

# Utilization and Conversion of Discarded Lobster Shells into Valuable Biopolymers - A Pilot Study

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**Abstract:** Chitin is one of the most abundant polysaccharides and can be made by combining shells of crabs, shrimp, lobsters, and fish. This study used discarded lobster shells as a raw material to produce a biopolymer. As part of the process, TiO<sub>2</sub> was incorporated into the film in order to increase tensile strength. As compared to the control sample without TiO<sub>2</sub> (3.34 MPa), the experimental sample with TiO<sub>2</sub> showed improved tensile strength (3.96 MPa). Bio composites containing chitin and chitosan have been shown to produce thin, labile, textured films with good apparent textures. Upon tensile testing, it was determined that the material produced was sturdy enough for use as packaging and medical equipment. The prepared samples were analyzed in a variety of ways to understand their properties. In the FTIR analysis, it is evident that certain functional components are present that are responsible for the plasticity of the material. A study conducted on pathogenic *Escherichia coli* and *Staphylococcus aureus* indicated that the film had antimicrobial properties, as indicated by the zone of inhibition between 13 mm and 11 mm in samples with TiO<sub>2</sub>, respectively, and 11 mm and 10 mm in samples without TiO<sub>2</sub>. According to the physical degradation test, the produced biopolymer was 100% degraded when compared to a synthetic plastic (12%), which makes it the best choice for creating a multipurpose material. In addition to its biodegradability, biocompatibility, and anti-microbial properties, this material is suitable for use as biomedical interfaces and packaging materials.

**Keywords:** Biodegradability, Biomaterial, Chitin, Chitosan, Lobster shells, TiO<sub>2</sub>

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## 1. Introduction

There are 63 billion tonnes of plastic waste in the world, and only 9% of plastic is recycled globally. For the purpose of combating the global crisis of plastic pollution, some scientists are currently developing a cost-effective and efficient method for utilizing copious shell waste. It is becoming increasingly important to make biodegradable choices, as most plastics end up in landfills for more than hundreds of years. Researchers have found that they may be able to solve two problems concurrently (pollution caused by plastic and food waste) by converting shells trash into biodegradable plastic. (Steffen, 2019)

Marine invertebrates, insects' exoskeletons, fungi and algae's cell walls contain copious amounts of chitin, the second most abundant organic material after cellulose. Crustacean shells are a major commercial source of chitin

because they contain a high content and are readily available (Ray, 2019; Xu, 2020). Several huge amounts of lobster shells are dumped into the environment every year, which can be used to recover chitin and produce chitinases to degrade heaps of chitin waste (Jabeen, 2021). In crustacean shells (shrimp, crab, squilla and krill), chitin accounts for 15–40%, protein for 20–40%, calcium and magnesium carbonate for 20–50%, and minerals for 15% (Khoushab, 2010 & Mohan, 2021). Chitin and proteins make up the exoskeleton of lobsters, along with calcium and carotenoids. In the exoskeleton, chitin accounts for approximately 75% of the organic fraction (Fabritius, 2009 & Gayathri 2017). There is a high amount of chitin in the head and shell of giant tiger shrimp – 39% and 36.5%, respectively. Only lobster shell is capped by chitin, which comprises 88% to 97% (Das, 1996 & Buendia, 1999). Since lobster shell powder contains 21.6% chitin, the chitin yield of lobster shell powder was

