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Extraction Of Chitosan From *Aristeus Antennatus* Using *Punica Granatum* Peel Extract And Its Utilization In Fruit Preservation

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ABSTRACT

Chitosan, a natural biopolymer known for its antimicrobial and biodegradable properties, has gained attention as an eco-friendly material for food preservation. This study explores the extraction and utilization of chitosan from *Aristeus antennatus* (Red shrimp) shells combined with *Punica granatum* (pomegranate) peel extract for extending the shelf life of grapes. Chitosan was extracted using hydrochloric acid and sodium hydroxide through demineralization, deproteinization, and deacetylation, followed by physicochemical, antimicrobial, antifungal, FTIR, and HPLC analyses. Pomegranate peel extract was prepared using ethanol extraction, then characterized by phytochemical screening, antioxidant assays, and GC-MS analysis. The combined coating was applied to grapes, and its effectiveness was evaluated through spoilage analysis, total bacterial growth, phenolic and flavonoid content, and antioxidant activity. Treated fruits exhibited reduced microbial spoilage, higher antioxidant retention, and longer shelf life, indicating that this natural coating is a promising and sustainable alternative for fruit preservation.

Keywords: Chitosan,, Deproteinization, FTIR, *Aristeus antennatus*

INTRODUCTION

Shelf life of food is the time a food item stays safe, nutritious, and of good quality under certain storage conditions. Keeping food safe, preventing food waste, and maintaining nutritional value all depend on having an optimal shelf life for food products. Temperature, humidity, packaging, and handling practices contribute significantly to the shelf life of food (Tarlak *et al*., 2023). Fruits and vegetables are foods made

up of vitamins, dietary fiber, phytochemicals, antioxidants, and minerals, whose intake is associated with various health effects like the promotion of human body immunity and lowering the risk of cardiovascular and cancer diseases, which are crucial to human nutrition. Fruits and vegetables are most commonly consumed but difficult to handle across the supply chain as they are living tissues whose physiological processes, like CO2 evolution and O2 uptake during respiration, are active even after harvest. Fruits and vegetables are also high in water content, hence they are highly perishable commodities (Perez-Vazquez *et al*., 2023).

The *Aristeus antennatus* (red shrimp), is a significant deep-sea crustacean species that inhabits the Mediterranean and Atlantic. It is a member of the Aristeidae family and Malacostraca class, which comprises most crustaceans. As much as 4548% of the shrimp is discarded as waste, primarily the shell and head. In order to conserve the environment, it is crucial to transform this waste into products (Carbonell *et al*., 1999). Shrimp shells primarily contain chitin, proteins, and mineral salts and thus represent an inexpensive source of chitin. Chitin recovery typically includes two major steps: demineralization and deproteinization, which may be carried out by biological or chemical means. The biological process is greener and more sustainable, but the chemical process is used widely in industries due to its efficiency and affordability (Kandile *et al*., 2018). Chitin is the second most abundant polysaccharide after cellulose on Earth and is composed of three sorts of reactive functional groups: an amino group at C-2, and both the primary and secondary hydroxyl groups at the C-3 and C-6 positions, respectively(Hamed *et al*., 2016). Chitosan is a naturally occurring substance obtained from chitin by deacetylation. It possesses numerous beneficial properties, including the fact that it is harmless to the body, biodegradable in the environment, antibacterial, and adhesive to mucous membranes. Chitosan is obtained from shrimp and crab shells, which are generally by-products of the seafood industry. With these shells, chitin and chitosan can be recovered as valuable products and minimize waste and harm to the environment. Shrimp shells are typically composed of 30%–40% chitin, while crab shells contain around 15%–30% (Pakizeh *et al*., 2021). The traditional procedure for chitosan production normally involves three consecutive chemical treatment steps, but the process may include four to remove accompanying substances. The steps involve demineralization, deproteinization, and deacetylation of shell waste (Boarin-Alcalde *et al*., 2016). The degree of deacetylation is a significant parameter that affects the ultimate properties of chitosan. A higher degree of deacetylation gives rise to a more soluble and more reactive material, whereas a lower degree of deacetylation gives rise to a less soluble, more crystalline chitosan. Such versatility in manufacturing process makes it possible for chitosan to be designed to meet particular needs, and it is thus an extremely versatile product. In addition, antimicrobial action is ascribed to chitosan when the amino groups are cationic in nature, i.e., antimicrobial action of chitosan is greater at low pH (Goy *et al*., 2009).

Pomegranate has been entered into China's catalog of "homology of medicine and food." The roots, flowers, fruits, peel, seeds, and other organs of pomegranate can be used as medicinal materials. Pomegranate (*Punica granatum*) is a subtropical and tropical fruit with very nutritious compounds. Byproducts in huge quantities are produced during processing of pomegranate while getting juice, jams, and wine, among other products. It is a nutrientdense fruit that has been held in esteem for its medicinal virtues for centuries. High in antioxidant, polyphenols, flavonoids and tannis pomegranate peel has recently received a lot of attention due to its health and skincare advantages. Traditionally it has been used for its anti-inflammatory, antimicrobial, anticancer activity and food preservation (Drinić *et al*., 2020). *Punica granatum* peels may serve as a natural substitute for artificial preservatives in meat products, helping to extend shelf life while maintaining quality. Adding pomegranate peel extracts offers benefits like improved texture, longer freshness, enhanced color stability, and increased antioxidant properties. This addition helps manufacturers respond to consumer demand for healthier and more appealing natural ingredients in meat products (Gullón *et al*., 2020). *Punica granatum* peel is rich in numerous health benefits due to the presence of several beneficial chemicals such as anthocyanins, gallic acids, ellagic acid, tannins, catechins, and flavonoids. Studies indicate that the peel contains higher amounts of active ingredients compared to other fruit parts. The peel's compounds such as ellagic acid and punicalagin have the potential to induce cell death in cancer cells by destroying their DNA and inhibiting their growth. Also, *Punica granatum* peel may raise significant antioxidant levels in the body and lower dangerous substances such as malondialdehyde (Abdelrahman et al., 2024). Extraction is the process of separating certain components from the raw material of a plant through the use of several analytical techniques. There are various traditional and green techniques for extracting phenolic compounds. Traditional techniques utilize a high volume of solvents, much manual effort, and are, therefore, less consistent. Green extraction processes are designed to solve some of the problems associated with traditional methods by facilitating faster extraction times, improved energy efficiency, greater mass and heat transfer, smaller equipment, and fewer steps in processing (Singh *et al*., 2023). *Punica granatum* peel, a waste product of pomegranate juice, is a natural source of valuable materials. It contains bioactive materials such as polyphenols, which are beneficial to health (Siddiqui *et al*., 2024).

