracted from 2 Tunisian crustacean species (Zouhour et al., 2011) [40]. A crucial analysis of potential sources of polysaccharide and Chitosan terminated that shrimp, prawn, and crab wastes area unit the principle supply of polysaccharide and Chitosan (Wassila Arbia et al., 2011) [36]. Their area unit bound reports that polysaccharide and Chitosan extracted from the present sources will be utilized as phytohormone and supplements that show no toxicity.

Food has been thought of as healthy food for humans and its by-products may be utilized for the production of an added product like enzymes, xanthophylls, chitin/chitosan, and glucosamine (Shahidi, F. and R. Abuzaytoun, 2005) [29] (Kim, S.K. and J. Venkatesan, 2014) [13]. Chitosan is basic sugar and part deacetylated compound of glucosamine obtained from polysaccharides by alkalescent deacetylation (Guibal, E., 2004) [9]. Chitosan consists of β -(1-4-2- acetamido-2-deoxy-D-glucose) units and when polysaccharide it's the second exuberant biopolymer on earth. Chitosan has been utilized in many agricultural, food protections in medical specialty and antibiotics applications as drug delivery systems or in drugs formulations (Muzzarelli, R.A.A., 2011) [21].

The objective of the study is to utilize the shell waste of the commercially necessary crab, mussel and white shrimp mythical being *Serrata* to supply a vital biopolymer that is subjected to analysis to make sure the standard of the soluble polysaccharide and Chitosan.

In recent years, polysaccharides and Chitosan tried to be flexible and promising biopolymers. The employment of those biopolymers is in numerous fields. They need a vital role as natural alternatives having some biological properties and a few specific applications like drug delivery, tissue engineering, efficient food, food preservative, accelerator immobilization, waste water treatment, molecular fixing, and metal Nano composites. The molecular mechanism of the biological properties like biocompatibility, mucoadhesion, permeation enhancing result, anticholesterolemic, and antimicrobial has been a region of interest for several researchers (Inmaculada et al., 2009) [11].

In India, the crab and shrimp area unit are the foremost necessary crustacean seafood. White shrimp shell is a by-product of the food processor with many thousands of tons it contains four-hundredth carbonate, 35% macromolecule, and chitin (Meyers et al., 1990) [18]. So, the employment of crustacean shell wastes may be utilized in business, food process, biomedicine, biotechnology, cosmetics, and agriculture and conjointly environmental issues will be solved (Sadek et al., 2002) [26]. Fish wastes area unit one among the foremost necessary atmosphere issues that have to attach a growing interest particularly within the times of its harmful effects on the environment, human health and safety and even additional importantly; this can be the way to get obviate them. However, these residues area unit a supply of value sources by increasing the take pleasure in them which needs the event of associate degree integrated management system. Therefore, nice attention has been paid to the employment of crustacean shell wastes therefore, the target of

this study is to extract and characterize chitosan from shrimp, mussel shell wastes and crab shell and so as to explore potentialities for his or her utilization.

Materials and methods:

Collection of sample:

The shells of *Scylla serrate* (mud crab), *litopenaeus setiferus* (white shrimp) and *modiolus modiolus* (horse mussel) were collected with the help of the local fisherman from mallipattinam coast, Pattukkottai taluk of Thanjavur district, Tamilnadu. (10°16'N, 79°19'E) The collected shells were scraped free of slack tissue, washed with water, boiled and dried under the sun for 8 hrs. Afterward shells were pulverized and sieve (60.80 mesh) for the powder extraction of chitin and chitosan. The samples were stored in a closed container prior to use.

Chitin extraction:

Deproteination (DP)

(Sagheer et al., 2009)^[4] Were utilized to deproteinize, demineralized, and deacetylation shell wastes. The sample was then deproteinized with 300ml of 1N NaOH at eighty °C for twenty-four-hour with constant stirring. The NaOH was changed intermittently and therefore the sample was washed with water when before adding contemporary NaOH. Once twenty-four hours the sample was filtered. The sample filtrate was washed as before and dried.

Demineralization (DM)

Samples from the deproteination method were supplemental with one.0 M HCl within the quantitative relation 1:16 (w/v) and allowed to face for twenty-four hours (Puvvada et al., 2012)^[23] with hydrogen ion concentration price ranged hydrogen ion concentration one.0-2.5 at the temperature (~25° C). After that, the answer was filtered and therefore the samples were washed with water till Neutral hydrogen ion concentration was achieved (pH6.5-8.0). The samples were then dried underneath the sun for six hours and so the drying method was continuing exploitation associate degree kitchen appliance at 80°C till constant weight was obtained. The dried sample is currently called a chitin.