almost complete (94% of total chitin). Based on the results, chitin was recovered from lobster shells in a relatively high percentage (~94%). According to Oduor-Odeto, Struszezyk, and Peter (2007), ornate rock lobster (*Panulirus ornatus*) chitosan yielded 74.3 %. (Wei, 2020). There is approximately 1012 tonnes of chitin wastes accumulated in oceans every year as a result of aquatic products that are composed of organisms loaded with chitinous matter (Jahangiri, 2019; Ray, 2019; Sun, 2019). Plastic, like chitin, is a polymer, which is made of molecules attached by repeating units (NatashahHitti, 2019). Natural polymers such as chitin are found in products that we discard every day, such as seafood, insect shells, and fungi walls. A versatile material can be produced from chitin. (Steffen, 2019). The most common method of disposing of claws and legs is to throw them back into the ocean or into landfills. There are many uses for these shells, including eco-friendly packaging and food products. The shells have chitin and chitosan which are both biodegradable materials. A number of chemical and biological applications are achieved through the use of chitin or its deacetylated derivative chitosan (Muzzarelli, 2009). Chemically modifying crustacean shells to eliminate protein and calcium residues, chitin is produced by treating them with acids, alkalis, and heat. (Percot, 2003). These polysaccharides can be made use to produce and characterize various biopolymers for different applications. Hence this study was undertaken to establish a protocol for a biomaterial production from discarded lobster shells.

### 1.1. Bioplastics

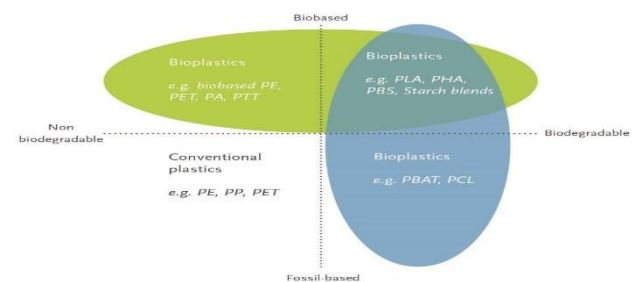
Bio-based: The phrase 'bio-based' refers to a substance or product that is (in part) generated from biomass (plants). Using cellulose-based materials, such as corn, sugarcane, or any other, bioplastics can be made. The benefits of bioplastics: Bioplastics have various advantages in the hunt for novel material solutions, while keeping the objective of sustainable production and use in mind. Moisture accelerates the disintegration process and accelerates decay or breakdown in the presence of oxygen and light. Polybutylene succinate (PBS), polyvinyl alcohol (PVOH/PVA) Polycaprolactone and Polybutyleneadipate terephthalate (PBAT) are examples of polybutylene compounds. They almost all degrade when exposed to UV light from the sun at high temperatures while some are degradable when exposed to high temperatures at industrial scale. Furthermore, these plastics can be recycled organically, meaning they decompose more quickly when discarded. Due to the fact that they contain no chemicals or toxins, they are also non-toxic. Plastic waste falls into two categories: Thermoplastics that are recyclable include PET, HDPE, LDPE, PP, PVC, and PS. Plastics that cannot be recycled (Thermoset & other non-recyclable plastics): laminated and multilayered plastics, bakelite, polycarbonate, nylon, etc. A plastic can be classified into seven types, such as PET, HDPE, PVC, LDPE, PP, PS, etc (Ghadge, 2022). Due to their large molecular size, monomers are considered biochemically

inert units of plastic polymers (Modak, 2022). A lot of bio-waste can be recycled into compost, and biogas can also be produced from it as a renewable source of energy. 13% of the waste stream is composed of traditional plastics, accounting for 32 million tonnes of trash a year. Only 9% of these plastics are recycled (Prelims, 2022). The recycling of plastics includes process of sorting, cleaning, shredding, melting and remoulding of the materials (Khanal, 2022). The bioplastic prevents erosion, runoff, and vegetation establishment, which makes it an environmentally friendly method of waste disposal (Dili, 2022).

### 1.2. Bio-based, non-biodegradable technical / performance polymers

Many specific polymers are included in this large group, including bio-based polyamides (PA), polyesters (e.g., PTT, PBT), polyurethanes (PUR), and polyepoxides. (Figure 1)

**Figure 1:** Material coordinate system of bioplastic (Ottoni, 2018)



Fibers used in textiles (seat covers, carpets), foams used in automotive seats, casings, cables, hoses, and covers are some examples of common technical applications. Typically, their operational life is several years. Therefore, biodegradability is not a desirable property, and therefore they are referred to as durables.

