Investigating the potential of polysaccharides extracted from *Sargassum cinereum* for intelligent food packaging.

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "**Investigating the potential of polysaccharides extracted from *Sargassum cinereum* for intelligent food packaging"** is based on the results of investigations carried out by me in the Discipline of Biotechnology at the School of Biological Sciences and Biotechnology, Goa University under the Supervision of Dr. Samantha Fernandes D'Mello and the same has not been submitted elsewhere for the award of a degree Or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations / experimental or other findings given in the dissertation.

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Date:8 April 2024

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COMPLETION CERTIFICATE

This is to certify that the dissertation report "**Investigating the potential of polysaccharides extracted from *Sargassum cinereum* for intelligent food packaging**" is a bonafide work carried out by Mr. Hari Arun Marathe under my supervision in partial fulfilment of the requirements for the award of the degree of Masters of Science in the Discipline of Biotechnology at the School of Biological Sciences and Biotechnology, Goa University.

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PREFACE

The increasing crisis of plastic waste pollution and the consequential spoilage of food during shipping and logistics have emerged as pressing global concerns, demanding immediate attention and innovative solutions. This dissertation delves into the synthesis and application of a sustainable alternative: a biopolymer film tailored for intelligent food packaging. By harnessing the abundant crude alginate derived from *Sargassum cinereum*, the study involves the extraction process of alginate, while standardizing the concentrations of glycerol, wax, and pH indicators crucial for enhancing the film's properties. Comprehensive characterization utilizing techniques such as FTIR, XRD, TGA, SEM, along with rigorous tests including biodegradability, water vapor transmission, and antioxidant evaluations, have been conducted to elucidate the film's efficacy and suitability. Moreover, the practical application of the biopolymer film is scrutinized through observations of colour changes post-food spoilage, offering a promising stride towards sustainable packaging solutions.

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ABBREVIATIONS

- ℃ - Degree Celsius
- mL- Milli liter
- mg -Milli gram
- h -Hour
- g- Gram
- wt. - Weight
- % - Percentage
- Min- Minutes
- MPa - Megapascal
- DPPH -2,2-diphenyl-1-picrylhydrazyl
- Abs - Absorbance
- CMC - Carboxy methyl cellulose
- PVA - PVA (polyvinyl alcohol)
- CS - Chitosan
- ATH- Anthocyanin
- XRD- X-Ray Diffraction
- SEM- Scanning electron microscope
- FTIR- Fourier-transform infrared spectroscopy

ABSTRACT

This dissertation investigates the pressing global challenges of plastic waste accumulation and food wastage, particularly prevalent in India where approximately 68.8 million kilograms of food are wasted annually, representing 7 % of the world's total. A substantial portion of this waste stems from inadequate real-time food freshness information provided by current food data labels. To address this issue, the study focuses on the development of intelligent packaging strategies utilizing bioplastic materials. Specifically, a biopolymer film derived from polysaccharide crude alginate, incorporating alizarin as a pH indicator, is synthesized for intelligent food packaging applications. The research includes comprehensive characterization of the biopolymer film and rigorous testing to evaluate its efficacy in food preservation. This study contributes to the ongoing efforts to reduce plastic waste and reduce food wastage through innovative packaging solutions.

CHAPTER - I

1. INTRODUCTION

1. INTRODUCTION

1.1 BACKGROUND

The proliferation of plastic and micro (nano)plastic pollution poses a grave threat to the sustainability of our world's economy, society, and environment. Over the past fifty years, the utilization and disposal of plastic have surged dramatically, particularly in the realm of single-use plastic packaging. Unfortunately, many of these single-use plastic items end up in landfills or incinerators after only a few uses if they are recyclable at all. Effectively tackling the issue of plastic and micro (nano)plastic pollution requires concerted action on a global scale. This entails reimagining production processes, promoting innovative recycling technologies, and implementing stringent waste management policies (Walker et al., 2023). Researchers from all around the world are currently showing a great deal of interest in bioplastics that are made from organic and natural biopolymers like cellulose, starch, proteins, lactic acid, hydroxy alkenoates, and other substances found in plants or microbes. (Mostafa, et al., 2018). The capacity of bioplastics to biodegrade that is, to break down enzymatically into inorganic compounds or biomass with the help of different microorganisms is one of its main advantages (Tamnou et al., 2021).

Despite these challenges, various strategies exist for the collection and recycling of single-use plastic packaging. The accumulation of such packaging in soil and water bodies poses a serious risk to ecosystems, highlighting the urgency for the food and beverage industry to transition away from conventional petroleum-based packaging towards more sustainable alternatives. One innovative solution to this dilemma is the production of bioplastics (BPs), which mimic the functionality of traditional plastics while being categorized as biodegradable materials. This means that they break down

into carbon dioxide, water, and biomass under suitable environmental conditions. Bioplastics are crafted from biopolymers sourced from biomass, including proteins, cellulose, and starch, either in their natural or modified forms. This shift towards bioplastics represents a promising step toward mitigating the environmental impact of single-use packaging within the food industry. (Porras et al., 2024)

Bioplastics are made from a variety of biomaterials, including vegetable oils, maize, potatoes, wood, food waste, and cereal crops. Bioplastics made of starch are now the most common, followed by bioplastics made of polylactic acid (PLA), poly-3-hydroxybutyrate (PHB), polyamide 11 (PA 11), and organic polyethylene (PE). Seaweed-based bioplastics have been a promising development recently. Seaweeds have a lot of promise because of their high production, affordability, simplicity of growing in natural settings, capacity to adapt to different climates, and year-round availability for harvest. (Lim et al., 2021)

Algae are a broad category of autotrophic organisms that can have single or multicellular structures. Macroalgae, especially seaweeds, have more potential for the manufacturing of bioplastics than microalgae, such as spirulina dregs, which are difficult to extract. Seaweeds are highly advantageous due to their vast biomass, versatility in many conditions, affordability, simplicity of production in their native habitats, and year-round harvestability.

Seaweeds include natural polysaccharides that are widely used in food technology, biotechnology, microbiology, and medicine. In spite of this, the plastics sector has not yet fully used these polysaccharides' potential. Their ability to form carbon-containing sugar polymers from renewable biomass makes them very promising for the production of superior biodegradable bioplastics. The goal is to create

environmentally acceptable bioplastics that are non-toxic, inexpensive, and have tensile strength and chemical resistance on par with or better than conventional polymers. Because of their special characteristics, seaweeds provide a novel way to produce bioplastics.

Based on pigmentation, seaweeds are divided into three groups: green (Chlorophyta), brown (Phaeophyta), and red (Rhodophyta). These groups of seaweeds are found in maritime environments and are essential to marine ecosystems. (Rajendran, et al., 2012) Food packaging plays a crucial role in preserving quality, ensuring safety, preventing contamination, and prolonging the shelf life of food products. Traditionally, materials like paper, metal, glass, and plastics are employed for these purposes. Plastic packaging, in particular, stands out due to its affordability and robust mechanical properties, making it extensively utilized in the food industry. However, the prevalence of single-use plastic packaging contributes significantly to the millions of tons of plastic waste that evade collection systems and end up in landfills or waterways, posing a grave environmental threat. This is because the chemical composition of plastic packaging hinders natural degradation processes. (Gade et al., 2013)

Intelligent food packaging systems represent an innovative solution geared towards overseeing and communicating the state of food and its surrounding environment throughout transportation and storage processes. These advanced systems are equipped to monitor and report vital indicators related to food quality, thereby offering real-time insights to producers, retailers, and consumers. By tracking and recording critical parameters, such as temperature, humidity, and freshness levels, these packaging systems ensure a dynamic feedback loop that enhances overall food safety and quality assurance throughout the supply (Cheng et al., 2022)

1.2 AIM AND OBJECTIVE

Aim:

To explore the polysaccharides extracted from *Sargassum cinereum* for intelligent food packaging.

Objectives:

1. To collect brown seaweed from the coast of Goa and extract the polysaccharide alginate.
2. To synthesize a seaweed-based biopolymer film.
3. To partially characterize the synthesized biopolymer film.
4. To assess the application of the biopolymer film in intelligent food packaging.

1.3 RESEARCH HYPOTHESIS

Polysaccharides extracted from *Sargassum cinereum* can be effectively utilized in the development of intelligent food packaging materials.

It is hypothesized that the biopolymer films synthesized from these polysaccharides will demonstrate desirable properties such as enhanced biodegradability, moisture barrier capabilities, and sensitivity to food spoilage indicators. Additionally, it is anticipated that incorporating specific additives, such as pH indicators, into the biopolymer films will further enhance their functionality, allowing for real-time monitoring of food freshness and potentially reducing food waste. Through comprehensive characterization and testing, it is expected that the biopolymer films will exhibit promising performance, offering a sustainable solution to address the challenges of conventional plastic packaging in the food industry.

1.4 SCOPE

This thesis aims to comprehensively explore the potential of polysaccharides derived from *Sargassum cinereum* for intelligent food packaging applications.

The research will involve the extraction and characterization of polysaccharides from *Sargassum cinereum.* Subsequently, the synthesized polysaccharides will be utilized in the development of innovative packaging materials, as biopolymer films with a particular emphasis on enhancing their intelligent functionalities. These functionalities may include responsiveness to food quality indicators. The performance of the polysaccharide-based packaging materials will be thoroughly evaluated through a series of tests, including mechanical testing, barrier properties assessment, and compatibility with food products. By exploring the use of polysaccharides from *Sargassum cinereum* in intelligent food packaging, this thesis will contribute to the advancement of sustainable and innovative packaging technologies with potential applications in the food industry.

CHAPTER - II

2. LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 Seaweed:

Approximately 500,000 species are found in and are supported by the seas, meaning that over 75% of all known species live in saltwater. Among them are algae, which can live in both freshwater and saltwater (Guiry, et al 2012). Algae are often divided into two groups: macroalgae and microalgae. This research mainly focuses on macroalgae however both have the potential to contribute to the development of seaweed bioplastics. The macroalgae group that includes seaweeds is identified by three primary colours: brown (Phaeophyta), red (Rhodophyta), and green (Chlorophyta). Seaweed is used for a variety of purposes other than as food. They work in several industries, including plastics, paper, medical, biofuel, fertilizer, wastewater treatment, and manufacturing of paper (Rao et al., 2018). Seaweeds develop more quickly than land crops, which makes them extremely prolific (Siew-Moi, et al., 2017). Seaweeds have captured considerable attention due to their diverse range of applications, spanning from food, energy generation, to the production of materials such as plastic and paper. This heightened interest is directly attributed to the inherent composition and characteristics of seaweeds. In general, the dry-weight composition of seaweeds typically consists of approximately 50% total carbohydrates, 1-5 % lipids, and 7-73 % minerals across all three groups. Protein content tends to be lower in brown seaweeds, ranging from 4-24 %, while red and green seaweeds exhibit higher protein content, ranging from 8-47 % (Sedayu et al., 2019).