Chitosan, a bioactive polysaccharide extracted from chitin, has gained widespread popularity in the food sector due to its natural antibacterial and biocompatible nature, making it a perfect material for use as edible coatings. Chitosan coatings have impressive functional properties when blended with Punica granatum extracts that contain bioactive compounds such as polyphenols and essential oils with respect to antibacterial and antioxidant activities.

(Chaparro-Hernández *et al*., 2019).

MATERIALS AND METHODS

COLLECTION OF SAMPLES:

Fresh samples of *Aristeus antennatus* (Red shrimp) were obtained from Royapuram Fishing Harbour in Chennai. This species was verified by Dr. S. Poojasri of Biodeavour Research Laboratory, and *Punica granatum* (Pomegranate) was also collected from Kovai Pazhamudir Nilayam in Alapakkam, verified by Dr. K. N. Sunil Kumar of the Siddha Central Research

Institute.

EXTRACTION OF THE CHITOSAN FROM *ARISTEUS ANTENNATUS*

Aristeus antennatus (Red shrimp), 1kg were peeled, washed, and cleaned. It was dried in incubator at 22°C for 3 days. After drying shells were blended into a powder using a blender. There are three main steps involved in chitosan production:

DEMINERALIZATION

20 g of shell powder was weighed and mixed with 1M HCl (prepared in the ratio of 7.29 g:200mL). The solution was added to the shell powder and stirred using a magnetic stirrer for 24hrs at room temperature. The residue was filtered and washed with distilled water to eliminate impurities until it reached pH neutral, then add 20mL of ethanol and stir well. The residue was dried at 50°C for 24hrs in the hot air oven. Once the residue had dried, the powder was weighed.

DEPROTEINIZATION

The demineralized shell powder with a weight of 7.87g was dissolved in 1M NaOH (which was pre-prepared in the ratio of 4g:100mL). The above solution was blended with the shell powder and stirred by a magnetic stirrer at a temperature of 70°C for 4hrs. The residue was filtrated, picked up, and washed with distilled water to clear impurities until it reached pH neutral, and 20mL of ethanol was added and stirred well. The residue was dried at 50°C for 24hrs using the hot air oven. After drying the residue, the powder was weighed.

DEACETYLATION

The 3.94g of deproteinization shell powder was dissolved in 12.5M of NaOH (30g:60mL ratio) and allowed to cool down and stored frozen at 80°C in an ultra-freezer for 24hrs. Remove the ultra-freezer mixture and store it in cooldown after agitating the mixture with a magnetic stirrer at 115°C for 6 hrs. The residue was filtrate collected and distilled water was used to wash the residue and get rid of the impurities until it reached

pH neutralization, then the residue was dried under 50°C for 2 days using the hot air oven. After drying of the residue, the powder was weighed (Kandile *et al*., 2018)**.**

PUNICA GRANATUM PEEL EXTRACTION

1 kg of Punica granatum (pomegranate) was purchased from the supermarket. The fruit was peeled, chopped into small pieces, washed, and dried in a hot air oven at 50°C for 3 days. The peel was ground into powder after drying using a blender. Then, 10g of the powder was blended with 100mL of ethanol in a conical flask, which was shaken for 5 days. The mixture was filtered and then evaporated with a hot air oven at 40°C for 2 days to get dried extract, the yield of which was calculated (Abdul-Rahman *et al*., 2021).

PHYSICOCHEMICAL ANALYSIS OF CHITOSAN FROM *ARISTEUS ANTENNATUS*

DETERMINATION OF YIELD OF CHITOSAN

The yield of chitosan was calculated by comparing the raw material weight to the raw material weight to the weight of chitosan produced after treatment using the following formula (Vigneshwari *et al*., 2018).

$$\textbf{Yield \%} = \frac{\text{Weight of chitosan}}{\text{Weight of raw material}} \times 100$$

SOLUBILITY TEST

A 0.2g quantity of deacetylated chitosan was blended with 10mL of 1% acetic acid solution and stirred at room temperature overnight for 24 hours. The solution was monitored for any residual undissolved chitosan. If it was present, samples were centrifuged at 3000rpm for 10-15 minutes to discard undissolved particles. The samples that were separated were weighed and dried in a hot air oven at 50°C for a few days, then reweighed. The solubility test was performed using a formula (Hosney *et al*., 2022).

Solubility Test %

$$= \frac{\text{Dissolved chitosan} - \text{Undissolved}}{\text{Initial weight of chitosan}} \times 100$$

DETERMINATION OF MOISTURE CONTENT

0. 2g of a chitosan sample was weighed in a pre-weighed dish. The sample was dried at a hot air oven at 50°C for three days. Upon drying, dry weight was recorded. The moisture content was calculated by using a specific formula (Vigneshwari *et al*., 2018).

Moisture content %

$$= \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100$$

DEGREE OF DEACETYLATION

To make chitosan by titration, 0.2g of dry chitosan is taken and dissolved in 0.1M HCl in a proportion of 0.1mL to 30mL, stirring for 30 minutes. Next, 5-6 drops of methyl orange are added, which makes the solution red. The solution is titrated with 0.1M NaOH, made at a proportion of 0.1g to 25mL, until it turns orange. The amount of deacetylation is determined by a formula (Ravi Kumar *et al*., 2000).