### 1.3. Lobster shell into bioplastic

Spiny lobster (*Palinuridae*) and Slipper lobster (*Scyllaridae*) are India's most popular fishing resources and are shipped worldwide. During commercial scale processing, an immense amount of exoskeleton is generated. As a result of frequent discarding of shells in the sea or along coasts, bad odors and biogenic amines are produced due to decay, which threatens the balance of the coastal ecosystem (Kelleher, 2005 & Xu, 2008). Processing facilities that process lobster or other crustaceans are exposed to significant environmental risks under the current waste management procedures. Among other minerals and chemical compounds, the lobster's exoskeleton comprises chitin, proteins, calcium, and carotenoids. About 75% of the exoskeleton's organic content is composed of chitin (Fabritius, 2009). To combat this problem, along with utilizing the vast amounts of shells discarded from lobster processing operations, an eco-friendly alternative to the extraction of chitin

physiochemically is being developed, an environmental obligation in order to reduce pollution and ensure sustainability. In addition to performing an essential biogeochemical role, chitinolytic microbes recycle polysaccharide chitin, the second most abundant polysaccharide made up of 1-1,4 N-Acetyl glucosamine units. There is a possibility that chitin is the only source of carbon and nitrogen for these microbial communities. As chitin is degraded enzymatically by microorganisms in chitin-rich conditions, chitinases, proteases, and other enzymes are released.

## 2. Materials and methods

### 2.1. Sample collection

The raw materials used in this present study includes exoskeleton of lobster, which was collected from the near by fish market. The samples were dried under room temperature for 7days and ground well to make it into a powder form.

#### Chitin and Chitosan extraction

For the isolation of chitin, the exoskeletons were treated with sodium hydroxide (4% v/v) for 3 hours at 100°C and stirred well for 3 hours in 250ml beakers. Following the treatment, samples were filtered and allowed to cool at room temperature for 60 minutes.

#### Demineralization

For demineralization process 50g of the exoskeleton was treated with 100ml of Hcl at a strength of 2M and concentrations of 0.5% & 1.0%.The samples were soaked and boiled using water bath for 3hrs, as a result of the minerals present (mostly calcium carbonate) being removed.To reduce the amount of alkali, and alkali earth metals, the demineralization process was used (Abatyough, 2022).In order to decompose the albumen into water soluble amino-acids, a demineralised lobster shell sample is treated with 50ml of 20% NAOH solution for 2 hours. Afterwards, demineralized water is used to wash the sample, and it is filtered. Following the extraction of chitin, deacetylation is used to convert it to chitosan.

#### Deacetylation

For deacetylation process 100 ml of 50% NaOH is added to the sample and boiled at 100°C for 3h. Then the sample was filtered and cooled for 60min at room temperature. In order to retain the solid matter, the chitosan, the samples were continuously washed with the cooled water and filtered.

#### Film preparation

The obtained chitosan is mixed with water, polyvinyl alcohol and 5% acetic acid and stirred well until the gel formation. Then the solution is poured in a Petri plate and dried at room temperature for 5 hours. Then the film is removed from the plate.

#### FTIR

For determination of the preliminary structures of the films and to compare the functional groups and chemical bonds between the films. The FTIR spectra were recorded

from 4000 to 400 cm<sup>-1</sup> at a resolution of 1cm<sup>-1</sup> using FTIR spectrometer.

#### Tensile strength

The test is performed to determine how much force is required to pull the bio plastics to the point where they break. ASTM- D638-77 has been used as the standard method to determine the mechanical properties of bioplastics in this test (Norhafezah, 2018).

#### Biodegradation test

Bio plastic samples with dimensions of 2 cm x 2 cm were put at a depth of 5 cm in compost soil and left for 48 hrs. The buried samples are removed, which were then weighed. The biodegradation test was carried out at room temperature with a relative humidity of 35–40°C.

