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EXTRACTION, CHARACTERIZATION, ANTIMICROBIAL ACTIVITY OF CHITOSAN EXTRACTED FROM CRAB SHELL AND PREPARATION OF CHITOSAN-BASED BIOPLASTIC FILM FOR FOOD PACKAGING

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ABSTRACT

A novel synthesis of eco-friendly bioplastic film made of chitosan extracted from crab shell is presented in the current study. Crab shell use can result in a reduction in marine waste management. The extracted chitosan has a broad variety of applications in the food industry as a food preservative due to its antibacterial function. A novel procedure for preparing chitosan from crab shells was developed. In the present study, chitosan extracted from crab shell through deproteinization, demineralization and followed by deacetylation. Characterization of extracted chitosan was done by FTIR, and SEM was used to record the photomicrograph image of extracted chitosan. The antibacterial activity of extracted chitosan was studied against *Staphylococcus aureus* and *Escherichia coli*. The bio-plastic film was synthesized using the extracted chitosan by adding glycerol as plasticizer along with citric acid, and agarose to the mixture to develop bio-plastic film. FTIR vibrations patterns showed stretching of hydroxyl (O-H), amine (N-H) and carbonyl (C-O) groups which indicated the presence of chitosan. The change in the morphology of samples was followed by SEM. It is concluded that the procedure developed in the present work showed that the extracted chitosan is suitable for the preparation of ultra-thin, labile, bioplastic film with good apparent texture. The advantages of the developed product could be its biodegradability, biocompatibility, and effective use as biomedical interface.

Keywords: Chitosan, Antibacterial activity, Biocomposite, Crab shell, Bioplastic film.

1. INTRODUCTION

The greatest losses in food are due to microbial alternations. Many chemical and physical processes have been developed to preserve food quality. Among such processes adequate packaging is a fundamental factor in the conservation and marketing phases. Thus, packaging plays a prominent role in maintaining food quality [1].

The term antimicrobial packaging encompasses any packaging technique(s) used to control microbial growth in a food product. These include packaging materials and edible films and coatings that contain antimicrobial agents and also techniques that modify the atmosphere within the package. In recent years, antimicrobial packaging has attracted much attention from the food industry because of the increase in consumer demand for minimally processed, preservative-free products. Reflecting this demand, the preservative agents must be applied to packaging in such a way that only low levels of preservatives comes into contact with the food. The film or coating technique is considered to be more effective, although more complicated to apply. A greater emphasis on safety features associated with the addition of antimicrobial agents is perhaps the next area for development in packaging technology. Antimicrobial packaging has been touted as a major focus in the next generation of 'active' packaging. Active packaging is the packaging system which possesses attributes beyond basic barrier properties, which are achieved by adding active ingredients in the packaging system and/or using active functional polymers. When the packaging system acquires antimicrobial activity, the packaging system (or material) limits or prevents microbial growth by extending the lag period and reducing the growth rate or decreases live counts of microorganisms [2].

The primary goals of an antimicrobial packaging system are (1) safety assurance, (2) quality maintenance, and (3) shelf-life extension, which is the reversed order of the primary goals of conventional packaging systems. Nowadays food security is a big issue and antimicrobial packaging could play a role in food security assurance.

Edible films or coatings have been investigated for their abilities to retard moisture, oxygen, aromas and solute transports. It is one of the most effective methods of maintaining food quality. Edible and biodegradable films are not meant to replace the synthetic packaging films [3]. Usually film-forming substances are based on proteins, polysaccharides, lipids and resins or a combination of these [4].

Crab is a decapod crustacean. There are about 4500 species crabs that come in many different sizes and colours. About 30-40% of crustacean shell waste consists of protein, 30-50% calcium carbonate and 20-30% chitin [5]. These proportions vary with species and season. Chitin and its derivative, chitosan, are hydrating agents.

Chitosan is mostly obtained from the exoskeleton (food waste) of crustacean shells. It is a linear polysaccharide consisting of (1,4)-linked 2-amino-deoxy-β-D-glucan, and deacetylated derivative of chitin, which is the second most abundant polysaccharide in nature just after cellulose. Chitosan has been proved to be nontoxic, biodegradable, biofunctional, biocompatible and possess antimicrobial characteristics [2].

Chitosan can be used in various fields such as pharmaceutical industry, food, health, agriculture, textiles and so on. Also, it can be used as a coagulant in wastewater treatment, moisturizer/facial creams, seed coatings, metal ion adsorbents, anti-cancer/anti-tumor, blood cholesterol control, additional components of animal feed, biopesticides, contact lenses, plaque inhibitors on teeth, accelerator of the healing of wounds and bones, fat solvents, food preservatives, food stabilizers and colours [6]. These properties make chitosan a very attractive biomaterial. Due to its bio-preservation efficacy against food borne pathogens it can be used as a biodegradable and bio-preservation packaging material for food wrap and other products.