Seaweed is a promising bioresource for the development of bioplastics, according to recent studies. One of the first people to look into alginate as a possible material for food packaging was Rimundo (Rinaudo et al., 2014).

Several seaweed genera are used in film production. Examples of seaweeds are as follows: for red seaweeds, *Macrocystis, Laminaria, Ascophyllum,* and *Lessonia;* for green seaweeds, *Ulva, Codium,* and *Enteromorpha;* and brown seaweeds, *Eucheuma, Gracilaria, Porphyra, Gelidium,* and *Pterocladia* (Thiruchelvi et al., 2020). Due to their elevated polysaccharide content, seaweeds are well-suited to produce polysaccharide films. However, it's important to note that polysaccharides from different seaweed phyla vary not only in their chemical properties but also in their functionalities (Tanna et al., 2018). Rather than depending on seaweed derivatives, raw seaweed specifically, *Kappaphycus alvarezii* can be used directly in the manufacturing of plastics, according to recent studies. This discovery makes the plastic edible in addition to offering a more economical choice (Sudhakar et al., 2020).

Green seaweeds typically possess a higher cellulose content within their cell walls and tend to store carbohydrates in the form of starch, specifically amylose and amylopectin (Moral et al.,2019). Red seaweeds are widely used in many different industries, such as food, agriculture, cosmetics, and biomedical uses. Common polysaccharides found in red seaweeds include xylan, mannan, carrageenan, cellulose, agar, and floridean starch (Goyanes et al., 2017).

2.2 Brown Seaweed:

The brown seaweeds, classified under the phylum Ochrophyta and belonging to the class Phaeophyceae, stand out as the most extensive and advanced group among seaweeds. The unique brown hue of these seaweeds is attributed to the presence of a pigment called fucoxanthin (Generalić et al.,2019). The *Sargassum* sp. are abundant,

starting from the low tide at low tide and half-moon down. These algae live attached to rocks or chunks of coral and can be lifted from the substrate during big waves and washed away to the sea surface or washed up on the top of the beach (Chaldun, et al.,2023). Brown seaweed is renowned for its phenolic compounds, which are acclaimed for their antioxidant properties. Among its components, salicylic acid (SA) has been noted for its ability to mitigate the impacts of diverse abiotic stressors like high temperatures, soil salinity, and drought (Aina, et al., 2022). Brown seaweed is widely used in the food, fertilizer, and medicine industries. *Saccharina* sp., *Himanthalia* sp., *Laminaria* sp., *Ascophyllum* sp., *Undaria* sp., and *Sargassum* sp. are a few prominent species of brown seaweed (Sudhakar et al., 2018).

2.3 Polysaccharide:

The principal structural polysaccharide discovered in the cell walls and intercellular matrix of brown seaweeds is alginic acid. Alginic acid provides these seaweeds with the flexibility and mechanical resilience necessary for thriving in marine environments, offering resistance to water pressure (Sudha, et al., 2017). Alginic acid is the most abundant polymer found in brown seaweeds in terms of amount. Blocks of successive fl-1,4-linked D-mannuronic acid and blocks of consecutive e-1,4-linked L-guluronic acid make up its unbranched chains polymannuronic acid-containing alginic acid is generally present in intercellular spaces and immature cell wall tissue. On the other hand, polyguluronic acid-enriched alginic acid is typically found within the actual cell wall (Elizabeth et al., 1979). The polymer can comprise three distinct block types: segments that are solely composed of consecutive Ms, consecutive Gs, or a combination of both M and G units arranged randomly. Typically, the ratio of M to G is maintained at 1:1. However, the specific proportions of M and G units, as well as

their distribution along the polymer chain, are subject to variability influenced by factors like the species of algae, environmental growth conditions, seasonal variations, and the particular segment of the algae being considered (Usman,.et al., 2017). In addition to alginate, Carina et al. have included additional polysaccharides that are isolated from brown, red, and green seaweeds to further our understanding of seaweed polysaccharides for food packaging applications (Carina, et.al.,2021). Furthermore, research by Pacheco et al., Zhang et al., and Zanchetta et al. sheds light on the general state of seaweed polymers and their characteristics, providing important knowledge for the manufacturing of diverse plastics (Zanchetta, et al.,2021) (Zhang, et al., 2021) (Pacheco, et al., 2022).

2.4 Bioplastic:

Biopolymers have garnered extensive attention in the research realm for the development of packaging materials (Ramesh & Tharanathan, et al.,2003). Polysaccharides stand out as among the most prevalent naturally occurring biopolymers. Consequently, significant attention has been directed towards the exploration of polysaccharide-based bioplastics sourced from biomass in recent years (Kabir et al., 2020). Various polysaccharides, including cellulose and starch sourced from agricultural resources, chitin and chitosan obtained from byproducts of marine food processing, pullulan derived from microorganisms, alongside numerous other polysaccharides sourced from diverse natural reservoirs, have demonstrated promising capabilities in bioplastic formation (Mohammed et al., 2021).

During the synthesis of bioplastics, specific additives like plasticizers, antioxidants, and antimicrobial agents have been integrated to augment their functional

characteristics (Sivakanthan et al., 2020). Compatibilizers are also essential in biopolymer blends because they contain components that are compatible with all the polymers involved, making the polymers easier to bond together. This leads to an enhancement in the mechanical strength of bioplastics made from heterogeneous biopolymer blends (Balqis et al., 2017). The most common plasticizer used in the manufacturing of bioplastic films from seaweed and other biopolymers is glycerol. Its addition improves the deterioration process, increases extensibility and flexibility, and helps to improve the synthetic film's elongation characteristic (Evon et al., 2020). The bioplastic prepared using sodium alginate and starch exhibited a water absorption capacity ranging between 53 % and 63.36 %, along with a tensile strength value varying from 0.006 to 0.028 MPa (Ismayadi, et al., 2020). Castillo and his colleagues talked about the diverse qualities of starch and chitosan, emphasizing their amazing film-forming capabilities and biodegradability. Because these polymers work well in active and smart packaging, they are widely used in a variety of food-related applications. Their study highlights how these packaging options may actively monitor food product conditions and give customers access to real-time information about them (Castillo. et al., 2017).The bioplastic prepared from wheat starch have shown high elongation of 42.69 % and a tensile test results of 205.4 Mpa (Saiful et al., 2019). The sample created with gelatin/CMC/agar and 2.5 % glycerol emerged as the most favourable formulation, ideal for potential application in food packaging. It exhibited such as the lowest water vapor permeability and the highest biodegradability rate, outperforming other samples tested (Yaradoddi et al., 2020). Although chitosan-based bioplastic exhibits a notable capacity for water absorption, experimental results indicate that it is unsuitable for liquid storage (Bilgen et al., 2022). The research has showcased the feasibility of producing biodegradable trays using cassava starch and

corn husk residues, incorporating additives like glycerol, potassium stearate, and guar gum at different concentrations. Analyses involving FTIR, SEM, XRD, and TGA affirmed that the composition comprising 85 % cassava starch and 15 % corn husk flour demonstrates favourable physical and chemical properties (Aguirre et al., 2023).

2.5 Intelligent food Packaging:

Pereira et al., investigate the field of food packaging, with a special emphasis on time-temperature indicators, which they refer to as an element of intelligent packaging. Their study highlights the significant impact of temperature variations on food quality and presents a tool for continuous food condition monitoring. Their study's goal was to develop and evaluate a temporal temperature indicator (TTI) using a polymer composed of PVA and chitosan that had been infused with anthocyanins. This TTI is a useful instrument for detecting changes in packaged food pH levels brought on by exposure to incorrect storage temperatures (Pereira et al., 2015). Vo et al., have effectively fabricated pH-indicative films comprised of PVA/CS/ATH. These films were cast from hydrogels by blending 1 % PVA solution and 1 % CS solution in different volume ratios. They incorporated ATH as an indicator and supplemented it with STPP as a cross-linker during the process (Vo et al., 2019). pH indicators offer potential as effective components in intelligent packaging systems, given their ability to detect pH changes which often accompany food spoilage. With consumer inclination towards natural alternatives over synthetic chemicals, and concerns regarding contact between colorants and food items, the quest for natural and food-grade pH indicators holds significant importance in the advancement of intelligent packaging systems for food (Etxabide et al., 2021). Alizarin, sometimes called Turkey Red or 1,2-dihydroxyanthraquinone in chemistry, is a naturally occurring food

colouring that dissolves in alcohol. It is taken out of the madder plant's roots (Sun, et al., 2018).

The molecular composition of alizarin comprises three benzene rings or conjugated orbitals, each adorned with hydroxyl groups. As the pH fluctuates, the molecular structure of alizarin undergoes alterations, facilitating the linkage of hydroxyl groups to carbonyl oxygen atoms. This configuration enables the transfer of protons through an intramolecular hydrogen bond (Jen, et al., 2017). Ezati (2019) created pH-sensitive markers using starch and cellulose, with alizarin acting as a natural color. They found that over a pH range of 2 to 11, these indicators displayed color changes, moving from yellow to purple to dark red. Interestingly, color shifts in the indicators were easily visible to the human eye when they were between a pH of 7 and 9. Additionally, color stability tests revealed that, across a variety of storage settings spanning two months, alizarin-incorporated starch-cellulose showed stronger stability at 4 ℃and 25 ℃ than alizarin-coated starch cellulose biopolymer film (Ezati et al., 2019). Ezati et al. formulated two varieties of pH-responsive smart gas sensors through the integration of alizarin into CMC and CNF polymers. Their findings indicated that alizarin (1 wt.%) was uniformly dispersed within the polymer matrix, as confirmed by XRD and FTIR analyses. Furthermore, the incorporation of alizarin resulted in enhancements to the UV barrier, antioxidant activity, and thermal stability properties of the indicator films (Ezati et al., 2020).