Degree of Deacetylation % =

$$\frac{(\text{Volume of NaOH} \times \text{Normality of Naoh})}{\text{Mass of chitosan sample} \times 0.16} \times 100$$

ANTIMICROBIAL ACTIVITY OF CHITOSAN

The chitosan extract was obtained by dissolving 0. 2g of chitosan powder in 10mL of 1% acetic acid solution. The solution was stirred at room temperature for 24 hours. The extract was screened by agar well diffusion method against *Escherichia coli*,

Staphylococcus epidermidis, and *Pseudomonas aeruginosa*. Bacterial pathogens were cultured on MHA agar plates and wells punched to add the chitosan extract in 0. 1mL aliquots. For 24-48 hours, incubation at 37°C was done before measuring the antimicrobial activity with the diameter of the inhibition zone around the well. The zone is larger as the effectiveness increases (Wang *et al*., 2024).

ANTIFUNGAL ACTIVITY OF CHITOSAN

Antifungal potential of chitosan extract was evaluated by agar well diffusion method against Aspergillus niger. Fungal pathogens were inoculated on a plate, to which 20mL of Sabouraud Dextrose Agar (SDA) was added. Wells were prepared in the agar, and 0. 1mL of chitosan extract was added. The antifungal activity was determined after incubation at 30°C for 24– 48 hours by the size of the zone of inhibition around the well. The bigger the zone, the greater the antifungal activity; the absence of a clear zone means chitosan is possibly not active against the microorganism under test (Meng *et al*., 2020).

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (CHITOSAN EXTRACT)

Chitosans were prepared by degrading them using $NaNO_2$ at room temperature. To prepare a 1% chitosan solution, combine 0. 2g of chitosan in 10ml of 1% acetic acid under stirring. Add 0. 1 M $NaNO_2$ dropwise and allow it to react for 3 hours, unless otherwise specified. Precipitate the chitosan after neutralizing the mixture with 1N NaOH to pH 8. 0. The chitosan is next centrifuged, washed with deionized

water, and dried to be analyzed by HPLC. The analysis includes a C18 reverse phase column and a UV-Vis detector at 230nm, with solvent A and B as the mobile phase. Prepare samples by dissolving chitosan in 0. 1% trifluoroacetic acid to a concentration of 1-5 mg/mL and filter through a 0. 45 µm filter. Fix HPLC conditions at a column temperature of 30°C, flow rate of 1. 0 mL/min, and injection volume of 20 µL. The overall run time is 20-30 minutes, and the information is analyzed with the software of the instrument to quantify each peak's retention time, area, and height for the quantification of chitosan (Ghosh *et al*., 2021).

FOURIER TRANSFORM INFRARED SPECTROSCOPY (CHITOSAN EXTRACT)

The FTIR spectrum of extracted chitosan was established based on the method. For preparation, 1% chitosan (prepared in the ratio of 0.2g:10ml) was dissolved in 1% acetic acid and dried. The powdered chitosan was well mixed with potassium bromide and prepared as KBr pellets. FTIR spectra were measured by using Perkin Elmer model Spectrum 400) in the range of 4000-450 cm^{-1}. Each spectrum is a mean of 64 scans with a resolution of 2 cm^{-1} (Mohammed *et al*., 2013).

PHYTOCHEMICAL ANALYSIS OF *PUNICA GRANATUM* PEEL EXTRACT

By utilizing standard protocol phytochemical evaluation was done in *Punica granatum* peel extract was analyzed to detect the presence of phytochemicals (Karthikeyan *et al*., 2019).

QUALITATIVE ANALYSIS OF *PUNICA GRANATUM* PEEL EXTRACT

ALKALOID

Dragendroff's test was conducted, to few mL of *Punica granatum* peel extract and 1mL dragendroff reagent was added in the test tube. Orange red precipitate indicates the presence of alkaloids.

FLAVONOID

1mL of *Punica granatum* peel extract was mixed with 2mL of 2% NaOH solution to the test tube. The red ring formed shows the presence of flavonoids.

TANNINS

Lead acetate test was performed, 0.5mL of *Punica granatum* peel extract was mixed with 1mL of lead acetate solution in the test tube. A white precipitate indicates the presence of tannins.

PHENOLIC COMPOUND

A ferric chloride test was performed, to few mL of *Punica granatum* peel extract, a few drops of neutral 5% ferric chloride solution was added. A dark blue colour indicates the presence of phenolic compounds.

QUANTITATIVE ANALYSIS OF *PUNICA GRANATUM* EXTRACT

TOTAL PHENOL CONTENT

Total Phenolic Content (TPC) of Punica granatum peel extract utilized by the solvent (ethanol). The extract was quantified by Folin-Ciocalteu's method; 20µL of the extract diluted to 980µL of distilled water and combined with 1mL of Folin-Ciocalteu's reagent (1:10 diluted with distilled water) and left to react for 15min. Subsequently, 1mL of sodium bicarbonate (7.5%) was added, and the resulting solution was left for incubation at room temperature for 30 minutes before measurement of the absorbance at 765 nm via UV spectrophotometer. TPC for Punica granatum peel extract was presented in mg gallic acid equivalent per mL of extract

(Zaki *et al*., 2015).

TOTAL FLAVONOID CONTENT

The Total Flavonoid Content (TFC) of Punica granatum peel extract utilized by the solvent (ethanol). The content of extract was found with aluminium chloride (AlCl3) colorimetric assay, 20µL extract was made 980µL to distilled water and to it add 1mL aluminium chloride (10%) was added and kept stand still for 6minute, thereafter add 1mL 1M sodium acetate solution into mixture and left to room temperature to be incubated for 30min and after absorbance is to be recorded 410nm through the help of UV spectrophotometer. TFC Punica granatum peel extract content is equivalent per ml to milligram quercetin (Hayat *et al*., 2020).