Chitosan production

Deacetylation (DA)

The deacetylation method was conducted by soaking dried polysaccharide ready from demineralization in a very 48% NaOH for forty-eight hours at temperature (~25°C). Once 2 days, the product is thought of as chitosan (Kumari & Rath, 2014)^[16]. Chitosan was washed with H₂O till neutral (pH6.5-8.0) and dried as delineated in deproteination and demineralization.

Physicochemical and characterization of extracted chitosan:

Yield of chitin and chitosan

The chitosan yield (%) was calculated because of the dry weight of the chitosan flakes relative to the wet weight of Sabah shrimp waste (Nouri et al., 2015)^[22].

$$\text{Yield (\%)} = \frac{\text{dry weight of the chitosan}}{\text{weight of shell waste (g)}} \times 100$$

Determination of Moisture and Ash Content in Chitosan

The Moisture content of extracted chitosan resolves following the strategy of (AOAC, 1990)^[5]. The water mass of the sample was calculable by drying 1g of the sample in very pre-weighed instrumentation and measured. The initial weight was noted. The instrumentation was placed in a very hot air oven appliance at 6000 for 6hrs till constant weight was obtained and final weight was additionally noted.

$$\text{Moisture (\%)} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100$$

Ash content of the sample was calculable following the strategy of (AOAC 1990)^[5] by incinerating 1g chitosan in pre-weighed tarred crucibles. The sample was heated within the muffle chamber at 600°C for an amount of 6 hours. The crucibles were allowed to cool down and weights of the residues were noted.

$$\text{Ash (\%)} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100$$

Water Binding capability (WBC)

WBC of chitosan was measured employing a changed technique of (knorr,1982)^[15], WBC was at first allotted by consideration a centrifuge tube containing zero.5g of the sample, adding 10ml of water, and mix on a vortex mixer for one min to disperse the sample. The contents were left at close temperature for thirty min with intermittent shaking for 5seconds in each ten min and centrifuged at three; 500rpm for twenty-five min. once the supernatant water was decanted of the tube was weighed once more. All experiments were triplicated. WBC was calculated as follows:

$$\text{WBC (\%)} = \left[\frac{\text{water certain (g)}}{\text{initial sample weight (g)}} \right] \times 100$$

Fat binding capability (FBC)

FBC of chitosan was measured with 3 kinds of oil e.g.: soybean, corn & flower employing a changed technique of (knorr, 1982)^[15]. FBC was at first allotted by consideration a centrifuge tube containing zero.5g of sample, adding 10ml of 1 of the oils and mix on a vortex mixer for 1min disperse the sample. The contents were left at close temperature for 30min with shaking for 5s in each 10min and centrifuged at

3500rpm for 25min. After the supernatant oil was decanted off the tube was weighed once more. Similar experiments were allotted with corn and flower oils. All experiments were allotted in triplicate.

$$\text{FBC (\%)} = [\text{fat certain (g)/initial sample weight (g)}] \times 100$$

Degree of Deacetylation

The DDA of the sample was determined per the strategy employed by (Brugnerotta et al., 2001) [7]. The A1320 was the height space of the band 1320 cm⁻¹, the A1420 was the height space of 1420 cm⁻¹ and A (1320) is the peak for the organic compound cluster and A (1420) is the peak for the methane series cluster.

$$\% \text{ DA} = (A1320 / A 1420) - 0.3822$$

$$0.03133$$

$$\% \text{ DDA} = 100 - \% \text{ DA}$$

Where, DDA = degree of deacetylation (%)

DA = degree of acetylation (%)

Microorganisms

The microorganism strains utilized within the biological assays were Gram-positive bacteria: staphylococci aureus (MTCC 3160), Bacillus subtilus (MTCC 441), and Gram-negative bacteria: Escherichia coli, (MTCC 732), Pseudomonas aeruginous (MTCC 1035), *Klebsiella pneumonia* (MTCC 3040). It obtained from microorganism kind culture assortment (MTCC) at the institute of microorganism Technology (IMTECH), Chandigarh, India. The check microorganisms were cultured on special culture media (nutrient agar slants). Incubation was at 37°C. The agar slants were maintained at 4°C.