CHAPTER –III

3. MATERIALS AND METHODOLOGY

3. MATERIALS REQUIRED

Chemicals Used

- Glycerol
- Ethanol
- Sodium alginate
- Starch
- Bees wax
- Hydrochloric acid
- Sodium carbonate
- Sodium hydroxide
- Isopropanol
- Ascorbic acid
- Alizarin Red S
- Bradford Reagent
- DPPH
- Bovine serum albumin
- Silica gel
- Calcium chloride

Apparatus

- SORVALL ST 8R refrigerated bench top centrifuge
- UV mini 1240 UV-Vis spectrophotometer
- Dynamometer
- pH meter (pH 700, Eutech Instruments, Thermo Fisher Scientific, India)
- Carbon Fiber Composites digital Caliper
- Autoclave
- Laminar Air Flow
- Biosafety cabinet
- Hot air oven
- Refrigerator
- ZESSIS EVO 18 scanning electron microscope (SEM)
- XRD SMARTLAB SEW
- FTIR - Bruker alpha 2
- STA 449 F3 Jupiter Thermal Analyz

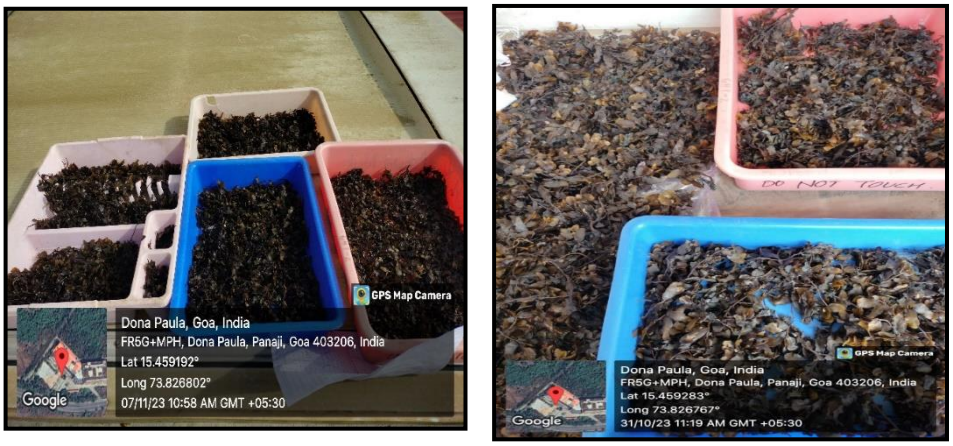
3. METHODOLOGY

3.1 Seaweed sampling and processing:

The brown seaweeds were collected from Odxel beach along the coast of Goa during low tide in the post-monsoon season (15.4539588N, 73.8297984E)). The samples were identified as *Sargassum cinereum* based on their morphological characters by Prof. Vijaya Kerkar from Botany Discipline, School of Biological Sciences and Biotechnology, Goa University. The seaweeds were then washed with tap water to remove any sand or other particles and were sun-dried as shown in Figure 2. Once completely dry, they were powdered and stored in the dark at room temperature.

A B

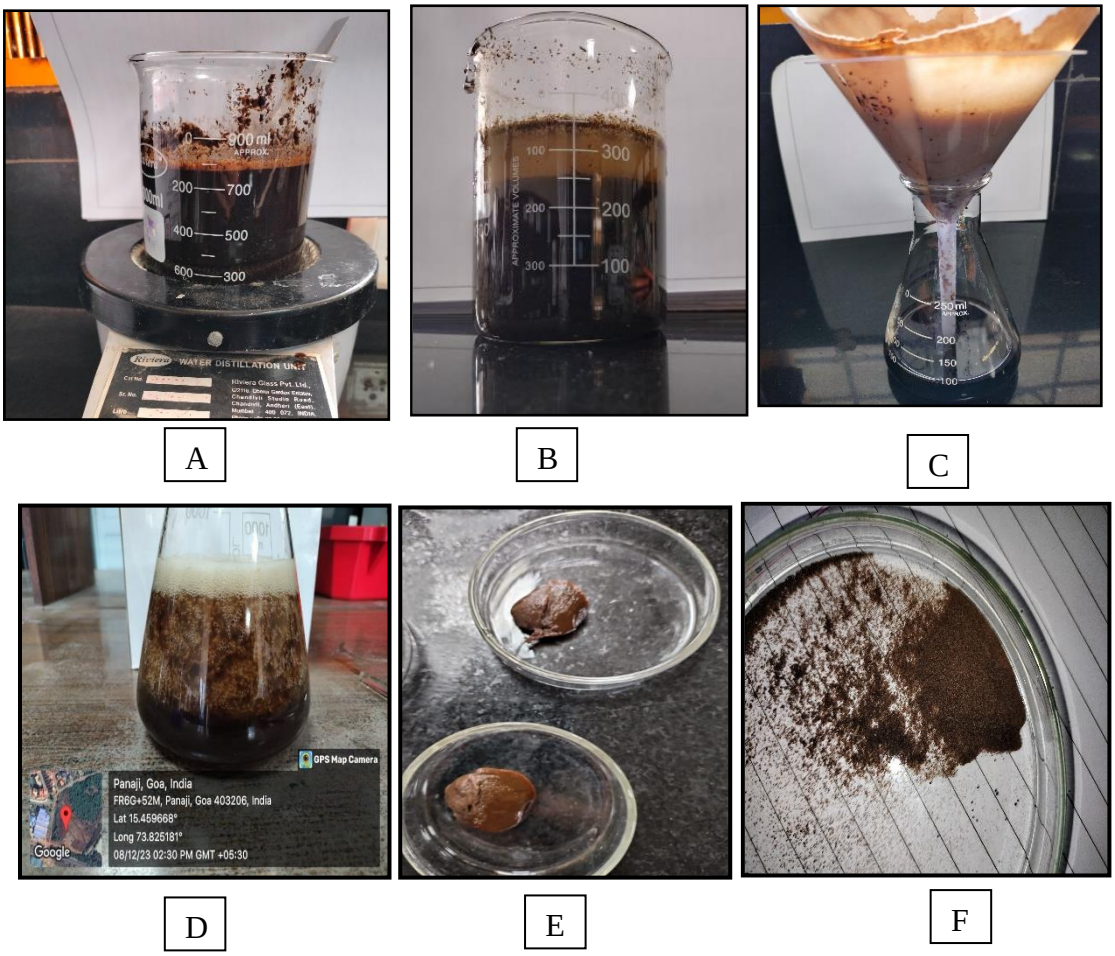
Figure 1: - (A) GPS image of sampling site (B) Collection of seaweed samples from the coast of Goa.



A B

Figure 2: - (A) & (B) Sun drying of *Sargassum cinereum* at room temperature.

3.2 Extraction of polysaccharide from seaweed:



A B C

D E F

Figure 3: Extraction of Sodium alginate from seaweed (A) Sample soaked in HC (B) Sample in 4 % Na_2CO_3 being heated (C) Filtration of solution (D) Formation of lumps after adding HCl (E) Crude alginate pellet after centrifugation (F) Dried powder of crude alginate.

Extraction of alginate from brown seaweed was carried out using the hot alkaline extraction method given by Widiyanti (2012) with a few modifications (Widyanti et al., 2012). Dried seaweed samples weighing 25 g were first cut into small pieces and immersed in a 1 % HCl solution for a duration of 1 hour. Subsequently, any excess HCl was removed, and the seaweed was submerged again, this time in a 4 % Na_2CO_3 solution. The mixture was then heated at 60 ℃ for 2 hours with continuous stirring as shown in Figure 3 (A). Following this, as shown in Figure 3 (B) the mixture was diluted with distilled water and left to stand for 30 minutes. The resulting mixture underwent filtration overnight using Whatman filter paper as shown in Figure 3 (C). The filtrate obtained was then adjusted to a pH of 2-3 by adding 5% HCl and left overnight to form foam lumps as shown in Figure 3(D).

These foam lumps were collected and treated with 1M NaOH (10 mL), allowing them to stand for 10 minutes. Subsequently, 99% Isopropanol was added to the sample at a ratio of 1:2 and left for another 10 minutes. The mixture underwent centrifugation at 5000 rpm at 4 ℃ for 10 minutes, after which the pellet was collected and dried. The yield of alginate was determined using the following method:

$$\text{Yield of alginate (\%)} = \frac{\text{weight of crude alginate}}{\text{weight of dried powder}} \times 100$$

3.3 Preparation of Alginate beads:

The preparation of Alginate beads followed a method based on Kaur (2018). (Kaur et al., 2018) with slight modification. Initially, 1 g of crude alginate polysaccharide sample was combined with 2 mL of distilled water and heated until the alginate completely dissolved. Subsequently, the mixture was transferred into a syringe and added drop by drop into a beaker filled with chilled 2 % $CaCl_2$ solution, monitoring the formation of beads. Following bead formation, they were carefully filtered, washed with distilled water, and dried using filter paper.

3.4 Synthesis of Biopolymer Film:

3.4.1 Standardization of glycerol concentration:

Glycerol served as the plasticizer in the preparation of biopolymer films, as described by Inayati et al. (2019). Eight concentrations of glycerol, ranging from 2.5 % to 20 %, were used to prepare biopolymer films.

Figure 4: - Biopolymer film prepared using different glycerol concentrations (%) (A) 2.5 % (B) 5 % (C) 7.5 % (D) 10 % (E) 12.5 % (F) 15 % (G) 17.5 % (H) 20 %

3.4.2 Standardization of wax concentration:

To enhance the hydrophobicity of the biopolymer film, wax was incorporated. Specifically, wax concentrations of 2.5 % and 5 % were tested for this purpose as shown in Figure 5.