ANTIOXIDANT ACTIVITY OF *PUNICA GRANATUM* PEEL EXTRACT

DPPH RADICAL SCAVENGING ASSAY

The antioxidant activity of Punica granatum peel extract was analyzed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. A 100µL of Punica granatum extract in solvent (ethanol) was diluted to 1900 µL of distilled water and mixed with 1 mL of 0.1 mM DPPH solution. The solution was incubated at room temperature in the dark for 30 minutes. The absorbance was determined at 517 nm using a UV spectrophotometer. The antioxidant activity was presented as a percentage inhibition of DPPH radical scavenging activity employing the formula as follows (Malviya *et al*., 2014).

Radical scavenging activity %

$$= \frac{\text{(A control – B sample)}}{\text{A control}} \times 100$$

PREPARATION OF EDIBLE COATING IN CHITOSAN FROM *ARISTEUS ANTENNATUS* WITH *PUNICA GRANATUM* PEEL EXTRACT

To prepare the edible coating solution, dissolve 0. 2 g of chitosan in 100 mL of 1% acetic acid, made by mixing 1 mL of acetic acid with 99 mL of distilled water. Stir the solution for 5 minutes and then continue for 3 hours at 40°C. Once cooled, blend 10 mL of this solution with 0. 2 µL of glycerol and shake for 10 minutes. Add 1 mL of Punica granatum extract and filter the solution (Kyriakidou *et al*., 2021). Before coating, wash the grapes with sterile water and air-dry them. Coat the grapes by immersing them in the solution, then air-dry for another hour. Control samples are left uncoated. Store approximately 20 grams of grapes for each treatment and monitor their microbiological characteristics and rotting of the fruit were measured over a period of 7-day (Popescu *et al*., 2022).

ENUMERATION OF TOTAL BACTERIAL COUNT FOR COATED AND UNCOATED GRAPES

The CFU was enumerated by the spread plate method. 4g samples of coated and uncoated grapes were ground in a sterile mortar and then blended with 90mL of sterile distilled water. A sterile pipette transferred 1mL of these samples into test tubes containing 9mL of sterile water to prepare dilutions from 10-1 to 10-6. Each plate was given 25mL of Nutrient Agar medium, and 100µL of every dilution was plated on the solid agar. The plates were incubated at 37°C for 24-48 hours, and colonies were counted after that. CFU/mL was determined to calculate microbial concentration with a certain formula

(Divyashri *et al*., 2024).

$$\textbf{CFU/mL} = \frac{\text{Number of Colonies} \times \text{Dilution Factor}}{\text{Volume of sample plated (ml)}}$$

QUANTITATIVE ANALYSIS OF COATED AND UNCOATED GRAPES
TOTAL PHENOL CONTENT

The Total Phenolic Content (TPC) was estimated by Folin-Ciocalteu's method for coated and uncoated grapes were homogenized separately with sterile distilled water. 100µL of samples was diluted to 900µL of distilled water and then mixed with 1mL of Folin-Ciocalteu's (FC) reagent (1:10 diluted with distilled water) left to react for 15minutes. Then 1mL of sodium bicarbonate (7.5%) was added to the mixture. Solution was incubated for 30 minutes at room temperature, then the absorbance at a wavelength of 765nm was taken in a UV spectrophotometer. TPC of uncoated and coated grapes extract was given as mg of gallic acid equivalent per mL of sample (Divyashri *et al*., 2024).

TOTAL FLAVONOID CONTENT

The Total Flavonoid Content (TFC) was assayed using aluminum chloride (AlCl3) colorimetric method for the coated and uncoated grapes were homogenized

separately in sterile distilled water. 100µL sample diluted to 900µL distilled water and 1 mL aluminium chloride (10%) was added and allowed to stand for 6miuntes, 1 mL of 1M sodium acetate was added to the mixture, and the mixture was incubated at room temperature for 30 minutes, the absorbance was then measured using a UV spectrophotometer at a wavelength of 410 nm. The TFC of coated and uncoated grapes extract was reported as mg of quercetin equivalent per mL of sample (Hayat *et al*., 2020).

ANTIOXIDANT ACTIVITY OF COATED AND UNCOATED GRAPES

DPPH RADICAL SCAVENGING ASSAY

The antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay in coated and uncoated grapes were homogenized separately in sterile distilled water. A sample of 100µl was diluted to 1900µl of distilled water and then added to 1ml of 0.1 mM DPPH solution. The reaction mixture was kept in the dark at room temperature for 30 minutes. Absorbance was recorded at 517 nm in a UV spectrophotometer. The antioxidant activity was calculated as percentage inhibition of DPPH radical scavenging activity using the formula (Robles-Sánchez *et al*., 2013).

RESULT AND DISCUSSION

COLLECTION OF SAMPLES:

Aristeus antennatus, commonly known as (Red shrimp), is a commercially important deep-sea crustacean found in temperate and warm oceans. Fresh samples were obtained from Royapuram Fishing Harbour in Chennai and confirmed by Dr. S. Poojasri of the Biodeavour Research Laboratory and *Punica granatum*, commonly called (Pomegranate), is a compact tree or shrub belonging to the family Punicaceae. The fruits were obtained from Kovai Pazhamudir Nilayam, Alapakkam, Chennai, and the peel was authenticated by Dr. K. N. Sunil Kumar, Department of Pharmacognosy, Siddha Central Research Institute, Hospital Campus, Arumbakkam, Chennai.

Fig 1: Collection of red shrimp and Pomogranate

EXTRACTION OF CHITOSAN FROM *ARISTEUS ANTENNATUS*

Chitosan was purified from 1 kg of Aristeus antennatus shells by a series of chemical treatments. The treatment involved removal of proteins with 1. 0 M NaOH, demineralization with 1. 0 M HCl, and deacetylation with 48% NaOH at room temperature for 48 hours. This produced a fine white powder of high purity, thus ensuring successful conversion from chitin to chitosan. The process successfully removed proteins and minerals, and the yield was consistent with previous reports, suggesting that Aristeus antennatus shells are a sustainable source of chitosan

(Boudouaia *et al*., 2019).