Crab shell is a waste material from different sites like restaurants, markets, and marine environment. Hence, the best way to avoid these problems is to utilize the waste material as a resource for the development of new biodegradable products like bioplastics. In recent years a lot of research works is going on for development of products from crab waste materials and new ways to process them. The objective of the present work is to investigate the potential usage of crab shells that is being discarded as waste, for extraction of chitosan to produce chitosan based antimicrobial films.

2. MATERIAL AND METHODS

2.1. Collection of samples

The crab shells were collected from local market in Coimbatore, Tamilnadu, India. The shells were washed and dried under sun light. The viscera and tissues were carefully removed and placed in hot air oven at 60°C for 24 hours. The sample was then blended with blender and crushed with a mortar to create crabshell powder. After the crab shell powder was obtained, the effort to extract chitosan from crab shells was attempted.

2.2. Extraction of Chitosan from crab shell

Chitosan extraction from crab shell involves three major steps such as deproteinization, demineralization and deacetylation. Takiguchi [7, 8] method was employed for deproteinization, demineralization and deacetylation to extract chitosan from shell wastes.

2.2.1. Deproteinization

The sample was deproteinized with 300ml of 1N NaOH at 80°C for 24 hours with constant stirring. The NaOH was exchanged intermittently, and the sample was washed with distilled water every time before adding fresh NaOH. After 24 hours, the sample was filtered. The sample filtrate was washed as before and dried. The weight was noted.

2.2.2. Demineralization

Deproteinized sample powder (20gm) was demineralised with 300ml of 2N HCl or 24 hours with constant stirring and thus filtered. The filtrate was again washed with distilled water and filtered till the liquid showed neutral pH. The filtrate was then dried in a vacuum dryer and weighed.

2.2.3. Deacetylation

Chitin was deacetylated with 40% NaOH, heated for 6hrs at 110°C in constant stirring then 10% acetic acid was added to the sample and stored for 12hrs at room temperature with constant stirring. The dissolved sample was reprecipitated by adding 40% NaOH at pH 10. The sample was then dialyzed by deionized water to a pH of 6.5 and centrifuged at 10,000 rpm for 10minutes and freeze dried.

2.3. Characterization of Samples

2.3.1. Fourier Transform Infrared Spectroscopy (FTIR)

The extracted Chitosan was characterized using FTIR analysis which aims to discover the functional groups that are found in it. It was obtained by comparing the FTIR spectrum produced during the process with the standard chitosan spectrum or by looking at the correlation map for the establishment of functional groups in the infrared spectrum.

2.3.2. Scanning Electron Microscope (SEM)

Morphology of samples was inspected using a Hitachi scanning electron microscope model TM-1000 operated at an acceleration voltage of 15 kV. The microscope is equipped with an energy dispersive spectroscopy (EDS) detector (Oxford instruments, Oxford, UK).

2.4. Antimicrobial activity of Chitosan from crab shell

Antibacterial activity was tested by agar well diffusion method using Muller-Hinton agar. Test bacterial strain used in this study were *Escherichia coli* and *Staphylococcus aureus*. Overnight culture of *E. coli* and *S. aureus* inoculated into Muller-Hinton agar using sterile cotton swab. The chitosan was dissolved in 1% acetic acid and the chitosan solution of various concentrations such as 20, 40, 100 (μg/ml) were added into the wells using micropipette, each well was loaded with 40μl of sample. It was incubated for 24 hours at 37°C and the zone of inhibitions was observed.

2.5. Preparation of chitosan based anti-microbial films

Chitosan was dissolved using 1% acetic acid. 5ml of this solution was then mixed with 10ml of glycerol, and stirred for 30 minutes at 60°C, and then 0.5g citric acid and 2g of agarose was added into the solution. The aliquot was spread over the aluminium tray and placed in an oven at 60°C for 24hours. After 24 hours, the mixture was checked to make sure that is fully dried and allowed it to cool.

3. RESULTS AND DISCUSSION

Chitin and chitosan naturally abundant polysaccharides are found particularly in the shell of crustaceans. It is white, hard, inelastic, nitrogenous polysaccharide, which forms the major source of surface pollution in coastal areas. It is a special biopolymer having specific properties including biodegradability, biocompatibility, and bioactivity. It is interesting not only as an abundant resource but also a novel type of functional material [9].

3.1. FTIR analysis of chitosan extracted from crab shell

The chitosan was extracted from the marine waste material crab shell by deproteinization, demineralization and deacetylation. The yield of chitin from the crab shell was found to be 29.31%. Percentage yield of chitosan from chitin was found to be 71.3.

