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SYNTHESIZING CHITOSAN FROM DIFFERENT CHITIN SOURCES AND IT'S APPLICATIONS

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ABSTRACT

Plastics have been a crucial component in our development but now we know the problems with its widespread use. Therefore, there's a need for greener alternatives that can replace plastics. Chitosan is a sugar derived from chitin the second most abundant biopolymer on earth, it can be used to make bioplastics and many more applications. Our objective was to synthesize chitosan from 4 different sources of chitin viz. Crab shell, fungi, cockroaches & prawn shells and study their properties in different applications. Chitin is converted to chitosan by 3 standard steps viz. Deproteinization, demineralization and deacetylation in that order. These steps involve incubation of chitin with sodium hydroxide and hydrochloric acids at different concentrations and temperatures. The presence of chitosan was confirmed with ninhydrin tests. The applications tested were Edible chitosan coating, Chitosan reinforced bioplastic, Antimicrobial activity, Chitosan-sodium alginate beads & Antioxidant activity. Chitosan showed promise in its antimicrobial, antioxidant and water treatment properties.

KEYWORDS: Chitosan, bioplastic, chitin, food coating, wastewater treatment.

INTRODUCTION

- Chitosan is a natural polymer derived from chitin, found abundantly in the exoskeletons of crustaceans such as shrimp, crab, insects and lobster.
- Chitin is the precursor of chitosan and the second most abundant polysaccharide after cellulose. An estimated 1 billion tons of it is produced in the biosphere every year therefore there's no scarcity in it's availability.
- Chitin from shrimps, crabs, insects, etc is present in the exoskeleton and is thrown away as waste therefore it can be procured at almost no cost and used for chitosan production.
- Making chitosan from chitin is fairly simple and can be made with ingredients and chemicals that are readily available.

Sources

We are using 4 sources for the purposes of this practical. They are-

1. Prawns Shell: These are used as a chitin source due to their abundance in seafood processing industries, making them a cost-effective and sustainable option.
2. Crab Shell: These are rich in chitin and are readily available from crustacean processing, making them a convenient source for chitin extraction.
3. Cockroaches: Cockroaches have chitin-rich exoskeletons and we are using them as an alternative source.
4. Fungus (Mucor): Fungal sources like Mucor provide chitin and these can be obtained in bulk from fermentation industries that utilize fungi.

PRAWN

CRAB

COCKROACH

MUCOR

MATERIALS REQUIRED

❖ SOURCES OF CHITIN

- PRAWN SHELL
- CRAB SHELL
- COCKRAOACHES
- FUNGUS-MUCOR

❖ CHEMICALS

- SODIUM HYDROXIDE
- HYDROCHLORIC ACID
- ACETIC ACID
- NINHYDRIN
- METHANOL

❖ HOT PLATE MAGNETIC STIRRER
❖ GLASSWARE (TEST TUBES, BEAKERS & FLASKS)
❖ STRAINER
❖ CENTRIFUGE

METHOD

Chitosan is produced by sequential treatment of it's precursor chitin with different chemicals. This process is divided into 4 steps- Deproteinization, Demineralization, Decolorization (optional) & Deacetylation.

A) DEPROTEINIZATION

The deproteinization step is crucial in chitosan production because it removes proteins from chitin by alkaline treatment with sodium hydroxide, which is essential for obtaining high-quality chitosan. This process ensures that chitosan maintains its purity and desired properties, such as biocompatibility and biodegradability. Without proper deproteinization, chitosan may contain impurities that can affect its functionality and performance in various applications, including biomedical, pharmaceutical, and environmental fields. 1M sodium hydroxide (NaOH) in 1:3 solid to liquid ratio [g/ml] was used for deproteinization with 1 hour of continuous stirring at 80°C on the magnetic stirrer.

B) DEMINERALIZATION

The demineralization step is of paramount significance in chitosan production as it eliminates mineral salts from chitin, thereby enhancing the purity and quality of the resulting chitosan. Mineral salts, such as calcium carbonate and magnesium carbonate, are naturally present in chitin, sourced mainly from crustacean shells. Demineralization is typically achieved through acid treatment or chelating agents, which dissolve and remove these salts. This process not only improves chitosan's biocompatibility and biodegradability but also allows for better control over its chemical and physical properties.

Demineralization is vital for tailoring chitosan to specific applications in industries like pharmaceuticals, biomedicine, cosmetics, and environmental sectors. For instance, in pharmaceuticals, demineralized chitosan exhibits improved drug delivery capabilities due to its enhanced solubility and interaction with active compounds. In biomedicine, demineralized chitosan is utilized in tissue engineering and wound healing applications for its ability to promote cell adhesion and regeneration. 1M Hydrochloric acid (HCl) in 1:4 solid to liquid ratio [g/ml] was used for demineralization with 1 hour of continuous stirring at 80°C on the magnetic stirrer.