PUNICA GRANATUM PEEL EXTRACTION PREPARATION

Peel extract was obtained from 1 kg of Punica granatum by peeling, washing, drying, and grinding peels into powder. The powder was extracted using 100 mL ethanol on a rotary shaker for 5 days. Upon filtration, the extract was evaporated for further examination. Ethanol was used for its efficacy to extract phenolic compounds from plant materials. Nevertheless, the extended extraction time could have resulted in partial degradation by hydrolysis, oxidation, or ionization. These results underscore the necessity of optimizing extraction conditions to maintain compound integrity (Sai-Ut *et al*., 2023).

PHYSICOCHEMICAL ANALYSIS OF CHITOSAN FROM *ARISTEUS ANTENNATUS*

DETERMINATION OF YIELD OF CHITOSAN

The yield of chitosan from *Aristeus antennatus* shells was 15.3%. This is within the range reported in earlier research works with yields ranging from 13% to 19%. Yield variations are determined by species type, extraction process, and processing conditions. The finding affirms the efficacy of the employed extraction process (Elaraby *et al*., 2024).

SOLUBILITY TEST

Aristeus antennatus shell-derived chitosan was 66% soluble, indicating it is moderately soluble. The value indicates that the percentage of deacetylation can be less than 70%, which would influence its functional properties. The solubility can be influenced by parameters such as NaOH concentration, reaction time, and temperature in deacetylation. The optimization of these parameters could improve solubility and extend the application of chitosan (Carrera *et al*., 2023).

DETERMINATION OF MOISTURE CONTENT

The water content of *Aristeus antennatus* shell chitosan is 10%, and it is acceptable to commercial criteria as regards stability and safety. Though good-quality chitosan typically contains less than 10% water, this percentage is acceptable. The moisture content according to other research is 4%-7%, which should be better in terms of quality. Low water content is necessary to preserve structure, function, and shelf life and to minimize microbial contamination. Therefore, the 10% moisture content shows that the chitosan extracted is suitable for a wide range of high-quality applications (Alca Ahing *et al*., 2016).

DEGREE OF DEACETYLATION

The degree of deacetylation (DDA) of *Aristeus antennatus* shell chitosan was determined to be 70. 3% by an acid-base titration method. The DDA level is significant as it influences the solubility, bioactivity, and general application of chitosan. Although commercial chitosan might have greater DDA values, variations occur due to sources, extraction processes, deacetylation procedures, and titration methods. The DDA value obtained indicates that the extracted chitosan has functional potential for a range of applications (Dutta & Priyanka *et al*., 2022).

ANTIMICROBIAL ACTIVITY OF CHITOSAN

Chitosan from *Aristeus antennatus* shells exhibited various degrees of antimicrobial activity against three bacteria:

Escherichia coli, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*. The highest effect was against *P. aeruginosa* with an inhibition zone of 23 mm, then *E. coli* with 18 mm, and the lowest against *S. epidermidis* with 16 mm. This suggests that chitosan is a broad-spectrum antimicrobial agent, most effective against *P. aeruginosa*. Earlier research indicates that chitosan kills bacteria by destroying their membranes. The reduced efficacy against *S. epidermidis* could be attributed to its more protective layer. Generally, shrimp-derived chitosan is a potential natural antimicrobial agent (Sahariah *et al*., 2023).

ANTIFUNGAL ACTIVITY OF CHITOSAN

Chitosan extracted from *Aristeus antennatus* shells did not inhibit *Aspergillus niger* and was weakly active against fungi under the test conditions. This implies that chitosan effectiveness is either strainspecific or may differ. Conditions such as pH sensitivity, molecular weight, or a lack of proper chemical modification may be the cause of this activity failure. Previous studies suggest that enhancing these factors can potentially make chitosan more antifungal, especially against resistant species such as *A. niger* (Meng *et al*., 2020).

CHARACTERIZATION

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (CHITOSAN EXTRACT)

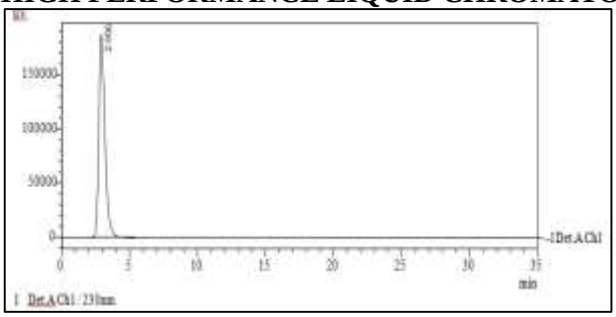

Fig 2: HPLC analysis of chitosan extract

The HPLC result of chitosan from *Aristeus antennatus* shell exhibited a distinct peak at 2. 906 minutes (fig 2), showing high purity without any observed impurities. The applied method provided excellent separation and repeatability, verifying the proper extraction and purification of chitosan. The findings were consistent with known properties of chitosan (Ghosh *et al*., 2021).

FOURIER TRANSFORM INFRARED SPECTROSCOPY (CHITOSAN EXTRACT)

The FTIR spectrum of *Aristeus antennatus* shell chitosan verifies its functional groups and structure was shown in (fig 3). The broad peak at 3289.92 cm^{-1} reflects O-H and N-H stretching, which is characteristic of hydroxyl and amine groups.

The peak at 1656.3 cm^{-1} reflects C=O stretching (amide I), indicative of residual acetyl groups. Peaks at 1377 cm^{-1} and 1308.2 cm^{-1} are indicative of C-H bending and amide III (C-N and N-H), verifying the polymer's amine structure. The 1025.91 cm^{-1} peak indicates C-O stretching, verifying the polysaccharide ring structure. These findings agree with literature, verifying partial deacetylation and successful extraction (Vigneshwari *et al*., 2018).

