DEVELOPMENT OF BIODEGRADABLE NANOCOMPOSITE FILMS FOR EXTENDING THE SHELF LIFE OF GINGER AND CORN

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DECLARATION

I, hereby declared that the presented work in the thesis entitled “**Development of Biodegradable Nanocomposite Films for Extending the Shelf Life of Ginger and Corn**” in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of Dr. Madhuri Girdhar, working as Assistant Professor, in the Division of Research and Development of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled **Development of Biodegradable Nanocomposite Films for Extending the Shelf Life of Ginger and Corn** submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the School of Bioengineering and Biosciences, is a research work carried out by Zeba Tabassum, 11919632, is bonafide record of her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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Abstract

The creation of biodegradable nanocomposite films appears as a viable option in a world struggling with the environmental problems brought on by non-biodegradable plastics and the requirement to assure food security through increased shelf life. This research work conducts a thorough investigation of this novel approach with the goal of advancing both sustainable packaging techniques and the preservation of perishable items. Plastic waste is accumulating at an alarming rate, and its negative effects on ecosystems and human health have highlighted the need for a swift switch to more environmentally friendly options. Biodegradable polymers have become a popular choice, and combining them with nanoparticles may improve their functional qualities, making them appropriate for packaging. The main goal of this study is to design biodegradable nanocomposite films with specialized features to handle the complicated issues associated with perishable goods preservation. This thesis has 5 chapters namely: (i) introduction, (ii) review of literature, (iii) objectives, materials and methods, (iv) result and discussion and (v) summery and conclusion. The study starts off with a thorough literature analysis, that offers information on the current state of biopolymers, nanomaterials, and their potential for cooperation in sustainable fabrication of packaging materials. Barrier performance, mechanical strength, moisture regulation, and antibacterial activity are important features considered here. Reviewing literature led to material selection followed by starting of experimental section. Chitin, a naturally occurring polysaccharide found in the shells of crustaceans like prawns and crabs as well as in the cell walls of fungi, is the source of the biopolymer chitosan. Due to its natural film forming ability, inherent antimicrobial property, biodegradability, biocompatibility, and adaptability, chitosan has recently attracted a lot of interest for its possible use in the creation of bioplastics. Overall, chitosan-based bioplastics show promise as a long term and sustainable replacement for traditional plastics. But in order to fully realize their promise and overcome current constraints, just like with any new technology, more research and development is required. A natural biopolymer called xanthan gum [$(C_{35}H_{49}O_{29})_n$] is created when the bacteria *Xanthomonas campestris* ferments carbohydrates. Due to its distinct rheological characteristics, it is frequently employed as a thickening, stabilizing, and emulsifying agent in the food and industrial sectors. Xanthan gum can play a number of functions when it comes to improving the properties of other biopolymers, including improving stability, film forming capacity, compatibility, and miscibility. To acquire the desired qualities in the finished product, however, experimentation and optimization are often necessary. Successful formulation also depends on potential interactions and compatibility between the biopolymers. Montmorillonite (MMT) nanoclay is a common type of nanoparticle used in the production of nanocomposite packaging films. The development of bioplastics and biocomposites packaging has drawn substantial attention to nanoclays, particularly montmorillonite (MMT). Traditional plastics made from petroleum can be replaced by bioplastics, which are more environment friendly. Bioplastics can have their mechanical, thermal, barrier, and biodegradable qualities improved by adding nanofillers, which makes them appropriate for a variety of packaging applications. On the other hand, numerous studies have also shown that natural polysaccharides and their composites comprising nanofillers like nano ZnO particles, have average to outstanding parameters in terms of antibacterial property, superior thermal stability, increased hydrophobicity, declined solubility, and even in certain instances show better barrier ability than commercial packaging. So, the purpose of the current research was to create chitosan and xanthan gum composite incorporating ZnO nanoparticle or montmorillonite nanoclay, in various ratios, and to analyze their functional as well as nano structural characteristics for possible application in degradable food packaging that have not been previously investigated or published. To create a variety of film compositions, the films were created using solvent casting techniques, altering biopolymer ratios, and sequentially adding nanomaterials (different %). Comprehensive characterization of the synthesized biodegradable nanocomposites is an important aspect of our research. Thickness of all the films were measured through FE-SEM analysis, which shows increase in the amount of components and their ratios, led to an increasing trend in their thickness. Opacity of all the films also followed the same rule. FE-SEM analyzed surface morphology of the films, indicating presence and distribution pattern of nanofillers in the biopolymer blend matrix. EDX and mapping was done to investigate elemental analysis and their distribution. Tensile testing was used to evaluate mechanical properties and show how the introduction of nanomaterials affects the strength and flexibility of films. FTIR analysis revealed intermolecular

interactions. TGA analysis, furthermore revealed that all the films possessed thermal stability almost similar to the control. Measurements of oxygen permeability and water vapor transfer rates were used to assess barrier performance. Due to the tortuous path effect and increased material density, the nanocomposites have better barrier characteristics than the neat biopolymer. Presence of nanomaterials proved to enhance UV light absorbance, which acted as UV shield. Additionally, the films' water uptake behavior was examined to give light on their ability to control the moisture content of packed perishable goods. Evaluation of the nanocomposite films' biodegradability is done in addition to their functional performance. Tests of standard biodegradation were carried out indoor by burying in natural soil. The films showed a notable amount of disintegration over the testing period for the first two months, demonstrating their potential to reduce the buildup of plastic trash. Inhibiting microbial development is one of the key elements of perishable products preservation. In order to do this, antimicrobial efficacy related to the nanocomposites was investigated. The films demonstrated substantial inhibition against both gram strain bacteria *Staphylococcus aureus* and *Escherichia coli*, along with fungi representative *Candida albicans*. Inherent antimicrobial property of chitosan, along with utilization of nanofillers are thought to damage microbial cell membranes and prevent their growth. The optimized film ratio (chitosan and xanthan gum blend comprising 3% ZnO nanoparticles) was finally subjected to additional antimicrobial efficacy test against 3 more microbial strains, shelf-life studies, and toxicity test. This film exhibited significant antimicrobial efficiency against gram positive bacteria *Bacillus subtilis*, gram negative bacteria *Pseudomonas aeruginosa*, fungi *Aspergillus niger*. For shelf-life studies, ginger and corn were chosen as representative of perishable items. Ginger slice and corn seeds were wrapped with the best ratio film, and observed for one week. The fabricated film with best ratio prevented spoilage of the perishables. For assurance of safety, the cytotoxicity of the optimized film among all the manufactured packaging materials were evaluated. In conclusion, by providing a thorough analysis of the fabrication of biodegradable nanocomposite films in an effort to improve the shelf life of perishable goods, this thesis makes a contribution to the field of sustainable packaging. The study emphasizes how these films have the ability to address important issues in food preservation, such as barrier qualities, mechanical

strength, moisture regulation, and antibacterial activity. One can obtain a comprehensive approach to sustainable packaging by combining biopolymers with nanomaterials. 3% ZnO incorporated chitosan-xanthan gum composite turned out to be the best film among all in regarding its reliability, consistency, strength, flexibility, antibacterial efficacy, improved resistance to water vapor, oxygen and UV; as well as low toxicity. The research opens the door for the packaging sector to adopt biodegradable nanocomposite films, providing a promising means of reducing plastic pollution and ensuring the integrity and safety of perishable goods.

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"Indeed, Allah is with the patient." (Quran, Surah Al-Baqarah, 2:153)

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This thesis work is dedicated to my loving husband, Mostafizur Rahman, who has been a constant source of support and encouragement during the hardship and difficult times. Words cannot express my gratitude. I’ve learnt so many things from him. He always inspired me that education and hard work never fails. I could not have undertaken this journey without him and this endeavor would not have been possible without his support. His willingness to provide a nurturing home environment allowed me to focus on my research and studies and his shared responsibilities lightened my load, making this achievement possible. I’m extremely grateful to him for his unwavering support in every step/stage, for keeping belief in me, for being always there for me in every thick and thin and for giving me wings to fly.

This accomplishment is not only mine, but it belongs to our family as a whole. In my baby girl’s words “abbu ammu and iqra together family”. Their support has been my foundation, I am thankful for their sacrifices during my academic pursuits and I look forward to celebrating many more milestones together.

This work is also dedicated to those girls who lives in a small village in Baramba, West Bengal, and don’t get the opportunities to complete their education. I, myself being the first girl to study science, first one to do Masters and PhD from that village, hopes that I can inspire and encourage them with more work and researches.

I, here acknowledge all of my family members, including my parents, in laws, and other relatives, for their love, prayers and encouragement.

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Abbreviation used

S. No.	**Abbreviation**	**Full Form**
1.	C	Chitosan film (pure)
2.	CX	Chitosan-xanthan gum blend
3.	CXM1	Chitosan-xanthan gum-1 % montmorillonite nanocomposite
4.	CXM3	Chitosan-xanthan gum-3 % montmorillonite nanocomposite
5.	CXM5	Chitosan-xanthan gum-5 % montmorillonite nanocomposite
6.	CXZ1	Chitosan-xanthan gum-1 % nano ZnO nanocomposite
7.	CXZ3	Chitosan-xanthan gum-3 % nano ZnO nanocomposite
8.	CXZ5	Chitosan-xanthan gum-5 % nano ZnO nanocomposite
9.	UV	Ultra Violet
10.	OTR	Oxygen Transmission Rate
11.	OP	Oxygen Permeability
12.	WVTR	Water Vapor Transmission Rate
13.	WAC	Water Absorbance Capacity
14.	TGA	Thermogravimetric Analysis
15.	FTIR	Fourier Transform Infrared spectroscopy
16.	FE-SEM	Field Emission Scanning Electron Microscopy
17.	MMT	Montmorillonite
18.	PHBV	Poly(3-hydroxybutyrate-co-hydroxyvalerate)
19.	LDPE	Low-density polyethylene
20.	LLDPE	Linear low-density polyethylene

CHAPTER I

INTRODUCTION

1. Introduction

A recent analysis from the Food and Agriculture Organization estimates that each year 1.3 billion tons of food are wasted. Food waste comes from the food industry and post-harvest agricultural processing, which is an extreme risk to both the pre-market and post-market sites (Ananno et al., 2021; Blakeney 2019; Ranganathan et al., 2020). Food waste also has an ethical angle, as a large proportion of population around the world is starving, still huge amount of food goes wasted (FAO, 2010). According to a study, the packaging of food items may be responsible for around 20– 25% of household food waste. The packaging related waste is mostly caused by some packaging related factors, such as larger and difficult-to-open packages, as well as waste from expired "best before" dates (Williams et al., 2012).

The amount of packaged food consumed has significantly increased recently. The market for packaged foods worldwide was estimated to be worth $1.9 trillion in 2020 and is projected to expand at a 5% yearly rate to reach $3.4 trillion by 2030 (Kan & Miller, 2022). Two million tons in 1950 to 381 million tons in 2015, the yearly worldwide plastic manufacturing increased dramatically during the previous six decades and nearly 40% of all plastic consumption is accounted for packaging (Kan & Miller, 2022). Packaging is the largest contributor to the world's non-degradable plastic garbage, accounting for over 50% of the total weight (Ncube et al., 2021). Plastic packaging is widely used and discarded, raising serious environmental issues, with negative consequences on ecosystems as well as human well-being. Their persistent nature is one of the main problems, as they take hundreds of years to disintegrate. This causes the discharge of dangerous chemicals and microplastics, which contaminate the food chain, the soil, and water supplies. Due to its heavy reliance on fossil fuels, the manufacturing of plastic packaging contributes significantly to greenhouse gas emissions. Its resources' extraction, refinement, and transportation all result in the release of greenhouse gases, aggravating climate change. When plastic packaging is disposed off by burning it, hazardous chemicals and air pollutants are released, lowering air quality and endangering human health. Poor waste management practices and a lack of recycling facilities contribute to plastic pollution in both urban and rural areas (Kabir et al., 2020; Ncube et al., 2021; Nwafor & Walker, 2020; Okunola et al., 2019; Thushari & Senevirathna, 2020; Verma et al., 2016).

Considering the negative effects of traditional plastic packaging on the environment, eco-friendly substitute packaging is absolutely necessary, which will meet the technical requirements, along with the ability to extend duration of their shelf lives. Adopting sustainable alternatives can cut carbon emissions, lessen pollution, and protect ecosystems, encouraging a more resilient and environment friendly approach to packaging solutions.

1.1 Background and Significance of Food Packaging

Food safety and preservation are the top priorities in the food business and enterprises, and the importance of packing is directly related to these issues. Food packaging safeguards perishables from outside influences that can improve their quality, safety, and shelf life. Problems with food waste includes foodborne infections, and the need to ensure that food reaches consumers in the best possible condition, are very important issues to consider. Food goods are protected by packaging from harmful elements such air, moisture, light, germs, and physical damage. For instance, whereas moisture can result in mold and other microbial growth, oxygen and UV light can promote oxidative reactions. A controlled environment is created by packaging materials with the right barrier qualities, minimizing interactions between the food and its surroundings and preserving the food's integrity. Effective food packing considerably lowers the risk of foodborne illnesses by preventing the introduction of dangerous substances and microbes. It guarantees that the food keeps its flavor, texture, and nutritional content for the duration of its shelf life. This is especially important for perishable goods like fresh produce, dairy products, and meat since they are prone to contamination and spoiling. Additionally, packaging is essential for avoiding physical damage during handling and shipping. Food items are protected by packaging against impacts, crushing, and bruising, retaining their quality and appearance, until they are consumed. Packaging not only safeguards food goods but also gives consumers important details including expiration dates, nutritional information, allergen warnings, and storage recommendations. In addition to ensure correct handling and consumption of the product, clear and accurate labelling helps consumers to make right decisions. Food packaging minimizes food waste, cuts down on financial losses, and promotes sustainable practices by maintaining the quality and freshness of the items (Ashfaq et al., 2022; Marsh & Bugusu, 2007; Shaikh et al., 2021). Suitable characteristics and types of a food package are shown in figure 1.1 & 1.2.

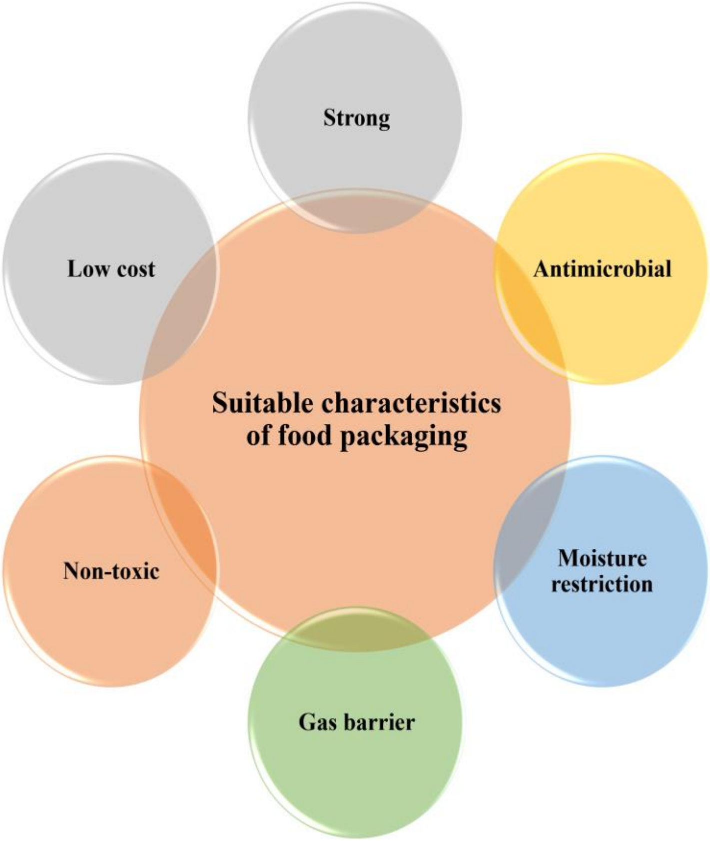


Figure 1.1: Suitable characteristics of food packaging

The food sector has been transformed by new packaging technologies that provide creative ways to improve product quality, safety, and convenience. As customer demands and preference change, these cutting-edge technologies take advantage of developments in materials science, engineering, and nanotechnology to meet their requirements and preferences while simultaneously promoting sustainability and minimizing environmental effect. Incorporating nanoparticles into the packaging matrix to create nanocomposite materials enhances the barrier characteristics, mechanical strength, and antibacterial qualities. By extending the shelf life of perishables and lowering the requirement for preservatives, these materials have the possibility to minimize food wastage. The need of sustainable packaging is rising as awareness over plastic waste and its effects on the environment grows. Unlike traditional plastics, biodegradable/compostable packaging are derived from renewable resources, offer eco-friendly approach.