C) DEACETYLATION

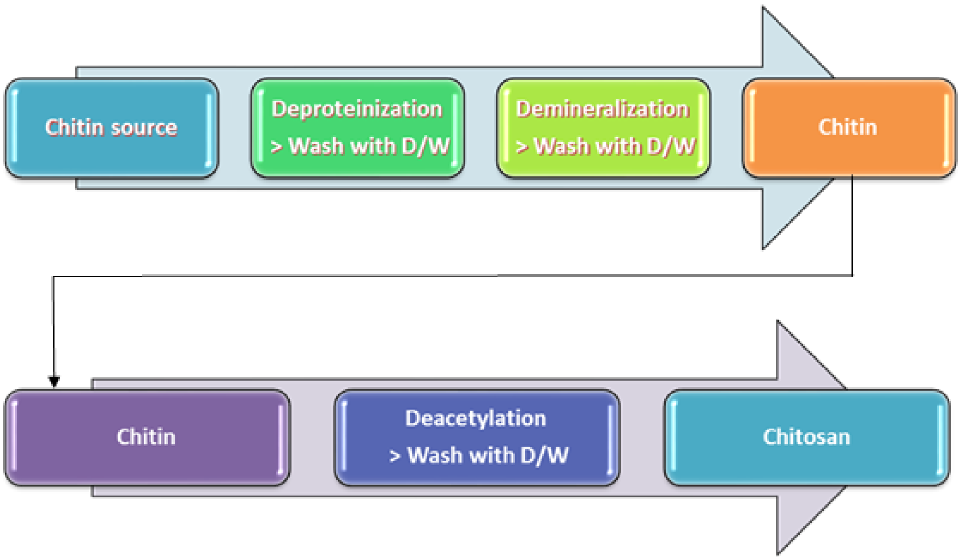
The deacetylation step holds significant importance in chitosan production as it transforms chitin into chitosan by removing acetyl groups from the polymer chain. This process enhances the biological activity and functional properties of chitosan, making it suitable for a wide range of applications. Deacetylation is typically achieved through alkaline hydrolysis or enzymatic methods, where chitin is treated with alkaline solutions or enzymes to break the acetyl bonds.

The degree of deacetylation (DDA) plays a crucial role in determining chitosan's properties, including its solubility, viscosity, charge density, and bioactivity. Higher DDAs result in more positively charged chitosan molecules, which are advantageous for applications like drug delivery, wound healing, and biomedical uses due to their enhanced interaction with cells and molecules.

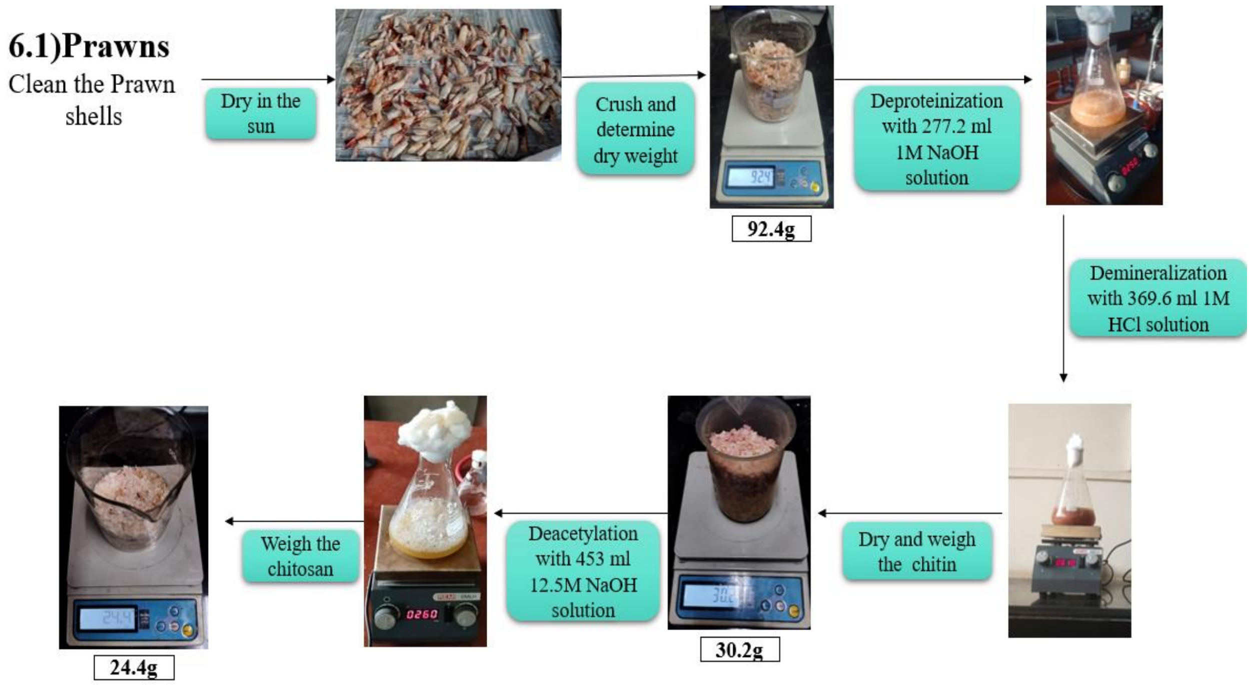
In pharmaceuticals, deacetylated chitosan is prized for its ability to encapsulate and deliver drugs effectively, improving drug solubility and bioavailability. In biomedical fields, deacetylated chitosan is used in tissue engineering, regenerative medicine, and implantable devices due to its biocompatibility and cell-adhesive properties.

The quality of the chitosan is directly proportional to the degree of deacetylation, which depends on the alkali concentration, source of chitin, temperature, and time under deacetylation. 12.5 M sodium hydroxide (NaOH) in 1:15 solid to liquid ratio [g/ml] was used for deacetylation with 2 hours continuous stirring at 80°C on a magnetic stirrer.