A B

Figure 5: - Biopolymer film prepared by two concentrations of wax (%), (A) 2.5 % (B) 5 %

3.4.3 Standardization of pH indicator concentration:

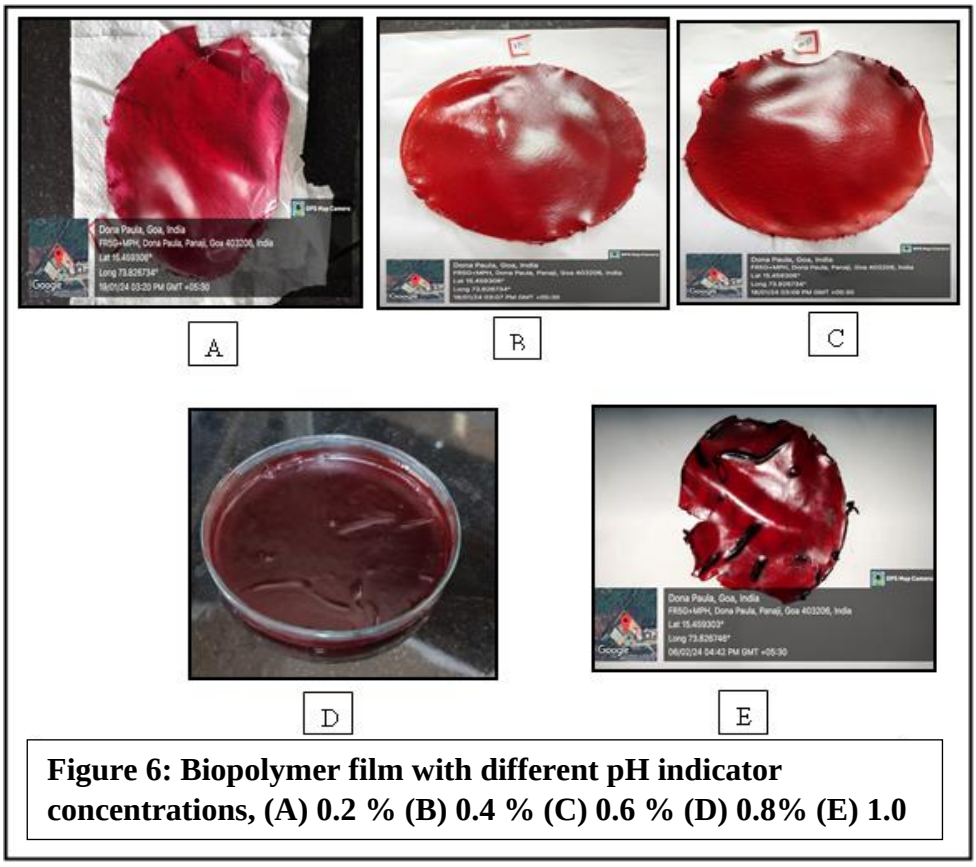


Figure 6: Biopolymer film with different pH indicator concentrations, (A) 0.2 % (B) 0.4 % (C) 0.6 % (D) 0.8% (E) 1.0

Alizarin pH indicator was applied by coating its solutions onto biopolymer film, following the method outlined by Ezati et al. (Ezati et al., 2019). Five different concentrations of the pH indicator, ranging from 0.2 % to 1.0 %, were tested for their colour change.

3.4.4 Synthesis of Biopolymer film:

The commercial alginate biopolymer film and crude seaweed alginate biopolymer film were made by using solution casting method (Thiruganasambanthan, et al., 2022). 1 g of commercial alginate and crude seaweed alginate was mixed with 20 mL distilled water and heated till the alginate dissolved completely. To this, starch was added in a 1:4 (starch: alginate) ratio. Then, 4 mL of glycerol was added. The solution was then brought to a boil and poured into Petri plates and left undisturbed in the oven at 100 ℃ till dry. After drying, the biopolymer film was coated with pH indicator and kept for drying in the oven for 12 hours.

3.5 Characterization of the Biopolymer Film

3.5.1 Physical Properties

3.5.1.1Tensile Strength:

The tensile strength of the commercial alginate biopolymer film and seaweed biopolymer was done with the help of a dynamometer (Gabriel et al.,2021). The tensile strength of the film was calculated as:

$$\text{Tensile strength } (N/m^2) = \frac{\text{Applied Force}}{\text{Surface Area}}$$

3.5.1.2 Thickness:

The thickness of the biopolymer film was measured using digital Carbon fibre composites digital caliper. The thickness of five random spots on biopolymer film were recorded (Teixeira et al., 2017).

3.5.2 Water vapor transmission rate:

The water vapor transmission rate was evaluated following the method outlined by Amirah et al. (Amirah et al., 2023). In accordance with this procedure, silica gel was placed within a glass vial, which was subsequently sealed with a biopolymer film. The sealed vial was then placed inside a desiccator containing 10 mL of water in a crucible. The setup of the apparatus was done as shown in Figure 7. After a duration of 24 hours, the initial and final weights of the film were recorded for analysis. The water vapor transmission rate was then determined using the formula mentioned below:

$$\text{Water vapor transmission rate } (g/m^2/24\text{hours}) = \frac{Wi - Wf}{TA}$$

where W_i- initial weight of the biopolymer film (g), Wf- final weight of the biopolymer film (g), T- time (h) and A – surface area of biopolymer film (m^2).

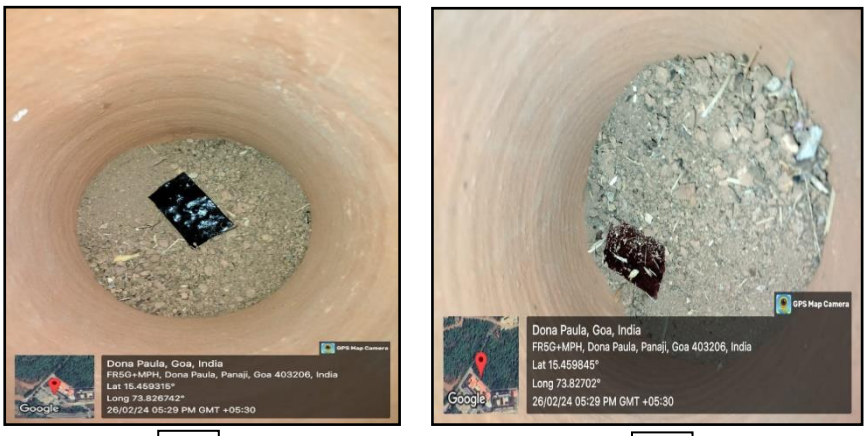
A B

Figure 7: - Set up arranged for water vapour transmission rate.

3.5.3 Biodegradation Test:

The examination of the biopolymer film biodegradation was conducted using the Soil Burial Test (SBT), as described by Nissa et al. (Nissa et al., 2019). For this test, biopolymer film samples measuring 3 ×1.5 cm were initially weighed (W_o) and subsequently buried in soil. Following a 28-days incubation period, the samples were retrieved and weighed again (W_f). The percentage weight loss was then determined using the formula:

$$\% \text{ Weight loss} = \frac{Wo - Wf}{Wo} \times 100$$



A B

Figure 8: - Samples buried in soil for biodegradation test, (A) Crude alginate Biopolymer film (B) Commercial alginate biopolymer film.

3.5.4 Morphological and elemental composition of the biopolymer film:

3.5.4.1 Scanning Electron Microscope Analysis:

The surface morphology of the biopolymer films underwent examination using a ZESSIS EVO 18 scanning electron microscope (SEM). Prior to SEM analysis, the samples were cut into pieces measuring 1×1 cm and coated with a thin layer of gold via sputter coating.

3.5.4.2 X-Ray Diffraction (XRD):

The XRD analysis were used to observe elemental composition of the biopolymer films. For this analysis the XRD SMARTLAB SEW was used and pieces of sample were cut and mounted on the sample holder.

3.5.4.3 Fourier Transform Infrared Spectroscopy (FTIR):

FTIR analysis was conducted using a Bruker alpha 2, operating within the range of 4500 to 0 wavenumbers, for biopolymer film samples. This spectroscopic technique was employed to identify and characterize the functional groups present in the samples, to compare the functional groups present in commercial alginate biopolymer film and crude seaweed alginate biopolymer film, facilitating the determination of the type of plastic synthesized in the study (Ismail et al., 2016).

3.5.5 Thermogravimetric analysis (TGA):

TGA was conducted using a STA 449 F3 Jupiter Thermal Analyzer apparatus, employing open aluminium pans under a nitrogen atmosphere. Measurements were carried out by gradually increasing the temperature from room temperature to 150 ℃ at a rate of 5 ℃ per minute.

3.5.6 Assessing the Antioxidant potential of the biopolymer Film:

The Antioxidant properties of the biofilms were measured using the DPPH radical scavenging activity protocol by Blois, (1958) with few modifications. 100 mg of biopolymer film samples were mixed with 5 mL of 70 % ethanol. From this stock 1.5 mL was taken and diluted with 1.5 mL of 70 % ethanol. To this, 1 mL of DPPH reagent was added, and the tubes were kept for incubation at R.T in the dark for 30 min. Absorbance was measured at 517 nm and compared to a standard graph prepared using Ascorbic acid.

Table 1: Antioxidant test by DPPH method

Stock - 20mg/mL, Diluent - 70% ethanol.

Sample	Amount of unknown (mL)	70% ethanol (mL)	Volume of DPPH Reagent (mL)	Incubation at R.T. for 30 minutes	O.D at 517nm
Blank	0	3	1		
Crude alginate Film	1.5	1.5	1		
Commercial alginate Film	1.5	1.5	1		

3.6 Application of Biopolymer Films:

The ability of the biofilm to change its color as an indicator of food spoilage (due to the change in pH) was tested. The commercial alginate biopolymer film and the crude alginate biopolymer film were cut and sealed using a plastic sealer. Shrimp were sacrificed and placed in the packets and sealed completely as shown in Figure 9 [(B), (C)]. The packaged bags were kept at three different temperatures i.e. freezer, room temperature and ice box for 48 hours. After 48 hours, the color change was recorded, and the shrimp samples were homogenised. Protein estimation was done by Bradford method (Kruger et al., 2009).