#### Antimicrobial activity

The antibacterial activity was tested against human pathogens by agar well diffusion method. Bacteria was grown in NB medium and incubated for 24 hours at room temperature. Petri plates were sterilized by keeping them in autoclave at 121°C for 30 minutes. MacConkey agar medium was prepared and poured on petri plates. After solidification, the bacterial culture was swabbed on agar medium using sterilized buds. The samples were placed in the agar. A 24-hour incubation period was conducted at 37°C. Zones of inhibition in mm were measured to determine the antibacterial activity. The strongest inhibition zones were those above 15 m, moderate zones were those between 8 and 15 mm, and weak zones were those between 1 and 8 mm.

## 3. Results and discussion

In the presence of endo and exochitinases, chitin is hydrolyzed to N-Acetylglucosamine, whereas chitosan and an acetyl group are formed by chitin deacetylase (Goody, 1990).Many plant tissues generate chitinases, and these enzymes are principally responsible for the protection against biotic stresses (Punja, 1993; Mauch, 1988). Chitinolytic bacteria and fungi, which break down chitin and its derivatives, are used as agricultural biocontrol agents since they are key components of bacterial peptidoglycan, fungal cell wall's and insect cuticles (Ordentlich, 1988). The extraction of chitin from crab shells has been studied previously by microbial breakdown. When additional whey, lignocellulose and starch are added to the lactic acid fermentation, shrimp heads are more easily ensilated (Fagbenro, 1996). A probiotic curd and microorganisms from crustacean gut microbiomes were used to separate chitin from shrimp waste (Rao, 2000).As the crab and shrimp shells were demineralized and deproteinized by microbial processes, a liquid fraction rich in proteins and minerals was formed, along with an insoluble chitin fraction that was retained in the sediment (Jung, 2007; Xu, 2008).Earlier studies had studied the shells of shrimp and crab, not lobster shells. But this study explored how discarded lobster shells might be degraded through biodegradation within the local economy and environment.

### 3.1. Fourier transform infrared spectroscopy (FT-IR) analysis

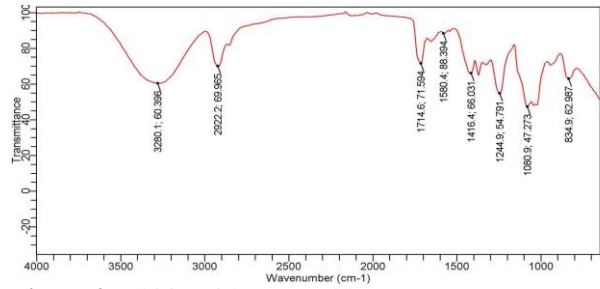
**Table 1:** Chitin with pva. (Sample 1)

Peak Number	Wave number (cm <sup>-1</sup> )	Intensity	Compound
1	834.92368	62.98713	C-H out of plane
2	1080.92797	47.27267	C-O
3	1244.93084	54.79126	C-N stretch
4	1416.38838	66.03082	C-H bending
5	1580.39124	88.39394	N-H bending
6	1714.57541	71.59374	C=O
7	2922.23286	69.96534	C-H stretch
8	3280.05730	60.39634	N-H bending

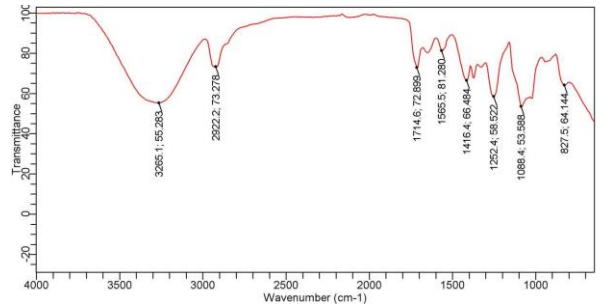
**Table 2:** Chitin with pva and titanium dioxide. (Sample 2)

Peak Number	Wave number (cm <sup>-1</sup> )	Intensity	Compound
1	827.46900	64.14360	C-H out of plane
2	1088.38265	53.58757	C-O-C stretch strong
3	1252.38551	58.52162	C-N stretch strong
4	1416.38838	66.48397	C-H bending strong
5	1565.48189	81.28032	NH bending
6	1714.57541	72.89855	C=O
7	2922.23286	73.27774	C-H stretch strong
8	3265.14795	55.28293	NH stretch