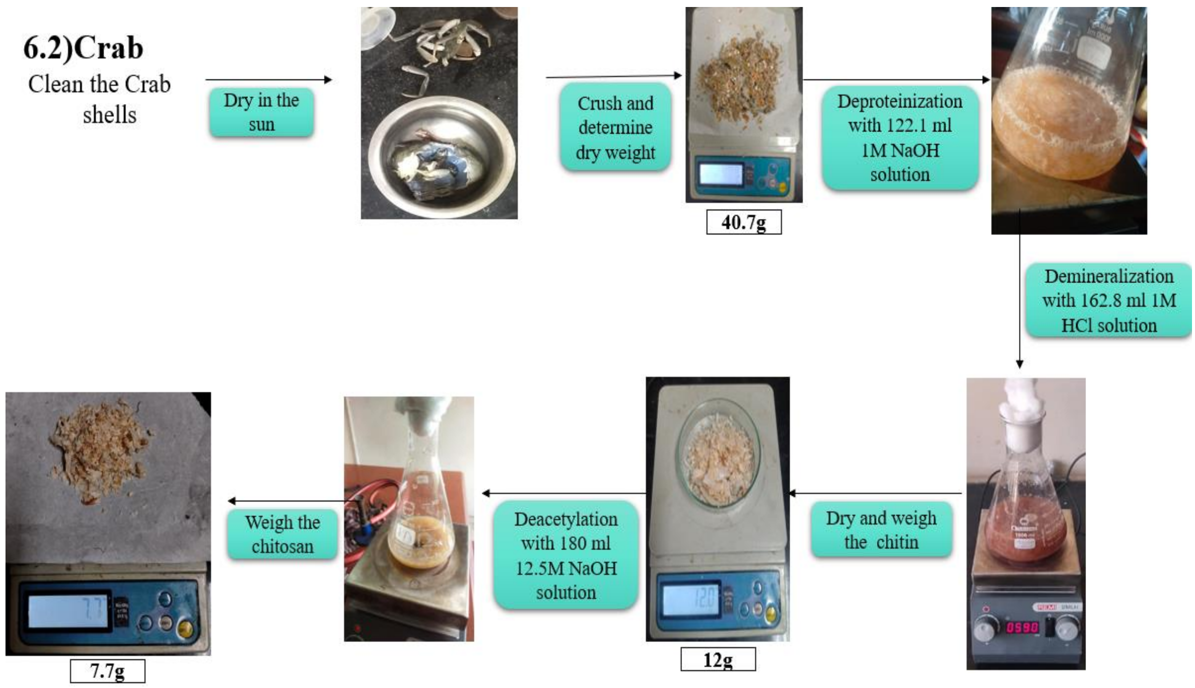
STANDARD PROCEDURE



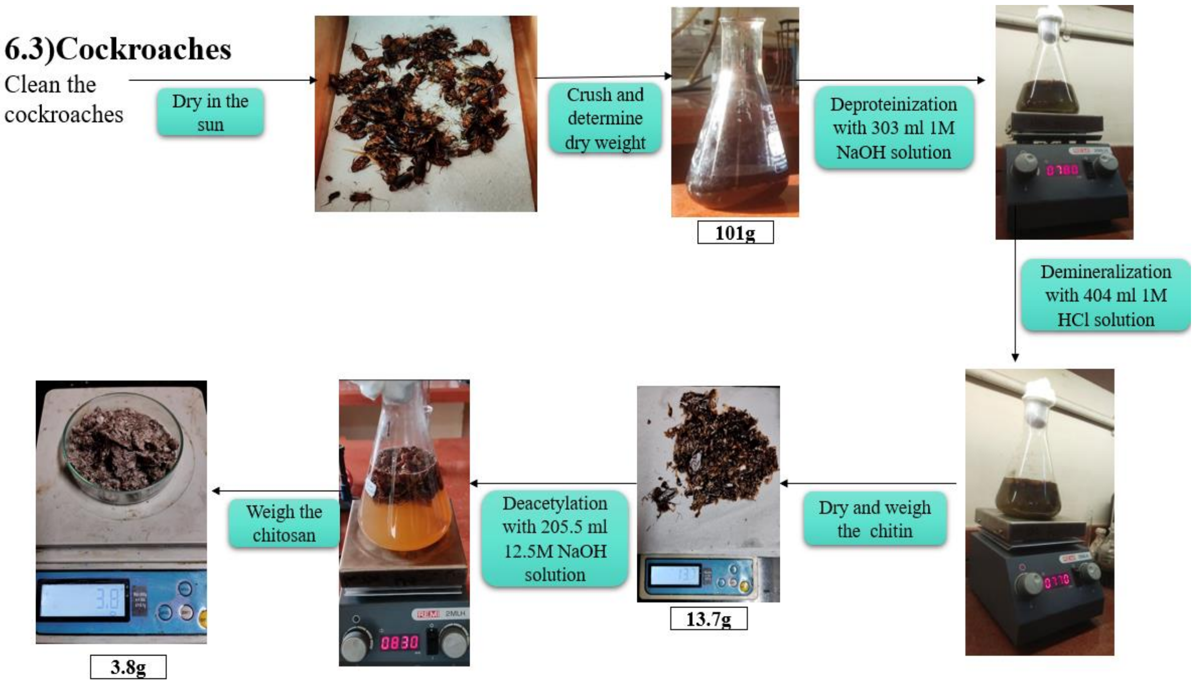
Chitosan is extracted from all 4 sources i.e Prawn shells, crab shell, cockroach & mucor fungi following the same standard procedure but the quantities of reagents used varies depending on the quantities of raw material (chitin sources) used.