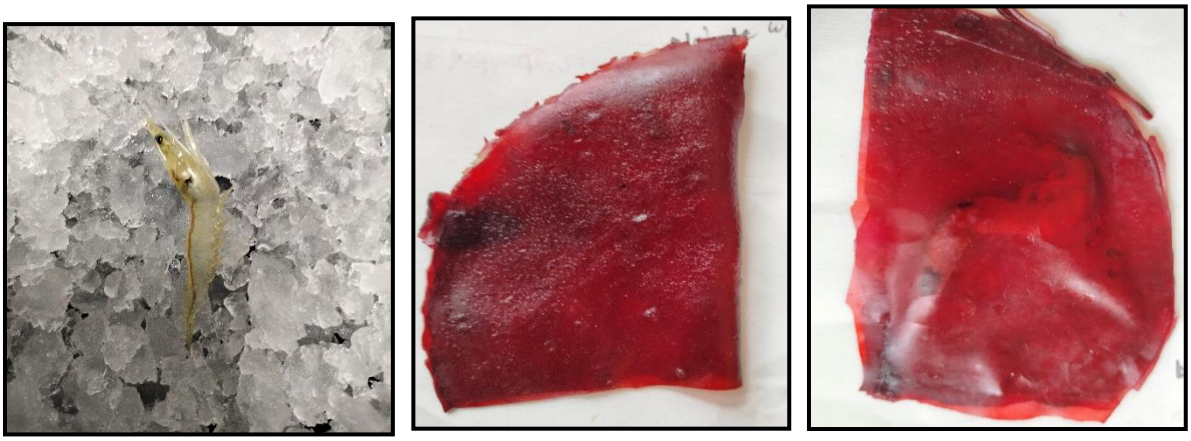


Figure 9: - Application of biopolymer film (A) Shrimp before packaging (B) Pouch made from biopolymer film (C) Shrimp package in biopolymer film

Table 2: Protein estimation by Bradford method

Stock- 100mg/mL Diluent- Distilled water

Shrimp sample	Amount of sample (mL)	Amount of distilled water (mL)	Amount of reagent (mL)		
Before packaging	0.1	0.9	3	Incubation at room temperature for 10 minutes	O.D at 595 nm
	0.1	0.9			
	0.1	0.9			
After incubation at room temperature	0.1	0.9			
	0.1	0.9			
	0.1	0.9			
After incubation at Freezer	0.1	0.9			
	0.1	0.9			
	0.1	0.9			

CHAPTER - IV

4. RESULTS AND DISCUSSION

4.1 Extraction of polysaccharide alginate:

Polysaccharide was obtained from brown seaweeds *Sargassum cinereum* through the hot alkali extraction process, yielding 39.2 % polysaccharides from *Sargassum* sp. The commercial extraction of alginate involves five key processes: acidification, alkaline extraction, solid-liquid separation, precipitation, and drying. The pivotal stage is alkaline extraction, constituting the actual extraction phase. Upon exposure to a sodium carbonate solution following acidification, insoluble alginic acid converts into soluble sodium alginate, entering the aqueous phase (Abraham et al., 2018). In the extraction process, alginic acid salts initially convert into free alginic acid through proton exchange with a strong acid (such as HCl). Subsequently, the neutralization of insoluble alginic acid with an alkali occurs to produce water-soluble sodium alginate, which is then recovered from the extraction solution by precipitation with hydrochloric acid, calcium chloride, or alcohol before undergoing drying and grinding (Alba & Kontogiorgos, 2018).

Davis et al. (2004) reported that the hot alkaline extraction method yielded 24.5 % alginate for *S. fluitans* and approximately 20.5% for *S. oligocystum* (Davis et al., 2004). According to a study by Rhein-Knudsen et al., (2017) the yield of alginate from *Sargassum* was 86.37 %. The yield alginate from *Sargassum* sp. was 16.9 % in a study by Torres, et al., (2007). A comparison with their study indicates that the results obtained here demonstrate a lower yield than Rhein-Knudsen et al., (2017) and higher yield than Torres, et al., (2007).

4.2 Confirmatory test for Alginate:

Figure 10: Ca-Alginate beads from Polysaccharide extracted from *Sargassum* sp.

The crude polysaccharide solution came into contact with cold $CaCl_2$, it exhibited the formation of bead-like structures as shown in Figure 10. This observation suggests that the polysaccharide in question was likely sodium alginate.

Sodium alginate has the ability to create firm gels through the influence of calcium ions or other multivalent cations. The bead formation process occurs through spherification, wherein alginate and calcium ions interact to establish cross-linked bonds among the macromolecules (Sharma, al., 2017).

4.3 Hydrophobicity of the biopolymer sample:

Figure 11: Hydrophobicity of the film (A) Control without wax (B) 2.5 % wax concentration (C)5 % wax concentration

Table 3: Water contact angle of biopolymer film samples

Sr. No	Wax Concentration (%)	Water contact angle (degree)
1	0	36
2	2.5	59
3	5	64

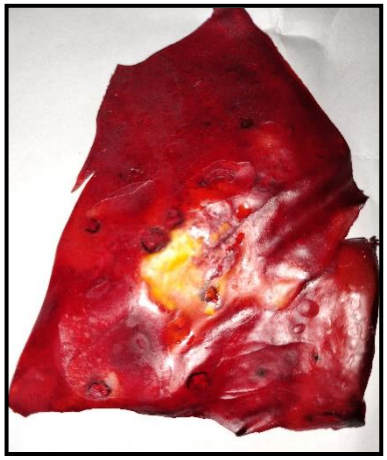
Table 3 indicates that the biopolymer film containing 5 % wax concentration exhibits greater hydrophobicity compared to the 2.5 % wax concentration and the control. The absence of wax in the biopolymer film led to water absorption due to interactions between starch, glycerol, and water molecules. Conversely, the inclusion of wax in bioplastics created a barrier that repelled water molecules, attributed to factors such as physical roughness, surface configuration, temperature, humidity, and adsorbed substances (Yasuda et al., 1994). According to literature, the water contact angle of alginate biopolymer film was reported to be 42° (Pereira et al., 2013), while another study incorporated chitin into starch-based bioplastic to enhance hydrophobicity. They found that a 4 % chitin concentration yielded a superior water contact angle of 72° (Dawam Abdullah et al., 2020).

4.4 Colour Change of pH indicator:

Table 4: Colour change of biopolymer film at different concentration of pH indicator

Concentration of pH (%)	Colour
0.2	Red
0.4	Red
0.6	Red
0.8	Yellow
1.0	Yellow

4.5 Colour Change of pH indicator:



A

B

Figure 12: Colour change after exposing to acidic solution, (A) Commercial alginate biopolymer film (B) Crude alginate biopolymer film

Table 4 shows that the 0.8 % and 1.0 % concentration of pH indicator showed the colour change after exposing it to acidic solution.

This result states that the high concentration of pH indicator (0.8 % and 1 %) shows an increase in the visibility of color change (from red to yellow) within the biopolymer film. Similar results were reported by Khan et al., that concludes that a higher concentration of the indicator leads to a more intense color change reaction. Hence, subtle pH changes in the biopolymer film would be more easily detectable (Khan et al., 2023).

4.6 Synthesized Biopolymer Films:

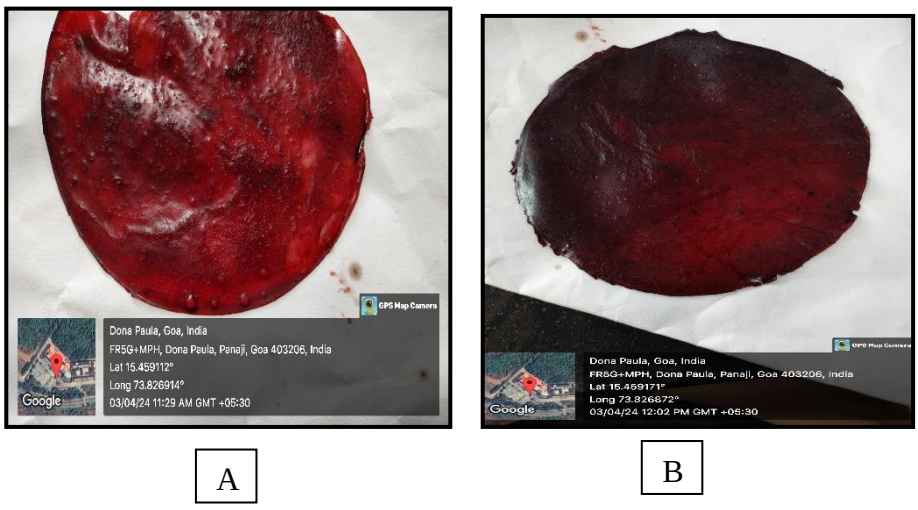


Figure 13: Biopolymer films (A) Biopolymer film prepared from commercial alginate (B) Biopolymer film prepared from crude alginate

4.7. Characterisation of the Biopolymer film:

4.7.1 Physical properties

4.7.1.1 Tensile strength:

The Tensile Strength was calculated using the given formula (as mentioned in 3.5.1.1) and recorded as seen in Table 5.

Table 5: Tensile strength of the biopolymer film

Glycerol Concentrations (%)	Tensile strength (N/m^2)
2.5	75.67
5	96.56
7.5	125.67
10	147.47
12.5	189.45
15	225.18
17.5	290.76
20	**334.67**

The tensile strength of the alginate biopolymer film and seaweed biopolymer film with 20 % glycerol concentration was calculated to be 324.675 N/m^2 and 303.345 N/m^2.

The bioplastic fabricated using a combination of sodium alginate and starch demonstrated a tensile strength ranging between 6000 N/m^2 and 2800 N/m^2, as reported by Ismayadi et al. (2020). Conversely, Marangoni et al. noted a tensile strength of 1290 N/m^2 for the sodium alginate and starch film. A comparison of the findings suggests that the bioplastic in the given study exhibited lower tensile strength, likely due to the incorporation of wax and a lower concentration of polysaccharide compared to the studies conducted by Marangoni et al. (2022) and Ismayadi et al. (2020).

4.7.1.2 Thickness:

The thickness was measured and recorded as seen in Table 6.

Table 6: Thickness of biopolymer

Samples	Thickness(mm)
Crude alginate Biopolymer film	0.28 ± 0.16
Commercial alginate biopolymer film	0.21 ± 0.05

In a study conducted by Zinina (2023) it was demonstrated that a biofilm composed of alginate and glycerol had a measured thickness of 0.06 mm. The thickness of packaging films plays a crucial role in ensuring the safety of food products. Adding to this, Norajit et al. (2010) found that a bioplastic film made from alginate and glycerol exhibited a thickness of 0.070 mm. The significance of film thickness can contribute to pest resistance and gas permeability, as noted by Guidara et al. (2020). An increase in layer thickness may enhance these properties. Despite the biofilm produced in the current experiment with extracted polysaccharide being thicker compared to the findings of Norajit et al. (2010) and Zinina (2023) it suggests potential advantages in terms of providing improved barrier protection for packaged food items (Zinina et al., 2023).

4.7.2 Water vapor transmission rate:

The Water vapor transmission rate was calculated using the given formula (as mentioned in 3.5.2) and recorded as seen in Table 6

Table 7: Water vapor transmission

Parameters	**Seaweed film (in grams)**	**Standard alginate film (in grams)**
Initial weight of the empty vial	21.09	21.09
Weight of vial with silica gel	22.3	22.3
Initial weight of vial with silica gel+ film	23.8	23.9
Final weight of vial with silica gel+ film after 24 h	24.3	24.0
Difference = final and initial weight of the vial	0.5	0.1

The water vapor transmission rates (WVTR) of the commercial alginate biopolymer film and the crude alginate biopolymer film were determined to be 0.1061 g/m²/24hours and 0.5307 g/m²/24hours, respectively. WVTR serves as a standard metric for evaluating films' resistance to moisture transmission, with lower values indicating superior moisture protection. In a study by Hartiati et al. (2021), different concentrations of alginate and starch were utilized in the preparation of biopolymer films, resulting in a WVTR of 7.19 g/m²/hr. A comparison of this result with the provided data reveals a lower water vapor transmission rate and better moisture protection in the given study.

