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Production of Biopolymer and Preparation of Soap Using Chitin and Chitosan from *Brachyura* Shell

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Abstract: Biodegradable polymers are large macromolecules which are composed of polysaccharides such as starch, cellulose, chitosan, alginate and proteins. Chitin and chitosan were extracted from *Brachyura (*crab) shells using acid and alkaline treatments followed by decolorization. The functional group present in samples was detected by using FTIR. The proximate analysis such as carbohydrates, calcium and iron were determined for both chitin and chitosan which are found to be 4.1 mg and 5.7 mg; 120 mg and 153 mg; 54 mg and 70 mg, respectively. Both chitin and chitosan have antibacterial effects against *Bacillus, Streptococcus, E. coli, Klebsiella, Staphylococcus aureus,* and *Pseudomonas.* Bioplastic production using chitin and chitosan and biodegradation assay was analyzed. 2.7 g of glycerol-based bioplastic was reduced to 2.3 g on day 35 with the optimum rate of weight loss of 14.81%. Chitosan soap has a good odour and has more foam at pH 7. The tensile strength and water absorption of biopolymers were analyzed showing that gelatin-based chitosan biopolymers have great ability in absorbing water. The current study reveals the feasibility of this marine crustacean's shell waste being converted into useful biodegradable packaging materials as well as soap that can work for sustainable development and reduce environmental pollution.

Keywords: FTIR, Antibacterial, Biopolymer, Biodegradation, Proximate Analysis, Crab

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Introduction

Natural plastics are petrochemicals made from fossil fuels like coal, natural gas, and oil**.** Synthetic plastic materials used commonly are polyester terephthalate (PET), high-density plastic (HDPE), polyvinyl chlorine (PVC), low-density plastic (LDPE), propylene (PP), polyurethane (PS or Styrofoam) (Harshvardhan and Jha, 2013). Ethylene and propylene combined with other chemicals are used for preparing plastic material. These polymers are all highly designed materials

with exact physical qualities, despite their low cost (Geyer *et al.,* 2017). 18% of plastic polymers are recyclable, 24% are burned globally and remaining 58% are non-renewable either in landfills or are released into the environment for a very long time and creates pollution. Bio polymers are macromolecules composed of several repeating units synthesized from vegetable fats and oils, corn starch, straw, woodchips, sawdust, recycled food waste. They are eco-friendly and can be used instead of synthetic polymers. Some bioplastics are obtained by processing directly from polysaccharides such as starch, cellulose, chitosan and alginate; proteins such as soy protein, gluten and gelatin while others are chemically synthesized from sugar derivatives such as lactic acid; lipids such as oils and fats from either plants or animals (Ezeoha, 2013). Biopolymers have a variety of functions in the pharmacological and food sectors, including palatable films, fluid emulsion packaging components, medical implants such organs, scaffolds of cells for wound treatment, and dressing materials. The utilization of biopolymers reduces the production of municipal solid waste, carbon dioxide emissions, and the need for petroleum-based resources. Chitosan can be used in water filtration, and degrades heavy metals, and dyes. Organic biopolymers such as chitin and chitosan are widely used in a variety of industries, encompassing biological medicine, medication, groceries, farming, cosmetics, grooming products, and the environment. Chitosan can be applied in wound healing process since it can adhere to fibrinogen causing clotting of blood and hemostasis (Feng Peipei *et al.*, 2021). Chitosan is found in the shells of crustaceans, such as lobsters, crabs, shrimp, insects, and fungi. The combination of glucosamine and 1-4-linked N-acetylglucosamide makes up chitosan as a straight-chain polysaccharide. It reduces environmental stress in plants due to drought and soil deficiencies. It strengthens seed vitality, increases production and deterioration of crops. Chitosan acts as a growth enhancer in plants, biopesticide**.** One of the most beneficial components of cell membranes in fungus and the cuticles in arthropods can bind metal ions and proteins, can be nano formulated into particles, miniature capsules, or tiny fibers, promote plant growth and development, and can confer stress tolerance and resistance to infections in plants (Sanjanwala *et al.,* 2022). Chitosan are biodegradable and as biocompatible membranes in implants include the regeneration of skin, liver, nerve, ligament, cartilage, and tendon. *Cryptosporidium parvum*, prevalent in babies, immunocompromised people, and young ruminants. Chitosan supplementation *in vivo* study using mice, protects against *C. parvum* infection by up and down regulation as well as decreased production of IFN- and TNF controls *C. parvum* infection. The utilization FAF-Drugs4, Swiss ADME, and Pre ADMET tools to forecast the oral bioavailability of chito-oligomers, as well as Swiss ADME, Pre ADMET, ADMETSAR 2.0, and ENDOCRINE DISRUPTOME tools to predict their pharmacokinetic profiles, Tox tree for carcinogenicity and mutagenicity revealed that chito-oligomers has pharmacological effect and less toxic effects on humans (Durackova, 2010). The current study focuses on extraction of chitin and chitosan from *Brachyura* (crab) shells using chemical treatments. The extracted chitin and chitosan were characterized by proximate analysis, FTIR spectroscopy. Preliminary biofilm production was carried out using chitin and chitosan to explore the feasibility of this marine crustacean's shell waste to get converted into useful biodegradable packaging materials that can work for sustainable development and soap was prepared using chitosan. By recycling the biological waste into a renewable resource can reduce environmental pollution and preparation of soap.