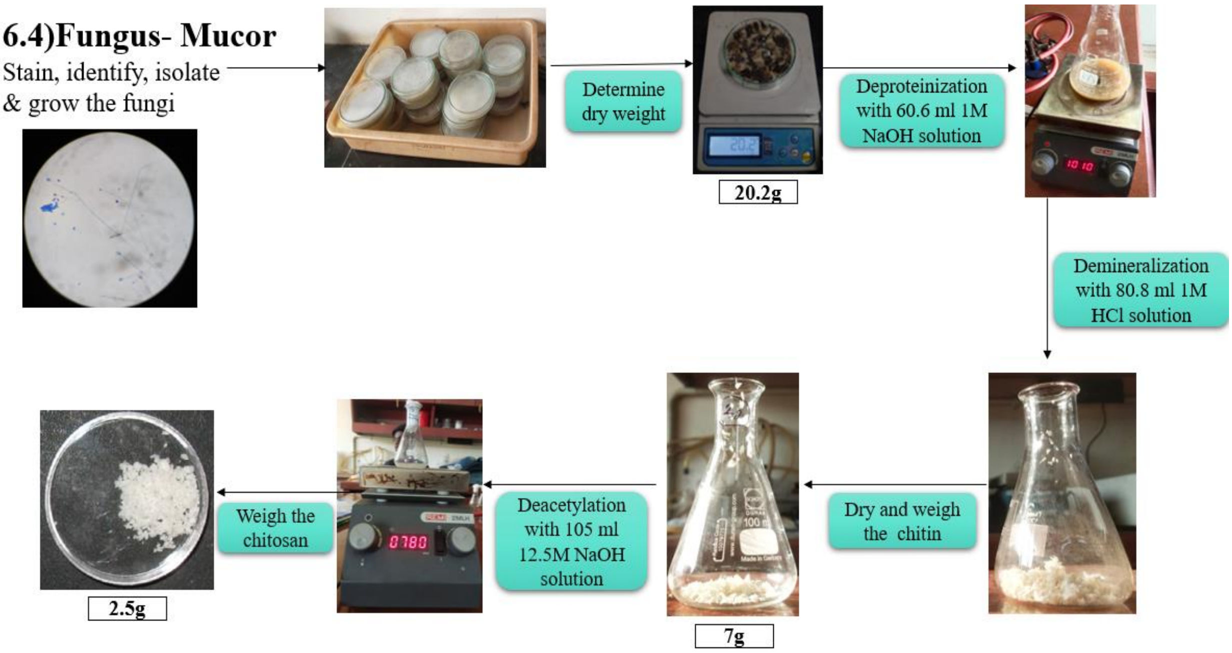


6.2)Crab



6.3)Cockroaches





TEST TO CHECK PRESENCE OF CHITOSAN-NINHYDRIN TEST

The Ninhydrin test is a qualitative and semi-quantitative method commonly used in laboratories to detect the presence of primary amines, specifically the amino group (-NH2) in the molecule. Ninhydrin reacts with primary amines to form a purple-colored complex known as **Ruhemann's purple.** This reaction is commonly used to detect the presence of amino acids, proteins, and other compounds containing primary amine groups.

Prepare Chitosan solution: 0.1 g in 1 ml of 1% acetic acid solution.

↓

Prepare Ninhydrin Solution: 0.2 g in 10 ml acetone.

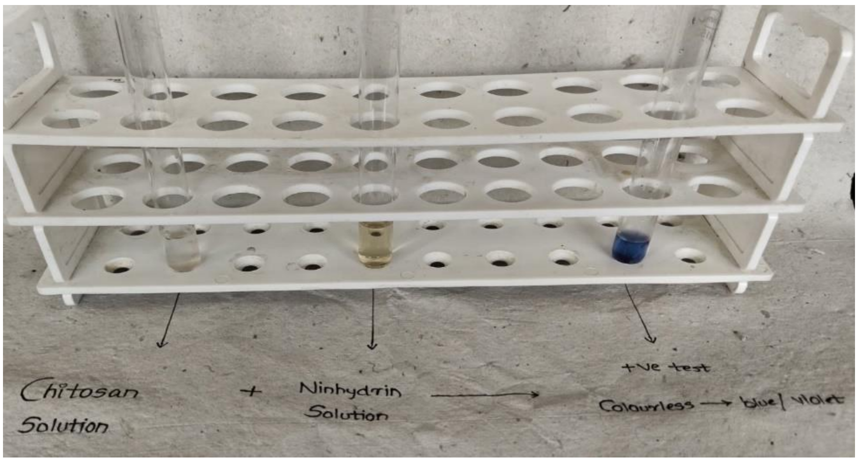
↓

In a test tube: Add 1 ml of chitosan solution and 2-3 drops of ninhydrin solution.

↓

After heating, observe the color change in the solution.

RESULT- All sources showed **purple color** indicating presence of free amino terminals and chitosan.