The interaction between two composite films was determined using FTIR spectroscopy. (a) As of 3280.05cm<sup>-1</sup>, broad peak indicated N-H bending ; peak at 2922.23cm<sup>-1</sup> (C-H stretching); at peak 1714.57cm<sup>-1</sup> indicates the presence of (C=O) and peak at 1580.39cm<sup>-1</sup> (N-H bonding); peak at 1416.38cm<sup>-1</sup> (C-H bonding); at 1244.93cm<sup>-1</sup> presence of C-N stretch strong; peak at 1080.92cm<sup>-1</sup> (C-O) and peak at 834.92cm<sup>-1</sup> indicates the presence of C-H out of plane . (a) PVA and (b) TDO has around similar peaks in which the starting broad peak at 3265.14cm<sup>-1</sup> indicates the presence of N-N stretch; peak at 2922.23cm<sup>-1</sup>, 1714.57cm<sup>-1</sup>, 1565.48cm<sup>-1</sup>, 1416.38cm<sup>-1</sup>, 1252.38cm<sup>-1</sup>, 1088.38cm<sup>-1</sup> and 827.46cm<sup>-1</sup> are similar to PVA (a) sample. From this analysis, it was evident that the type of bioplastic obtained from these study is polyester substantiated with the presence of 5 functional groups.



**Figure 2:** Chitin with pva (Table 1)



**Figure 3:** Chitin with pva and titanium dioxide (Table 2)

### 3.2. Tensile strength

**Table 3:** Tensile strength of chitin with pva (Sample 1)

	Maximum load [N]	Tensile strength [MPa]	Elongation at break [%]
1	2.33	3.34	88.32
Mean	2.33	3.34	88.32

**Table 4:** Tensile strength of chitin with pva and titanium dioxide (Sample 2)

	Maximum load [N]	Tensile strength [MPa]	Elongation at break [%]
1	2.33	3.96	69.78
Mean	2.33	3.96	69.78

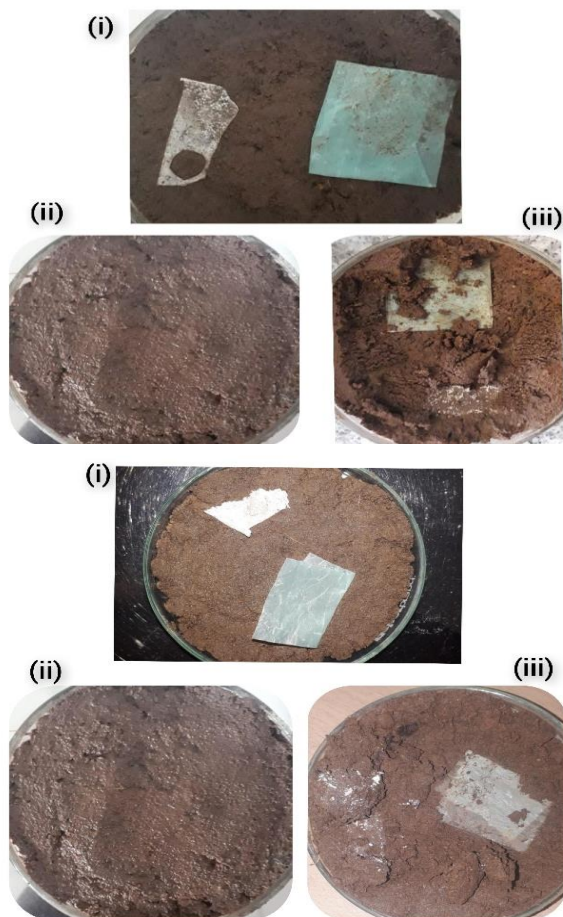
Table 3 & 4 show the mechanical properties of chitin with pva (sample 1) and chitin with pva and Titanium dioxide (sample 2). The result of Titanium dioxide with pva and chitin (sample 2) has greater mechanical strength than chitin with pva (sample 1) but less flexibility. The elongation of chitin with pva is reduced from 88-69 when it changed at chitin with pva and Titanium dioxide (sample 2) due to TiO<sub>2</sub> nanoparticle. The behaviour is due to the anti plasticization property. As more TiO<sub>2</sub> particles are incorporated into the sample, the elasticity of the sample is reduced, resulting in more rigid, stiff and harder (Hayeemasae, 2015).