Materials and Methods

The shell of *Brachyura* was obtained at a local fish market in Ambattur, Chennai, Tamil Nadu, India during the month of August 2022. The *Brachyura* shell was washed 2 to 3 times with tap water,

then with distilled water, dried at 60°C for 24 h, shells were later pulverized and kept at - 4°C till use.

Extraction of Chitin and Chitosan:

Demineralization:

The shells were ground and soaked slowly in 1 N Hydrochloric acid solution for 6 h at room temperature to remove the calcium salts with a solid/solvent ratio of 1:15 (w/v) and incubated for 4 to 6 h. With the help of Buchner funnel, a decalcified shell was collected on the Whatman No.1 filter paper. Washed the resulting solid with deionized water until it is neutralized and the samples were dried for about 24 h at 60°C and weighed.

Deproteinization:

Demineralized samples were treated with 3% sodium hydroxide solution in the ratio of 1:10 and kept in a preheated hot air oven at 121° C for 30-40 min. The contents were filtered with help of Whatman No.1 filter paper and washed thoroughly with deionized water to remove sodium hydroxide. Later the samples were dried in a hot air oven for 24 h at 60°C.

Decolorization:

Chitin residue was further treated with 60% Sodium hypochlorite solution in the ratio 1:10 and mixed thoroughly. The decolorized chitin sample was washed thoroughly with distilled water for about more than 3 times. Then it was dried in a hot air oven at 60°C for 24 h.

Deacetylation:

The extracted crab chitin was treated with 50% sodium hydroxide in the ratio of 1:15 and kept in a hot air oven at 121°C for 4 h. The samples were washed with distilled water to remove the saline and were dried and weighed.

Physico Chemical Analysis:

Determination of ash content:

Two grams of chitin and chitosan were placed in two distinct crucibles. The crucibles were weighed and placed in a preheated oven at 100°C for 3 h before being transported to a muffle furnace to burn fully into ashes. It was then cooled and weighed, and the ash content of the samples was determined**.**

Determination of moisture content:

Chitin and chitosan (1 g each) were kept in a crucible and baked at 100°C for 3 h. After that, it was cooled and weighed. The procedure was repeated two or three times until a steady weight was reached. The moisture content of the sample was determined using a standard procedure (Singh *et al.,* 2017).

Estimation of carbohydrate by anthrone method:

100 mg of Chitin and chitosan was added to 5 ml of 2.5 N Hydrochloric acids and heated for 30 min at 65°C, the samples were allowed to cool and neutralized with sodium bicarbonate. The amount was raised up to 100 ml with distilled water, centrifuged and supernatants were used in the estimation of carbohydrate using anthrone and the standard solution is glucose**.**

Estimation of calcium:

Ash of each 5 g of chitin and chitosan was dissolved in a few drops of 0.1 N Hydrochloric acid and the solution was built up to 100 ml in a standard flask. 20 ml of this solution was collected and brought up to 40 ml by adding 20 ml of 4% ammonium oxalate and stored 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 100 ml of 2 N sulphuric acid and heated to a comfortable temperature (60°C). The released Oxalic acid was then titrated against potassium permanganate. The titration result was documented and the quantity of calcium contained in a specific sample was determined (Balu *et al*, 2019)**.**

Estimation of Iron:

1 g of sample was weighed and kept in a muffle furnace for 5 h for ash. It was then dissolved in 0.1 N Hydrochloric acid and was made up to 100

ml with the same in standard flask. 3 ml of sample was taken in a test tube labeled as F_1 and F_2. 1.5 ml of 0.1N Sodium sulphite solution was added to all the tubes followed by 1.5 ml of dipyridyl reagent. The contents of the tubes were mixed well for uniform mixing and kept in a boiling water bath for 10 min and then cooled. The intensity of the pink color developed was read at 540 nm.

Antioxidant activity:

The DPPH scavenging activity of the sample was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Briefly, 0.4 mM solution of DPPH in methanol was prepared and 2 ml of this solution was added to different concentrations of sample and was allowed to stand at room temperature for 20-30 min, and then absorbance was read at 517 nm against blank samples.

Antibacterial activity:

The antibacterial potential of sample chitin and chitosan were evaluated against gram-positive bacteria (*Staphylococcus aureus, Bacillus, Enterococcus*) and gram-negative bacteria (*Escherichia coli, Klebsiella, Pseudomonas*) using Agar well diffusion technique. 30µl of extracts at varied concentrations was applied to corresponding wells. The plates were placed in the refrigerator for 30 min to enable the extracts to diffuse effectively into the agar. Then, the plates were incubated at 37°C for 24 h under aerobic conditions. After incubation, confluent bacterial growth was noticed by zone formation.

FTIR analysis:

Identification of chemical constituents and elucidation of structures of compounds was carried out with Fourier transform infrared (FTIR) spectrometry. The powder was placed in a FTIR spectrophotometer (FTIR 8400S, Shimadzu, Japan). The FTIR spectrum of all the experimental runs were recorded.