4.7.3 Biodegradation test:

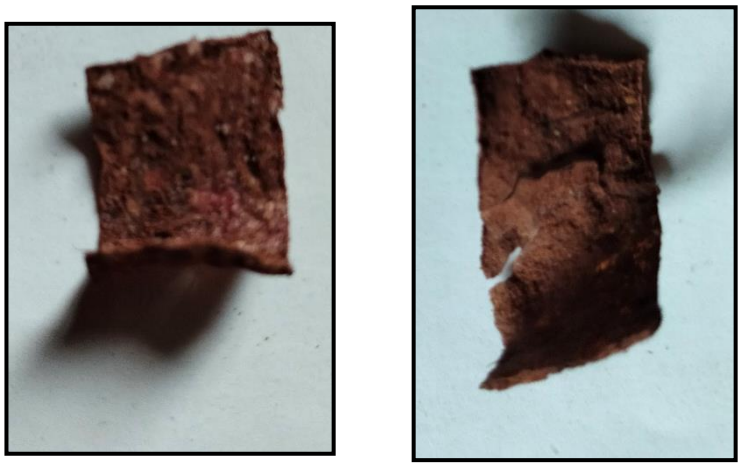


Figure 14: - Biopolymer film after 28 days incubation. (A)Commercial alginate biopolymer film (B) crude alginate biopolymer film

The percentage of weight loss in the commercial alginate biopolymer film and crude alginate biopolymer film was calculated to be 86.24 % and 87 % respectively.

Nissa et al., (2019) utilized the Dip Hanging Method and Soil Burial Test (SBT) to assess the biodegradation of starch-based bioplastics. Their findings revealed a notable 30 % weight loss in the samples subjected to the SBT. Consequently, they deemed the SBT as a highly effective quantitative method for investigating biodegradation processes (Nissa, et al., 2019).

4.7.4 Morphological and elemental composition of the biopolymer film:

4.7.4.1 Scanning Electron Microscopy (SEM):

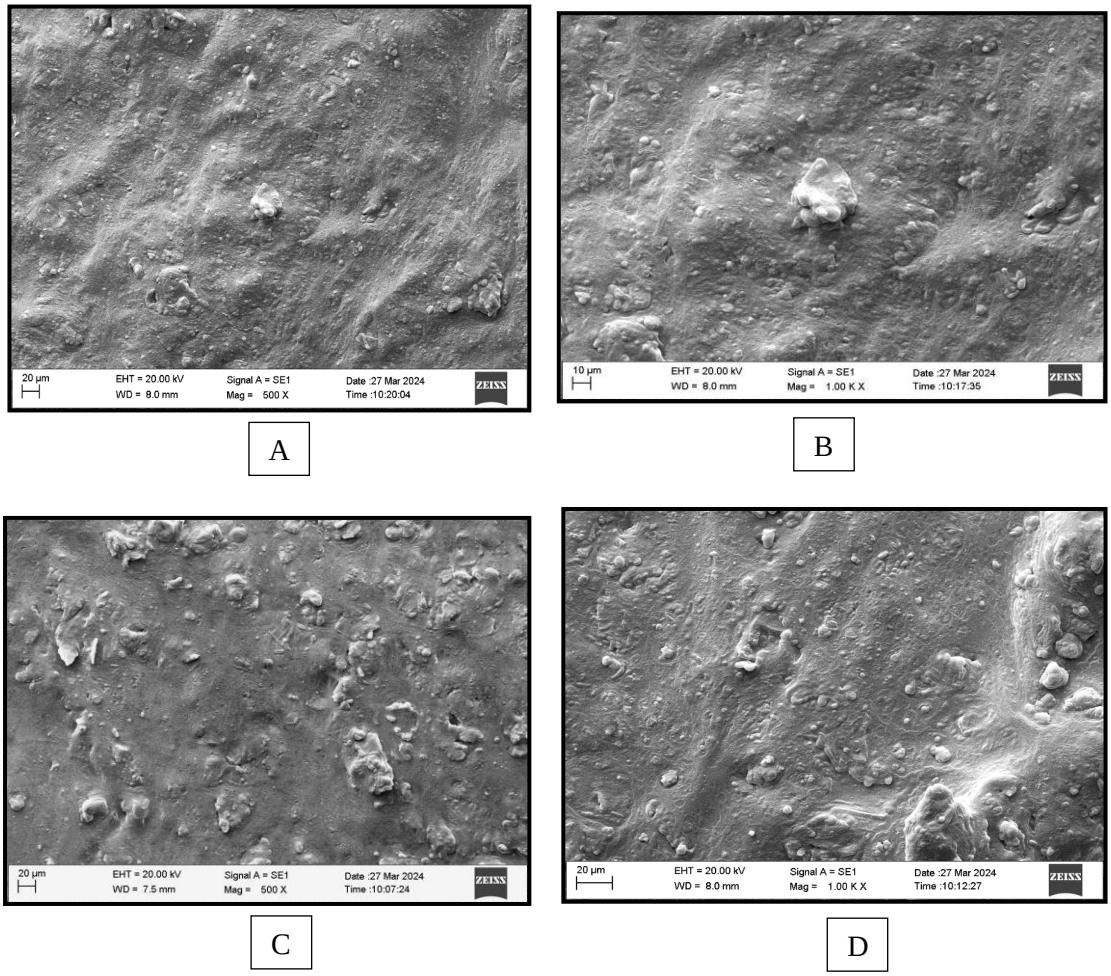
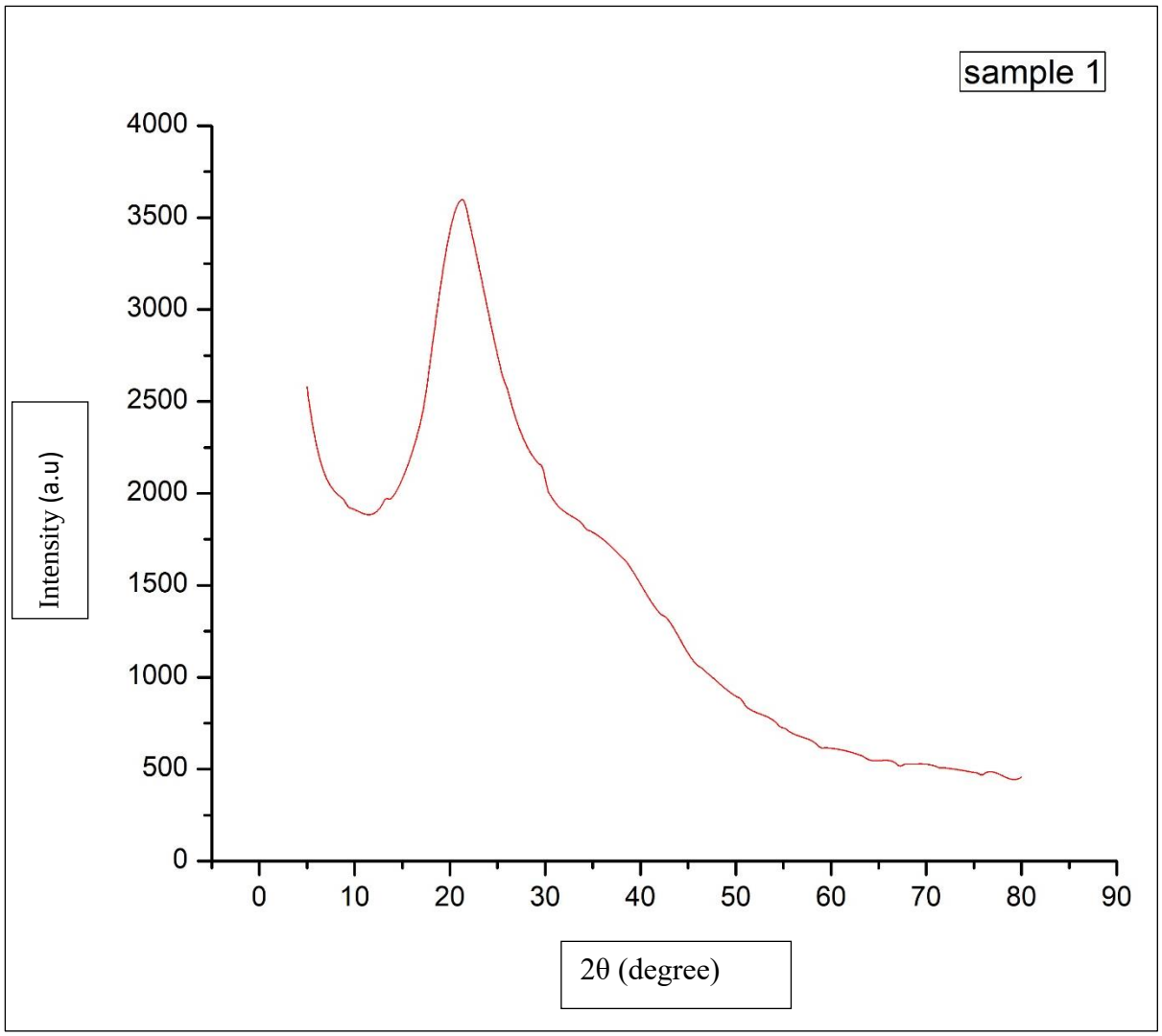


Figure 15: SEM images of biopolymer film (A) Commercial alginate film at 500X (B) Commercial alginate film at 1000 X (C) Crude alginate film at 500X (D) Crude alginate film at 1000 X

Scanning electron microscopy images were analysed to examine the morphology of the biofilms. The comparison revealed that the surface of the commercial alginate sample appeared smoother than that of the crude alginate biopolymer film, with small particles observed on the surface of the latter. This difference in smoothness can be attributed to the production process. Commercial alginate biopolymer films are typically synthesized from purified alginate extracted from seaweed sources, resulting in a more uniform polymer composition. In contrast, crude alginate biopolymer films may contain impurities or varying alginate compositions, which can impact the overall smoothness of the film surface (Stephen and Phillips, 2016).