RESULT AND DISCUSSION

CHITIN SOURCES	PRAWN	CRAB	COCKROACH	MUCOR
SOURCE WEIGHT(g)	92.4	40.7	101	20.2
CHITIN OBTAINED(g)	30.2	12	13.7	7.0
CHITOSAN OBTAINED(g)	22.4	7.7	3.8	2.5
YIELD PERCENTAGE	24	18	3.8	12

Prawn shells were observed to be the **best source** of chitosan with 24% yield followed by crab, cockroach & Mucor with 18%, 12% & 3.8% yield.

APPLICATIONS

ANTIMICROBIAL ACTIVITY

Chitosan exhibits significant **antimicrobial activity**, making it a promising candidate in various industries. Its antimicrobial properties stem from several factors:

1. **Positive Charge:** Chitosan's amino groups impart a **positive charge at acidic pH**, allowing it to interact with negatively charged microbial cell membranes, disrupting their structure and function.
2. **Size and Structure:** The molecular size and degree of deacetylation influence chitosan's antimicrobial effectiveness. Smaller chitosan molecules with higher deacetylation degrees tend to exhibit stronger antimicrobial activity.
3. **Mechanisms:** Chitosan can inhibit microbial growth through mechanisms such as cell membrane disruption, intracellular enzyme inactivation, and DNA damage.
4. **Broad Spectrum:** Chitosan's antimicrobial activity extends to a wide range of microorganisms, including bacteria, fungi, and some viruses.

These properties make chitosan valuable in various applications, including food preservation, wound healing products, medical devices, and agricultural formulations. Its natural origin, biocompatibility, and biodegradability further enhance its appeal for antimicrobial use, presenting a sustainable alternative to synthetic antimicrobials.

PROCEDURE

1g chitosan from 4 sources dissolved in 10ml of 1% acetic acid solution

↓

2 nutrient agar plates were bulk seeded with *E. coli* and 5 wells were punched in the 2 agar plates with a sterile borer.

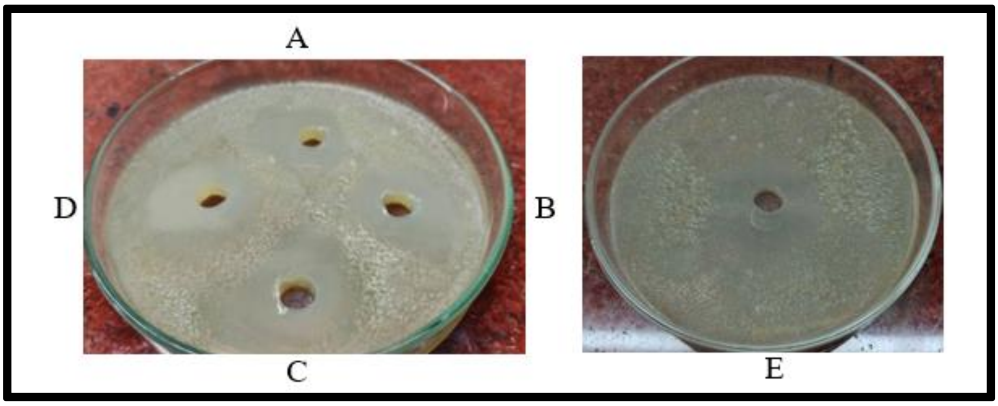
↓

Fill 1 well with 0.1 ml 1% acetic acid and other 4 with chitosan solutions from 4 different sources

This acts as **control** and done to determine the antimicrobial activity of 1% acetic acid in absence of chitosan

↓

Incubate the plates at 37°C for 24 hours



Resultant zones of inhibitions
1% Acetic acid (A)- 4 mm
Fungus- Mucor (B)- 8mm
Prawns (C)- 12mm
Cockroach (D)- 9mm
Crab (E)- 11mm

CONCLUSION

- This verifies the antimicrobial activity of chitosan.
- Maximum antimicrobial activity was found to be in prawns followed by crab, cockroach & Mucor.

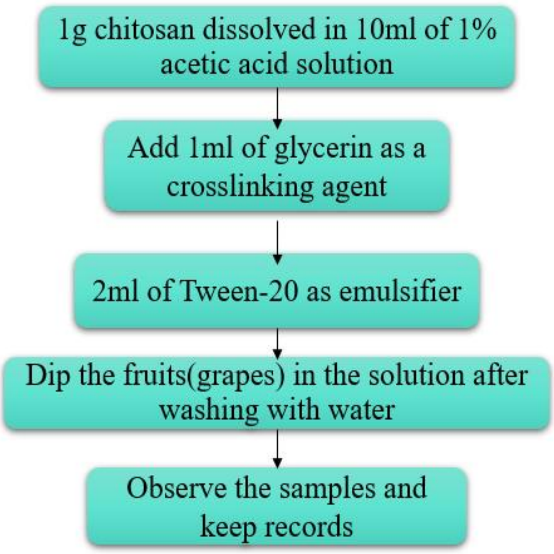
EDIBLE CHITOSAN COATING

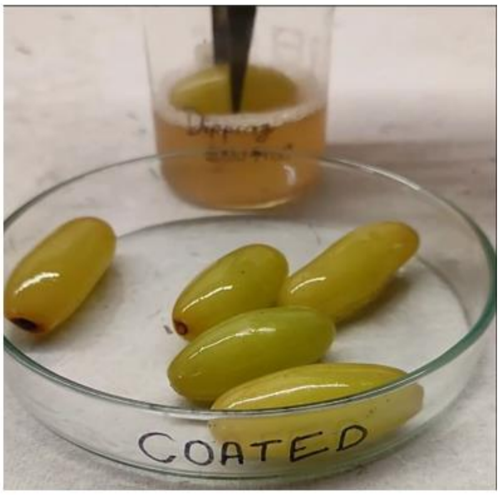
Chitosan is increasingly utilized as an **edible coating** in the food industry due to its versatile properties and benefits:

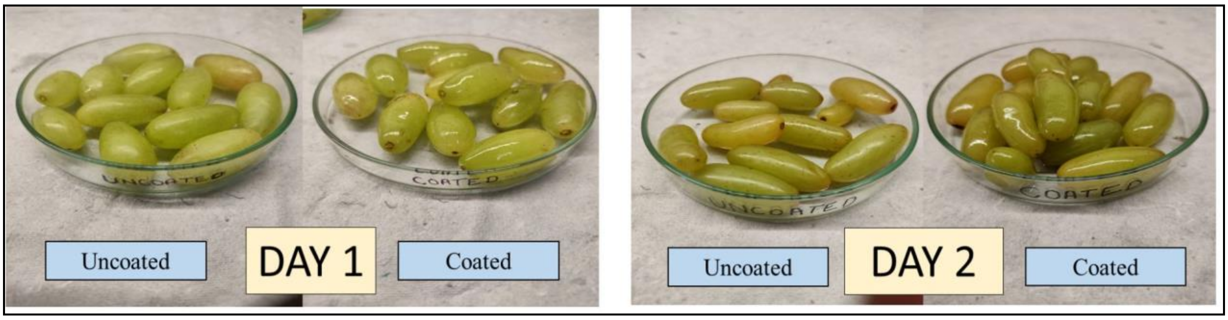
1. **Barrier Function:** Chitosan forms a protective barrier on food surfaces, reducing moisture loss, gas exchange, and microbial contamination. This extends shelf life and preserves food quality.
2. **Antimicrobial Activity:** Chitosan's inherent antimicrobial properties inhibit the growth of bacteria and fungi on coated foods, enhancing their safety and reducing the need for chemical preservatives.
3. **Biodegradability:** Being derived from natural sources like shrimp and crab shells, chitosan is biodegradable and environmentally friendly compared to synthetic coatings.
4. **Enhanced Appearance:** Chitosan coatings can improve the visual appeal of fruits and vegetables by maintaining their color, texture, and freshness.
5. **Functional Additives:** Chitosan coatings can be enhanced with additives like antioxidants, antimicrobials, and vitamins, offering additional benefits such as improved nutritional value and extended storage stability.

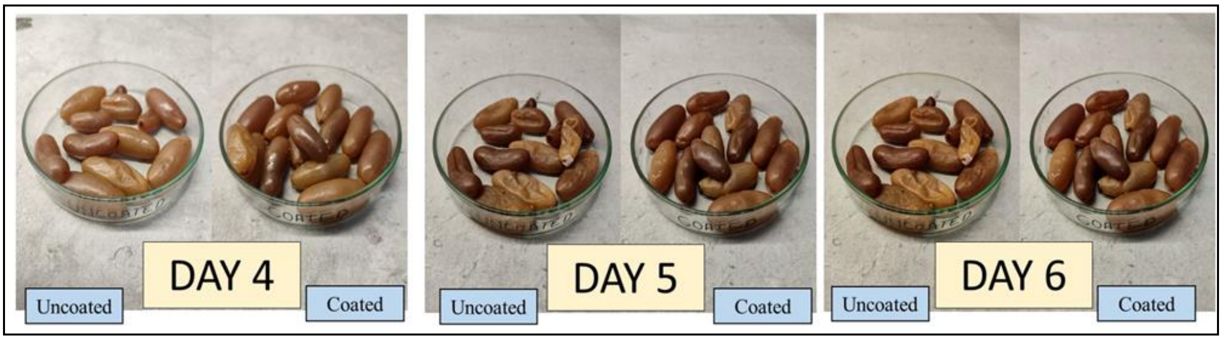
Overall, chitosan-based edible coatings are a sustainable solution for food preservation, enhancing food safety, quality, and consumer appeal while reducing the reliance on synthetic additives.