Production of Biopolymer:

Protocol I:

1 g of Chitosan was weighed and mixed with 97 ml distilled water and 3 ml glacial acetic acid and boiled for 15 min at 70° C. To the solution added 0.8 % glycerol and 1g of polyethylene glycol (PEG). The mixture was later used to prepare biopolymer and kept at room temperature for 2-3 days. Different concentrations of gelatin (0.8, 1 and 1.6 ml) were added to 1 g of chitosan to prepare the biopolymer.

Protocol II:

1 g of Chitosan was weighed and mixed with 97 ml distilled water and 3 ml glacial acetic acid and boiled for 15 min at 70° C. To the solution added 10 ml of 10 % dissolved gelatin and 1 g of PEG was added. The mixture was later used to prepare biofilm and kept at room temperature for 2-3 days. The different concentrations of chitosan and gelatin were used for the analysis in the ratio of 9:1, 8:2, 5:5, respectively.

Tensile Strength (*TS*):

After the production of bioplastic, the tensile test was conducted to determine the mechanical properties of bioplastic on Tensile Strength. Control, Protocol I film and Protocol 3 film samples with dimensions of 100 mm x 30 mm from three different types of bioplastics were prepared for the ability of the film to resist applied pressure. Finally, all data were calculated. The graph was plotted to compare the results of the different types of bioplastics.

Water Absorption (*Penjumras et al., 2015*):

This test is to determine the water absorption behavior on bioplastic. To test the bioplastic, 50 mm x 50 mm dimensions are required and pre-weighed, then place it in petripate /beaker and add 15 ml of distilled water and the weight of the film was noticed periodically at first hour, second hour, third hour, 24th hour. Water absorption capacity of the film was determined in triplicate and calculated using a formula:

$$\% \text{ Water absorption capacity} = [(W_t - W_o) / W_o] \times 100$$

Where, W_t (g) is the weight of bioplastic at the time being; W_o (g) is the initial weight of bioplastic before being immersed in distilled water.

Degradation Properties:

Soil Degradation:

Bio polymers prepared using chitosan and glycerol were used for this analysis. The samples were weighted using weight balance and recorded as an initial data. Next, the samples were buried for 35 days in natural soil. The sample was washed with distilled water and dried in an oven at 60˚C for 24 h. After 24 h, the sample was weighted and the data was recorded. The following equation was used to calculate weight loss;

$$\text{Weight Loss (\%)} = [(W_o - W_t) / W_o] \times 100$$

Where, W_o - Initial weight of bioplastic before buried in soil (g); W_t- Final weight of bioplastic after the time duration (g).

Microbial Degradation:

Bio polymers prepared using sample-1 (chitosan and glycerol) and sample-2 (chitosan and gelatin) were used for this analysis. The samples were weighted and recorded as an initial data. Next, the samples were immersed to the *Pseudomonas* culture for 5 days. After 5 days, the samples were washed in distilled water, weighed and the data was recorded. The following equation was used to calculate weight loss:

$$\text{Weight Loss (\%)} = [(W_o - W_t) / W_o] \times 100$$

Where, W_o - Initial weight of bioplastic before implanting in the culture (g); W_t - Final weight of bioplastic after the time duration (g).

Soap Preparation:

Soaps are the sodium salts or potassium salts of stearic acids or any other fatty acids. They are prepared by the saponification process, which is, reacting the oil which contains triglycerides with caustic soda to give the soap. Coconut oil was heated to precisely 35°C, added 1-2 drops of the chosen fragrance, 9 M sodium hydroxide solution was added followed by 1 g of chitosan and added Lauryl sulfate. The liquid thickened to a pudding-like consistency as it transformed, gradually becoming smoother and opaque. The addition of 2-3 drops of the desired food colouring was followed by the addition of roughly 1/8 teaspoon of stearic acid to function as a hardening agent for the liquid soap and pour the mixture into a carefully chosen mould form. Later the quality of the soap was determined.

Results and Discussion

The Crab shell procured from local fish market of Ambattur, Chennai, India was washed thoroughly in tap water and then with distilled water, dried, powdered and stored in - 4° C until use. The yield of 1 kg weight of wet crab shell sample after drying was 570 g.

Extraction of Chitin and Chitosan:

100g of powdered crab shell was deproteinized, demineralized and decolorized shell was carried out by chemical method to obtain chitin about 25 g was retained and the remaining chitin residue deacetylated to chitosan. The chitin and chitosan were stored for further analysis in the refrigerator until use (Fig. 1).

Proximate Analysis:

1 g of chitin and chitosan were weighed separately, kept at 100°C for 3 h. The steps were repeated for 2 to 3 times till the sample is in constant weight. 2 g of chitin and chitosan were weighed separately, heated over at 100°C for 3 h and kept in a muffled furnace till the substance was completely ashes, cooled and weighed. The amount of calcium present in chitin and chitosan extracted from the crab shell was found to be 120 mg and 153 mg, respectively. The iron content in the ash of chitosan was found to be higher than chitin ranges from the 54-70 µg/g. The amount of carbohydrate in chitin and chitosan was found to be 4.1 mg and 5.7 mg in 100 ml of sample, respectively. The carbohydrate content of chitosan was found to be higher and as the concentration increased the amount of carbohydrate present in the sample also