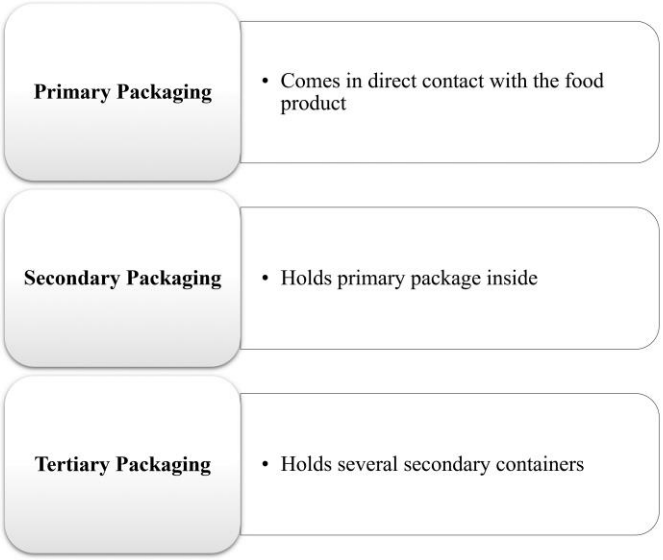


Figure 1.2: Main 3 types of packaging

Changes in consumer demand, technical development, and a growing emphasis on sustainability and environmental responsibility will all going to have an impact on future trends in food packaging. The food industry is attempting to get around these challenges while searching for innovative answers that will meet consumer demands, ensure product safety, and have the least possible negative effects on the environment; in order to eliminate single use plastics and encourage recycling and composting, as consumers and regulatory organizations are increasingly pressing for sustainable packaging alternatives. Packaging materials that are biodegradable, compostable, and edible are becoming more popular as feasible eco-friendly alternatives. As, for both customers and the industry, food safety is still a major priority, packaging must adhere to laws governing food contact and have effective barrier qualities to prevent contamination. However, balancing convenience and sustainability is still difficult task (Kabir et al., 2020; Khalid & Arif, 2022; Saha et al., 2020; Siracusa & Rosa, 2018).

1.2 Historical Prospects of Food Packaging

The packaging of food has undergone remarkable transformations over time, aligning with the evolving lifestyles of people, as depicted in Figure 1.3. During the nomadic period of human history, food was consumed wherever it was found. As humans transitioned to settled communities during the Stone Age, there were emergence of preserving leftover food practice in natural vessels. These vessels included hollow tree trunks, hollow stone pieces, animal skin, organs, leaves, and shells. Subsequently, humans gradually developed earthen pots, bamboo containers, and grass-woven baskets for storage of food. The ancient Egyptians introduced the concept of glass containers, while in China, the production of paper from mulberry bark provided a novel packaging strategy. The demand for long-lasting food preservation gained prominence during the time of Napoleon Bonaparte, leading to the discovery of the canning method by Nicolas Appert. This innovation enabled the preservation of food for extended periods. In the 1800s, commercial packaging entered the market in England, propelled by Peter Durand's patent for tin-coated iron cans used for preserving food. Within a decade, canned food found extensive usage, including its supply in large quantities to the Royal Navy. The advent of plastics revolutionized food packaging due to their low permeability to oxygen and water vapors, enhancing food preservation (Risch, 2009).

Following World War I, there was a notable emergence packaging material including molded glass, cardboard boxes, metal cans, and cellophane into the market. Subsequently, after World War II, the "use and throw" strategy led to the popularity of aluminum foil and plastics (Robertson, 2019; Rossa et al., 2022). Prior to these developments, packaging was primarily limited to luxury goods. In the 21st century, the packaging industry has gained global popularity. Food packaging has evolved beyond being a mere container for storing food; it now plays a pivotal role in influencing the food quality. With increasing environmental concerns, growing attention has been shown in the application of biodegradable polymers as an alternative to petroleum-derived packaging. These sustainable materials offer several advantages, including the replacement of non-biodegradable packages, increased food shelf life, and reduced environmental pollution by minimizing solid waste. In summary, the packaging of food has continually evolved throughout the history to cater the changing needs and preferences of individuals. From rudimentary natural vessels to sophisticated modern packaging, the industry has progressed significantly. The current focus on biodegradable polymers exemplifies the ongoing efforts to enhance food packaging's sustainability while ensuring food quality and minimizing environmental impact.

Figure 1.3: Evolution of packaging

1.3 Conventional Food Packaging Materials

Packaging materials play a pivotal role in safeguarding products during transportation, storage, and retail distribution. The widespread usage of traditional packaging materials over time has aided in the expansion of the world economy. Materials for packaging are essential in today's consumer driven economy because they make it easier to carry goods securely and effectively. Main conventional food packaging materials includes glass, paper, metal, and plastics. Conventional packaging materials have traditionally been utilized due to their cost effectiveness and functionality. Glass, paper, metal, and plastics are the primary materials used in conventional food packaging (Tajeddin & Arabkhedri, 2020). Disadvantages associated with these materials shown in figure below (figure 1.4). However, concerns about environmental sustainability have recently motivated the investigation of greener options.

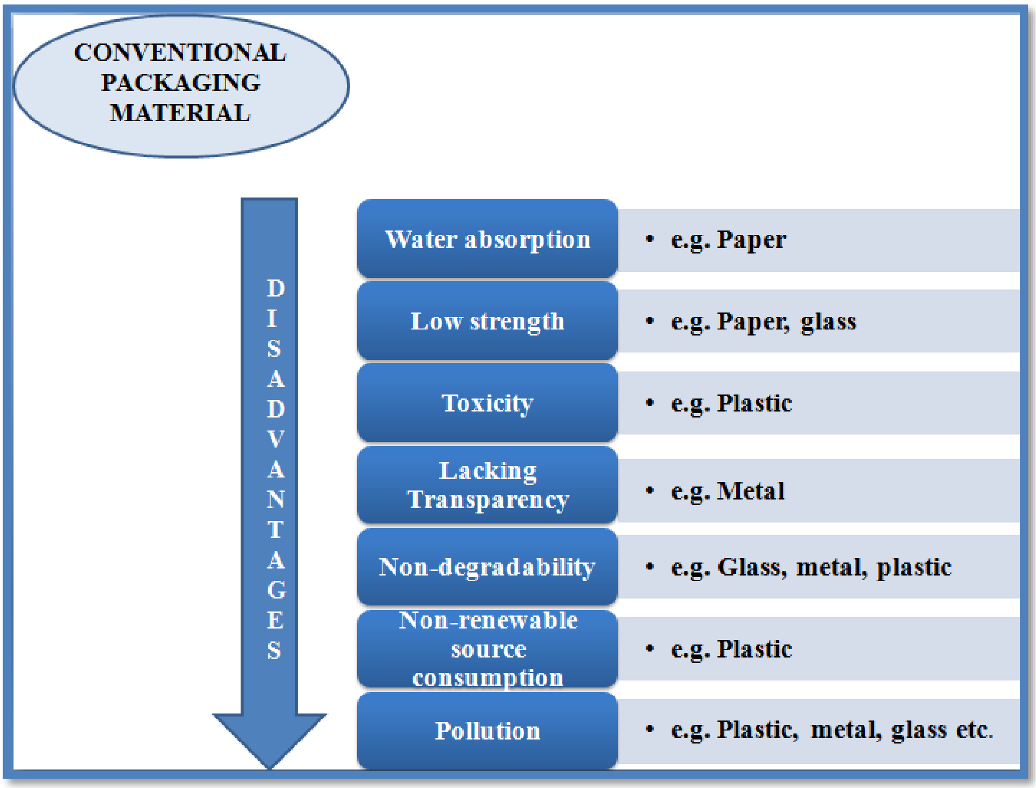


Figure 1.4: Drawbacks of traditional packaging materials

1.4 Environmental Impact of Conventional Packaging

Product protection, shelf-life extension, and distribution ease are all significantly impacted by packaging. However, the extensive use of conventional packaging materials has brought up questions over how they may affect the environment.

Although glass is recyclable, its weight and energy requirements during production and shipping result in increased carbon emissions. Despite the fact that glass is inert and does not release dangerous chemicals into the environment, the collecting and transportation of glass debris for recycling can be expensive and energy intensive (Gallucci et al., 2021; Robertson, 2019).

Metal packaging includes aluminum and steel cans, for example, have a high recycling rate and use very little energy during production. Nevertheless, the extraction and processing of metals can have a negative impact on the environment, resulting in things like habitat destruction and water contamination. In some areas, the removal of bauxite for the manufacturing of aluminum is linked to deforestation (Deshwal & Panjagari, 2020; Rossa et al., 2022).

Paper and cardboard are more environmentally friendly option, as they are renewable and biodegradable materials. However, the process of making paper from trees uses a lot of water and energy, which contributes to deforestation and the release of greenhouse gases. Paper recycling can lessen these effects; however, contamination and low recycling rates are still problematic (Robertson, 2019).

Plastic has definitely shown itself to be the greatest option among all the traditional packaging materials for a variety of purposes. Its unmatched qualities have solidified its place in a variety of industries as a packaging material. Over the last six decades, plastics have grown to be an essential and required component of our everyday lives, owing to their adaptability, broad variety of qualities, chemical composition, and countless application.

Plastics exceed other materials for packaging (paper, wood, cloth, and metals) in terms of accessibility, mechanical versatility, resilience, minimal weight, and economic viability. Even though plastic was formerly thought to be innocuous and inert, years of dumping plastic led to a number of issues. The pollution caused by non-biodegradable plastic garbage is now universally recognized as a significant environmental issue (Chinomso Iroegbu & Ray, 2022). In particular, plastics among conventional packaging production increase greenhouse gas emissions. These emissions worsen environmental issues and have negative effects on climate change. Furthermore, concerns regarding the sustainability of materials like metals and polymers made from fossil fuels are raised because they deplete finite resources.

1.4.1 Plastic Pollution

Due to their adaptability, durability, and affordable production, plastics, such as polyethylene, polypropylene, and polyethylene terephthalate, dominate the packaging industry. They have exceptional barrier qualities that shield the packaged goods from being harmed by water vapor, gases, and light (Tajeddin & Arabkhedri, 2020).

Because plastic packaging is not biodegradable, it has grown to be a huge environmental concern. Ecosystems and wildlife have significantly threatened by plastic packaging waste. Plastic pollution from improper disposal, harms human health, ecosystems, and marine life. Plastic garbage that is not properly disposed off ends up in landfills and oceans, where it takes hundreds of years to degrade (Thushari & Senevirathna, 2020). Many times, marine animals mistake plastic for food, which results in ingested deaths. Microplastics, tiny pieces of plastic, have also gotten into aquatic food systems, endangering marine biodiversity and possibly making it to human food supplies. Plastic packaging manufacturing utilizes a lot of fossil fuels, which increases greenhouse gas emissions and consequences regarding climate change. Additionally, the depletion of precious resources is caused by the shockingly low recycling rates for plastic consumption (Chinomso Iroegbu & Ray, 2022; Tabassum, Mohan et al., 2023). Around 6.3 billion tons of plastics were generated between 1950 and 2018, just 9% of which were recycled and 12% were burned (Okunola et al., 2019). Packaging accounts for the biggest amount of plastic usage (26% of total volume), reported by the International Energy Agency, and is expected to continue growing over the following years, potentially increasing to 318 million tons annually by 2050, which is more than the current size of the plastics industry (Shen et al., 2020). The fact that it is typically single-use kind, and there is extreme difficulty in the whole process of recycling, packaging comes under severe categories among plastic waste. Ecologically sound disposal methods for plastic or fuel-based polymers are still in their infancy. As a result, their wastes frequently leak harmful compounds and continue to be sources of pollution. It has been estimated that 800 metric tons of polymeric waste had been burned over the past 65 years (1950-2015). Although typical polymeric wastes may only be totally degraded through thermal treatment, burning produces harmful pollutants

(Kasmuri et al., 2022; Verma et al., 2016). Plastic has a carbon footprint that ranges from production to combustion of about 6 kg CO_2/kg plastic (Norman et al., 2022). Emitted greenhouse gas from the manufacture to disposal of plastics are predicted to reach 1.34 gigatons annually by 2030 and 2.8 gigatons annually till 2050, drastically exhausting the rest of the carbon budgets worldwide and endangering increase in global temperatures of 1.5°C to 2°C by 2100. The issue will deepen if the plastics industry intends to increase manufacturing on such a massive scale. The World Economic Forum predicts that between 2030 and 2050, the production and usage of plastics would expand at a 3.8% annual rate, then declining to 3.5% annually (Shen et al., 2020). For all these reasons mentioned, several nations around the world are posing ban on the use of plastic packaging, specifically on single use plastic (Tabassum, Mohan et al., 2023).

In summary, plastic packaging has unquestionably improved convenience and effectiveness in our daily lives, but it also has negative consequences on the environment that cannot be ignored. Society must actively look for sustainable solutions and realize the terrible effects of plastic on the eco system and health. We can all work together to reduce the negative consequences of plastic packaging and work towards a greener, more sustainable future by adopting eco -friendly products and supporting sustainability (Tabassum, Mohan et al., 2023).

1.5 The Need for Sustainable Packaging Solutions

Traditional packaging materials have long been effective at protecting goods and promoting trade. However, because of their negative effects on the environment, we must move towards sustainable alternatives. In modern times, concerns about the environmental effects and long-term viability of food packaging have grown significantly. As worldwide awareness of environmental challenges rises, consumers, businesses, and politicians are looking for sustainable packaging solutions that reduce the ecological impact and contribute to a future that is more sustainable. Conventional packaging materials, which frequently come from non-renewable resources like plastics derived from petroleum, have a huge negative impact on resource depletion, pollution, and climate change. Ecosystems and wildlife are seriously threatened by the everincreasing amount of single use packaging trash, much of it ends up in landfills, oceans, or as litter. By implementing eco-friendly procedures throughout the package lifecycle, sustainable packaging seeks to resolve these problems. Sustainable packaging solutions minimize negative environmental effects, save energy, and cut greenhouse gas emissions by utilizing eco-friendly designs and materials. For instance, utilizing marine, fish, or seafood waste to fabricate food packaging offers several socio-economic benefits. Firstly, it addresses the environmental issue of waste management by repurposing materials that would otherwise contribute to pollution. This recycling reduces the burden on landfills and minimizes the environmental impact of waste disposal from the fishing and seafood processing industries. Secondly, it promotes sustainability by creating value from what was previously considered waste, leading to a more efficient use of resources. Thirdly, it can contribute to economic growth by establishing new industries or products derived from these waste materials, thus generating employment opportunities and boosting local economies. Additionally, using biodegradable and environmentally friendly packaging materials derived from marine waste aligns with consumer preferences for sustainable and eco-friendly products, enhancing market competitiveness and potentially increasing market share for businesses adopting these practices. Overall, the financial growth experienced by fishermen, vendors and others in similar profession through the utilization of marine/fish/seafood waste underscores the importance of sustainable practices in both economic and environmental contexts. By adopting sustainable packaging, brands may create long term cost savings through lower material usage and waste disposal costs while also aligning with the values of their environmentally concerned customers. Businesses are also becoming aware of the financial and reputational advantages of implementing sustainable packaging practices as customer knowledge of and demand for environmentally friendly products rises. In addition, to helping firms engage with the idea of their environmentally concerned customers, adopting sustainable packaging promotes long term cost savings through lower material utilization and waste disposal costs. (Kabir et al., 2020; Shaikh et al., 2021; Siracusa & Rosa, 2018). The focus is shifting towards comprehensive life cycle approaches, embracing the ideas of circular economy and extended producer responsibility (EPR), which are about reducing waste and making companies responsible for their products' impact even after they are sold.

There's also a growing emphasis on a fair transition, which means making sure that as we make changes for the environment, we also think about fairness and include everyone in decision-making. This includes protecting people's rights and making sure that everyone benefits from these changes. We can create a future where packaging materials adhere to the ideas of environmental stewardship without sacrificing utility and economic growth through innovation, regulation, and conscious consumer choices.

1.6 Introduction to Nanotechnology in Food Packaging

Since the last decade, tremendous progress in the domains of nanotechnology transformed numerous scientific and industrial fields including the food sector. Since Nobel laureate American physicist Richard P. Feynman introduced "nanotechnology" in his famous address "There's Plenty of Room at the Bottom" during the American Physical Society's annual meeting at Caltech in 1959 (Feynman RP, 1960), it has become an exciting area of science and a growing sector of techno economic research. Richard Feynman pioneered the concept, while Norio Taniguchi established the term "nanotechnology" in 1974. The word "nano" originates from Greek language nanos or the Latin word nanus, both of which denote "dwarf" (Kolahalam et al., 2019). Nanotechnology is the manipulation and arrangement of molecules smaller than 100 nm, which can provide unique features by reducing particle size and raising contact surface area and reactivity. Researchers in the field of nanoscience worldwide are actively studying these unique attributes to develop innovative and upgraded tool through green processes. It is anticipated that this emerging field of science would aid in boosting both industrial output and the economy (Sharma et al., 2017). The essential building block of nanotechnology is nanoparticle. Nanoparticles have unique physical, chemical, mechanical, and biological properties because of their huge surface area and nano size range of 1 to 100 nm with one or more dimensions (according to ISO and ASTM standards). Due to their potential for a vast variety of uses in the fields of food packaging and coating, catalysis, pharmacology and health care, including tissue engineering, drug delivery, and medical implants (Liu & Kim, 2012), cosmetics, textile, agriculture, energy-based research, aerospace, electronics, sensors, and other environmental preservation (Baig et al., 2021;

Khan et al., 2019), they are good candidates for a variety of industrial and daily uses. Nanotechnology has played a major role in the technical advancements in food packaging in the twenty-first century by enhancing its characteristics for shelf-life extension of food products. This has become a separate priority study field as a result of the encouraging reports for nanotechnology interventions in food packaging. Food packaging consisting of nanomaterials, offers better mechanical and barrier capabilities, as well as food preservation, and is likely to be met with favorable feedback from the consumer market (Ashfaq et al., 2022; Sharma et al., 2017). Functional nanoparticles have demonstrated significant rise in strength, stability, and resilience of packaging materials, hence extending the duration of the storage time of food goods (Ashfaq et al., 2022; Shukla et al., 2019).