4.7.4.2 X-Ray diffraction Analyses:



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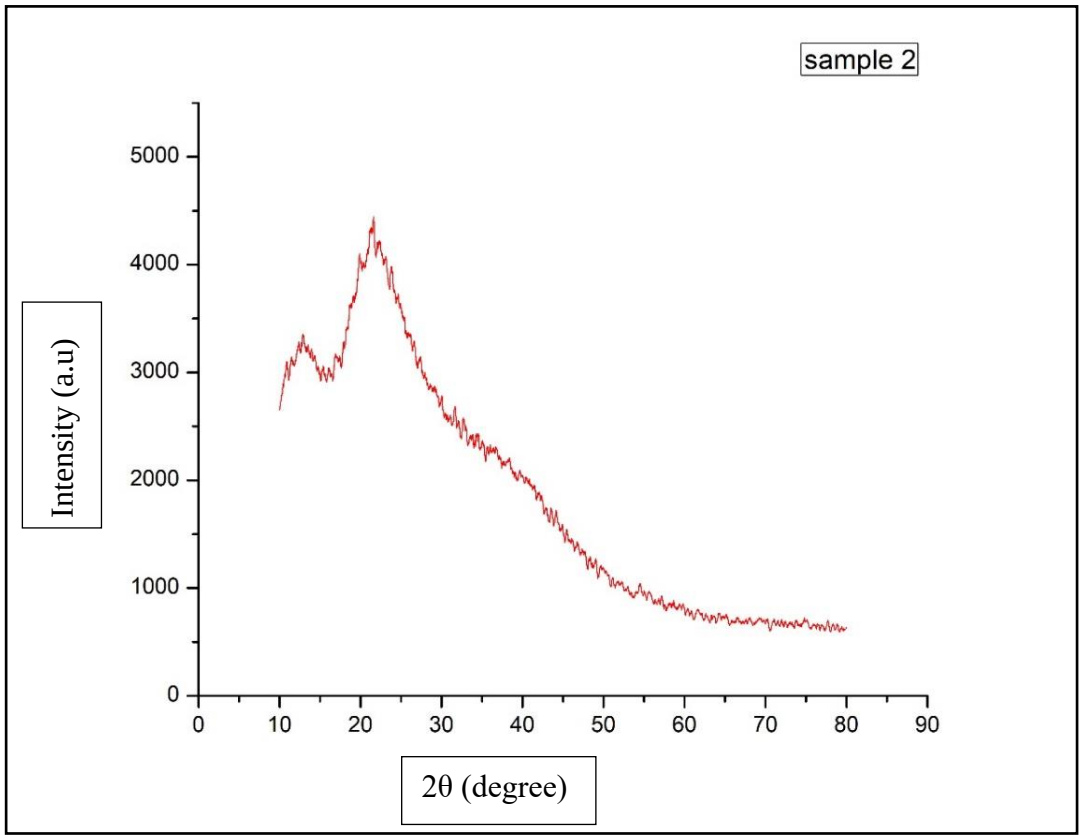


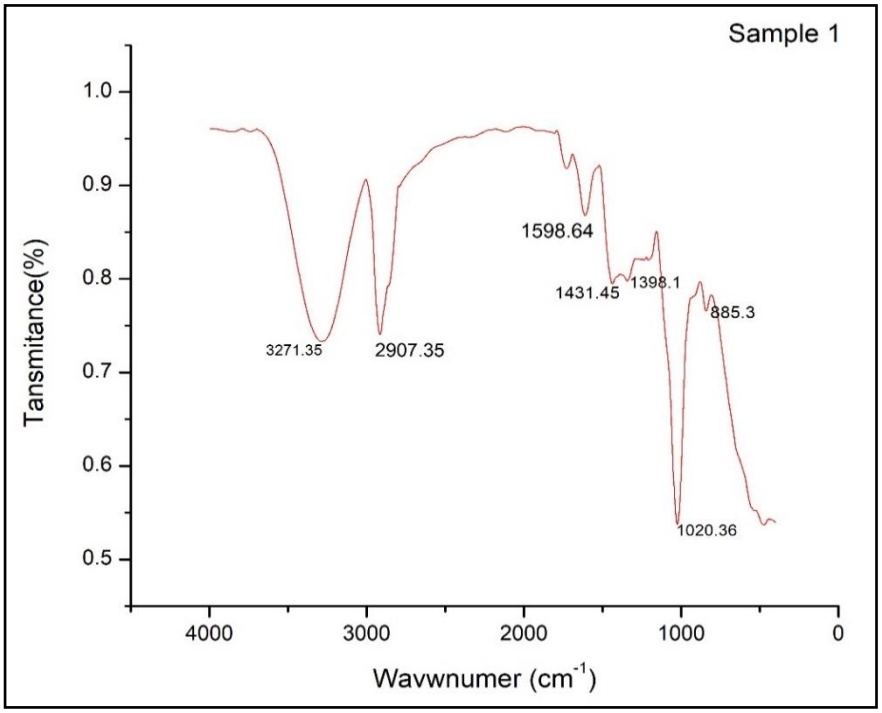
B

Figure 16: XRD analysis (A) Commercial alginate film (B) Crude alginate film

In a recent investigation conducted in 2021, the X-ray diffraction (XRD) analysis of sodium alginate derived from *Sargassum latifolium* revealed the presence of three distinct and prominent crystalline diffractions observed at 2θ values of 20.51°, 21.04°, and 29.73°(Dalal et al., 2021). Interestingly, when examining the XRD patterns of commercial alginate biopolymer, a peak was identified at 21.04°; however, this peak lacked sharpness. This could potentially be attributed to the relatively low crystallinity of the sample and the exposure of the polysaccharide to elevated temperatures during the synthesis of the biopolymer film. Conversely, the XRD profile of crude alginate biopolymer film displayed a spiked line graph with a minor peak at 2θ value of 20.51°. Notably, the presence of diffuse scattering in the analysis indicated the amorphous nature of the sample.

4.7.4.3 Fourier-transform infrared spectroscopy (FTIR) analysis:

The FTIR data was analysed using Origin 8 Software (https://www.originlab.com/) and the wavenumber values were compared with the FTIR database (https://instanano.com/all/characterization/ftir/ftir-functional-group-search). The wave number, along with the associated functional groups and its nature were listed down as seen in Table 7 & 8.



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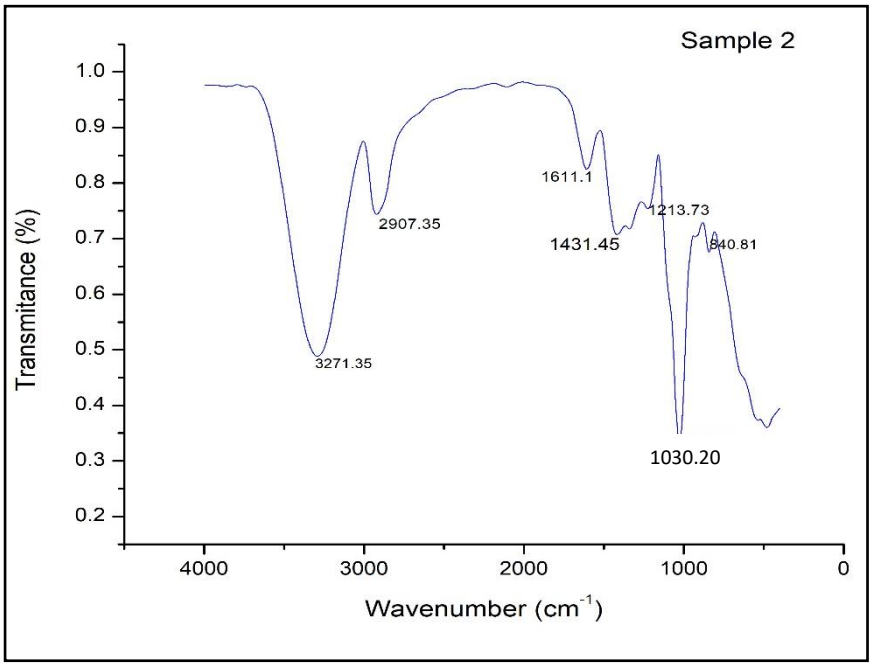


Figure 17: FTIR graph (A) Commercial alginate biopolymer film (B) Crude alginate biopolymer film

Sample 1

Table 8: FTIR data of commercial alginate

Wavenumber (cm^{-1})	Functional group	Nature
3271.35	O-H	alcohol
2907.35	O-H	carboxylic acid
1598.64	N-H	amine
1431.45	C-H	aldehyde
1398.1	O-H	carboxylic acid
1020.36	C-N	amine
885.3	C=C	alkene

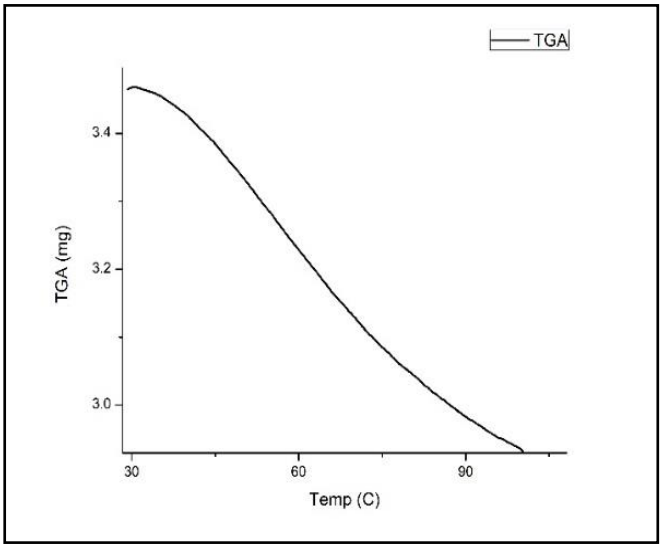
Sample 2

Table 9: FTIR data of Crude alginate biopolymer film

Wavenumber (cm^{-1})	Functional group	Nature
3271.35	O-H	alcohol
2907.35	O-H	carboxylic acid
1611.1	N-H	amine
1431.45	C-H	aldehyde
1213.73	C-N	amine
1033.20	S=O	sulfoxide
840.81	C=C	alkene

In the study conducted by Nazia Rahman (2017) on the Fourier-transform infrared spectroscopy (FTIR) analysis of alginate biopolymer, it was observed that the biopolymer exhibited the presence of several functional groups including C-H, C-O, C=O, and C-H-O groups (Rahman et al., 2017).Both the commercial alginate and crude alginate biopolymer films exhibited similar functional groups, with one notable distinction being the presence of the S=O group exclusively in the crude alginate compared to the commercial alginate biopolymer film. Upon comparison of their respective Fourier-transform infrared spectroscopy (FTIR) spectra, it was observed that the absorption of radiation in the commercial alginate biopolymer film was greater than that in the crude alginate biopolymer film. This difference in absorption could potentially be attributed to the higher concentration of these specific functional groups present in the commercial alginate biopolymer film (Petit & Madejova, 2013).