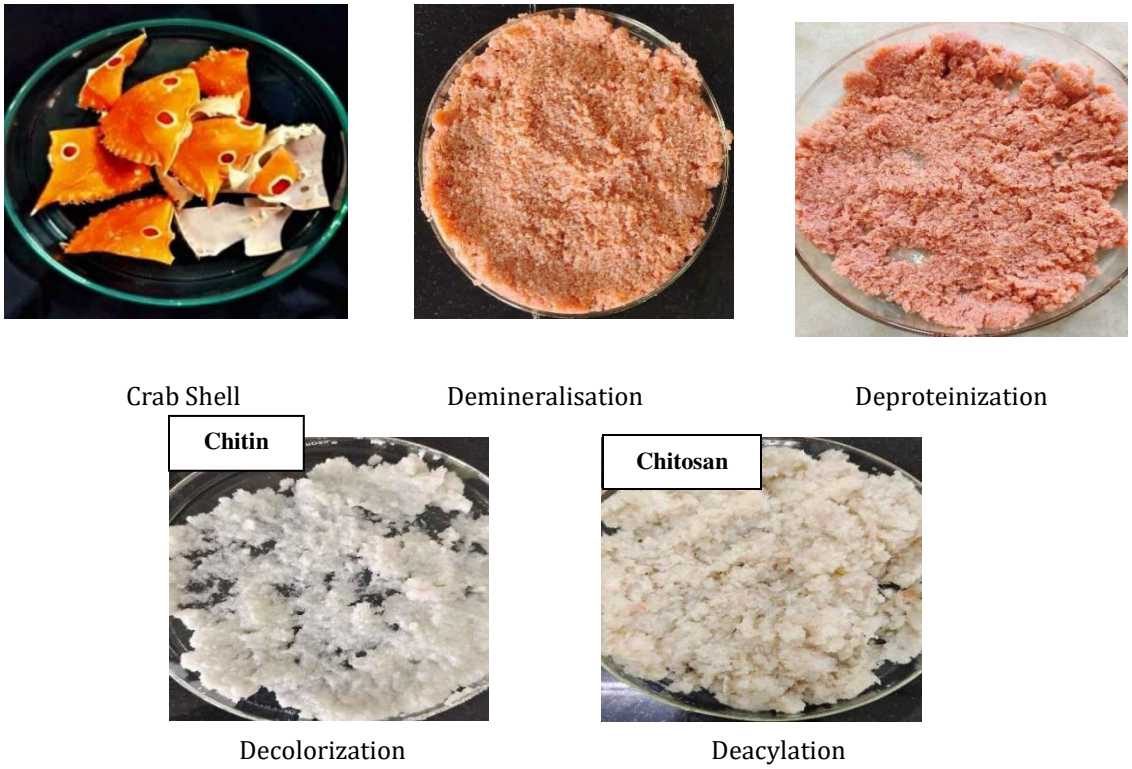


Fig.1: Extraction of chitin and chitosan from crab shell

Table 1: Physico Chemical and Proximate analysis of Chitin and Chitosan crab shell

SAMPLE	MOISTURE (%)	ASH (%)	Carbohydrates (mg/dl)	Calcium (mg)	Iron (mg/g)
Chitin	34	98	4.1	120	54
Chitosan	38.4	100.5	5.7	153	70

increased. The iron content in chitin and chitosan was found to be 54 mg and 70 mg in 100 ml of the sample respectively (Table 1). Chitin extracted from the crab shell contains 2.12% moisture content, 1.56% ash and 4.18% nitrogen. The total amount of calcium, magnesium, zinc, iron, copper and manganese was 26.84, 50.49, 21.80, 12.68, 0.22 and 1.17 mg/kg, respectively.

Antioxidant activity (*DPPH Assay*):

DPPH free radical scavenging activity was carried out for chitin and chitosan samples using standard ascorbic acid, and beta-carotene. Chitin has more antioxidant activity than Chitosan and as the concentration increases, percentage inhibition is also increased (Table 2; Fig. 2). Utilizing organic probiotics, the production of chitin and carotenoids from crab biowaste has been studied. The inoculum concentrations varied from 1 to 10%, the glucose content from 0 to 15%, and the incubation times varied from 0 to 72 hours for the fermentation studies. It is possible to quickly assess the antioxidant activity using the model of stable DPPH free radicals. Studies showed that DPPH is a stable free radical that can accept an electron or OH to transform