1.7 Biodegradable Nanocomposite Films: An Innovative Approach

In the field of sustainable materials, biodegradable nanocomposite films represent a cutting-edge and creative strategy. These films offer a multipurpose platform for a variety of applications by combining the benefits of biodegradable polymers with the integration of nanoparticles. The addition of nanomaterials gives the films special qualities like improved mechanical strength, barrier characteristics, thermal stability, and regulated biodegradability. Additionally, these cutting-edge biodegradable nanocomposite films tackle the serious environmental issues brought on by traditional plastic films, which frequently linger in the environment for extended periods of time and cause pollution and ecological harm. The films are created to spontaneously degrade into non-toxic components using biodegradable polymers and nanoparticles derived from sustainable sources, minimizing the environmental effect and fostering circular economy. Advanced processing methods, such as solvent casting, extrusion, or electrospinning, are used to create these films in order to ensure the nanofillers are evenly distributed and aligned within the biopolymeric matrix. Additionally, in order to improve the qualities and capabilities of these nanocomposite films, researchers are developing brand new bio-based nanomaterials and investigating already existing ones. Overall, sustainable nanocomposite films represent a fascinating and promising direction in the creation of environmentally friendly materials with a wide range of uses, including packaging, agriculture, healthcare etc. The continuous study in this area has enormous promise for promoting sustainable growth and reducing the environmental impact of contemporary businesses and technologies (Kumar et al., 2020; Ramos et al., 2018; Tabassum, Girdhar et al., 2023; Tabassum, Mohan et al., 2023).

While environment friendly alternatives show promising applications, in respect to the issues including cost, scalability, and consumer awareness need to be resolved. To promote the adoption of eco-friendly packaging practices, governments, businesses, and consumers must work together, so as to overcome the drawbacks resulting from the plastic alternatives.

The current study aims to prepare and characterize bio-nanocomposite films using chitosan and xanthan gum as the matrix, incorporating surface-modified montmorillonite (MMT) and zinc oxide (ZnO) nanoparticles in different ratios. The objectives include analyzing the structural morphology of the nanocomposites, evaluating their water vapor and gas barrier properties for packaging suitability, conducting biodegradation and toxicity tests, assessing antimicrobial activity against bacteria and fungi, and studying the shelf-life of perishables when packaged with these films.

CHAPTER II

REVIEW OF LITERATURE

2.1 Literature Review

2.1.1 Biopolymers

Sustainable materials are in high demand due to advancements made in creation of an environmentally friendly world. Bio-based polymers and their blends represents a sustainable alternative that has the potential to significantly replace fossil fuel-based polymers and mitigate their harmful effects. Harnessing biopolymers offers a great hope for preserving natural ecosystems and preventing further ecological devastation. Biopolymers, in accordance with the Inter-governmental Panel on Climate Change (IPCC), could help limit global warming to 1.5 ºC by reducing emissions of CO_2 up to 20% (IPCC, 2018). Recent trends in natural biopolymer research have shown a positive trend in terms of worldwide long-term strategies for sustainable development (Sun et al., 2022). Biopolymers have drawn a lot of interest due to their distinctive qualities, inexpensiveness, being biodegradable, non-toxic, biocompatible, economically viable, supply convenience, and broad area of uses (Choudhary et al., 2022). Evonik, a German company, for example, has recently created degradable biopolymer for extended release of drug (R. Song et al., 2018). Other research also endorsed biopolymer-based advanced biomedical applications (Bashir et al., 2014; Jeevanandam et al., 2022; Mohan et al., 2021; Nathanael & Oh, 2020). Furthermore, it has been reported that some natural microbial biopolymers can be utilized effectively in single use supplies, for example, sanitary pads, diapers etc. (Sinha, 2018). Additionally, it has been noted that current research trends are concentrated on developing substitutes for conventional food packaging, especially biopolymers appearing to be a potential option (Tabassum et al., 2023).

Figure 2.1 represents different sources of these biopolymers. Biopolymers are a class of polymers that come from microbial, synthetic, and natural sources. Natural biopolymers are made from renewable plant or animal resources, such as proteins (such as collagen, silk) and polysaccharides (such as cellulose, chitosan). Chemical processes are used to create synthetic biopolymers like polyhydroxyalkanoates (PHA) and polylactic acid (PLA) from renewable feedstocks like maize or sugarcane. Microorganisms produces microbial biopolymers like exopolysaccharides and bacterial cellulose. As biotechnology and material science research develops, biopolymers have the potential to replace synthetic polymers and contribute to a future that is more ecologically friendly.

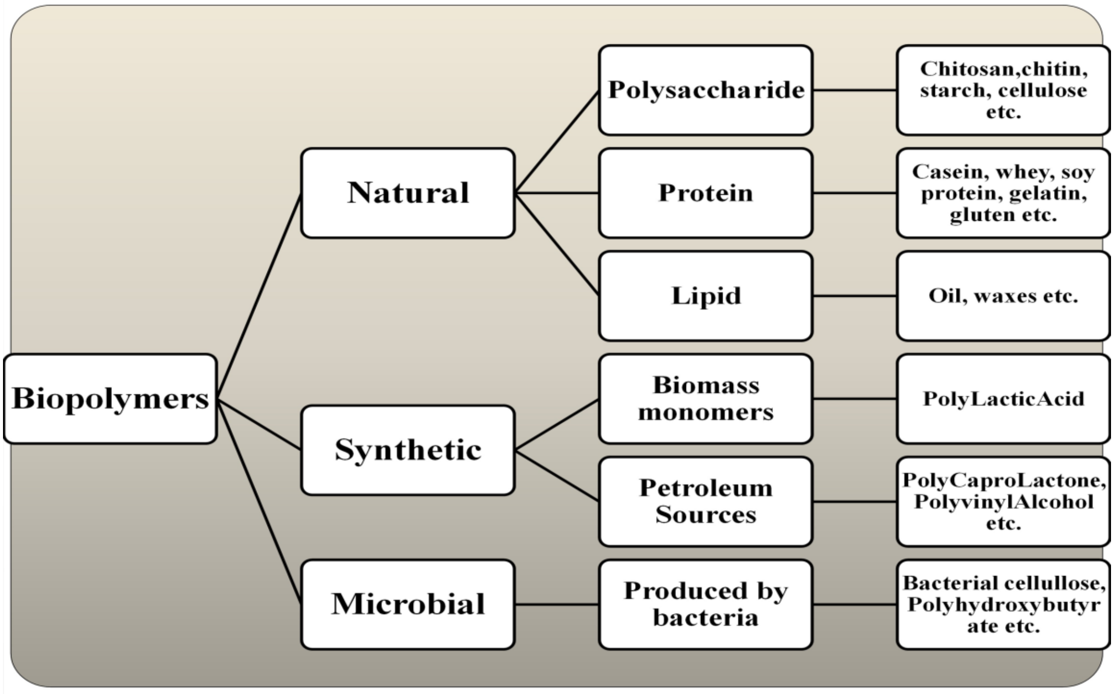


Figure 2.1: Different Sources of Biopolymers (Theagarajan et al., 2019)

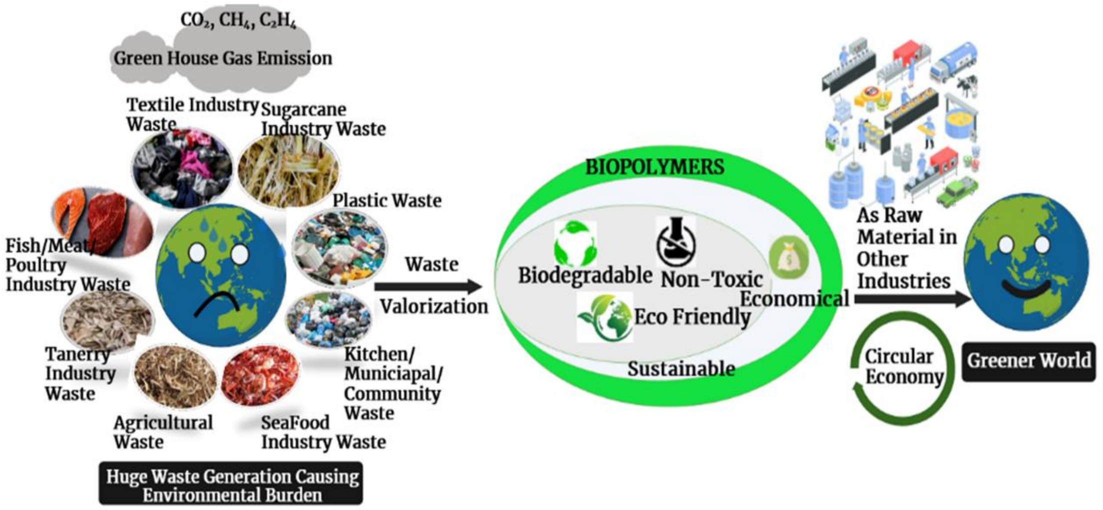


Figure 2.2: Biopolymers promoting sustainability for a greener planet

Biopolymers made from waste leftovers perform better than those made from other sources in terms of both expense and sustainability (Yaashikaa et al., 2022). Biopolymers, according to a number of researchers in this subject, are a better material than fuel-based plastics or other traditional materials (Silva & Blumberga, 2019).

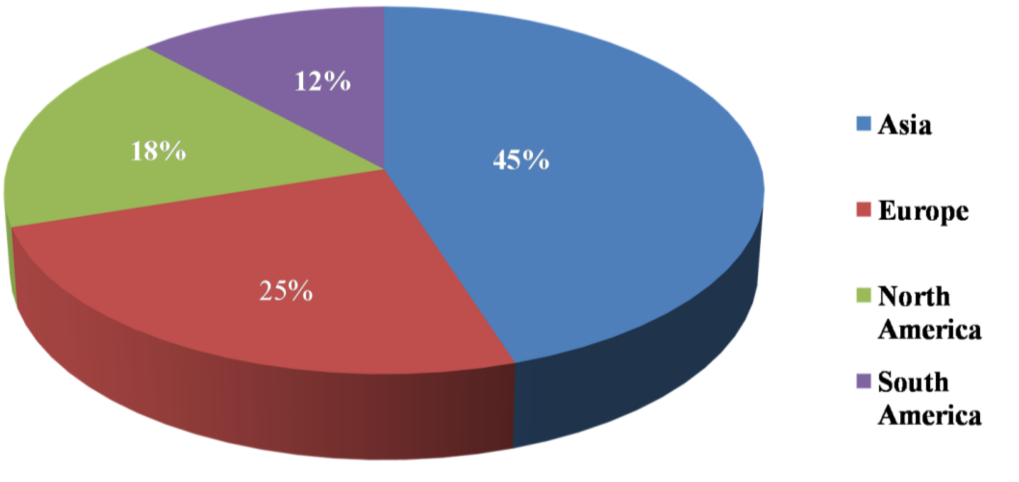


Figure 2.3: Global Production of bioplastic (Shaikh et al., 2021)

In recent years, there has been a substantial rise in research focused on biobased polymers due to their potential practical applications. This surge in research is driven by the growing awareness of environmental issues and the need for sustainable materials. Biobased polymers offer a promising solution as they are derived from renewable resources and are biodegradable, reducing the dependency on fossil fuels and minimizing environmental harm. This increased research effort signifies a shift towards more eco-friendly and sustainable material choices in various industries.

From 2000 to 2002, yearly publication on bio-based polymers was just getting started. There were 67 articles published in total, and despite the small number of articles during this time, they were getting frequently cited. From 2003 to 2017, there was a rapid increase in research in this field, with 1,694 publications altogether. 2018 through 2021, it was experiencing decent expansion, with 1,746 articles published overall, around 437 articles published on average each year, 76,852 citations overall, and 19,213 citations on average per year. In this meantime, each year the number of articles published peaked at 623, with 27,546 citations (Y. Sun et al., 2022). Biopolymer market size, on the other hand, was 6.95 billion dollars in 2018 and is anticipated to increase to 14.92

billion dollars by 2023, at a compound annual growth rate (CAGR) of 16.5% (Globe Newswire, 2019). Even leading modern service industries like Samsung and Apple are also opting to employ biopolymers in their products and packaging (Kabir et al., 2020). The European Union is the world's largest user of biopolymers, as an outcome of strict laws and popular demand to adopt biopolymers to mitigate the detrimental effect on environment caused by fossil fuel based polymers. The biopolymer market of Asia-Pacific region is projected to expand consistently, as a result of growing environmental awareness and international demands for pollution reduction (Kabir et al., 2020).

2.1.2 Biopolymer Blends, Nanofillers and Bionanocomposites

Biopolymers often blended with other polymers or nanoparticles are incorporated for practical applications due to several key reasons. Firstly, this process enhances their mechanical strength and durability, making them suitable for a variety of products. Secondly, blending improves specific properties like barrier performance, crucial for packaging to preserve perishable items. Nanocomposites offer unique advantages such as increased surface area, improved thermal stability, and sometimes enhanced antimicrobial effects, making them valuable in advanced fields like medicine or electronics. Furthermore, these blends and nanocomposites overcome individual biopolymers' limitations, like brittleness or processing challenges, broadening their potential applications across multiple industries.

Biopolymers generally have less resistance to mechanical force and chemical stability, when compared to the polymers derived from petroleum (Choudhary et al., 2022). Scientists have worked hard to overcome such limitations in order to compete with plastic in practical applications. Biopolymer blends are an innovative strategy which brings together the special qualities of various biopolymers to produce improved material performance. Scientists can customize the thermal, mechanical, and barrier properties of biopolymers by blending them according to the needs of particular applications. Other than biopolymers, plasticizers, plant extracts or oils, nanoparticles, and other ingredients have also been included in this approach (Nastasi et al., 2022; Vieira et al., 2011; Zubair et al., 2022).

Particles at the nanoscale known as nanofillers are revolutionizing many different sectors. Owing to large surface area to volume ratio, they have special qualities that improve mechanical, thermal, and barrier properties of biopolymer matrices (Heiligtag & Niederberger, 2013; Sanità et al., 2020). Nevertheless, an expanding class of materials called bionanocomposites combines biopolymers with nanomaterials to produce cutting-edge composites. The thermal, mechanical, and barrier properties of biopolymer matrices are improved significantly by addition of nanofillers like nanoparticles, nanoclays, and nanotubes. Remarkable characteristics of bionanocomposites include higher strength, decreased gas permeability, and enhanced antimicrobial activity. Biopolymers and nanofillers work together synergistically to create materials with several uses, including packaging for food, biomedicine, and environmental remediation. These biocompatible and sustainable composites provide promising answers to today's materials science problems, helping to create a greener and more technologically advanced world (Ashfaq et al., 2022; Sharma et al., 2017).

Reducing repulsive forces within biopolymer chains presents an intriguing alternative for enhancing the characteristics of polymer blends (Sadasivuni et al., 2020). Soy waste pulp and thermoplastic starch can be used to make single use goods, packaging and many more (Behera et al., 2022). Oats and barely hull fractions used in paper production results in comparable standards and quality set by commercial paper used in food packaging (Kärkönen et al., 2022). Certain unconventional raw materials from animals, like feathers (Aranberri et al., 2017) and horns (Kumar et al., 2017), have been explored by researchers as fillers. To enhance the longevity of perishables items, it’s crucial to restrict the movement of oxygen. Some of the bionanocomposites have demonstrated superior abilities in reducing oxygen permeability rates, outperforming the LDPE. This improved performance can be attributed to elevated molecular interaction and blockage of space by nanoparticles (Indumathi et al., 2019). Biopolymer blends and nanocomposites synergistic effects open the door to sustainable solutions that address present problems with plastic waste management and advance a more environment friendly future.

2.1.3 Enhancement of packaging properties

Antimicrobial Activity

Customers look for healthy and reliable food products that abide by stringent safety regulations for prevention of the food borne transmission of infectious diseases. Among the primary reasons behind food quality degradation is microbial growth, which results in food discoloration, the development of off flavors, textural changes, and nutritional content loss. These effects shorten the food shelf life and raise the risk of infection brought on by food. Antimicrobial packaging has a significant role in extending the storage time of food products in addition to suppressing or eradicating the pathogenic microorganisms. By evaluating the inhibition zone, antimicrobial activity of packaging film is typically evaluated using the well diffusion approaches (İlk et al., 2018; Murali et al., 2019; Rodrigues et al., 2020). The amino group of chitosan, which confers natural antioxidant and antibacterial action, plays a crucial part in the biopolymer's ability to function. According to various scientists, the positively charge upon the amino group of chitosan binds with the negatively charged cell wall of microbes, alter structure of membrane, leading to release of intercellular components, and eventually cause cell destruction. Acidic environment generally induces this phenomenon (Ke, Deng et al., 2021). According to another theory, chitosan binds to enzymes in microbial cell and creates an obstruction on the active center, which further slows down microbial development (Divya et al., 2017). Low molecular weight chitosan enters through the cell wall of microorganisms and adhere to DNA, inhibiting production of mRNA and protein and ultimately causing death (Kravanja et al., 2019). Chitosan with a high molecular weight can generate an impenetrable coating on the microbial cell, prohibiting nutrient absorption (Kravanja et al., 2019; Verlee et al., 2017). Moreover, chitosan chelates the ions, minerals, nutrients etc. in the environment essential for living and may also limit mitochondrial ATP synthesis (Ke et al., 2021).