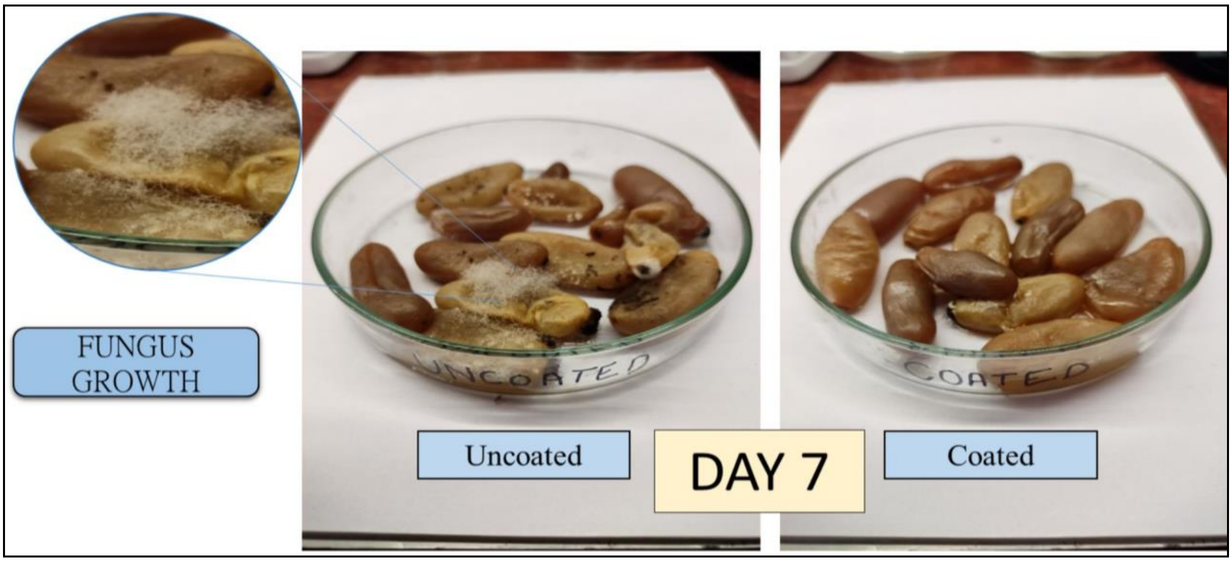
PROCEDURE











OBSERVATION

- As the days progress the uncoated sample appears to **shrink more** than the coated sample.
- On day 7 there is appearance of **fungi** on the surface of uncoated sample.

RESULT- This verifies the antimicrobial and moisture locking properties of the chitosan coating.

CHITOSAN REINFORCED BIOPLASTIC

Chitosan bioplastic is an innovative and sustainable alternative to traditional petroleum-based plastics, leveraging the abundant and renewable chitin from crustacean shells. This bioplastic embodies several advantages that align with modern environmental and economic priorities.

Firstly, its biodegradability sets it apart, as chitosan naturally degrades into non-toxic compounds, mitigating the long-term environmental harm caused by conventional plastics. This characteristic is especially critical in combating plastic pollution and preserving ecosystems.

Moreover, chitosan bioplastic's renewable source makes it an environmentally sound choice. By utilizing a resource readily available from marine waste, it reduces the strain on finite fossil fuels and promotes sustainability.

The versatility of chitosan bioplastic is noteworthy, as it can be molded into various forms, from packaging materials to disposable utensils and agricultural mulch, catering to diverse applications.

Furthermore, its biocompatibility is a significant advantage, allowing for safe use in food packaging, medical devices, and biomedical implants without adverse effects on human health.

By opting for chitosan bioplastic, industries contribute to a reduced environmental impact and a shift towards a circular economy, where resources are utilized efficiently and renewed responsibly.

PROCEDURE

2g chitosan dissolved in 10ml of 1% acetic acid solution

↓

Add 1g of glycerin & 2g of starch

↓

Solution is homogenized at 680 rpm at 60°C

↓

This solution is then poured into a container

Result

- The plastics obtained were **Flaky and weak** in mechanical strength.
- More experiments can be done to obtain a usable plastic with good mechanical strength.

CHITOSAN SODIUM ALGINATE BEADS

Chitosan sodium alginate beads are composite microspheres created by combining **chitosan**, a biopolymer derived from chitin, and **sodium alginate**, a polysaccharide extracted from seaweed. These beads possess unique properties and find applications in various fields:

1. **Drug Delivery:** The controlled-release properties of chitosan sodium alginate beads make them valuable in drug delivery systems. The beads can encapsulate drugs, protecting them from degradation and enabling sustained release for improved therapeutic efficacy.
2. **Biomedical Engineering:** These beads are used in tissue engineering and regenerative medicine due to their biocompatibility. They can encapsulate cells, growth factors, or bioactive molecules, providing a conducive environment for tissue regeneration.
3. **Food and Agriculture:** Chitosan sodium alginate beads are employed in food industries for encapsulating flavors, colors, or nutrients, enhancing food quality and shelf life. In agriculture, they can serve as carriers for biopesticides or fertilizers, promoting sustainable crop management.
4. **Environmental Applications:** These beads are utilized in wastewater treatment for the adsorption of pollutants or heavy metals, contributing to environmental remediation efforts.

Overall, chitosan sodium alginate beads are versatile materials with applications in drug delivery, biomedical engineering, food and agriculture, and environmental sciences, highlighting their significance in various industries.

Chitosan exhibits remarkable **chelation properties** and can be used to remove heavy metals from waste waters.

Chitosan's chelating activity arises from it's free amino terminals which provide a binding site for metal ions.

We are testing the heavy metal absorption of these chitosan beads which can be used for heavy metal absorption.

PROCEDURE

1g chitosan dissolved in 10ml of 4% acetic acid solution

↓

Add 100ml of 2% sodium alginate solution

↓

Keep on magnetic stirrer until completely homogenized

↓

Dropwise addition into 0.2M calcium chloride solution to form beads

Homogenization

Dropwise addition

Chitosan Beads

HEAVY METAL ADSORPTION BY CHITOSAN BEADS

This test is done to check the chelation activity and heavy adsorption activity of chitosan beads.