The extracted chitosan was subjected to FITR analysis to identify the presence of functional groups and chitosan. Fig.1 and table 1, show the infrared spectrum of chitosan. A strong band in the region 3291-3361 cm^{-1} corresponds to N-H and O-H stretching, as well as the intramolecular hydrogen bonds. The absorption bands at around 2921cm^{-1} and 2877cm^{-1} can be attributed to C-H symmetric and asymmetric stretching, respectively. These bands are typical characteristics of polysaccharide and are found in other polysaccharide spectra, such as xylan [10], glucans [11] and carrageenans [12]. The presence of residual *N*-acetyl groups was confirmed by the bands at around 1645 cm^{-1} (C=O stretching of amide I) and 1325cm^{-1} (C-N stretching of amide III), respectively.

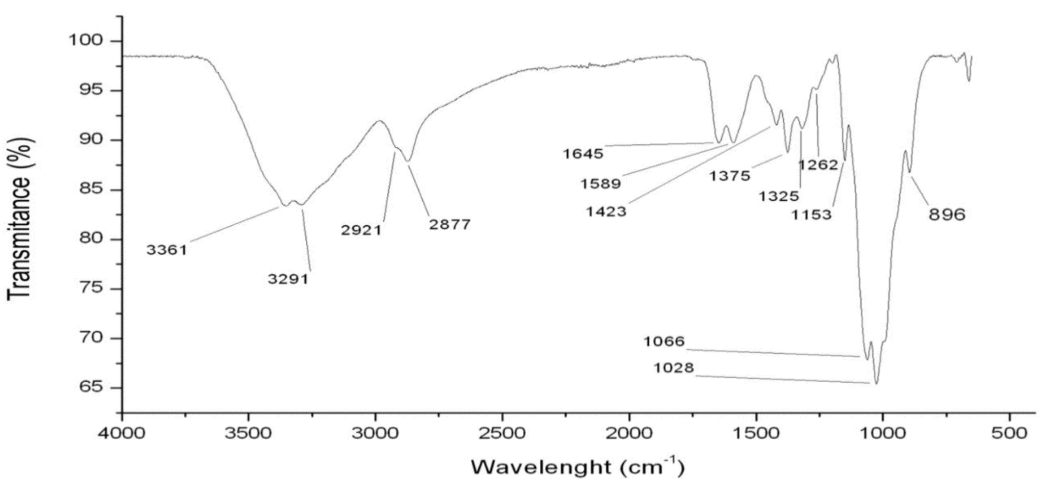

Fig. 1: FTIR spectrum of extracted chitosan from crab shell

A band at 1589cm^{-1} corresponds to the N-H bending of the primary amine. The CH_2 bending and CH_3 symmetrical deformations were confirmed by the presence of bands at around 1423 and 1375 cm^{-1}, respectively. The absorption band at 1153 cm^{-1} can be attributed to asymmetric stretching of the C-O-C bridge. The bands at 1066 and 1028cm^{-1} correspond to C-O stretching. All bands are found in the spectra of samples of crab shell extracted chitosan reported by other studies [13, 14]. GAGs are sulfated, and the presence of sulfate groups covalently bonded to the polysaccharide may be confirmed in the infrared spectra by the presence of very strong bands in the region around 1260-1270 cm^{-1} [15]. In the spectrum obtained from chitosan (Fig. 1, and Table 1), the signal at 1260cm^{-1} is very small and, therefore, does not correspond to sulfate groups, thus ruling out contamination of chitosan by GAGs. The signal at 1260cm^{-1} was assigned as the bending vibrations of hydroxyls present in chitosan [14]. The signal at 896 cm^{-1} corresponds to the CH bending out of the plane of the ring of monosaccharides.

Table 1: FTIR Spectral Peaks of chitosan extracted from crab shell

S. No.	Wave Number(cm^{-1})	Possible Assignment of a Absorption Band
1	3291	NH stretching
2	3361	OH stretching
3	2921-2877	CH (asymmetric and symmetric stretching)
4	1645	C-O stretching of amide-I
5	1325	C-N stretching of amide-III
6	1589	NH bending of primary amine
7	1423	CH_2
8	1375	CH_3
9	1153	Asymmetric stretching of C-O-C bridge
10	1066-1028	C-O stretching
11	1260-1270	Sulphate group
12	896	CH bending

3.2. SEM analysis of extracted chitosan

SEM was used to record the photomicrographic image of the extracted chitosan. A small volume of chitosan suspension was taken for SEM analysis on electron microscope stub. The morphology of chitosan was semi crystalline in structure and the fiber structure on the chitosan surface was smoother and denser. The surface morphology of chitosan is related to its crystal structure [16]. The SEM micrograph fig. 2 showed the presence of chitosan and its surface morphology. The crystalline size of extracted chitosan was 7.8mm.