### 3.3. Biodegradation test

**Table 5:** Biodegradation test of sample 1 & sample 2

Weeks	Percentages of Degradation		
	Sample 1	Sample 2	Commercial Plastic
1 <sup>st</sup> week	27%	29%	0%
2 <sup>nd</sup> week	51%	53%	2%
3 <sup>rd</sup> week	79%	81%	5%
4 <sup>th</sup> week	100%	100%	12%

Chitin with PVA (sample 1) and chitin with PVA in Titanium dioxide (sample 2) weight loss with burial time, the weight loss of these film increases. On 1<sup>st</sup> week (sample 1), film degraded more than 27% of their initial weight and (sample 2) film degraded up to 29%. And on the 2<sup>nd</sup> week (sample 1) & (sample 2), films degraded up to half of its initial weight. After 3<sup>rd</sup> week (sample 1) & (sample 2), degraded up to 80%. And finally at the 4<sup>th</sup> week (sample 1) & (sample 2) completely degraded. When compared to our bioplastic, the normal commercially available green plastic degraded half of its initial weight during the 4<sup>th</sup> week. Commercially available green plastics decompose in six months to two years, far faster than traditional plastic (Emmanuelle, 2022).



**Figure 4:** (a, b) a, b (i) Sample 1 & 2 is placed inside the compost soil with commercial plastic. a, b (ii) The samples are enclosed inside the compost soil. a, b (iii) After 4 weeks the final degradation result of the samples.

### 3.4. Antimicrobial activity

**Table 6:** Antimicrobial activity of chitin with PVA (Sample 1) and chitin with pva titanium dioxide (Sample 2)

Test Organism	Zone of Inhibition (mm)	
	Sample 1	Sample 2
<i>Escherichia coli</i>	11	13
<i>Staphylococcus aureus</i>	10	11

The result after 24hr incubation is shown in Table 6. The inhibitory effect was calculated using the clear zone surrounding the circular film disc. The outside diameter of film bioplastic was used to calculate the clear zone diameter. Bioplastic films showed a good anti-bacterial activity against both gram positive (*S.aureus*) and gram negative (*E.coli*) bacteria. For the antimicrobial activity, Commercial biodegradable plastics are used as control. The result showed that pva in Titanium dioxide (sample 2) bioplastic shows a maximum zone of inhibition against *E.coli* compare to *S.aureus*. pva in Titanium dioxide (sample 2) bioplastic seems to have more effective antimicrobial activity against gram negative (*E.coli*) bacteria than gram positive (*S.aureus*) bacteria. The commercial biodegradable plastic did not show any antimicrobial activity.

### 4. Conclusion

This study investigated preliminary methods for extracting chitin from lobster shells using chemical methods. Based on observations of the trial experiments and the texture and color of the material obtained, it concludes that commercial grade chitin is similar to the product obtained in this study. FTIR analyses were conducted on two samples chosen based on visual observation and assumed physical properties, which indicates chitin synthesis to be successful. By interpreting FTIR results, it shows that lobster shells can be successfully used as raw materials to synthesize chitin. It is also possible to use chitin in the production of value added products by observing films formed from it. As a biomedical interface, this product can be effectively used as it is biodegradable, biocompatible, and bio compatible. Various industries, medical fields, and pharmaceuticals can get benefit from lobster shell chitin. This finding provides an opportunity to enhance the potential value of the discarded lobster shell into valuable biopolymer.

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