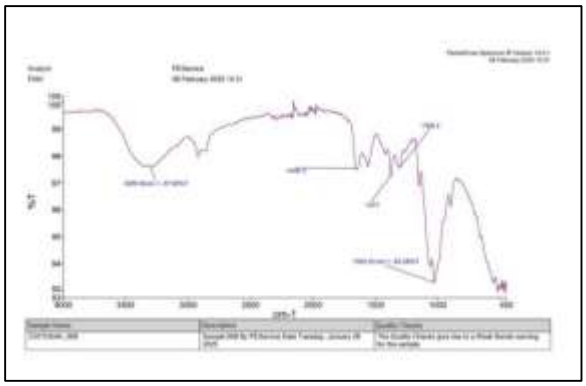

Fig 3: FTIR analysis of chitosan extract

PHYTOCHEMICAL ANALYSIS OF *PUNICA GRANATUM* PEEL EXTRACT

The phytochemical screening of *Punica granatum* peel extract indicated that it is rich in bioactive compounds such as alkaloids, flavonoids, tannins, and phenols.

QUANTITATIVE ANALYSIS OF *PUNICA GRANATUM* PEEL EXTRACT

TOTAL PHENOL CONTENT

The *Punica granatum* peel extract contains a total phenol content of 9.928 mg/mL of gallic acid equivalent, exhibiting significant antioxidant activity. Phenolic compounds are responsible for the neutralization of free radicals and defense against oxidative stress. The high content of phenolics most probably describes its antioxidant activity and justifies earlier work regarding pomegranate peel's beneficial health aspects. Variation in extraction procedures and pomegranate types may influence phenolic content, but their uniform presence attests to the success of the extract in antioxidant protection (Salim *et al*., 2023).

TOTAL FLAVONOID CONTENT

The Punica granatum peel extract has a total flavonoid content of 4. 372 mg/mL of flavonoids, that is, quercetin. Flavonoids have been shown to have antioxidant properties and can minimize oxidative stress by eliminating free radicals. This indicates that the extract has therapeutic potential and corroborates previous research in isolating *Punica granatum* peel as a rich source of flavonoids, highlighting flavonoids' significance in its antioxidant activity (Derakhshan *et al*., 2018).

ANTIOXIDANT ACTIVITY OF *PUNICA GRANATUM* PEEL EXTRACT

DPPH RADICAL SCAVENGING ASSAY

The DPPH assay revealed that the ethanolic extract of Punica granatum peel was 19. 6% inhibitory for free radical activity at a concentration of 100 µL, which proves it has high antioxidant potential. The finding proves that the extract has the capability to neutralize free radicals because of its high contents of polyphenols and flavonoids. In contrast, pomegranate seeds contain a reported DPPH inhibition of 10– 15%, so the peel extract is a stronger natural antioxidant source. This highlights the significance of the peel in combating oxidative stress (Elfalleh *et al*., 2012)

PREPARATION OF EDIBLE COATING IN CHITOSAN FROM *ARISTEUS ANTENNATUS* WITH *PUNICA GRANATUM* PEEL EXTRACT

Fresh grapes were washed with sterile water, air-dried, and immersed in a chitosan-pomegranate coating solution (fig 4), and then dried again before storage. Coated grapes were stored at room temperature with uncoated control samples. Microbial spoilage was observed on days 0, 7, and 14. Coated grapes had delayed spoilage and less microbial growth than controls. This establishes the coating as a physical barrier

and antimicrobial agent. The chitosan-pomegranate combination was successful in inhibiting microbial growth, which prolonged the storage life of fresh fruits and vegetables (Duan *et al*., 2019).

Fig 4: Coating solution

COATED GRAPES UNCOATED GRAPES

DAY 1 SPOILAGE ANALYSIS

DAY 7 SPOILAGE ANALYSIS

DAY 14 SPOILAGE ANALYSIS

ENUMERATION OF TOTAL BACTERIAL COUNT FOR COATED AND UNCOATED GRAPES

Microbial analysis according to the spread plate technique exhibited considerably lower numbers of colonies from coated grapes (3×10^2 CFU/mL) than from uncoated grapes (7×10^3 CFU/mL). Bacterial growth was slight and was encountered only at 10^{-1} dilution level. Microbial load was successfully reduced through the edible coating by preventing access of contaminants in the form of a barrier. The same evidence was documented within studies involving the use of chitosan-FA coatings in fish, establishing its antimicrobial efficacy. The decline in microbial level is due to chitosan's capacity to interfere with bacterial membranes

and modify intracellular pH. This is an indication of the coating's function in enhancing food safety and shelf life (Remya *et al*., 2022).

QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF COATED AND UNCOATED GRAPES

TOTAL PHENOL CONTENT

Phenolic content determination indicated that the uncoated grape extracts contained a higher concentration (2.275 mg/mL) than the coated grape extracts (1.732 mg/mL), both on a mg gallic acid equivalent per mL basis. This implies that the edible coating could minimize the extractable phenolic compounds in the grapes. Other such research has documented a reduction of total phenolic content in coated fruits over time through interactions between the coating matrix and phenolic compounds. Such interactions might lower the availability or solubility of phenolics upon extraction, resulting in lower measurable levels for coated samples though having the capacity to be preserved within the fruit matrix (Panwar *et al*., 2024).

TOTAL FLAVONOID CONTENT

Flavonoid levels were lower in coated grapes (0.386 mg/mL) compared to uncoated grapes (0.406 mg/mL), which was determined as quercetin equivalents. This indicates a decrease due to coating. Another study indicated that FD-CHP-CO coatings maintained flavonoids to a larger extent during storage. The coating can restrict extraction but ensures retention of compounds throughout a period of time. Therefore, it becomes a factor in antioxidant retention (Divyashri *et al*., 2024).

ANTIOXIDANT ACTIVITY OF COATED AND UNCOATED GRAPES

DPPH RADICAL SCAVENGING ASSAY

The DPPH radical scavenging activity was marginally greater in uncoated grapes (58.21%) compared to coated grapes (56.42%), reflecting slightly higher antioxidant activity. This slight difference implies that the coating restricts access to extractable antioxidants immediately. Yet, comparable results in chitosan-coated strawberries reflected reduced antioxidant degradation upon longer term storage. The coating provides a protective layer, slowing oxidative loss. It therefore maintains longterm preservation of antioxidants in coated fruit (Petriccione *et al*., 2015).