3.3. Antimicrobial Activity

The antimicrobial activity of the extracted chitosan was noticeable against *Staphylococcus aures* (Gram positive) and *E. coli* (Gram Negative) bacteria examined in the current study (Table 2). Every bacterium was exposed to different concentration of the extracted chitosan (25-100μg/ml). The antimicrobial activity of chitosan against *S. aureus*, showed a greater inhibition zone as 9mm at a concentration of 100μg/ml. The antibacterial activity of extracted chitosan against *E. coli* was notably high at 75μg/ml (6mm), which did not increase further with high chitosan concentration. Overall, 75 and 100μg/ml concentration of the chitosan tested were efficient in terms of zone of inhibition against of *S. aures* (Gram positive) and *E. coli* (Gram Negative) bacterial strains studied.

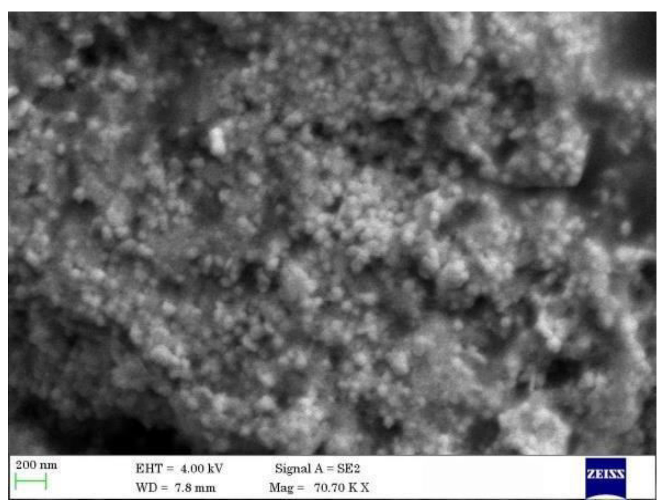

Fig. 2: SEM image of chitosan extracted from crab shell

These results apparently present the antimicrobial activity of chitosan extracted from crab shell against

S. aures (Gram positive) and *E. coli* (Gram negative). Earlier studies indicated that chitosan and its products have shown to have a greater influence on the cell wall degradation. Gram-positive bacteria (*S.aureus, B. subtills, Sarcinia lutea*) were much more susceptible to chitosan treatment than Gram-negative bacteria (*E. coli, Serratia marcescens*) [17]. Increased antimicrobial activity has been witnessed against various strains of fungi, Gram-positive, Gram-negative bacteria, especially for *S. aureus* and *E. coli*, with chitosan having a lower DA or higher number of free amino groups [18].

Table 2: Antimicrobial activity of chitosan extracted from crab shell

Species	Zone of inhibition				
	Control	25 (μg/ml)	50 (μg/ml)	75 (μg/ml)	100 (μg/ml)
S. aureus	11	1	3	8	9
E. coli	4	--	3	6	6

3.4. Bioplastic film using crab shell chitosan

The bio plastic film was synthesized with use of chitosan extracted from crab shell. Glycerol was used as plasticizer, citric acid and agarose was added to the mixture to develop bio-plastic film. The bio-plastic film formed using chitosan is observed to be labile with good texture and transparency (Fig.3). Due to its antimicrobial activity against *E. coli* and *S. aureus*, it can be used as a biofilm to cover the food material like meat to prevent the food borne pathogens due to its bio preservation efficacy.

Fig. 3: Chitosan based Bio plastic film

4. CONCLUSION

A novel synthesis of eco-friendly bioplastic film from chitosan from crab shell is presented in this paper. Based on FTIR interpretation, it can be concluded that present study successfully extracted chitosan from waste raw material such as crab shells. The SEM micrograph showed the presence of chitosan and its surface morphology. The extracted chitosan also showed notable antibacterial activity against *E. coli* and *S. aureus*. Due to its antimicrobial activity against gram-positive and gram-negative bacterium, it can be used as a bio film to cover the food material like meat to prevent the food borne pathogens due to its biopreservation efficacy. The visual observation of bioplastic film from chitosan is also indicative of the possible utilization of chitosan into value added product. The advantages of this product are, it is biodegradable, bio compatible, non-toxicity and can be effectively used as biomedical interface. From the current study, it is clearly understood that the chitosan could be a very effective bio-preservative agent and the uses of marine sources for chitosan extraction will help to reduce the marine waste.

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Conflict of Interest

The authors declare no conflict of interest.

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