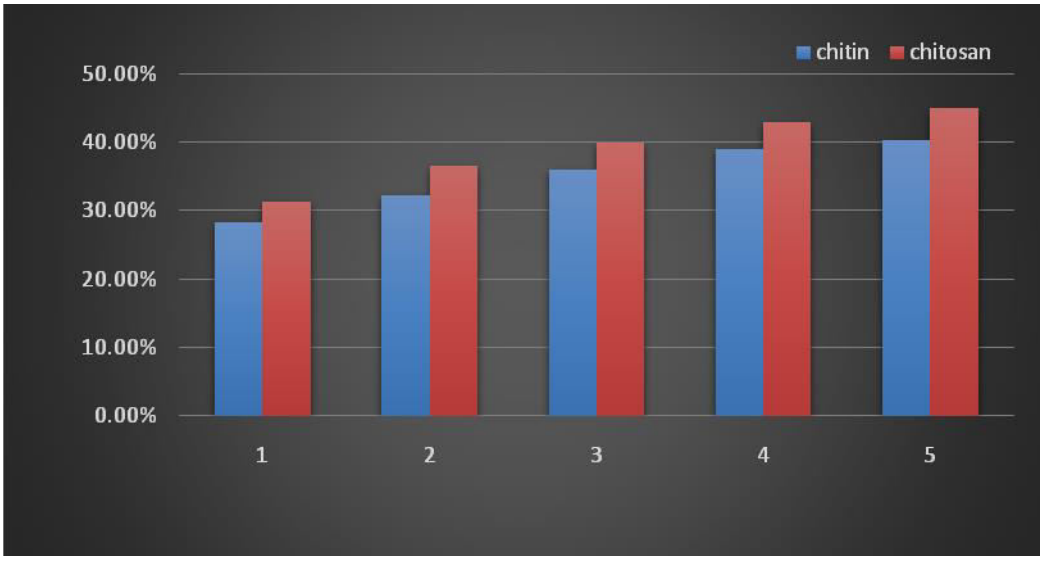


Fig. 2: Antioxidant activity of chitin and chitosan from crab shell. (x axis concentration µg; y axis % inhibition).

Table 2: Antioxidant Activity of Chitin and Chitosan from crab shell

S. No.	Sample	Concentration (µl)	Equivalent to Ascorbic acid (µg)	Equivalent Beta-Carotene (µg)
1	Chitosan	10	0.36	0.22
		20	0.39	0.23
		30	0.48	0.28
		40	0.49	0.29
		50	**0.52**	0.36
2	Chitin	10	0.31	0.14
		20	1.23	0.22
		30	1.34	0.3
		40	1.51	0.4
		50	**1.71**	0.5

into a stable diamagnetic molecule. The α-amylase and β- glucosidase enzyme inhibition is effective for chitosan and exhibits anticoagulant potential. Chitosan could be used in pharmaceutical industry and tissue engineering (Balu *et al.,* 2019). Antioxidant activity of extract exhibited higher phenolic and flavonoid contents than aqueous extract. This is due to its hydrogen or electron donating and direct free radical scavenging properties. Commercial Chitosan when grafted with dimeric α, β-peptides bearing two benzyloxyethyl side chains has antioxidant properties.

Antibacterial activity:

The antimicrobial activity of chitin and chitosan was determined by agar well diffusion method. Chitin and chitosan at different concentrations were loaded in Muller Hinton agar plates and incubated for 24 h at 37 °C. Chitosan has effective antibacterial activity compared to that of chitin, as the concentration increases the zone of

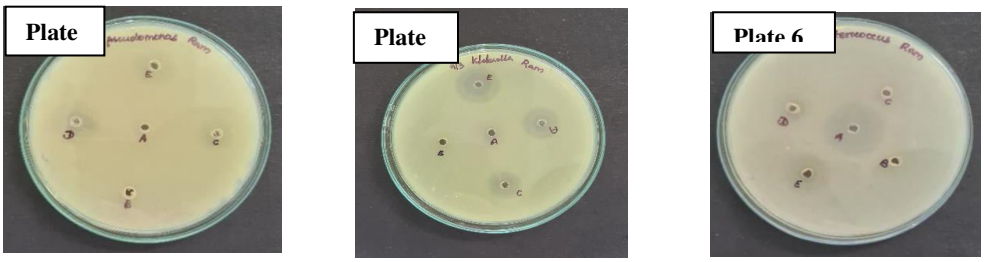


Fig. 3A

Fig. 3B

Plate 1 –*Bacillus*; Plate 2 *Staphylococcus aureus*; Plate 3- *Escherichia coli*;
Plate 4- *Klebsiella*; Plate 5 – *Pseudomonas*; Plate 6-*Enterococcus*

Fig. 3: Antibacterial Activity of Chitin (3A) and Chitosan (3B)

inhibition also increases. The chitin and chitosan are considered as a potent antibacterial effect against *Bacillus, E. coli,* and *Klebsiella,* organisms (Table 3; Fig. 3). Chitin and chitosan extracted from crab shells are biodegradable and they can be widely applied in medical and pharmaceutical industries and they are alternate to commercial chitin and chitosan (Gaikwad *et al.*, 2015). Enzymatic and chemical extraction of chitin and chitosan from marine organisms and its waste products has potent antibacterial, antifungal, antitumor and antioxidant (Islem Younes and Marguerite Rinaudo, 2015). Synthetic curcuminoid compounds like 1,7-bis-(4-hydroxy-3-methoxy-phenyl)-1,6-heptadiene-3,5-dione that contains 4-OH phenolic group possess higher

Table 3: Antibacterial Activity Chitin and Chitosan from Crab shell

Pathogens	Concentration (µl)	Zones of Inhibition (mm)	Zones of Inhibition (mm)
		Chitin	Chitosan
Escherichia coli	Positive control	15	15
	Negative control	-	-
	10	7	7
	20	8	11
	30	9	13
Klebsiella	Positive control	9	9
	Negative control	-	-
	10	6	7
	20	8	9
	30	9	10
Staphylococcus aureus	Positive control	12	12
	Negative control	-	-
	10	5	7
	20	8	8
	30	9	10
Pseudomonas	Positive control	16	16
	Negative control	-	-
	10	4	8
	20	6	9
	30	8	12
Bacillus	Positive control	15	15
	Negative control	-	-
	10	3	7
	20	6	8
	30	7	9
Enterococcus	Positive control	19	20
	Negative control	-	-
	10	4	5
	20	5	7
	30	8	10