Various research studies support the notion that chitosan-based blends or nanocomposites can effectively protect both the packaging material and the enclosed food from microbial attacks. This positive outcome includes slowing down pathogenic microbial growth and improving the performance of chitosan-based bio packaging. Charoensri and teammates claimed in their research that strong positive charges on their fabricated bionanocomposite films interacted with the negatively charged bacterial cell walls, which resulted into enhanced antimicrobial activity (Charoensri et al., 2021). Sanuja and group disclosed that the nanocomposite film comprising chitosan, clove oil, MgO nanoparticles had good antibacterial activity against the gram-positive bacteria *Staphylococcus aureus*. The phenol reactive group of eugenols in clove oil, the functional groups of chitosan and filler MgO, all are responsible to interact with the bacterial cell membrane and further inhibiting their growth (Sanuja et al., 2014). Other nanocomposite systems, such as chitosan-TiO_2, were able to disrupt the microbial membrane and cause cellular material leakage (Zhang et al., 2017). Similarly, silver nanoparticles exhibit bacteriostatic action (Abreu et al., 2015), ZnO nanoparticles proved to have antimicrobial activity (Rahman et al., 2017), TiO_2 (Ahmadi et al., 2019), metallic nanoparticles (Ag, Cu, S) (Sánchez-López et al., 2020), metal oxide nanoparticles (ZnO, TiO_2, CuO) (Liaqat et al., 2022) are capable to pose antimicrobial efficacy. Alekseeva et al.'s research revealed, the greater the lysis zone, the higher the concentration of clay in the composite film, which influences its antibacterial effect. Antimicrobial effectiveness towards *Escherichia coli* and *Staphylococcus aureus* found strong in hydroxyethyl cellulose-bentonite with over 1 percent nanomaterial. However, the lysis zone was absent from the pure hydroxyethyl cellulose film (Alekseeva et al., 2019). Some investigations also show that there are no evident inhibition zones when testing antibacterial effect using the disc diffusion approach. For example, Li et al. proposed that to create an inhibitory zone, molecules should be immobilized in the matrix and not diffused (Li et al., 2019).

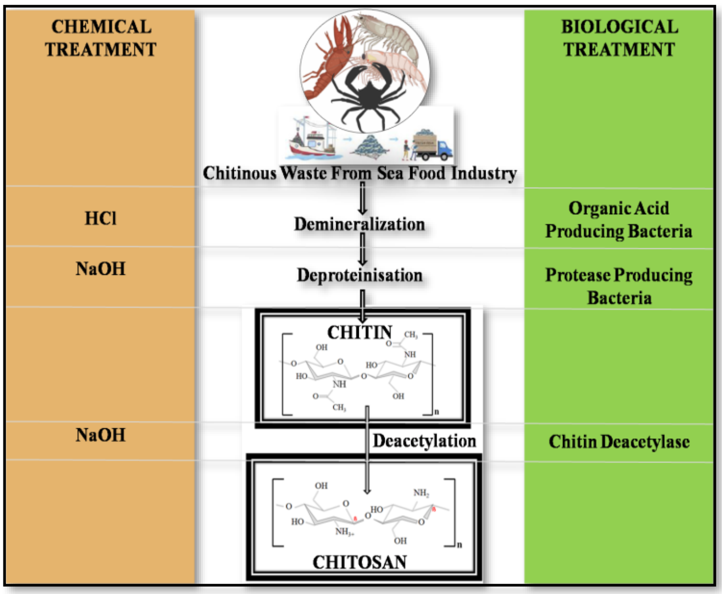


Figure 2.4: Using marine industry byproducts to produce chitin and chitosan

(Arnold et al., 2020)

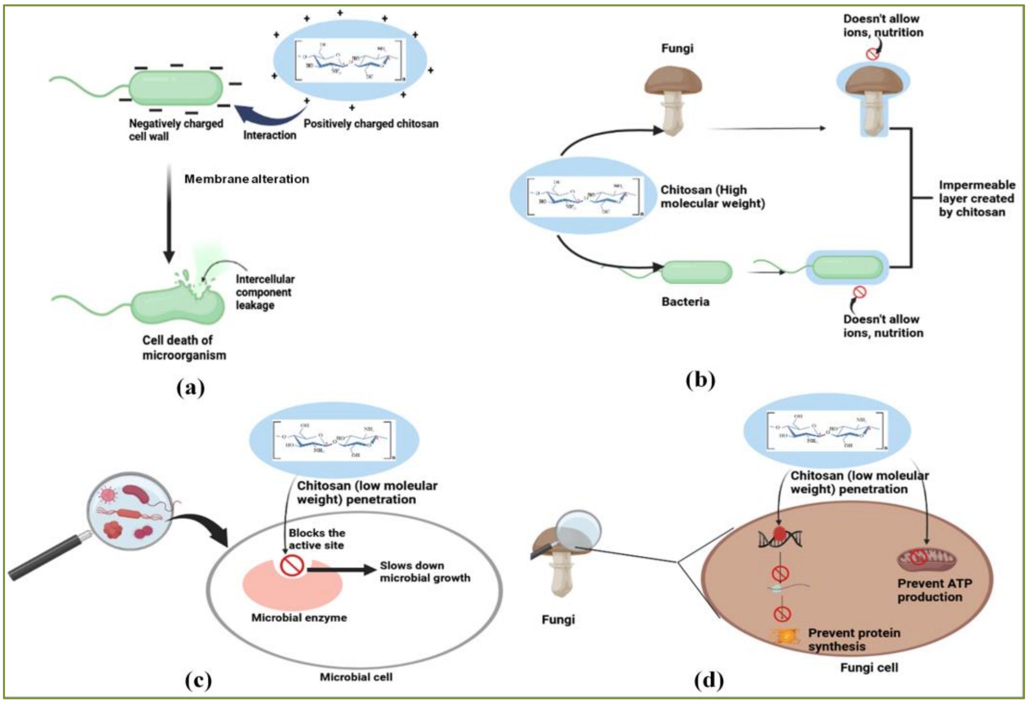


Figure 2.5: Antimicrobial action exhibited by chitosan

Table 2.1: Bionanocomposites and their antimicrobial activity

S. No.	Bionanomposite Films	Antimicrobial action against	References

		Gram-positive bacteria	**Gram-negative bacteria**	**Fungi**	
1.	Chitosan, clay, glycerol	NA	*Escherichia coli*	NA	(Kusmono & Abdurrahim, 2019)
2.	Silver nanoparticles/C30B/starch	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*	(Abreu et al., 2015)
3.	Bentonite-hydroxyethyl cellulose	*Staphylococcus aureus*	*Escherichia coli*	NA	(Alekseeva et al., 2019)
4.	Chitosan coated ZnO	*Staphylococcus aureus, Bacillus subtilis*	*Escherichia coli, Klebsiella pneumoniae*	NA	(Bharathi et al., 2019)
5.	Chitosan, Tween 80, rosehip seed oil, montmorillonite	*Bacillus cereus*	*Escherichia coli, Salmonella enterica*	NA	(Butnaru et al., 2019)
6.	ZnO particle, PHBV	*Listeria monocytogenes*	*Salmonella enterica*	NA	(Castro-Mayorga et al., 2017)
7.	Starch, polyaniline functionalized ZnO Nanocomposite	*Staphylococcus aureus*	*Escherichia coli*	NA	(Charoensri et al., 2021)
8.	Wheat gluten, nanocellul	*Staphylococcus aureus*	*Escherichia coli*	NA	(El-Wakil et al., 2015)

	ose, titanium dioxide				
9.	Chitosan, thyme oil, montmorillonite nanoclay	NA	*Escherichia coli*	NA	(Giannakas et al., 2020)
10.	Chitosan-Polyvinyl alcohol-ZnO Halloysite hybrid nanoparticles	*Staphylococcus aureus,* *Listeria monocytogenes*	*Escherichia coli,* *Salmonella enterica*	NA	(Giannakas et al., 2022)
11.	Chitosan, montmorillonite nanocomposite	*Staphylococcus aureus*	*Escherichia coli*	NA	(Han et al., 2010)
12.	Chitosan, Octadecylamine-Montmorillonite, *Nigella arvensis* Extract	*Staphylococcus aureus,* *Streptococcus mutans*	*Escherichia coli,* *Salmonella enterica*	NA	(İlk et al., 2018)
13.	Mahua oil-based polyurethane, chitosan, ZnO	*Staphylococcus aureus*	*Escherichia coli*	NA	(Saral et al., 2019)
14.	Nano-kaolin incorporated semolina	*Staphylococcus aureus*	*Escherichia coli*	NA	(Jafarzadeh et al., 2016)
15.	Gelatin/silver nanoparticle	*Staphylococcus aureus,* *Listeria monocytogenes,* *Bacillus cereus*	*Escherichia coli,* *Salmonella enterica*	NA	(Kanmani & Rhim, 2014)

16.	Acrylonitrile, acrylamide grafted on chitosan	*Staphylococcus aureus*	*Escherichia coli, Pseudomonas aeruginosa*	NA	(D. Kumar et al., 2018)
17.	Nano Au and gallium-doped hydroxyapatite	*Staphylococcus aureus, Staphylococcus epidermidis*	NA	NA	(Kurtjak et al., 2017)
18.	Chitosan–Agar/ZnO nanoparticles	*Staphylococcus aureus, Bacillus subtilis*	*Pseudomonas aureginosa, Klebsilla pneumonia*	NA	(Magesh et al., 2018)
19.	Nickel oxide nanoparticles into the chitosan-based film	*Staphylococcus aureus*	*Salmonella typhimurium*	NA	(Ardebilchi Marand et al., 2021)
20.	Lemon peel powder, xanthan gum, TiO_2–Ag nanoparticles	*Staphylococcus aureus*	*Escherichia coli*	NA	(Meydanju et al., 2022)
21.	Chitosan, nano ZnO	*Staphylococcus aureus*	*Escherichia coli*	NA	(Mujeeb Rahman et al., 2018)
22.	Chitosan, gelatin, Ag@ZnO	*Staphylococcus aureus*	*Escherichia coli*	NA	(Murali et al., 2019)
23.	Chitosan, *B. crataegina* seed oil and fruit extract	*Staphylococcus aureus, B. thuringiensis,*	*Escherichia coli, Proteus mirabilis,*	NA	(Kaya et al., 2018)

		Streptococcus mutans	*Proteus vulgaris, Pseudomonas aeruginosa, Enterobacter aerogenes, S. typhmurium*		
24.	Nano gold. Starch	*Staphylococcus aureus, Bacillus subtilis*	*Escherichia coli, Pseudomonas aeruginosa*	*Candida krusei, Candida viswanathii, Candida albicans*	(Sahoo et al., 2017)
25.	Cellulose acetate phthalate, chitosan, ZnO nanoparticles	*Staphylococcus aureus*	*Escherichia coli*	NA	(Indumathi et al., 2019)
26.	Carbon quantum dots containing nitrogen and phosphorus reinforced chitosan	*Staphylococcus aureus*	*Escherichia coli*	NA	(L. Wang et al., 2022)
27.	Chitosan with laponite immobilized Ag nanoparticles	*Staphylococcus aureus*	*Escherichia coli*	*Aspergillus niger, Penicillium citrinum*	(Wu et al., 2022)

28.	Sugarcane bagasse pulp-based cellulose nanofibrils, nisin hybrid	*Listeria monocytogenes*	NA	NA	(Yang et al., 2020)
29.	Carboxymethyl cellulose, arabic gum, gelatin, ginger extract–TiO_2	*Staphylococcus aureus, Listeria monoctogenes, Bacillus cereus*	*Salmonella typhmirum, Pseudomonas aeruginosa, Escherichia coli*	*Candida albicans*	(Youssef et al., 2021)
30.	Chitosan–citric membrane	*Staphylococcus aureus*	*Escherichia coli*	NA	(Zhuang et al., 2020)
31.	Chitosan and carboxymethyl cellulose, nano ZnO	*Staphylococcus aureus*	*Escherichia coli*	NA	(Zahiri Oghani et al., 2021)

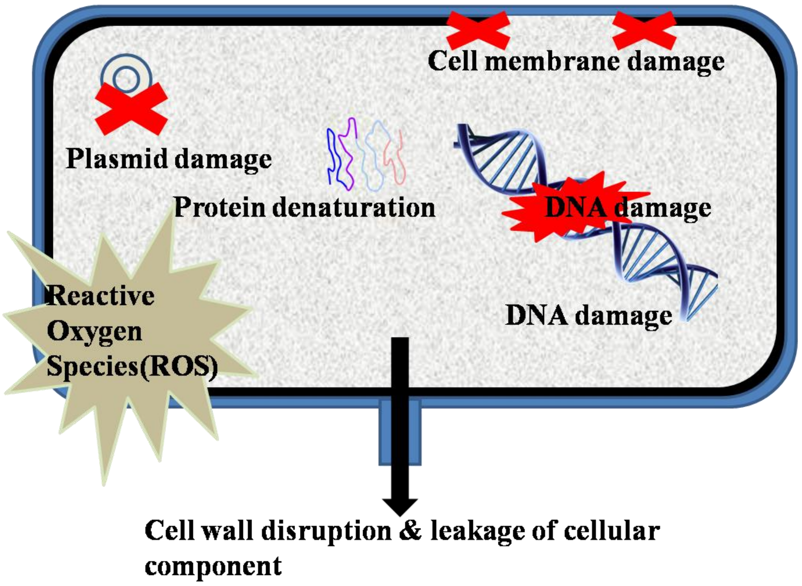


Figure 2.6: Antimicrobial action caused by nanocomposites

Mechanical Property

A standard food packaging material needs to be mechanically capable enough for its intended use in order to safeguard the food product throughout distribution, transit, or storage. The tensile strength of a material is the maximum amount of stress that it can withstand before failing. The tensile strength of a packaging film can be affected by the type of polymer, manufacturing circumstances, additions, alterations, fillers, and blends. The insertion of nanomaterials into a matrix comprising pure or blended polymers generally results in an efficient interaction that may boost tensile strength. The possibility of biopolymers in the food packaging industry has been extensively studied over the past ten years using metal and metal oxide nanoparticles such as Au, Ag, ZnO, TiO_2, SiO_2, etc. According to numerous studies, the amino group of chitosan and the metal oxide can make close contact and are highly compatible, which has significantly boosted the strength of the polymer films (Indumathi et al., 2019; Kustiningsih et al., 2019; Saral et al., 2019). FTIR spectrum revealed an association between the amino group of chitosan and the metal oxide causes the film to be stronger (Sanuja et al., 2015). Abdurrahim and his colleague created a chitosan-clay-glycerol composite and discovered that 5 % nanoclays facilitated intense electrostatic interaction in the $-NH_2$ and $-NH_3$ groups of chitosan that causes stress transfer between adjacent chains, greatly enhancing mechanical performance. The mechanical property enhancement was also caused by the uniform distribution of highly stiff clay particles and by ionic interactions connecting the clay and the polymer matrix (Kusmono & Abdurrahim, 2019; Naidu & John, 2020). Research also shows that the strength of the film was significantly enhanced by the evenly distributed nanohybrid fillers and intermolecular connections (Giannakas et al., 2022). Because of the increased hydrogen bonding between molecules, the chitosan neutralized with higher concentrations of NaOH and longer periods exhibited more stiff and compact structure (Chang et al., 2019). It was investigated and found that lower crystallinity can lead to lesser mechanical strength (Kaya, Khadem, et al., 2018). Furthermore, most of the time, tensile strength has been discovered to have a favorable association with an increasing proportion of nanofiller's weight percentage to a specific threshold. Furthermore, in the chitosan matrix, an increase in the weight percentage of nanomaterials has the potential to cause agglomeration, which causes local stress and is the cause of the mechanical performance decline (Abdurrahim, 2019; Saral et al., 2019).

Elongation at break is measurement that shows the amount of stretch a material can withstand as a percentage of its dimensions, prior break. In association with a material's superior tensile strength, a higher percentage in its elasticity typically denotes a higher-grade material. While strength indicates the material's resistance to rupture, elongation at break ensures that the packaging can endure deformation without tearing or failing catastrophically. This property is especially important for flexible packaging like films and pouches, where the material needs to accommodate product movement and handling during storage and distribution. Balancing strength and elongation at break ensure packaging integrity, preventing leaks, spoilage, and contamination of food products.