4.7.5 Thermogravimetric analysis (TGA):



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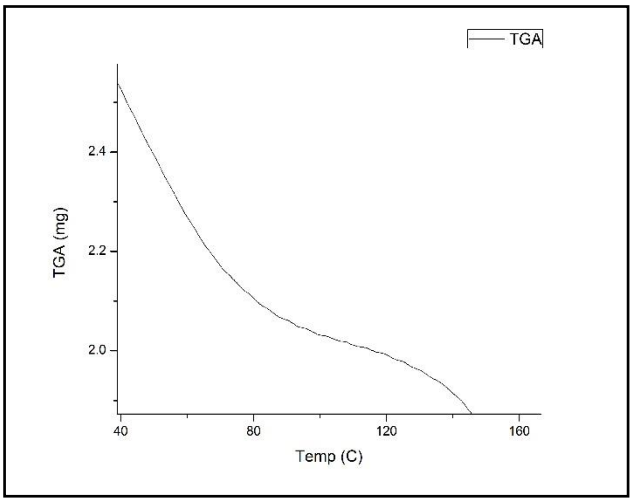


B

Figure 18: TGA analysis graph (A) Commercial alginate biopolymer film (B) Crude alginate biopolymer film

From Figure 18 the T50 % (temperature at which 50 % of weight loss occurred) for commercial alginate biopolymer film and crude alginate biopolymer film are at 60 ℃ and 80 ℃, respectively. This indicates greater stability of crude alginate biopolymer film than the commercial alginate biopolymer film. Commercial alginate is often subjected to purification procedures aimed at eliminating impurities and contaminants, thereby achieving a composition that is more uniform in nature. In contrast, crude alginate may harbour a greater concentration of impurities or exhibit variations in composition, factors that can significantly influence its thermal stability (Lee & Mooney, 2012).

4.7.6 Antioxidant Test:

The scavenging percentage of commercial alginate biopolymer film and crude alginate biopolymer was determined to be 12.60 % and 31.09 %, respectively. Free-radical scavenger antioxidants react with DPPH to produce DPPHH, which exhibits lower absorbance compared to DPPH due to a reduced hydrogen content. This radical form, relative to the DPPH-H state, induces decolorization characterized by a yellow tint as the accumulation of electrons rises (Baliyan et al., 2022).

A study demonstrated that the antioxidant potential of alginate biopolymer ranged between 30 % and 70 %. (Dysjaland et al., 2022) The antioxidant capability of alginate biopolymer film can be boosted through various methods, including: breaking the polymer chain, elevating the concentration of crosslinking agent, incorporating green propolis extract, and adding tannic acid. (Yerramathi et al., 2021).

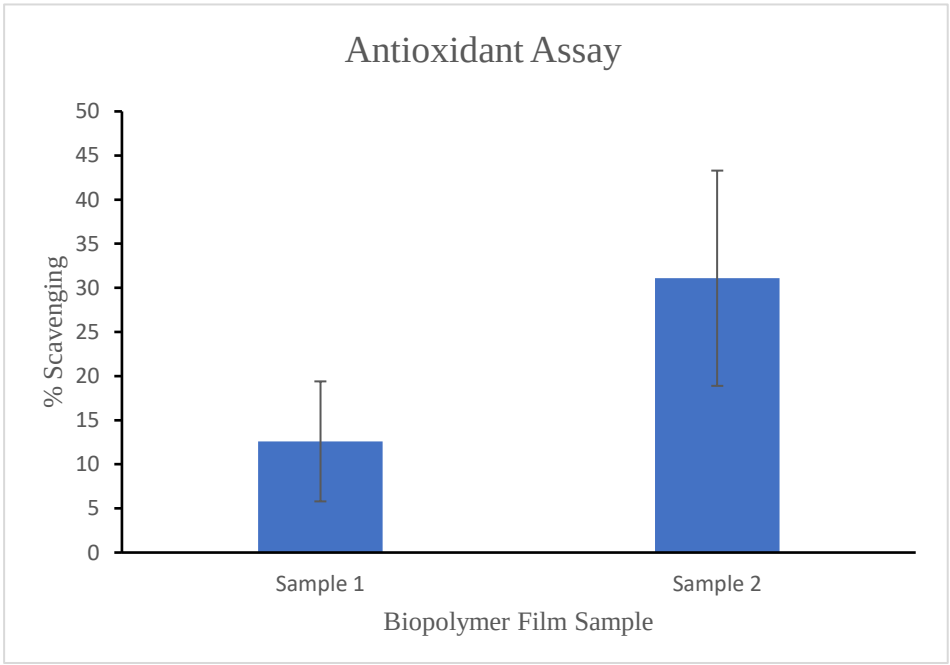
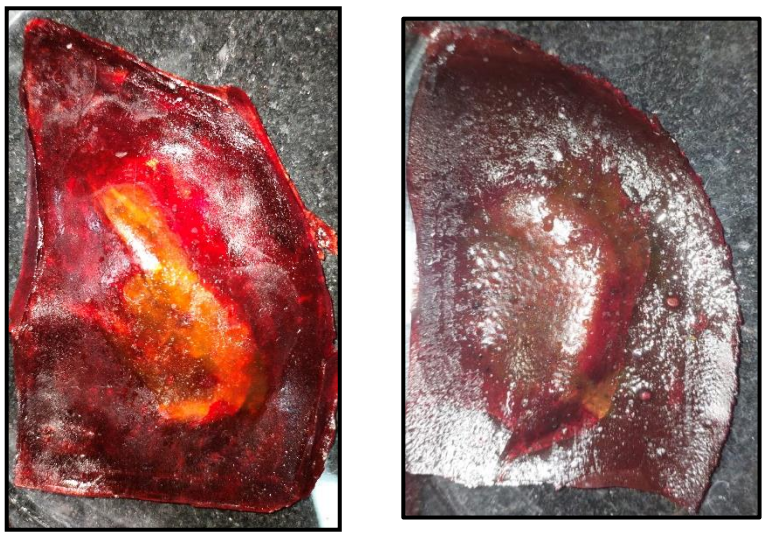


Figure 19: - Graph of antioxidant test, Sample 1- Commercial alginate biopolymer film Sample 2- Crude alginate biopolymer film

4.8 Application in food packaging:



A B

Figure 20: - Colour change after incubation for 48h. (A) Commercial alginate film (B) Crude alginate biopolymer film

Table 10: Protein analysis of shrimp

Shrimp	Concentration of protein
Before packaging	0.54 mg/mL
After 48 h incubation (Room temperature 23 ^{o}C)	0.45 mg/mL
After 48 h incubation (4 ^{o}C to 8 ^{o}C)	0.46mg/mL
After 48 h incubation (0 ^{o}C to -17 ^{o}C)	0.46 mg/mL

As seen in Table 9, the protein concentration was decreased from 0.54mg/mL to 0.45 mg/mL (23 ^{o}C), and 0.46 mg/mL (0 ^{o}C to -17 ^{o}C), 0.46 mg/mL (4 ^{o}C to 8 ^{o}C). A study carried out by Odeyemi et al., (2018) reported that spoilage of shrimp can result in a decline in its protein concentration. Consequently, over time, the overall protein concentration of the spoiled shrimp diminishes (Odeyemi et al., 2018).

It was noted that the colour of the bioplastic containing the shrimp changed from red to a slight yellow hue following a 48-hours incubation period. This alteration in coloration is attributed to the spoilage of the shrimp. Furthermore, research by Das and Mishra (2023) suggests that while the pH of fresh shrimp typically maintains a slightly basic or neutral range (7-7.5) spoilage can lead to a decrease in pH (4.5-5.5) This is due to the accumulation of acidic compounds produced by spoilage bacteria (Das & Mishra, 2023). These observations underscore the dynamic changes that occur in shrimp during the spoilage process, emphasizing the importance of monitoring both visual indicators, such as colour changes, and biochemical parameters, like pH levels, protein degradation for quality assessment in seafood.

CONCLUSION

- **Analysis of functional groups:** Research suggests that bioplastics derived from alginate polysaccharides typically contain hydroxyl and carboxylic groups. Through FTIR analysis of the provided biopolymer film, it was identified that the sample possesses hydroxyl groups at a wavenumber of 3271.35 cm^{-1} and carboxylic groups at 1398.1 $cm.^{-1}$Consequently, it can be interpreted that the given biopolymer film falls under the category of bioplastics.

- **Structural analysis:** Bioplastics such as polylactic acid (PLA) usually exhibit an amorphous nature due to the absence of organized molecular arrangements, thereby lacking a distinct crystalline structure. The amorphous structure of the synthesized biopolymer film, as revealed by XRD analysis, is consistent with the characteristics of bioplastics.

- **Biodegradability**: As per ISO 14855:1999 standards, the acceptable biodegradability threshold is at least 90 % total or 90 % maximum disintegration of a reference substance within six months. Comparatively, the observed 80 % weight loss within 28 days of the synthesized biopolymer films meet the biodegradation criteria for bioplastics.

- **Application**: The biopolymer film was used for intelligent food packaging. The pH indicator alizarin showed color change due to spoilage of food from red to yellow after incubation at room temperature (23 °C), freezer (0 to -17 °C) and in Ice box (4 °C to 8 °C) for 48 h. Further, the spoilage of food was confirmed by decrease in protein concentration from 0.54 mg/mL to 0.45 mg/mL and 0.46 mg/mL.

FUTURE PROSPECT

- Bleaching the crude alginate sample for better visibility of the color change.
- Characterisation of the biopolymer Film using Raman spectroscopy and determination of overall migration rate test
- Testing the application of biopolymer film for milk packaging.

APPENDIX

1. DPPH Reagent

Dissolve 1mg of DPPH powder in 13 mL Ethanol

2. Bradford Reagent

Dissolve 100 mg of Coomassie Brilliant Blue G in 50 mL of 95% ethanol. Then, add 100 mL of 85% phosphoric acid and 850 mL of distilled water.

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