Heavy metal solution was prepared (100 ml of 4% cadmium chloride solution)

↓

The solution was divided into 2 equal parts and 1 part was treated with 10g of chitosan beads for 30 min. the other acts as reference

↓

Check absorbance at 530nm

Result

- There was a **decrease** in absorbance of the treated sample indicating heavy metal adsorption by chitosan beads but the difference is **not significant**.
- More experiments with **variation in time of incubation and chitosan concentration** needs to be done for more concrete results.

Sample	Untreated	Treated
Abs at 530nm	0.13	0.09

ANTIOXIDANT ACTIVITY

The **antioxidant activity** of **chitosan** is a topic of significant interest due to its potential health benefits and applications in various industries. Chitosan exhibits antioxidant properties through several mechanisms:

1. **Free Radical Scavenging:** Chitosan can neutralize **free radicals**, which are reactive molecules that can cause cellular damage and contribute to aging and disease.
2. **Metal Chelation:** Chitosan can bind to **metal ions**, such as iron and copper, which are involved in generating harmful free radicals, thereby reducing oxidative stress.
3. **Enzyme Inhibition:** Chitosan can inhibit enzymes involved in oxidative processes, such as **lipoxygenase** and **cyclooxygenase**, further reducing oxidative damage.

The antioxidant activity of chitosan has implications in various fields:

- **Health and Nutrition:** Chitosan's antioxidant properties make it a potential ingredient in functional foods, dietary supplements, and nutraceuticals aimed at promoting health and combating oxidative stress-related conditions.

- **Cosmetics:** Chitosan's antioxidant activity is utilized in skincare products to protect against skin aging and damage caused by environmental factors.
- **Food Preservation:** Chitosan can be used as a natural antioxidant in food packaging materials to extend shelf life and maintain food quality.
- **Biomedical Applications:** In biomedical research, chitosan's antioxidant properties are investigated for their potential in wound healing, tissue engineering, and drug delivery systems.

Overall, the antioxidant activity of chitosan underscores its versatility and potential in promoting health, enhancing product stability, and contributing to various scientific and industrial advancements.

FORMULA: % radical scavenging activity =

$$\frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank}} \times 100$$

PROCEDURE

Prepare a 100mg/L ascorbic acid stock solution and 0.1mM DPPH solution

↓

Dissolve chitosan in methanol(1:20 ratio)

↓

Centrifuge at 2200 rpm for 10 mins and collect the supernatant

Tubes	Methanol	Sample	DPPH		Abs at 530nm	Radical scavenging activity(%)
Blank	5ml	0ml	0ml	Incubate for 30 min in dark	0.00	-
Reference(ascorbic acid)	1ml	0ml	3ml		0.61	-
Prawns	0ml	1ml	3ml		0.40	34.42%
Crab	0ml	1ml	3ml		0.42	31.15%
Cockroach	0ml	1ml	3ml		0.42	31.15%
Mucor	0ml	1ml	3ml		0.45	26.22%

RESULT

- This indicates the antioxidant activity of chitosan.
- Radical scavenging activity of prawn shell derived chitosan is found to be the most followed by crab, cockroach & Mucor.

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BIBLIOGRAPHY

1. "Extraction and Characterization of Chitosan from Shrimp Shells" written by *Nadia G. Kandile, Howida T. Zaky, Mansoura I. Mohamed, Abir S. Nasr, Yassmin G. Ali*, published by *Open Journal of Organic Polymer Materials, 2018; 8: 3.*
2. "OPTIMIZATION OF THE NINHYDRIN REACTION METHOD FOR THE ESTIMATION OF ACETYLATION DEGREE OF CHITOSAN" written by *Boros, Bianca-Vanesa; Menghiu, Gheorghita; Matica, Adina; Ostafe, Vasile. New Frontiers in Chemistry; Timisoara, 2017; 26(2): S4_OP4.*
3. "Study on the Physicochemical Properties of Chitosan and their Applications in the Biomedical Sector" written by *Digafe Alemu, Efrata Getachew, and Ajoy Kanti Mondal.*
4. "Chitosan microspheres/sodium alginate hybrid beads: an efficient green adsorbent for heavy metals removal from aqueous solutions" written by *El-houssaine Ablouh, Zouhair Hanani, Nadia Eladlani, Mohammed Rhazi & Moha Taourirte.*
5. "Chitosan-Based Coating with Antimicrobial Agents: Preparation, Property, Mechanism, and Application Effectiveness on Fruits and Vegetables" written by *Yage Xing, Qinglian Xu, Xingchen Li, Cunkun Chen, Li Ma, Shaohua Li, Zhenming Che and Hongbin Lin.*
6. "Characterization and Parametric Study on Mechanical Properties Enhancement in Biodegradable Chitosan-Reinforced Starch-Based

Bioplastic Film" wriiten by *Shiou Xuan Tan, Hwai Chyuan Ong, Andri Andriyana, Steven Lim, Yean Ling Pang, Fitranto Kusumo and Gek Cheng Ngoh*.

7. "Antimicrobial and antioxidant properties of chitosan and its derivatives and their applications" written by *Mohamed E. Abd El-Hack, Mohamed T. El-Saadony, Manal E. Shafi, Nidal M. Zabermawi, Muhammad Arif, Gaber Elsaber Batiha, Asmaa F. Khafaga, Yasmina M. Abd El-Hakim, Adham A. and Al-Sagheer*.