Positive Control- Antibiotics (Amoxycillin); Negative Control- Glacial Acetic Acid

potent antioxidants activity than the standard BHT and it is safe to use in consumer products. Chitin extracted using protease enzyme from *B. mojavensis A21* and *Bacillus subtilis* A26 frommarine source were tested for antimicrobial activity against gram positive organism such as *Micrococcus luteus, Staphylococcus aureus, Bacillus cereus* and gram-negative organism such as *Klebsiella pneumoniae, Escherichia coli, Salmonella typhi* shows inhibition except *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Chitosan acts as an antibacterial and antifungal component (Zhang *et al.*, 2015).

FTIR analysis:

Various types of chemical bonds present in molecules can be identified using FTIR Spectroscopy by producing an IR absorption spectrum; molecular finger. The functional group detected using FTIR is presented in Table 4 and Figure 4. The spectra located at 3426.66 cm^{-1} indicates the stretching vibration of aliphatic O-H, Absorption peak at 2967.58 cm^{-1} is C-H vibration, peak at 1654.98 cm^{-1} represents the carbonyl

Table 4: Functional Groups Detected Using FTIR in Chitin and Chitosan of crab shell

Bond	Type	Frequency range (cm^{-1})	Nature
O-H	Hydrogen bonded, Alcohol 's, Phenol	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

group, C=O from acetamide (-NHCOCH3), peak at 1535.39 and 1456.30 cm^{-1} indicating the bending vibration of –NH and stretching vibration of –CN from acetamide group, peak at 1048.35 cm^{-1} is the stretching vibration for –C-O-C- of the glucosamine ring and peak at 878.60 cm^{-1} is ring stretching a characteristic band for β-1, 4 glycosidic bonds are characteristic of chitin (Lima *et al.*, 2004).

Production of Bioplastic:

The bioplastic was prepared using the protocol I and II (Fig. 5). Protocol II was found to be a good bioplastic. In a high-shear cell, chitosans and other marine creatures form a coating that prevents other exfoliated biomolecules from passing through. Due its water-vapor permeability of the nano encapsulated chitosan film is markedly decreased, resolving a long-standing issue with the manufacturing of biopolymer nanofilms. Chitosan nanoparticles (NPs) have great potential as nanocarriers that encapsulate substances such as drugs or active compounds, deliver them to a specific place or site, and provide a controlled release. Chitosan NPs can serve as reinforcement elements in biodegradable plastics, in food packaging and in enhanced antimicrobial activity.

Tensile Strength (*TS*):

The bioplastic C 8:2 ratio sample is better composition than the C9:1 ratio sample. The tensile strength of both the samples shows that sample-II has more tensile strength than sample I. (Table 5; Fig. 6). Bioplastics prepared using chitin decreased the tensile strength and elongation at break slightly. Tensile strength of bioplastics without chitin addition is 7.8 MPa and elongation at break of 58.5 %. Bioplastics with 1-4 % chitin decreased tensile strength from 6.7 to 6.3 MPa. On the other hand, the elongation at break of chitin addition in bioplastics exhibits the reduction from 55.5 to 40.8 %**.** Chitin has strong intermolecular hydrogen bond and they exhibit weak interaction with starch molecules affects characteristics of bioplastics.

Water Absorption:

The water absorption study shows that gelatin-based chitosan biopolymer has great ability in absorbing water and depend on time (Table 6). The water-vapor permeability of the nano-encapsulated chitosan film is subsequently significantly reduced, solving one of the long-standing problems in the production of biopolymer nanofilms (Kumar *et al.*, 2016)**.** Chitosan bandage knitting with blood and seals laceration (Singh *et al.*, 2017). Chitosan hydrogel can used to heal burn, chronic diabetic wounds, and hydrofluoric acid burns. United States received approval in 2003 for chitosan films for medicine (Zhang *et al.*, 2015). Chitosan films are