CONCLUSION:

This research indicates that Aristeus antennatus chitosan and Punica granatum peel extract is a potent natural approach to maintaining fruit quality post-harvest. The chitosan possesses high purity, favorable properties, and excellent antibacterial activities, while the peel extract provides antioxidant and antimicrobial effects. The application of the materials to coat fruits prevents spoilage and microbial infection and extends shelf life compared to untreated fruits. The research suggests additional studies to improve antifungal activity and consider broader uses, long-term storage studies, consumer acceptability, and environmental effects. Low-cost production techniques and potential with nanotechnology may increase its application. Overall, it suggests the potential of such natural coatings in minimizing postharvest losses and enhancing food safety.

REFERANCE:

- Abdelrahman, K. N., Ghany, A. G. A. A., Saber, R. A., Osman, A., Sitohy, B., & Sitohy, M. (2024). Anthocyanins from pomegranate peel (Punica granatum), chili pepper fruit (Capsicum annuum), and bougainvillea flowers (Bougainvillea spectabilis) with multiple biofunctions: Antibacterial, antioxidant, and anticancer. *Heliyon*, *10*(11).
- Abdul-Rahman, S. M., & Abass, A. F. (2021, May). Preparation of edible films made from chitosan with pomegranate peel extract and study its barrier, mechanical and antioxidant properties. In *IOP Conference Series: Earth and Environmental Science* (Vol. 761, No. 1, p. 012122). IOP Publishing.
- Ahing, F. A., & Wid, N. (2016). Extraction and characterization of chitosan from shrimp shell waste in Sabah. *Transactions on Science and Technology*, *3*(1-2), 227-237.
- Boudouaia, N., Bengharez, Z., & Jellali, S. (2019). Preparation and characterization of chitosan extracted from shrimp shells waste and chitosan film: application for Eriochrome black T removal from aqueous solutions. *Applied Water Science*, *9*(4), 91.
- Carbonell, A., Carbonell, M., Demestre, M., Grau, A., & Monserrat, S. (1999). The red shrimp Aristeus antennatus (Risso, 1816) fishery and biology in the Balearic Islands, Western Mediterranean. *Fisheries Research*, *44*(1), 1-13.
- Carrera, C., Bengoechea, C., Carrillo, F., & Calero, N. (2023). Effect of deacetylation degree and molecular weight on surface properties of chitosan obtained from biowastes. *Food Hydrocolloids*, *137*, 108383.
- Derakhshan, Z., Ferrante, M., Tadi, M., Ansari, F., Heydari, A., Hosseini, M. S., & Sadrabad, E. K. (2018). Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. *Food and chemical toxicology*, *114*, 108-111.
- Divyashri, G., Swathi, R., Murthy, T. K., Anagha, M., Sindhu, O., & Sharada, B. (2024). Assessment of antimicrobial edible coatings derived from coffee husk pectin and clove oil for extending grapes shelf life. *Discover Food*, *4*(1), 181.
- Drinić, Z., Mudrić, J., Zdunić, G., Bigović, D., Menković, N., & Šavikin, K. (2020). Effect of pomegranate peel extract on the oxidative stability of pomegranate seed oil. *Food Chemistry*, *333*, 127501.

- Duan, C., Meng, X., Meng, J., Khan, M. I. H., Dai, L., Khan, A., & Ni, Y. (2019). Chitosan as a preservative for fruits and vegetables: a review on chemistry and antimicrobial properties. *Journal of Bioresources and Bioproducts*, *4*(1), 11-21.
- Dutta, J. (2022). A facile approach for the determination of degree of deacetylation of chitosan using acidbase titration. *Heliyon*, *8*(7). El-Araby, A., Janati, W., Ullah, R., Uddin, N., & Bari, A. (2024). Antifungal efficacy of chitosan extracted from shrimp shell on strawberry (Fragaria× ananassa) postharvest spoilage fungi. *Heliyon*, *10*(7).
- Elfalleh, W., Hannachi, H., Tlili, N., Yahia, Y., Nasri, N., & Ferchichi, A. (2012). Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower. *Journal of Medicinal Plants Research*, *6*(32), 4724-4730.
- Ghosh, N., & Dhepe, P. L. (2021). HPLC method development for chitin and chitosan valorisation chemistry. *Carbohydrate Polymer Technologies and Applications*, *2*, 100139.
- Goy, R. C., Britto, D. D., & Assis, O. B. (2009). A review of the antimicrobial activity of chitosan. *Polímeros*, *19*, 241-247.
- Gullón, P., Astray, G., Gullón, B., Tomasevic, I., & Lorenzo, J. M. (2020). Pomegranate peel as suitable source of high-added value bioactives: Tailored functionalized meat products. *Molecules*, *25*(12), 2859.
- Hamed, I., Özogul, F., & Regenstein, J. M. (2016). Industrial applications of crustacean by-products (chitin, chitosan, and chitooligosaccharides): A review. *Trends in food science & technology*, *48*, 40-50.
- Hayat, J., Akodad, M., Moumen, A., Baghour, M., Skalli, A., Ezrari, S., & Belmalha, S. (2020). Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of Marrubium vulgare L. from 2 different localities of Northeast of Morocco. *Heliyon*, *6*(11).
- Hosney, A., Ullah, S., & Barčauskaitė, K. (2022). A review of the chemical extraction of chitosan from shrimp wastes and prediction of factors affecting chitosan yield by using an artificial neural network. *Marine Drugs*, *20*(11), 675.
- Kandile, N. G., Zaky, H. T., Mohamed, M. I., Nasr, A. S., & Ali, Y. G. (2018). Extraction and characterization of chitosan from shrimp shells. *Open Journal of Organic Polymer Materials*, *8*(3), 33-42.
- Karthikeyan, G., & Vidya, A. K. (2019). Phytochemical analysis, antioxidant and antibacterial activity of pomegranate peel. *Res. J. Life Sci. Bioinform. Pharm. Chem. Sci*, *5*(1), 218.
- Kyriakidou, A., Makris, D. P., Lazaridou, A., Biliaderis, C. G., & Mourtzinos, I. (2021). Physical properties of chitosan films containing pomegranate peel extracts obtained by deep eutectic solvents. *Foods*, *10*(6), 1262.
- Malviya, S., Arvind, X., Jha, A., & Hettiarachchy, N. (2014). Antioxidant and antibacterial potential of pomegranate peel extracts. *Journal of food science and technology*, *51*, 4132-4137.
- Meng, D., Garba, B., Ren, Y., Yao, M., Xia, X., Li, M., & Wang, Y. (2020). Antifungal activity of chitosan against Aspergillus ochraceus and its possible mechanisms of action. *International journal of biological macromolecules*, *158*, 1063-1070.
- Mohammed, A. S. A., Naveed, M., & Jost, N. (2021). Polysaccharides; classification, chemical properties, and future perspective applications in fields of pharmacology and biological medicine (a review of current applications and upcoming potentialities). *Journal of Polymers and the Environment*, *29*, 2359-2371.