A crucial factor in the film's elongation during breakage is molecular contact (Butnaru et al., 2019). However, in many cases, a decrease in elongation at-break was observed alongside a boost in the strength. Films having more filler-matrix interactions are often tend to be less flexible (Jafarzadeh et al., 2016). Or, when two oppositely charged biopolymers form a polyelectrolyte complex, greater electrostatic association is formed, causing a decreased molecular mobility, and an turning the film brittle (de Morais Lima et al., 2017). Melro and fellow workers evaluated the influence of various acidic solutions on the properties of chitosan films and found that acetic acid containing chitosan films had the highest mechanical strength. Lactic acid, on the other hand, produces less durable films that must be evaporated at a temperature of 50ºC, indicating that the films have good flexibility but poor tensile strength (Melro et al., 2020). Jara and associates, on the other hand, declared the exact opposite finding while fabricating chitosan film with different concentrations and drying temperatures to test their influence on the qualities of the film. They discovered that drying the film at 40°C, in coupled with higher chitosan concentration, increased the tensile strength while decreasing elongation (Homez-Jara et al., 2018). It was investigated and found that plasticizers generally tend to decrease intra/inter molecular connections, allowing polymer chains to move over each other and so enhancing flexibility (Butnaru et al., 2019; Melro et al., 2020). Kaya and collaborators discovered very high flexibility with very low strength in their chitosan-based film while incorporating plant extracts (Kaya, Ravikumar, et al., 2018). They discovered similar results while developing edible chitosan films containing Berberis crataegina's fruit extract and seed oil. Even though adding oil can make materials less transparent (Kaya, Ravikumar, et al., 2018), oil droplets in the film disrupt the sample's continuous structure and weaker polymer-oil connections partially replace stronger interactions inside polymer structure, resulting in elevated elasticity (Li et al., 2019; Zheng et al., 2019).

Table 2.2: Mechanical property of chitosan-based blend and nanocomposites

S. No.	**Matrix**	**Blend**	**Nanofiller**	**Tensile Strength (MPa)**	**Elongation At break (%)**	**References**
1.	Chitosan	Gelatin	ZnO nanoparticles (2wt%)	30.87	27.53	(S. Kumar et al., 2020)
2.	Chitosan	Gelatin	Ag nanoparticles (0.1%)	21.19	27.23	(S. Kumar et al., 2018)
3.	Chitosan	Gelatin	Ag nanoparticles	25.8	4.34	(Ediyilyam et al., 2021)
4.	Chitosan	Sodium phytate	SiO_2 (0.1wt%)	32.65	NA	(Yu et al., 2021)
5.	Chitosan	NA	Ag nanoparticles (0.0075%)	27.67	NA	(Zhang et al., 2022)
6.	Chitosan	0.5% C. citratus Essential Oil	1% TiO_2 nanoparticles	62.46	4.81	(Hosseinzadeh et al., 2020)
7.	Chitosan	Gelatin	NA	31.87	29.77	(Haghighi et al., 2019)
8.	Chitosan	Black soybean seed	NA	23.24	73.88	(Wang et al., 2006)
9.	Chitosan	NA	Sodium montmorillonite	38.86	2.08	(Rodrigues et al., 2020)

			organically modified by octadecylammonium and ZnO nanoparticles			
10.	Chitosan	NA	0.1% TiO_2	12.35	2	(Kustiningsih et al., 2019)
11.	Chitosan	NA	Bacterial cellulose nanocrystals and 1% Ag nanoparticles	42.89	22.5	(Salari et al., 2019)
12.	Chitosan	Polyvinyl alcohol	ZnO Halloysite hybrid	109.1	6.1	(Giannakas et al., 2022)
13.	Chitosan	Polyvinyl alcohol	ZnO Montmorillonite hybrid	106.4	6.4	(Giannakas et al., 2022)
14.	Chitosan	Gelatin	Ag loaded ZnO nanoparticles	42	2.8	(Murali et al., 2019)
15.	Chitosan	Cassava starch-lysozyme	NA	3	58	(Ma et al., 2018)
16.	Chitosan	NA	5% TiO_2	39.4	16	(Li et al., 2019)
17.	Chitosan	3% Clove Oil	3% TiO_2	34.8	24.5	(Li et al., 2019)

18.	Chitosan		ZnO	41.73	32	(Mujeeb Rahman et al., 2018)
19.	Chitosan	Acorn starch-Eugenol	NA	16.8	66	(Zheng et al., 2019)

Barrier Property

Barrier properties include permeability of gases, water vapor, UV etc. which are critical elements in protecting the integrity of packaged food items. The flow of CO_2, O_2, H_2O etc. differs depending on the food matrix and packaging materials employed. Amount of permeability required varies on the types of food items. When it comes to the cellular respiration that occurs in fresh produce items, it is not optimal to totally stop gas flow and permeation. On the other hand, to prevent oxidation and decarbonation, carbonated drink containers should stop the release of oxygen and carbon dioxide to prevent oxidation and decarbonation (Robertson, 2006).

The water vapor transmission rate, which is often measured at 38°C and 90% relative humidity, is the volume of water vapor that moves across the material being packed per unit area and time. A variety of elements influence the barrier property, includes the water quantity, structure, morphology, activation agent, and polymer matrix. The formation of a dense structure by biopolymer blends and nanomaterials blockage of pore space in the matrix not only reduce moisture, may additionally boost hydrophobicity (Indumathi et al., 2019). Because clove oil is hydrophobic, it plays a critical role in chitosan, MgO combination in preventing passing of water vapor. Furthermore, effective interaction occurred between functional groups of chitosan and the OH-group found in eugenol, lessening the amount of H-bonds accountable for the hydrophilic feature (Sanuja et al., 2015). Chisenga and Li et al. made a similar statement, claiming that the lipid composition of essential oils can minimize moisture permeability in hydrophilic materials (Chisenga et al., 2020; Li et al., 2017).

Chitosan films readily absorb water vapor due to the presence of hydrophilic domains (Liu et al., 2021). The hydrophilic-hydrophobic component ratio of a film has a substantial impact on its barrier performance. The higher percentage of hydrophobic components in the film effectively limits moisture (Ma et al., 2021). It was discovered that the connection between glycerol hydroxyl groups and chitosan functional groups forms a network, which stops water from further interacting with the polar groups of chitosan and prevents water molecules from penetrating the films (Mujeeb Rahman et al., 2018). It was observed by researchers that the amount of ZnO nanoparticles in films is inversely related to their water vapor transmission rate. When nano ZnO particles are incorporated into the chitosan matrix, segmental mobility and porosity in the polymer surface are minimized, resulting in limited water vapor transmission (Chang et al., 2019). Polylactic acid biopolymer alone exhibits limited moisture resistance, even the addition of micro-crystal cellulose proved to be ineffective owing to its hydrophilic properties. The humidity barrier property of the composite usually shows notable improvements when nano clay is present (Dadashi et al., 2014). For example, the water-insoluble titanium dioxide nanoparticles can obstruct moisture channels, causing them to take longer to transit, and ultimately reducing the permeability of water vapor (Ahmadi et al., 2019). Regardless of the testing settings, Karkhanis and co-researchers found that moisture permeability values drop as the level of crystallinity in the polymer matrix increases (Karkhanis et al., 2018). Shogren made a similar claim about PHBV. To maintain the decreased rate of water vapor transport linked with the crystallinity of polymer, biopolymer material with a melting temperature higher than the projected use temperature should be chosen, because when the melting temperature is exceeded by heating, permeabilities decrease (Shogren, 1997). To summarize, the water vapor transfer rate is positively correlated with greater free volumes, reduced crystallinity, and higher polymer solubility characteristics.

The oxygen permeability coefficient [kg/mm^2/s/Pa] measures how much oxygen can travel through a substance per unit area and duration under pressure, which is typically measured at 25 °C with 0% relative humidity. Because oxidation and respiration rate in packed fruits or vegetables is affected by oxygen gas, the oxygen transmission of packing materials is crucial in food preservation. Inorganic nanoparticle reinforced polymeric composite generally have improved barrier properties due to the combination of two events. First of all, since impermeable nanoparticles are able to replace permeable polymers, there will be less space available for diffusion. Second, the distance a permeant covers in order to cross the film grows as it follows a twisted path around the impermeable nanoparticles. Indumathi and his peers discovered that combining polyurethane and chitosan reduced the permeability of the film by 36%, indicating a change in the polymers' inter-chain configuration as a result of their contact with one another (Indumathi et al., 2019).

It has been observed that adding nanoclays or nanoparticles to polymeric matrix affects gas permeability, acting as an oxygen barrier, reducing oxygen flow, and extending food shelf life (Giannakas et al., 2022). The oxygen permeation in ZnO-reinforced composite films was significantly lesser than the blended film. Increased molecule interaction with compact structures, as well as nanoparticle pore blockage, may possibly contribute to the significant drop-in oxygen transfer rate. When compared to typical food packaging materials (e.g., LDPE, LLDPE) the film containing 5wt percent ZnO nanoparticles exhibited an extremely low oxygen transmission rate (Indumathi et al., 2019). When magnesium hydroxide was inserted into chitosan matrix and neutralized with NaOH at pH 6, oxygen transmission rate and oxygen permeability decreased dramatically (OTR from 5313 to 7 cm^3 m^{-2} day^{-1} and OP from 192 841 to 287 cm^3 lm m^{-2} day^{-1} atm^{-1}). Karkhanis and colleagues made a similar claim, concluding that regardless of the testing conditions, the values of moisture permeability will decrease with a higher degree of crystallinity of polymer matrices when dealing with polylactic acid)/cellulose nanocrystals nanocomposite films (Karkhanis et al., 2018). Plasticizers tend to boost oxygen molecule mobility by creating extra space for oxygen via H-bond breakage. In contrast, the addition of nanoclay increases CO_2 barrier properties, resulting in a 50% reduction in permeability (Butnaru et al., 2019). The authors reported that increased crystallinity plays a significant effect in inhibiting the oxygen molecule route (Jančič et al., 2021). Similarly, because carbon dioxide might initiate deterioration reactions, it ought to be controlled to keep food fresh (Butnaru et al., 2019). When ZnO-modified parchment fibers were added to the polycaprolactone bionanocomposite, the CO_2 permeability coefficient decreased by almost 20%, indicating that inorganic nanoparticle-containing composites are crucial for superior gas barrier performance (Lepot et al., 2011). In contrast, Ahmadizadegan discovered in his research that the permeability of gases (H_2, N_2, CO_2, CH_4) gases rose as the amount of nanoparticles increased. The aggregation of nanoparticles that produce pores in the polymer matrix disrupts chain packing in the polymer matrix. Gas permeability can be higher due to increased diffusion and solubility coefficients caused by a rise of the overall free volume. An array of non-porous nanoparticles dispersed in glassy polymers showed a similar pattern (Ahmadizadegan, 2017).

Food packaging's capacity to block UV radiation is an essential feature that protects the stability and quality of packed goods. UV light, which is a part of sunshine, has the energy to start photochemical reactions and to cause oxidative degradation in different food components, which can result in unfavorable changes like nutrition loss, flavor changes, and color fading. Efficient UV light barrier qualities in food packaging materials operate as a protective barrier, restricting the penetration of dangerous UV radiation through the packaged materials. These barriers are often created by choosing UV resistant materials for packaging construction or by adding UV absorbing chemicals. The packaging protects both the sensory and nutritional value of the enclosed food by preventing UV light transmission, hence increasing shelf life and preserving customer safety. Thus, the UV light barriers property is essential for maintaining the general integrity and quality of packaged food goods during storage and distribution. LDPE and polypropylene, which make up the majority of petroleum based polymeric food packaging sheets, generally have weak UV light absorption (Indumathi et al., 2019; Saral et al., 2019).

Due to their exceptional capacity to improve ultraviolet blocking qualities, nanofillers have emerged as viable additives in food packaging materials. These nanoscale components are mixed with packaging polymers to produce nanocomposite films, including metal oxides (such as titanium dioxide and zinc oxide), carbon-based nanoparticles, and polymer nanoparticles. The addition of nanofillers improves the packing material's UV absorption and scattering characteristics, effectively reducing the penetration of harmful UV light. Because sensitive ingredients like vitamins, flavors, and nutrients are not photochemically degraded, the shelf-life of packaged meals is increased by this UV blocking feature. Additionally, the overall mechanical and barrier capability of the packing material are also significantly improved by the controlled dispersion of nanofillers within polymeric matrix. The incorporation of nanofillers into food packaging has a tremendous potential to improve food preservation and safety while lowering environmental consequences as nanotechnology develops.

Oxidized maize starch addition in chitosan matrix gives a push to increase UV barrier property (Hiremani et al., 2022). Compared to pure biopolymer, the ZnO nanoparticles reinforced chitosan-based nanocomposite film's ability to block UV light increased significantly with UV absorption at 280 nm. Additional absorption peaks at 360-380nm with high intensity was also observed. According to researchers, ZnO nanoparticles were mainly responsible here (Indumathi et al., 2019; Saral et al., 2019). Similarly, cellulose nanocrytals proved itself to enhance UV blocking ability of chitosan-based composite films (Yadav et al., 2020). Surface modified cellulose nanofibers with loading below 5% resulted in high UV light shielding in poly(vinyl alcohol) film (Niu et al., 2018). Cellulose nanocrystal-silver-alginate film showed great absorption in ultraviolet (200-400 nm) and visible light range (400-700 nm) (Yadav et al., 2019). ZnO alone and together with Mg quantum dots improved UV light shielding capabilities of zein film (Schmitz et al., 2020). Lignin has been used as a UV-blocker in a variety of materials for being a superior UV-B and UV-A light absorber because of its phenolic structure. The incorporation of lignin although can cause a decrease in the transparency of the films, however, it increases the capacity of UV absorption significantly (Avelino et al., 2019). Above 90.2% blockage in UV-A region and 96.5% in UV-B region were found in the film containing 4 wt% carbon quantum dots containing nanoparticles, however only 26.8% of visible light was filtered (Wang et al., 2021).

Nanofillers such as metal oxides, including titanium dioxide and zinc oxide, are highly efficient in scattering and absorbing UV radiation due to their compact size and extensive surface area. These nanoparticles are scattered throughout the packaging material, intercepting and scattering UV radiation before they can reach the packaged food. Furthermore, they have the capacity to directly absorb UV photons, transforming the energy into inert heat (Wildner & Drummer, 2019). The addition of nanofillers causes the packing material's refractive index fluctuations to rise. Multiple internal reflections of UV light are produced, as a result, it effectively traps and dissipates the energy within the material itself and lowers the quantity of UV radiation that enters the package (Z. Sun et al., 2022). At the nanoscale, quantum phenomena are more obvious. Quantum confinement effects, in which the behavior of electrons is constrained because they are contained within nanoscale structures. Enhanced UV absorption and scattering properties may result from this change in electronic properties (Ezati & Rhim, 2022). Nanofillers can have their surfaces altered to improve how well they interact with UV light. By adjusting the nanoparticles' absorption and scattering properties, functionalization of their surfaces can increase the effectiveness of UV blocking (Serpone et al., 2001). Nanofillers facilitate the formation of thin coatings with enhanced UV blocking characteristics. To increase UV protection, these films can be used as coatings on currently used packaging materials or integrated directly into multilayer packaging structures without materially changing the packaging's overall design (Schmitz et al., 2020). Utilizing before mentioned mechanisms, nanofillers successfully improve the UV blocking properties of food packing material, protecting the quality as well as long term durability of packed foods by minimizing UV radiation-induced deterioration.

Shelf-Life Enhancement

The amount of time that perishables can be consumed safely, nutritiously, and with acceptable quality is referred to as their shelf life. It relies on several aspects, e.g., the type of product, the processing methods, the packaging, and the storage circumstances. Fruits, vegetables, dairy products, and meat products are examples of perishables that subject to microbiological, enzymatic, and chemical degradation. It's essential to maintain the right temperature, humidity, and hygiene to increase their shelf life. By establishing a barrier of protection between the product and the outside environment, food packaging has a substantial impact on the perishables' ability to remain fresh. A suitable packaging material can stop moisture loss, oxygen exposure, and microbiological contamination. According to several published researches and patents, nanocomposite packaging is a promising development to considerably extends the shelf life of perishables. Numerous advantages can be developed by adding nano particles into the packaging matrix. Nanoparticles with antibacterial qualities, like silver, zinc oxide, or titanium dioxide, efficiently stop the microbial contamination, enhance the freshness of perishable goods. Additionally, increased barrier nanocomposite films minimize oxidation and spoilage by obstructing oxygen and moisture permeation. Additionally, the use of nanoclays improves mechanical stability and strength. A sustainable and effective method for prolonging the shelf life of perishables, lowering food waste, and assuring food safety is provided by the customized properties of nanocomposite packaging (Basumatary et al., 2022).