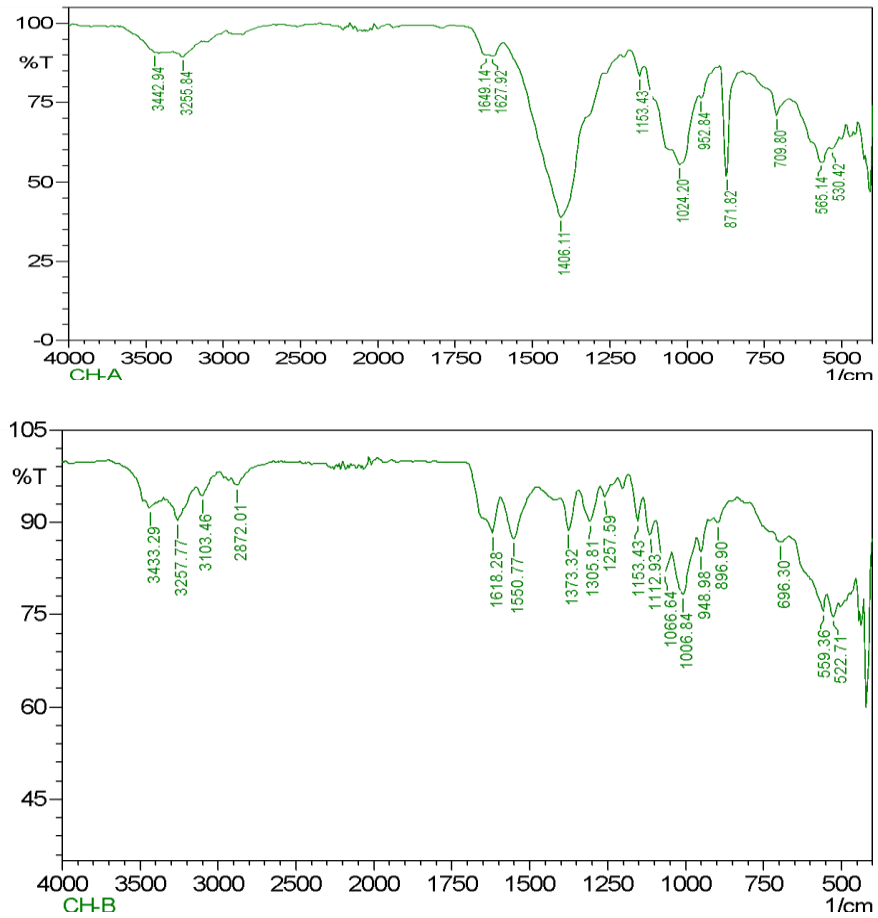
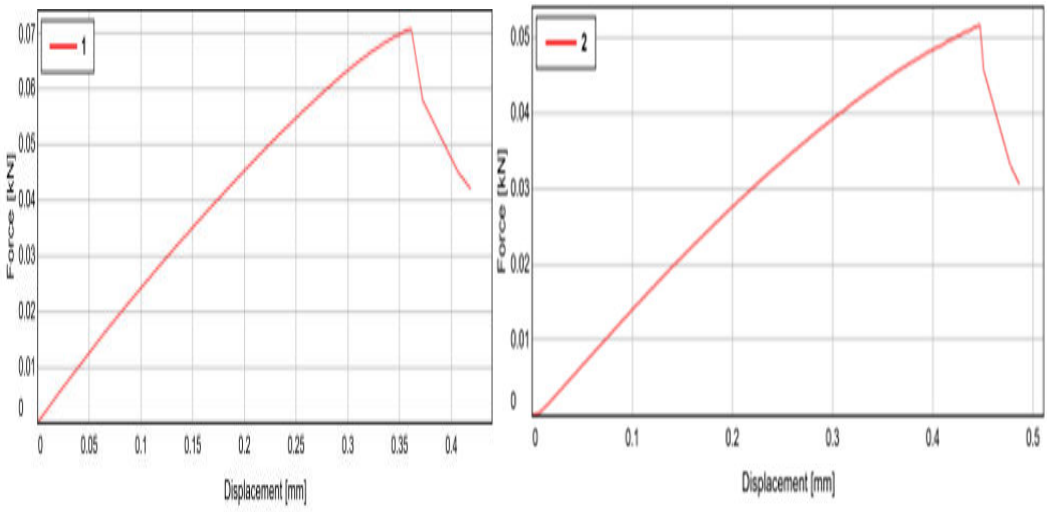
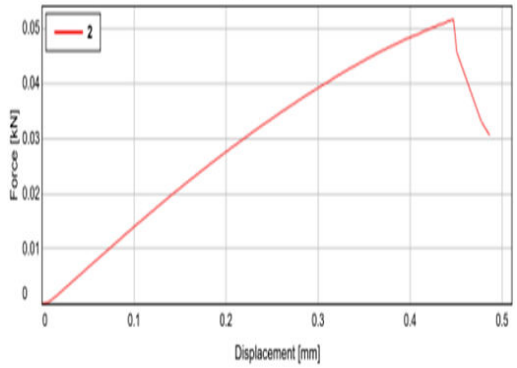


Fig. 4: FTIR analysis of Chitin (CH-A) and Chitosan (CH-B).

Fig. 5: Bioplastic prepared using chitosan and gelatin.



(a) Gelatin Polymer (b) Chitosan and Gelatin Polymer(9 :1)



(c) Chitosan and Gelatin Polymer (8 :2)

Fig. 6: Tensile Strength of polymers

Table 5: Tensile Strength chitosan of crab shell

Specimen label	Tensile stress at Break
Control	52.40
C 9:1	77.44
C 8:2	85.88

Table 6: Water Absorption chitosan of crab shell

Time (h)	% of Water Absorption Capacity
1	22.2
2	24.8
3	54
24	77

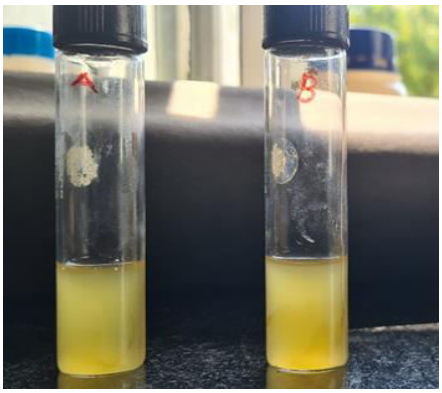


Fig. 7: Enzymatic Degradation (Specimen-A Glycerol mixed chitosan and Specimen-B Gelatin mixed chitosan).