Antibacterial activity (Disk diffusion technique)

Anti biogram was done by disc diffusion methodology (NCCLS, 1993; Awoyinka et al., 2007) [6] using sample. Petri plates were ready by running 30milliliters of sodium medium for the bacterium. The test organisms were inoculated on a coagulated agar plate with the assistance of a micropipette and unfold and allowed to dry for 10 minutes. The surfaces of media were inoculated with bacterium from a broth culture. A sterile cotton swab is a dipped into a homogenous microorganism takes a look at suspension and wont to equally inoculate the complete surface of the nutrient agar plate. Briefly, inoculums containing Escherichia coli, staphylococcus aureus, Bacillus subtilis, genus Pseudomonas aeruginosa and *Klebsiella pneumonia* of the bacterium were unfolded on nutrient agar plates for bacteria.

The antibacterial action makes up resulted by means that of (Holder IA and Boyce ST (1994)) [10]. By expanding DMSO as -ve management, samples of microorganisms' strains were ready in replication. Then, tested microorganism strains were incubated. Finally, medicine action was detected by measure the inhibition space (Agwa Het al., 2000) [3].

Measurement of zone of inhibition

The antimicrobial potential of check compounds was resulted on the premise of the mean diameter of the zone of inhibition around the disc in millimetre. The zones of inhibition of the tested microorganisms by the samples were measured employing a millimetre scale.

Results and Discussions:

Chitin was extracted from the shell wastes of *Scylla serrate* (mud crab), *litopenaeus setiferus* (white shrimp), and *modiolus modiolus* (horse mussel) followed by N-deacetylation to get chitosan. The yield proportion was given in Table. 1. In our study, from the 25g, the yield of chitosan from crab is forty-three.19% higher than shrimp and mussel. The give-up of chitosan from *Scylla Serrata* was thirty-eight. 23% (Kiruba et al. 2013) ^[14] and *Scylla tranquebarica* were ten.74% (Thirunavukkarasu and Shanmugam, 2009) ^[32] that were less in comparison with our chitosan give up from arthropod genus pelagicus. (Yen et al., 2009 and Yen M, Yang J, Manu J. 2008) ^{[38][39]} Have shown crab chitosan% were within the vary of 30-32%. Chitosan varieties had a wet content of 38.23, 0.82 and 1.49% for crab, shrimp, and mussel chitosan, severally. Our results have established the very best yield of chitosan from *Scylla serrate* shell wastes. Thus, chitosan, an economically and biologically valuable product, was effortlessly extracted from the low-value staples. Moisture content but 100 percent is a lot appropriate for business functions. The lower worth of the ash content indicates the purity of the sample that complete demineralization and deproteinization have occurred (Mohanasrinivasan et al. 2013) ^[20]. Ash content higher in mussel (38.72) than shrimp (0.64) and crab (1.8) respectively. Moisture and ash content were comparatively high in comparison with different studies (Sarbon et al. 2014; Walke et al. 2014 and Mohammed MH, et al., 2013) ^{[27][34][19]}. Chitosan is created from polysaccharides by partial N-deacetylation method with a powerful alkaline solution like caustic soda (Acharyalu et al. 2013) ^[12]. The quantity of chitosan extracted from the horse mussel within the current study is low in comparison to crab shells and shrimp shells (30– 36.7%). (Kiruba et al., 2013) reported a yield of 38.23% of chitosan from mud crab *Scylla Serrata*. The typical chitosan of 73.3 ± 4.5 and $71.6 \pm 5.1\%$ was derived from prawn and crab chitin, severally. Water Binding capabilities (WBC) of crab, shrimp, and mussel chitosan were 22.7, 1.22 and 40.5%, severally these results area unit in agreement (Mohanasrinivasan et al.). However beyond reported by (Cho et al., 1998) ^[8] the World Health Organization reported that blood corpuscle ranged from 458-805% for 5 commercial chitosan from shrimp and crab shell. Fat Binding capability (FBC) was 428.9, 333.0 and 196.0% for crab, shrimp, and mussel chitosan that in agreement with (Rout, S.K., 2001) ^[25] World Health Organization showed that FBC of chitosan and commercial crab chitosan for vegetable oil was 706 and 587%, severally. The DD of the 3 ready chitosan was (84.13, 96.0, and 62.17%) for crab, shrimp, and mussel chitosan (Table 1). The DD thinks about being a crucial parameter for the identification of chitosan16 explicit that DD analysis was affected the sort of analytical ways utilized, a form of instruments used and therefore the preparation of the sample.