Table 2.3: Extended shelf-life by biopolymer-based packages

S. No.	**Biopolymer and Composite films**	**Shelf life**	**References**
1.	Gelatin	Enhanced quality of a wide range of foods spanning from fish fillets to ground pork	(Etxabide et al., 2017)
2.	Gelatin, starch	The packaging notably reduced the overall presence and attack of microorganisms on marinated salmon significantly, by keeping them less than the permissible limits following 45 days while storing at 5 °C, Still, barrier of the package couldn’t stop weight loss	(Moreno et al., 2017)
3.	Polylacticacid, polyhydroxybutyrate	Extended storage duration of salmon in chilled environment by decreasing overall bacterial growth	(Ma et al., 2018)
4.	Chitosan, sodium montmorillonite, ginger essential oil	Abled to lengthen the shelf lives of fresh poultry meat	(Souza et al., 2018)

5.	Polylacticacid, sawdust particle	Pangasius fish fillets did not poses proliferation of bacteria for a period of 10 days	(Woraprayote et al., 2018)
6.	Chitosan, cellulose acetate phthalate, nano ZnO particles	Prolonged the freshness of black grapes by up to 9 days	(Indumathi et al., 2019)
7.	Chitosan, mahua oil based polyurethane, ZnO nanoparticles	Inhibited the growth of microorganisms and extend the freshness of sliced carrot for a duration of up to 9 days, suppressing the efficiency possessed by polyethylene bags	(Saral et al., 2019)
8.	Starch, glucose	The shelf life of cucumbers was increased to 1 month with minimal weight loss and retained levels nutritional content	(Patel & Panigrahi, 2019)
9.	Gallic acid, epsilon-polylysine hydrochloride, pullulan, carboxymethyl cellulose	Sea bass (*Lateolabrax maculatus*) fillet kept at 4 °C for a duration of 20 days, exhibit decreased protein deterioration, microbial proliferation, and lipid oxidation are suppressed	(Q. Li et al., 2021)
10.	Carboxymethyl cellulose, arabic gum, gelatin, garlic extract TiO_2 nanoparticles	Over a period of 3 weeks, while storing Nile Tilapia fish fillet at 4°C, microbial growth was controlled and weight loss rate was dropped	(Youssef et al., 2021)

11.	Chitosan, cassava starch, ZnO, pineapple leaf fibre,	Shelf-life studies on slice bread revealed the package prevented fungal growth over 30days of storage	(Armynah et al., 2022)
12.	Tea ground waste and furcellaran-carboxymethylcellulose	Capable of extending shelf life of salmon	(Jamróz et al., 2022)
13.	Collagen-carboxymethyl cellulose, Berberis lyceum root extract	Wrapped mushrooms in order to increase their longevity	(Ahmed et al., 2022)
14.	Xanthan gum, nano-*Litsea cubeba* essential oil	The robust antibacterial efficacy against *V. Parahaemolyticus,* accountable for quality of salmon at 4°C, played a pivotal role in extending the shelf life	(Cui et al., 2022)
15.	Gelatin, starch	Prolonged the freshness of cherry tomatoes, while also preserving the firmness, pH levels and also minimizing weight loss throughout 15 days storage time	(Rather et al., 2022)
16.	Collagen, zein, gallic, 8% gallic acid	Maintained quality of tilapia muscle and kept fresh for a minimum of two days	(Z. Song et al., 2022)
17.	Hydroxypropyl methylcellulose, xanthan gum	A qualitative enhancement was observed, as the rate of weight loss of banana was decreased from 22-28% to 12-20%	(Zheng et al., 2022)

18.	Acrylic monomers-vanillin	Significant antimicrobial action contributed to a 50% higher shelf life of packed meat products	(González-Ceballos et al., 2022)
19.	Xanthan gum-hydroxypropyl methylcellulose-tea polyphenols	After a duration of 8 days, a favorable outcome was achieved, with the vitamin C content of 127.81%	(Chen et al., 2022)
20.	Polybutylene adipate-co-terephthalate, thermoplastic starch, ZnO	Capable of prolonging storage duration of packed meat by over 3 days, when kept in refrigerated conditions	(Phothisarattana et al., 2022)
21.	Nano ZnO@Xylan, quaternized xylan/Polyvinylalohol	Maintained quality and kept cherry tomatoes fresh for up to 3 weeks	(H. Wang et al., 2022)
22.	Jackfruit seed starch, tamarind kernel xyloglucan ZnO nanoparticles	Postponed deterioration in tomatoes and maintained quality for extended period of time, while demonstrating antimicrobial action against representative of both gram strains	(Santhosh & Sarkar, 2022)

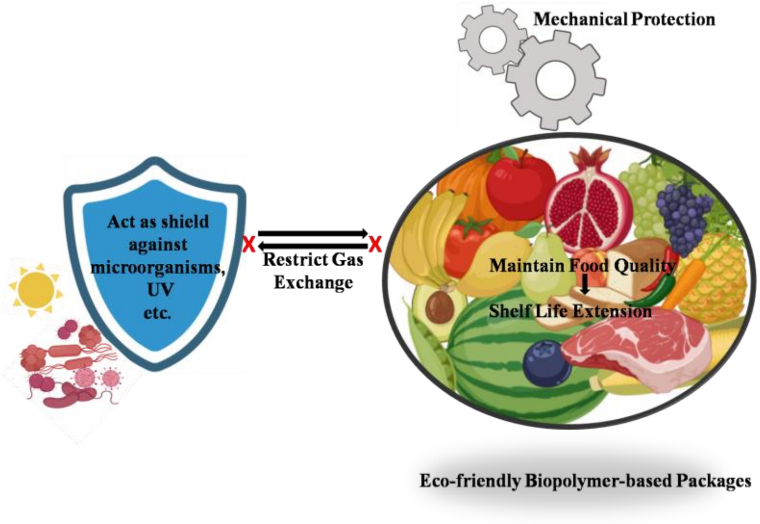


Figure 2.7: Shelf-Life extension by biopolymer-based packaging (Khalid & Arif, 2022; Saha et al., 2020; Westlake et al., 2022)

Biodegradability

The significant ecological and environmental benefits of biodegradable packaging make it a crucial alternative to replace plastics. Traditional packaging materials, including plastic made from fossil fuels, build serious ecological challenges to the earth. Biodegradable packaging is a more environmentally friendly option because it is made to decompose naturally and be consumed by microorganisms, which prevents trash from building up. The biodegradation process entails the enzymatic breakdown of complicated polymers into simpler chemicals that can be used as a source of energy and nutrition by microbes. Load on landfills and the amount of dangerous pollutants released into the environment can be lessen by using biodegradable packaging. Using biodegradable materials also promotes the adoption of a circular economy, where resources are conserved and recycled, helping to create a more sustainable world.

There is an urgent requirement for alternatives to conventional plastics to reduce the negative environmental impact of solid waste. The term “Biodegradability” describes the ability of a substance to decompose through the action of naturally existing microorganisms. Biodegradable materials can undergo degradation by soil microbes, resulting in the transformation into natural elements like water, carbon dioxide, methane, humus, and so on (Rachmawati et al., 2015). The rate at which polymers deteriorate is determined by a several variables, encompassing the variety of microorganisms in the vicinity, the temperature, moisture levels, and pH conditions of the surrounding environment, as well as the chemical composition and bonding.

$$\textbf{Biopolymers} \xrightarrow{\text{degradation}} \textbf{CO}_2 + \textbf{H}_2\textbf{O} + \textbf{Humus}$$

Biodeterioration, depolymerization, recognition, assimilation, and mineralization are all the phases in the biodegradation process (Shaikh et al., 2021).

The most frequent procedures for testing a polymer's biodegradability are the soil burial and the enzymatic test (Mangaraj et al., 2019). In case of soil burial test to check biodegradability, film samples were kept under soil and their mass loss is assessed at specified time intervals. Bacterial consortiums in soil and added compost in it typically have great influence on a film's biodegradation (Kaczmarek-Szczepańska et al., 2021).

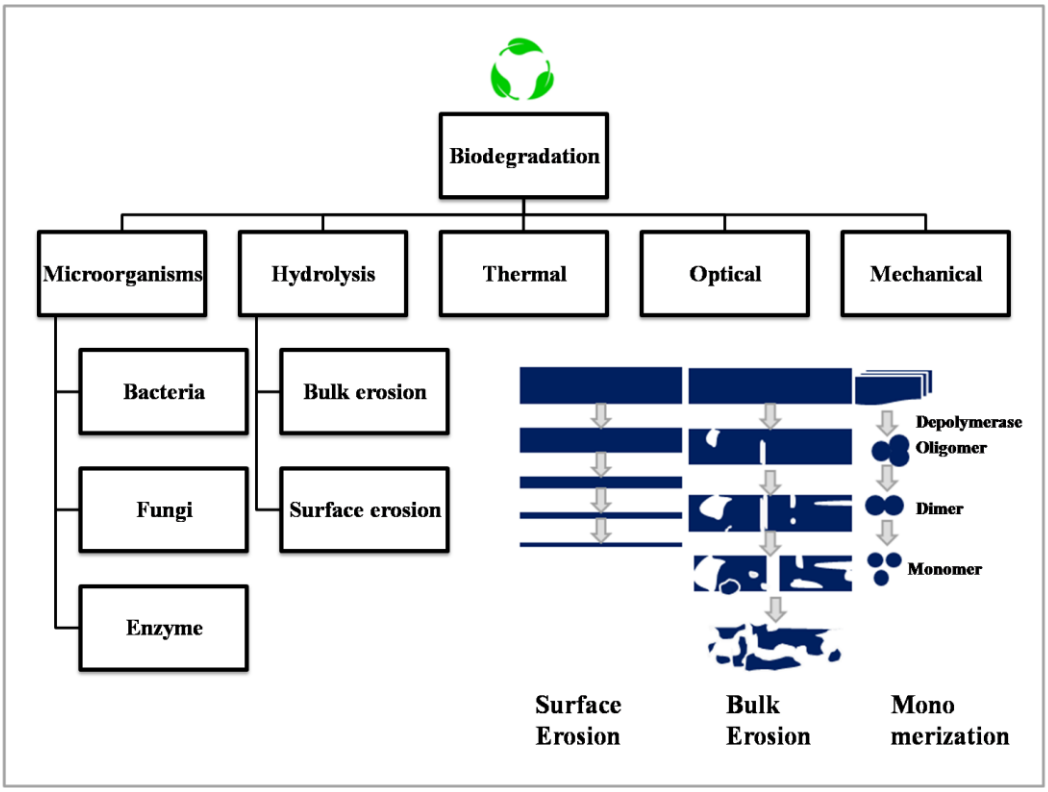


Figure 2.8: Different biopolymer degradation process (Kabir et al., 2020)

Biodegradation in natural environmental conditions, primarily leads to significant decline in mechanical capabilities in case of biopolymers of low molecular weight. Because pure chitosan film is hydrolysable, it loses weight at a faster rate because of its affinity for water. The polymer structure can readily allow soil moisture to infiltrate, causing weakening of the material and rendering it vulnerable to glycosidic chain hydrolysis. The reason behind faster degradability of a biopolymer like chitosan is due to its hydrophilic nature, high water absorption, and surface wettability (Indumathi et al., 2019). Typically, blends of biopolymers and bionanocomposites provide increased resilience compared to naive biopolymers. For instance, in comparison to pure biopolymer, which disintegrated totally, whereas biopolymer blend degraded roughly 70% and the nanocomposite had declined of 30-50% of its original weight within a month (Saral et al., 2019). Similar types of observation were also reported by Indumathi and co-workers (Indumathi et al., 2019). SEM scans indicated changes in its surface appearance, confirming that the film had suffered substantial structural disruption and had many uneven fragments on its surface, suggesting bacterial degradation due to erosion (Indumathi et al., 2019). Larger binding forces enhanced the durability of the nanocomposite film, to remain intact for an extended period of time before undergoing disassembly and decomposition totally after 5 months (Saral et al., 2019). In an aerobic biodegradation medium, starch breaks down at a rate of about 60% in ten days. Mixture of cassava starch and yerba mate completely degraded in the vegetal compost medium in only 6-12 days (Chisenga et al., 2020). In a different study, 85% of chitosan, polycaprolactone clay nanocomposite disintegrated in soil burial within 2 months, with a rapid breakdown procedure within the 1st 7days. According to another research, nanoclay concentration can delay the deterioration process, while compost can aid two or three percent increase in the rate of degradation (Swain PK et al., 2015). Ahmadizadegan's study of fungal degradation of polyimide, cellulose, TiO_2 bionanocomposite in the context of rot causing fungi T. versicolor rapid degradation, indicating that it might be quickly decomposed in natural conditions (Ahmadizadegan, 2017). Polylactic acid is a prominent biopolymer that has been studied extensively and utilized in biodegradable food package; the United States Food and Drug Administration has even approved it as a substance that can be used to wrap food. Polylactic acids do not deteriorate quickly due to their high glass transition temperature and melting point (Nilsen-Nygaard et al., 2021). Polylactic acid degrades through the absorption of water, ester cleavage and output, fragmentation of oligomers becoming soluble, and ultimately diffusion of soluble oligomers by bacteria (Sinha Ray et al., 2003). During the fungal degradation process, the biocomposite changed color and developed fractures. After 86 days in soil, FTIR measurements revealed the ester group bonds had dissociated (de Mesquita et al., 2016). However, nanomaterials could, nevertheless, contribute improving degradability if they are not dispersed sufficiently into the biopolymer matrix (Glaskova-Kuzmina et al., 2021).

2.2 Research Gap and Hypothesis

2.2.1 Research Gap

The predominance of non-degradable plastic and petroleum-based polymers in packaging of perishable food items, such as fruits and vegetables, raises serious environmental and health concerns. These materials have gained popularity because they are strong, flexible and convenient, but because of their lingering effects on the environment and potential health hazard, more environmental friendly materials are now required. Other conventional packaging materials also associated with such problem. Such as, paper bags tear, glass package breaks easily. Acidic food in aluminum can ruin the food quality, glass package doesn't decompose or degraded by microorganisms. According to the review of literature, chitosan, xanthan gum, montmorillonite nanoclay, and zinc oxide nanoparticles are a few more substances that have demonstrated interesting qualities for the creation of novel packaging solutions. However, the use of chitosan as a matrix, combined with xanthan gum, and modified with zinc oxide nanoparticles or montmorillonite nanoclay as a bionanocomposite material for packaging films has not been investigated yet. By developing and evaluating a novel degradable bionanocomposite membrane employing chitosan as the primary matrix, this suggested study aims to close this research gap. Chitosan's mechanical, barrier, and antibacterial properties are expected to be improved by blending it with xanthan gum and incorporation of zinc oxide nanoparticles and/or montmorillonite nanoclay. A sustainable packaging solution that takes into account both the environmental issues connected to traditional packaging materials and promoting long lasting shelf lives of perishables may be produced as a result of the development and characterization of such a bionanocomposite material. The study seeks to advance our understanding of sustainable packaging options by methodically examining the physical and chemical characteristics of this proposed bionanocomposite material. Chitosan being derived from waste of seafood industry, it could open the door for a more comprehensive approach to packaging design that takes into account both the ecological impact and functionality, fostering industrial symbiosis and lowering the environmental impact of the food packaging sector.

2.2.2 Hypothesis

Reviewing related literatures followed by research gap identification lets authors propose hypothesis that incorporating nanoparticles to degradable polymer-based matrices could improve the barrier qualities, mechanical strength, and antibacterial effectiveness of packaging films, thereby extending the shelf life of perishable foods by a significant amount. It is predicted that the combination of biodegradable polymers and nanomaterials, will result in a novel packaging solution that effectively restricts the entry of oxygen, moisture, and outside contaminants, reducing the processes of oxidation and microbial growth. Furthermore, the hypothesis also suggests, studying different composition and concentration of the nanoparticles as well as the polymer matrix by utilizing different instrumental characterization and in vitro tests will lead to optimize the best performance biodegradable nanocomposite film. This film is anticipated to be sustainable, will have increased mechanical strength, which can lessen physical damage during handling and transit, helping to maintain the overall quality of the final product. The theory also predicts that adding antimicrobial nanoparticles can have growth inhibiting effects on harmful and rotting microorganisms, hence extending shelf life even more.

The primary objective of the present study was to create composite films made by blend of chitosan and xanthan gum, incorporating ZnO nanoparticles and MMT nanoclay using solution casting method. The aim was to explore its functional qualities and nanostructure form for possible use in sustainable packages for food, an area that hasn’t yet been previously investigated or verified. These films were developed with the goal of significantly improving various packaging material attributes in order to face off with conventional plastic packaging. Since, chitosan and xanthan gum possess opposite charges, they were expected to interact effectively, potentially forming polyelectrolyte complex that could enhance mechanical properties of chitosan with respect to strength and elasticity. Moreover, introducing nano fillers was anticipated to improve mechanical capacity, thermal resistance, barrier property and antimicrobial efficacy. Consequently, it is anticipated that these composites could evolve into a novel, innovative and cutting-edge bio-based packaging with excellent biodegradability.