Table 7: Qualitative Analysis of Chitosan Soap

Test	Results
pH	7-acidic
Colour, Odour	Red, Pleasant
Foam Test	5cm height
Litmus test	Blue paper turns to Red

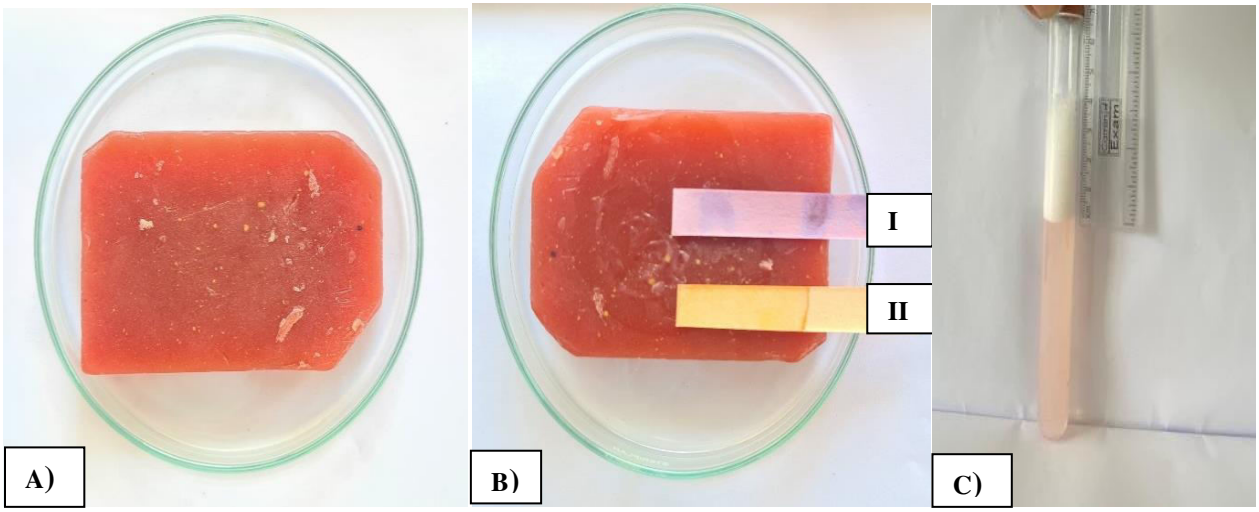


Fig. 8: (A) Chitosan Based Soap; (B) Test undergone for soap I- litmus test, II-pH test; and (C) Foam Test.

safe alternatives to preserve food products.

Soil Degradation:

The mass of glycerol-based bioplastic 2.7 g was reduced to 2.3 g on day 35 with the optimum rate of weight loss of 14.81%. Therefore, the glycerol-based bioplastic has a good ability to degrade under the soil. The mass of glycerol-based bioplastic was reduced 50% of weight and gelatin-based bioplastic was completely dissolved. Calcium carbonate, which makes up to 95% of eggshell, is followed by a little quantity of organic matrix (3.5%), which makes up the remaining 5%. Consequently, while the calcium carbonate is still present, the bioplastic is degraded by microbes. Due to its average rate of biodegradation, it may be inferred that by adding eggshells as a filler, the bioplastic is broken by microorganisms more slowly.

Enzymatic degradation:

The enzymatic degradability test of bioplastic

was determined after 5 days. Also, the initial mass of glycerol-based and gelatin-based bioplastic was recorded in sample I: 1.8 g and in sample II: 0.7 g and sample I reduced to 0.9 g on day 5 with the optimum rate of weight loss of 50% and sample II was completely dissolved (Fig. 7). Chitosan components undergoes break down using enzymes called chitinase. These enzymes are absent in mammals hence chitosan implantation causes it to gradually degrade over time and vanish entirely.

Soap Preparation:

Soap prepared by using chitosan has pleasant odour, has pH of 7, and has high foam production capacity (Table 7; Fig. 8). Chitosan is a type of antioxidant with applications in the pharmaceutical and tissue engineering sectors. Chitin and chitosan are marine-derived antibacterial, antifungal, anticancer, and antioxidant chemicals. Chitin, a natural polymer related to cellulose, has more applications than chitosan (Islem Younes and Marguerite Rinaudo, 2015).

Conclusion

The shellfish processing industry in India generates about 9.5 million tons of shell waste per year, environmental implication of disposal methods of such waste has created an alternative disposal. Chitin, chitosan can be extracted from crustacean by avoid dumping of shell waste in the environment. The increased use of natural chitin and chitosan is motivated by the fact that contrary to the petroleum derivatives, as it obtained from a sustainable and renewable source. It can be used as fiber, biofilm, affinity chromatography column matrix, gas-selective membrane, plant disease resistance promoter, anti-cancer agent, wound healing promoting agent, antimicrobial agent, preservation of fruit, cosmetics, artificial organs in pharmaceuticals and bioremediation.

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