- Pakizeh, M., Moradi, A., & Ghassemi, T. (2021). Chemical extraction and modification of chitin and chitosan from shrimp shells. *European Polymer Journal*, *159*, 110709.
- Panwar, A., Kumar, V., Dhiman, A., Thakur, P., Sharma, V., Sharma, A., & Kumar, S. (2024). Nanoemulsion based edible coatings for quality retention of fruits and vegetablesdecoding the basics and advancements in last decade. *Environmental Research*, *240*, 117450.
- Perez-Vazquez, A., Barciela, P., Carpena, M., & Prieto, M. A. (2023). Edible coatings as a natural packaging system to improve fruit and vegetable shelf life and quality. *Foods*, *12*(19), 3570.
- Petriccione, M., Mastrobuoni, F., Pasquariello, M. S., Zampella, L., Nobis, E., Capriolo, G., &

  Scortichini, M. (2015). Effect of chitosan coating on the postharvest quality and antioxidant enzyme system response of strawberry fruit during cold storage. *Foods*, *4*(4), 501-523.
- Popescu, P. A., Palade, L. M., Nicolae, I. C., Popa, E. E., Miteluț, A. C., Drăghici, M. C., ... & Popa, M. E. (2022). Chitosan-based edible coatings containing essential oils to preserve the shelf life and postharvest quality parameters of organic strawberries and apples during cold storage. *Foods*, *11*(21), 3317.
- Remya, S., Sivaraman, G. K., Joseph, T. C., Parmar, E., Sreelakshmi, K. R., Mohan, C. O., & Ravishankar, C. N. (2022). Influence of corn starch-based bio-active edible coating containing fumaric acid on the lipid quality and microbial shelf life of silver pomfret fish steaks stored at 4 C. *Journal of Food Science and Technology*, 1-12.
- Robles-Sánchez, R. M., Rojas-Graü, M. A., Odriozola-Serrano, I., González-Aguilar, G., & MartinBelloso, O. (2013). Influence of alginate-based edible coating as carrier of antibrowning agents on bioactive compounds and antioxidant activity in fresh-cut Kent mangoes. *LWT-Food Science and Technology*, *50*(1), 240-246.
- Sahariah, P., Kontogianni, G. I., Scoulica, E., Sigurjonsson, O. E., & Chatzinikolaidou, M. (2023). Structure-activity relationship for antibacterial chitosan carrying cationic and hydrophobic moieties. *Carbohydrate Polymers*, *312*, 120796.
- Sai-Ut, S., Kingwascharapong, P., Mazumder, M. A. R., & Rawdkuen, S. (2023). Optimization of ethanolic extraction of phenolic antioxidants from lychee and longan seeds using response surface methodology. *Foods*, *12*(15), 2827.
- Salim, A., Deiana, P., Fancello, F., Molinu, M. G., Santona, M., & Zara, S. (2023). Antimicrobial and antibiofilm activities of pomegranate peel phenolic compounds: Varietal screening through a multivariate approach. *Journal of Bioresources and Bioproducts*, *8*(2), 146-161.
- Salim, A., Deiana, P., Fancello, F., Molinu, M. G., Santona, M., & Zara, S. (2023). Antimicrobial and antibiofilm activities of pomegranate peel phenolic compounds: Varietal screening through a multivariate approach. *Journal of Bioresources and Bioproducts*, *8*(2), 146-161.
- Siddiqui, S. A., Singh, S., & Nayik, G. A. (2024). Bioactive compounds from pomegranate peels-Biological properties, structure–function relationships, health benefits and food applications–A comprehensive review. *Journal of Functional Foods*, *116*, 106132.
- Tarlak, F. (2023). The use of predictive microbiology for the prediction of the shelf life of food products. *Foods*, *12*(24), 4461.
- Thooyavan, G., & Karthikeyan, J. (2016). Phytochemical profiling and GC-MS analysis of Butea monosperma seed methanol extract. *Journal of Pharmacognosy and Phytochemistry*, *5*(5), 152.

- Vigneshwari, S., & Gokula, V. (2018). Extraction and FTIR characterization of chitosan from Portunus pelagicus (Linnaeus, 1758) shell wastes.
- Wang, L., Pang, Y., Xin, M., Li, M., Shi, L., & Mao, Y. (2024). Evaluating the antibacterial and antibiofilm activities of chitosan derivatives containing six-membered heterocyclics against E. coli and S. aureus. *Colloids and Surfaces B: Biointerfaces*, *242*, 114084.
- Zaki, S. A., Abdelatif, S. H., Abdelmohsen, N. R., & Ismail, F. A. (2015). Phenolic compounds and antioxidant activities of pomegranate peels. *International Journal of Food Engineering*, *1*(2), 73-76.