CHAPTER III – RESEARCH OBJECTIVES, MATERIALS And METHODS

3.1 Research Objectives

The objectives undertaken to accomplish the proposed work were:

1. Preparation of bio-nanocomposites with matrix of chitosan and xanthan gum utilizing surface modified MMT and ZnO nanoparticles in different ratio and their structural morphology analysis

2. Elucidation of water vapor and gas barrier property in the prepared nanocomposite film and its utility as packaging material

3. Analysis of prepared nanocomposite films for biological degradation and toxicity test

4. Investigation of antimicrobial activity of prepared bio-nanocomposite films against bacteria *Staphylococcus aureus, Escherichia coli* & fungi white mold and its shelf-life studies of ginger and corn

3.2 Materials

Table 3.2: Procured Materials

S. No.	Material	Company
1.	75% deacetylated chitosan (shrimp shell)	Loba Chemie Pvt. Ltd, India.
2.	Pure (food grade) xanthan gum	
3.	99.5% extra pure glacial acetic acid	
4.	Purified glycerol	
5.	Anhydrous calcium chloride ($CaCl_2$)	
6.	Sodium chloride (NaCl)	
7.	Zinc oxide (ZnO) nanopowder (particle size of less than 100nm)	Sigma Aldrich
8.	MMT/Montmorillonite nanoclay (surface modified with 35-45wt% dimethyl dialkyl amine)	
9.	DMSO (Dimethyl sulfoxide)	
10.	DMEM (Dulbecco's Modified Eagle Medium) High glucose	Himedia
11.	FBS (Fetal Bovine Serum)	
12.	MTT Reagent (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)	
13.	D-PBS (Dulbecco's Phosphate Buffered Saline)	

14.	Microbial cultures *Staphylococcus aureus* (MTCC no. 87), *Bacillus subtilis* (MTCC no. 2413), *Escherichia coli* (MTCC no. 443), *Pseudomonas aeruginosa* (MTCC no. 741), *Candida albicans* (MTCC no. 227), *Aspergillus niger* (MTCC no. 872)	The Microbial Type Culture Collection (MTCC) Chan`digarh
15.	Mouse fibroblast cell line (L929)	NCCS Pune

3.3 Proposed methodology

3.3.1 Fabrication of Films

The solution casting approach was used to prepare all of the films because it is affordable and easiest to carry out method for creating chitosan-based nanocomposite films. The solution casting process involves casting and followed by evaporation. Studies reveal that hydrophilic biopolymers like chitosan easily form homogenous dispersion.

Preparation of pure chitosan film (C)

Pure form of chitosan films were synthesized following the procedure outlined by Cazon and Vazquez (Cazón & Vázquez, 2019). To manufacture pure chitosan film (1%), one gram of chitosan flakes was dissolved in 100 ml of water-based solution containing 1% glacial acetic acid. The solution was left at room temperature on magnetic stirrer for one hour to ensure complete dissolution and the formation of clear solution. Further the solutions were casted in petri dishes and kept for drying.

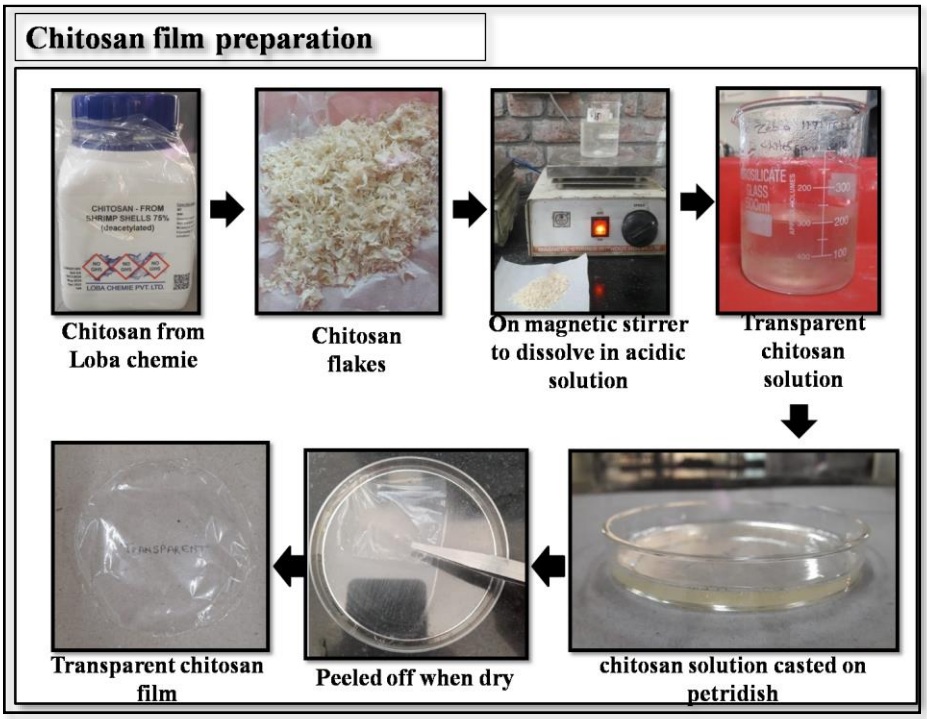


Figure 3.1: Chitosan film preparation

Preparation of Chitosan-Xanthan gum blend Film (CX):

By gradually dissolving the powdered form of xanthan gum in distilled water while constantly rotating with a magnetic stirrer using magnetic bead at ambient room temperature for 30 minutes, xanthan gum solutions were obtained (Xu et al., 2005). The blend of biopolymers i.e., chitosan and xanthan gum were fabricated using the approach outlined by Lima et al. (de Morais Lima et al., 2017). By administration of glycerol as a plasticizing agent at a concentration of 0.30g glycerol/g polymer, the chitosan and xanthan gum blend were crafted through homogenization at ten minutes. Subsequently, the ultimate homogenized solution was then cast onto a petri plate and allowed to air dry at ambient temperature. The dried film was then peeled off and kept in desiccator until further experiment.

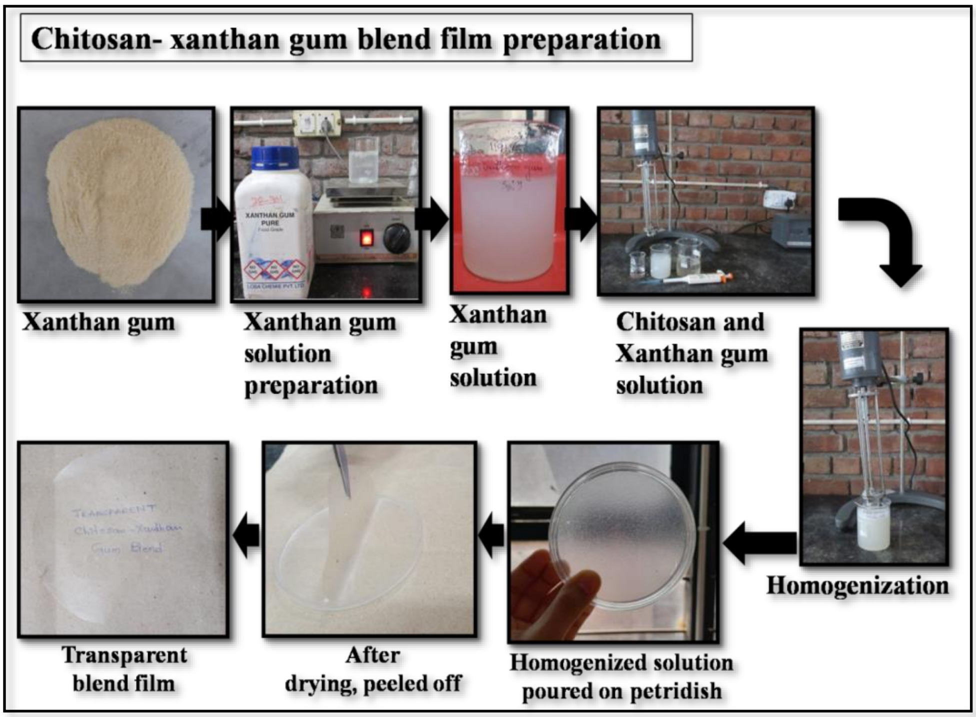


Figure 3.2: Biopolymer blend film preparation

For the blended polymer film, 0.5%, 1%, 1.5% of xanthan gum was added with the chitosan. With increasing amount xanthan gum resulted to less smooth, less transparent and brittle film as shown in figure below. So, further 0.5% of xanthan gum blend was chosen for future analysis.

Figure 3.3: Rejected blend film

Nanocomposite preparation: Two different types of nanocomposite films were prepared.

Preparation of Clay Nanocomposites (CXM):

First of all, for clay nanocomposite films, montmorillonite nanoclay was acid (1% glacial acetic acid) treated overnight and slowly added to the blended biopolymer solution, kept on magnetic stirrer for another 4 hours for proper mixing. Resultant solution was poured in petri dishes and kept to air dry at room temperature for next 2 days. The resultant thin film was peeled off carefully and kept in desiccator (Rodrigues et al., 2020; Xu et al., 2005).

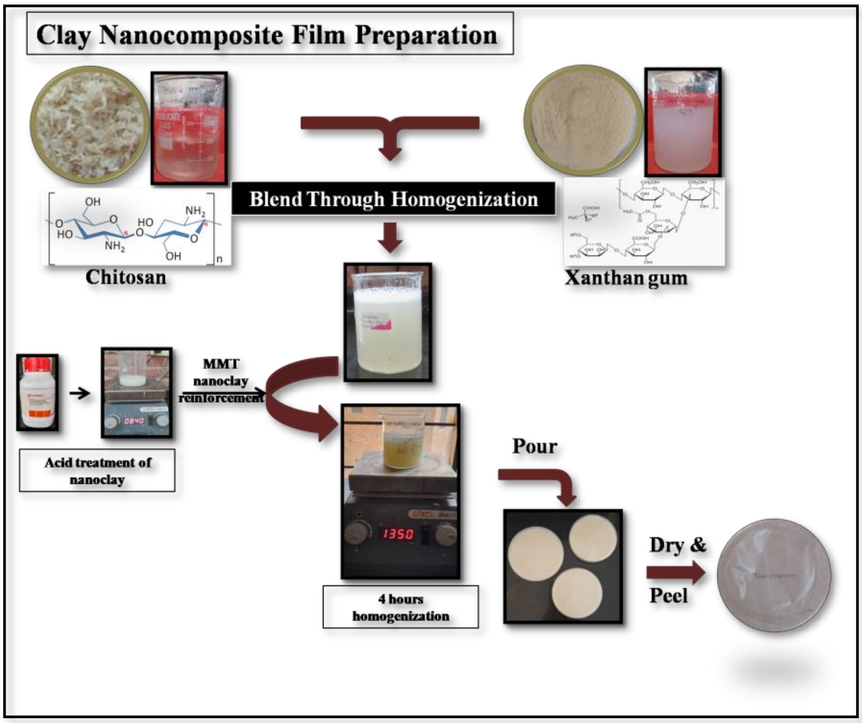


Figure 3.4: Clay nanocomposite film preparation

Preparation of ZnO nanocomposite (CXZ):

The films containing chitosan and xanthan blend incorporated with nano ZnO particles, were fabricated utilizing methodology based on related previous research, albeit with slight modification (Saral et al., 2019; Souza et al., 2020). To begin, a solution containing combined chitosan and xanthan gum were prepared and introduced with ZnO nanoparticles, which was further placed on a magnetic stirrer for 24 hours and subjected to around 10 minutes of degassing in ultrasound bath. The combined solution was then filled in petri plates and left to dry for a minimum of 48 hours. After the drying process, all the films were delicately removed and kept in a desiccator prior to the commencement of further experimentations (de Morais Lima et al., 2017; Indumathi et al., 2019).

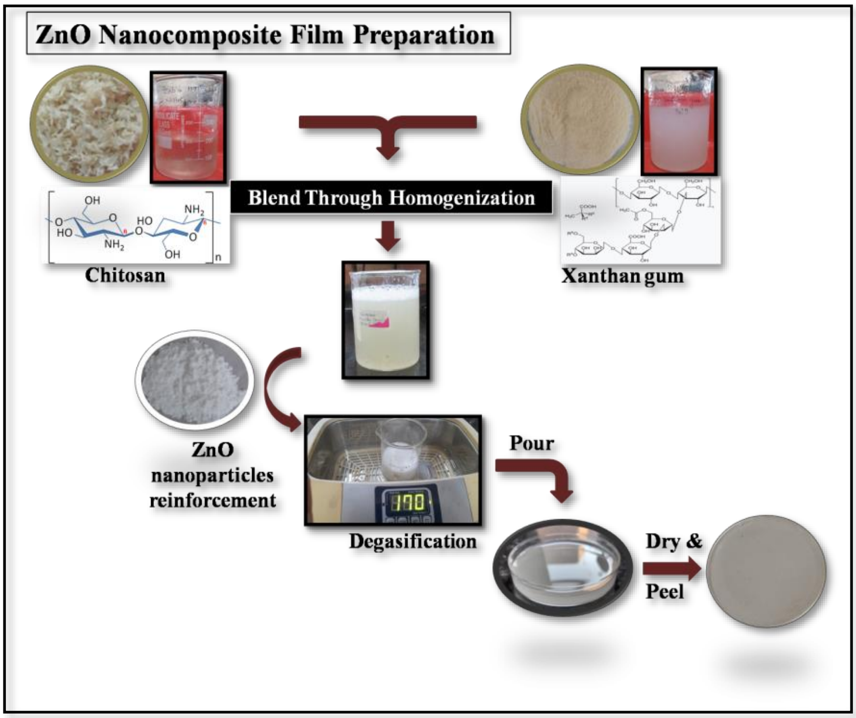


Figure 3.5: ZnO nanocomposite film preparation

When the films had dried, they were all carefully peeled off and stored in a desiccator until the next round of trials (de Morais Lima et al., 2017; Saral et al., 2019).

3.3.2 Characterization

Understanding the manufactured bioplastic films' physical characteristics, performance, and prospective applications requires a thorough investigation of their structural morphology. When it comes to bioplastic films, these substances generally come from renewable resources and have distinctive structural traits that affect their functioning and behavior. Such films' structural morphology can be thoroughly analyzed to reveal important insights that can direct future study, development, and optimization. In conclusion, a thorough knowledge of the characteristics and behavior of manufactured bioplastic films requires the investigation of structural morphology. The design of materials, process improvement, and future uses in sectors like packaging are all influenced by this information. This information guides the development of materials, the improvement of processes, and prospective uses in several industries, including packaging. For the analysis of structural morphology of newly created bio-nanocomposite membranes, different characterization was employed, such as field emission scanning electron microscopy, fourier transform infrared spectroscopy, thermogravimetric analysis, mechanical testing etc.

Thickness Measurement

As thickness of the film can impact on mechanical, optical or barrier property, thickness measurement becomes important parameter to consider. The measurement of thickness in newly fabricated bio-nanocomposites or bioplastics is a fundamental aspect of material characterization that holds significant scientific and practical implications. Accurately determining the thickness of these materials offers valuable insights into their structural properties, potential applications, and performances.

Thickness of all the thin films were investigated through FE-SEM by submitting the film samples in Central Instrumentation Facility, Lovely Professional University. Using Field Emission Scanning Electron Microscopy (JEOL), SEM images were captured at different locations along the sides of the film samples and the average thickness was measured (Tabassum, Girdhar et al., 2023).

Opacity

A bioplastic film's aesthetic appearance is directly impacted by its transparency or opacity. Materials may need to be transparent depending on the intended use, such as in packaging, in order to highlight the contents. Opacity may also be desired to obscure light where light blocking parameter is required. It is ensured that the film's aesthetic fits its intended usage by examining these features.

The opacity of the films was measured by using the following formula,

$O = Abs_{600}/X$

Where, O is the opacity, Abs_{600} is the absorbance of the film at 600 nanometer and X is the film thickness in millimeters (Indumathi et al., 2019; Yadav et al., 2019). Absorbance was taken using UV-Visible spectroscopy at 600nm by cutting the film in rectangular shape placing it in the cuvette.

FE-SEM, EDX, Mapping

Due to its high-resolution imaging capabilities and capability of researching nanoscale structures, Field Emission Scanning Electron Microscopy (FE-SEM) is particularly beneficial for examining the surface morphology. The examination of bionanocomposite packaging materials benefits from the high resolution of FE SEM as well as its capacity to provide thorough details about surface appearance, nanoparticle dispersion patterns, and elemental composition. For designing and engineering packaging materials with better performance and qualities, this knowledge is essential.

All the film samples were submitted in Central Instrumentation Facility, Lovely Professional University for analysis. To assess the appearance of the film surface through microscopic images, Field Emission Scanning Electron Microscopy (JEOL) was utilized for yielding visuals at various magnifications and accelerated voltages of 5 and 10 kV. To determine and characterize elemental composition, SEM was combined with EDX and mapping. To stop the surface being charged, a conductive coating of gold was applied to each film (Hiremani et al., 2022; Indumathi et al., 2019; Rodrigues et al., 2020; Saral et al., 2019; Soni et al., 2016; Tabassum, Girdhar, et al., 2023; Yadav et al., 2019, 2020).

Fourier-Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) is based on how materials interact with infrared light, it is an analytical technique used to pinpoint and assess the chemical structure or combination of various materials. FTIR spectroscopy was utilized to gain insight into the structures, bond formation and interaction between the materials of the manufactured packages.