The DD consider being an important parameter for the identification of chitosan (Ren et al., 2014) [24] stated that DD analysis was affected the type of analytical methods employed, type of instruments used and the preparation of sample.

Table1: Physicochemical properties of three types of chitosan (*Scylla serrate*, *litopenaeus setiferus* and *modiolus modiolus*)

S.No	Physicochemical properties	Crab chitosan (%)	Shrimp chitosan (%)	Mussel chitosan (%)
1	Yield	43.19	19.83	14.17
2	Moisture	38.23	0.82	1.49
3	Ash	1.8	0.64	38.72
4	WBC	22.7	1.22	40.5
5	FBC	428.9	333.00	196.0
6	DD	84.13	96.00	62.17

Table2: Antibacterial activity of chitosan from the shell wastes of *Scylla serrate* (mud crab), *litopenaeus setiferus* (white shrimp) and *modiolus modiolus* (horse mussel) against some pathogenic bacteria

S. No	Sample	<i>Staphylococcus aureus</i>	<i>Bacillus Subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginos</i>	<i>Klebsiella pneumonia</i>
1	Crab	18.00±0.0	14±1.00	37.2±0.00	22.8±0.00	19.0±0.00
2	Shrimp	16.00±1.00	15±0.00	30.0±0.00	18.0±0.00	17.0±0.00
3	Mussel	10.00±0.00	9.0±0.00	21.0±0.04	14.2±0.00	12.0±0.00

Values were expressed as Mean ± SD

The data of table [2] show the results of the antibacterial influence of shrimp shell, crab and mussel shell, that area unit resolved by anti-microbial resistance test ways beside totally microorganisms' strains e.g. *Bacillus*, *staphylococci aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* at sample concentrations 200 mg/ml, chitin extracts conferred bigger impact besides the verified strains than chitosan shell. It had been determined that the crab conferred huge diameter zones of inhibition beside *E. coli*, *P. aeruginosa*, *S. aureus*, *K.pneumonia*, *B.subtilis*, and with inhibition zones (Table 2). The shrimp shell chitosan showed moderate activity besides the total strains tested. The largest inhibition zones created by crab shells were beside *E. coli* with an inhibition space of 37.2mm. These findings were in accordance with (Shital S 2010)^[30] World Health Organization counselled that there's a nonstop and important necessity to work out recent anti-microbial materials with varied chemical compositions and novel mechanisms of action as a worrying increase within the prevalence of recent and re-evolving infectious diseases.

The antibacterial action of crab chitosan is bigger than that of shrimp and polysaccharide, all of them inhibit the expansion of cultured microorganisms, and this is often named that chitosan is wealthy with autonomic amines, which might connect with the negative charged remains of proteins carbohydrates, and

lipids found on the cell surface of gram-negative bacterium strains (Wu et al., 2006)^[37]. This is coordinated with the findings of (Tsai et al., 1999)^[33]. World Health Organization examined the potency of chitosan derived from shrimp with totally different concentrations as an matter of some strains of microorganisms as *Escherichia coli* (Liu et al., 2006)^[17]. Chitosan was found to be dose-dependent, which desires the growth-inhibitory influence for coincident binding of the plasma membrane to minor cellular constituents. Moreover, (Islam et al., 2011)^[12] established the exceptional anti-bacterial potency of chitosan derived from an explicit supply besides gram-negative (*Salmonella typhi*) and gram-positive strain (*Staphylococcus aureus*). Chitosan can be a crucial substance of medicines, that may well be besides infection of the bacterium. Chitosan had the simplest scavenging ability owing to its active amino and chemical groups (Wang et al., 2007)^[35].

CONCLUSION

It is all over that chitosan and polysaccharide area unit derived from natural sources that have several benefits like low-priced, well-endowed helpful and safe constituents. The present study equally conferred that various medicine may well be created extracting from chitosan and chitin substances that have excessive antimicrobial action. Also, it showed the likelihood of developing chitosan as a good substance for bacterium inhibition. By extracting chitosan from shell wastes, pollution because of the dumping of shell wastes on the coast is often reduced. This extracted chitosan is also widely utilized in biomedical specialty and pharmaceutical firms because of its versatile properties.

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