For above analysis, all the film samples were submitted to Central Instrumentation Facility, Lovely Professional University. FTIR spectroscopy was carried out in transmission mode within 400 to 4000 cm^{-1} range by utilizing Fourier Transform Infrared Spectrometer (Perkin Elmer: Spectrum 10 software, 0.5 cm^{-1} resolution) (Hiremani et al., 2022; Indumathi et al., 2019; Rodrigues et al., 2020; Saral et al., 2019; Soni et al., 2016; Tabassum, Girdhar et al., 2023; Yadav et al., 2019, 2020).

Thermogravimetric analysis

A key method for describing and comprehending the thermal stability and properties of materials, particularly bioplastics, is thermogravimetric analysis (TGA). The thermal characteristics, stability, and composition of bioplastics can all be learned by TGA analysis. This information is essential for creating, manufacturing, and choosing bioplastics for a range of uses while taking into account their distinct performances in natural environmental conditions. For the analysis, all the film samples were submitted to Central Instrumentation Facility, Lovely Professional University. Through thermogravimetric analysis, the thermal stability of each film was examined, and a corresponding derivative thermogravimetric (DTG) graph was created for each film. Thermogravimetric analysis was performed in a nitrogen atmosphere (20 ml/min) using a thermogravimetric analyzer (Perkin–Elmer) in the temperature range of 30.00°C to 500.00°C at a heating rate of 10°C/min. (Rodrigues et al., 2020; Soni et al., 2016; Yadav et al., 2019, 2020).

Mechanical Capability Analysis

To guarantee that packaging materials will be capable of withstanding all of the mechanical loads and situations they may experience throughout their lifecycle, from manufacturing and shipping to storage and usage, mechanical capacity analysis is crucial. This kind of analysis aids in assessing the structural reliability, robustness, and functionality of packing materials under various mechanical stresses. Tensile strength is the highest tensile (pulling) stress a material can withstand before cracking or rupturing. Elongation at break is a measure of a material's capacity for stretching before breaking. It is calculated by comparing the length of a sample material at the site of fracture to its initial length, and is represented as a percentage.

For the analysis, all the film samples were submitted to Central Research Facillity, IIT Delhi, Sonipat campus. A universal testing machine (Zwick Roell, Germany Static UTM Z010) was used to evaluate the mechanical attributes (tensile strength in MPa and elongation-at-break %) of the films at room temperature and at a cross head speed of 10 mm/min in accordance with the standards set by the ASTM (Hiremani et al., 2022; Indumathi et al., 2019; Rodrigues et al., 2020; Saral et al., 2019; Soni et al., 2016; Yadav et al., 2019, 2020).

Water Absorption Capacity (WAC) %

Packaging materials' ability to absorb water is a vital factor in guaranteeing safety and preservation of goods when considering practical application, due to the fact that moisture can cause deterioration, degradation, or a shorter shelf life.

The water absorption capacity was investigated by cutting them into 2cm×2cm pieces, measuring their initial weight (W_1) and completely submerging in deionized water for a period of 24 hours for sufficient water absorption. Following swelling, the film samples were taken out from water and remaining water was removed from the film surface using filter paper, and the ultimate weight (W_2) was noted. The following formula was used for assessing the films' water absorption capacity (Hiremani et al., 2022; Yadav et al., 2020): Water Absorption Capacity (%) = W_2-W_1/ W_1×100

3.3.3 Barrier Property: Moisture, Oxygen, UV

Water vapor transmission rate (WVTR)

Food packaging that prevents or inhibits the transmission of water vapor/moisture/humidity, between the packaged food and the outside environment is referred to as having a water vapor barrier. As excessive moisture transfer can result in spoiling, textural changes, and microbiological development, this barrier is crucial for maintaining the quality, freshness, and duration of storage time of various food products.

The water vapor transmission rate was determined following the ASTME96 method (Indumathi et al., 2019). To perform this assessment, the film samples were securely affixed to the opening of the glass vial with a diameter of 1 cm. Anhydrous calcium chloride ($CaCl_2$) was pre-weighed and kept in these vials. The vials were then put in a desiccator set to maintain relative humidity of 75±5% using a saturated sodium chloride solution. The vials were weighed both before and after a day, and the formula provided was used to figure out the film's WVTR using the provided formula:

WVTR ($g/m^2/day$) = W/S where W is the weight gain of the glass vial following a day and S denotes the exposed film surface area.

Oxygen Transmission Rate and Permeability

The capacity of packing material to stop gas entry or egress between the packaged food and the outside environment is referred to as gas barrier qualities in food packaging. These characteristics are essential for preserving the freshness and longevity of different food items. The gas barrier property in terms of oxygen barrier ability was estimated by conducting oxygen transmission test.

The analysis of oxygen permeability was conducted utilizing the methodology highlighted by other researchers in their studies (Hiremani et al., 2022; Yadav et al., 2020). To perform the test, 2cm×2cm film samples were wrapped around the opening of the glass vials and enclosed securely using a strip to seal. Afterwards, these vials were put inside a desiccator at room temperature. Over the course of three-days, the weight of each vial was measured daily. The following equations were utilized to estimate the oxygen permeability transmission rate (OPTR) and Oxygen Permeability (OP):

$OPTR = Slope \div Area\ of\ the\ film$

$OP = (OPTR \times L) \div \Delta P$

where ΔP symbolizes the difference in partial vapor pressure between dry atmosphere and pure water (0.02308 atm at 25 °C), and L stands for the average film thickness.

UV Barrier

A protective barrier that restricts or prevents the penetration of ultraviolet light from the sun or other UV sources is known as an ultraviolet (UV) barrier. A form of electromagnetic radiation with shorter wavelengths than visible light is ultraviolet radiation. While the production of vitamin D in human beings and plant photosynthesis depend on UV light, excessive UV radiation exposure can be damaging to materials and living things. When UV barriers are included into food packaging materials, packed food is kept safe, retains its nutritional value, and meets consumer standards for quality and freshness.

The Ultra Violet blocking ability of the fabricated packaging was evaluated with UV-visible spectrophotometer, spanning the wavelength range of 280 to 800 nm. The film samples were cut into rectangle shapes measuring 3 cm × 1 cm and placed in a quartz cuvette for spectrophotometric investigation. A cuvette that was empty, utilized as the control (Hiremani et al., 2022; Indumathi et al., 2019; Saral et al., 2019).

3.3.4 Biodegradation

In the course of natural process of biodegradation, bacteria, fungi, and other biological agents break down organic compounds into less complex ones. This procedure is necessary to recycle organic materials and cycle it back into the environment so that other living things can use it. In order to sustain the sustainability and balance of an ecosystem, biodegradation is essential.

Biodegradation assessment was conducted employing a technique previously reported by other researchers to investigate the degradability of biopolymer-based films via the films being buried in soil and measuring the reduction in weight (Hiremani et al., 2022; Indumathi et al., 2019). Every day, tap water was put in to the soil to maintain the desired levels of moisture and humidity, with any extra water drained out of the pot's bottom. The films were first weighed (W_i) and then buried in freshly dug soil that was gathered from the field in a big pot. Every seven days, the films' deterioration process was observed. To do this, the sample had to be carefully taken out of the soil, rinsed with distilled water to get rid of any remaining soil, dried in a desiccator, and then weighed (W_f). Here under given formula was followed to further assess the degradation or weight loss percentages for all the films.

Degradation % or Weight Loss % = $(W_i - W_f)/ W_i \times 100$

where W_i and W_f stand for the film sample's initial and final weights, respectively.

3.3.5 Antimicrobial Test

The antimicrobial effectiveness of the best 3 nanocomposite films among all the films was assessed against gram positive bacteria representative *Staphylococcus aureus*, gram negative bacteria representative *Escherichia coli* and fungi *Candida albicans* by well diffusion method. The optimized film was further subjected to investigation regarding antimicrobial efficacy against 3 more microbial strains i.e., *Bacillus subtilis* (gram positive bacteria), *Pseudomonas aeruginosa* (gram negative bacteria), *Aspergillus niger* (fungi).

Before conducting the tests, Mueller Hinton agar media was prepared and sterilized, followed by transferring into sterilized petri dishes and left to solidify. Once solidified, the test microorganisms were evenly spread and distributed on the surface of the agar plates. Circular pieces of both the control and the nanocomposites, measuring 1 cm in diameter were placed on the inoculated nutrient agar plates. Following that, these plates were incubated for 24 hours at 37°C for bacteria and 48 hours at 27°C for fungus. The diameter of the microbial inhibitory zones was evaluated after the incubation time (Indumathi et al., 2019; Jiao et al., 2017; Rodrigues et al., 2020).

3.3.6 Shelf-life studies

The capability of the film to safeguard the fresh ginger and corn, and to prevent it from spoilage, shelf-life studies or packaging suitability of the optimized film was evaluated. For further comparative analysis, fresh ginger and corn was wrapped into optimized film CXZ3 (chitosan-xanthan gum-3% ZnO nanocomposite), and also in open air as reference. They were kept in a plate that has been sterilized beforehand, left untouched and undisturbed. The evaluation was carried out through visual examination at a regular interval on the basis of appearance i.e., odor, texture and color (Indumathi et al., 2019; Moreno et al., 2017; Noshirvani et al., 2017; Patel et al., 2019; Saral et al., 2019; Souza et al., 2018).

3.3.7 Toxicity Test

Toxicity test is important as it is related with the safety of the food product. The packaging films will not be edible but expected to have no toxicity when food is packaged. Though non-toxic materials are chosen to synthesize novel packaging material, safety of the final product (the film with optimized ratio, taking into account all the preceding characterization findings) was tested. To check toxicity of the film, MTT assay ISO (the International Organization for Standardization) 10993:5 standard method was followed, as done by researchers like Indumathi and workmates. The MTT assay, is a popular colorimetric technique for determining the viability and cytotoxicity of cells. It is designed to assess the invitro studies of biological response of mammalian cells (cell line L929) by using suitable biological indicator (Indumathi et al., 2019).

3.3.8 Statistical Analysis

Each experiment’s data was collected in triplicates, and it was statistically analyzed. Using OriginPro 2021, One-way analysis of variance (ANOVA) and the Tukey test ($p \leq 0.05$) were used to find differences between the data’s mean values, which were shown as mean±Standard deviation.

CHAPTER IV - RESULTS AND DISCUSSIONS

4.1 Film Preparation

Total 8 different types of films were synthesized as mentioned in the table below (Table no. 4.2) and shown in figure 4.1. Pure chitosan film, chitosan-xanthan gum composite, chitosan-xanthan gum nanocomposite containing 1, 3, and 5% montmorillonite nanoclay, and 1, 3, and 5% ZnO nanoparticles. All of them were created using solution casting technique, as it is widely adopted for crafting nanocomposite films based on chitosan due to cost effectiveness and ease of preparation (Kim et al., 2022). Studies have indicated that extended drying times at lower temperatures lead to the restructuring of the materials in the solution, attributed to the van der waals forces and hydrogen bonding between the hydrophilic tails of the chitosan and the water molecules (Homez-Jara et al., 2018). Table 4.1 depicts the constituents and their proportion. Among the combined biopolymers, the one with 1% chitosan & 0.5% xanthan gum exhibited favorable qualities upon visual inspection. Conversely, the others (1% and 1.5% of xanthan applied to treat individual solutions of 1% chitosan) were deemed unsuitable due their reduced smoothness and transparency. The selected blended film was additional reinforcement employing 1, 3, 5 and 7% of ZnO nanoparticles and montmorillonite nanoclay. Nevertheless, increasing amounts of nano fillers more than 5 % resulted in lack of transparency (in montmorillonite nanoclay based composite films) and were stiff in nature (in nano ZnO particle based composite films). The remainder of the films underwent additional testing (Table no. 4.2). In consequence of the moisture evaporation from the film’s upper surface in the casting tray, all of them exhibited shinier appearance on their lower sides compare to the upper sides. This property was also observed by Zhou and fellow investigators in their fabricated film (Zhou et al., 2022).

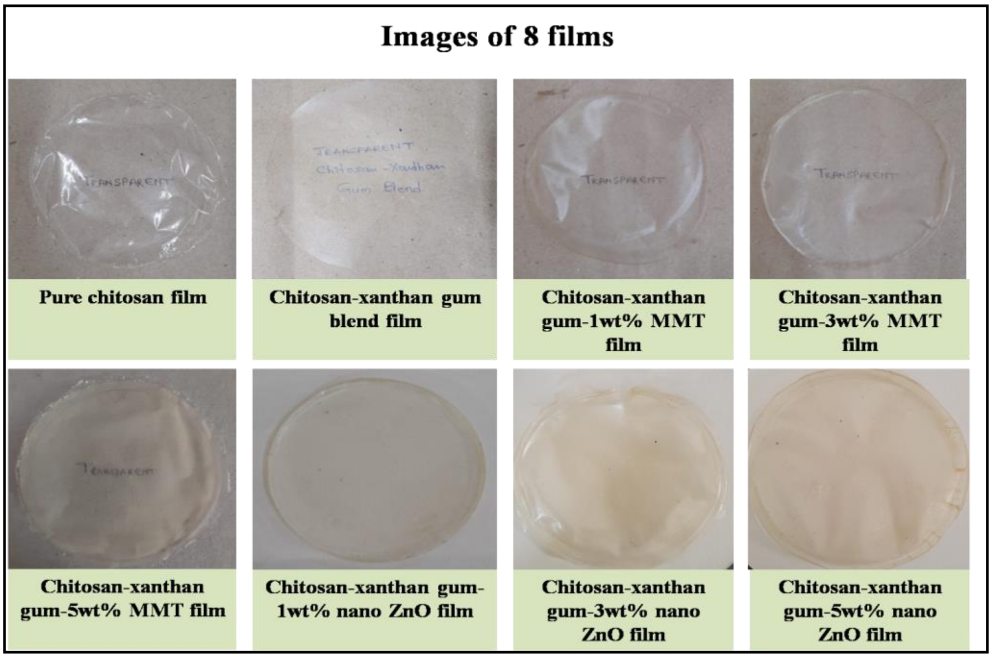
Table 4.1: Materials needed in production of every films and their ratios

S. No.	Chitosan	Xanthan gum	ZnO nanoparticles (%)	Montmorillonite nanoclay (%)	Remarks	Film Code

1.	1%	NA	NA	NA	Good film, transparent; Selected	C
2.	1%	0.5%	NA	NA	Good, transparent film; Selected	CX
3.	1%	1.0%	NA	NA	Decreased Transparency; Rejected	NA
4.	1%	1.5%	NA	NA	Brittle film; Rejected	NA
5.	1%	0.5%	1	NA	Good transparent film; Selected	CXZ1
6.	1%	0.5%	3	NA	Good transparent film; Selected	CXZ3
7.	1%	0.5%	5	NA	Good transparent film; Selected	CXZ5
8.	1%	0.5%	7	NA	Reduced transparency and stiffness; Rejected	NA
9.	1%	0.5%	NA	1	Good transparent film; Selected	CXM1
10.	1%	0.5%	NA	3	Good transparent film; Selected	CXM3
11.	1%	0.5%	NA	5	Good film, transparent; Selected	CXM5
12.	1%	0.5%	NA	7	Brittleness/stiffness; Rejected	NA

Table 4.2: Chosen films for further experiments and their code

S. No.	Code	Films
1.	C	Chitosan film (pure)
2.	CX	Chitosan-xanthan gum blend film
3.	CXM1	Chitosan-xanthan gum-1 % montmorillonite nanoclay film
4.	CXM3	Chitosan-xanthan gum-3 % montmorillonite nanoclay film
5.	CXM5	Chitosan-xanthan gum-5 % montmorillonite nanoclay film
6.	CXZ1	Chitosan-xanthan gum-1 % nano ZnO film
7.	CXZ3	Chitosan-xanthan gum-3 % nano ZnO film
8.	CXZ5	Chitosan-xanthan gum-5 % nano ZnO film



Images of 8 films

Pure chitosan film

Chitosan-xanthan gum blend film

Chitosan-xanthan gum-1wt% MMT film

Chitosan-xanthan gum-3wt% MMT film

Chitosan-xanthan gum-5wt% MMT film

Chitosan-xanthan gum-1wt% nano ZnO film

Chitosan-xanthan gum-3wt% nano ZnO film

Chitosan-xanthan gum-5wt% nano ZnO film

Figure 4.1: Digital photos of all the manufactured films

4.2 Characterization

Thickness Measurement

The thickness of the film is a significant component in determining its physical properties, including its optical and barrier properties. The thickness of the films did not change much when made of pristine biopolymer, combined biopolymers, or varied ratios of nanocomposite materials, but it did increase approximately 0.05 mm in thickness for various ratios. Table 4.3 lists the thicknesses that were measured. Figure 4.2 shows a favorable link between increasing the components ratio and thickness in both types of nanocomposite films.

Table 4.3: Thickness of all the films (Values are tabulated as Mean±Standard Deviation, n=3; Distinct letters within a column represent significantly varied values, at p≤0.05 using Tukey test)

S. No.	Films	Thickness (mm)
1.	C	0.05±0.003^{d}
2.	CX	0.06±0.01^{d}
3.	CXM1	0.06±0.0005^{cd}
4.	CXM3	0.07±0.008^{cd}
5.	CXM5	0.1±0.011^{a}
6.	CXZ1	0.08±0.003^{bc}
7.	CXZ3	0.09±0.005^{ab}
8.	CXZ5	0.1±0.005^{a}

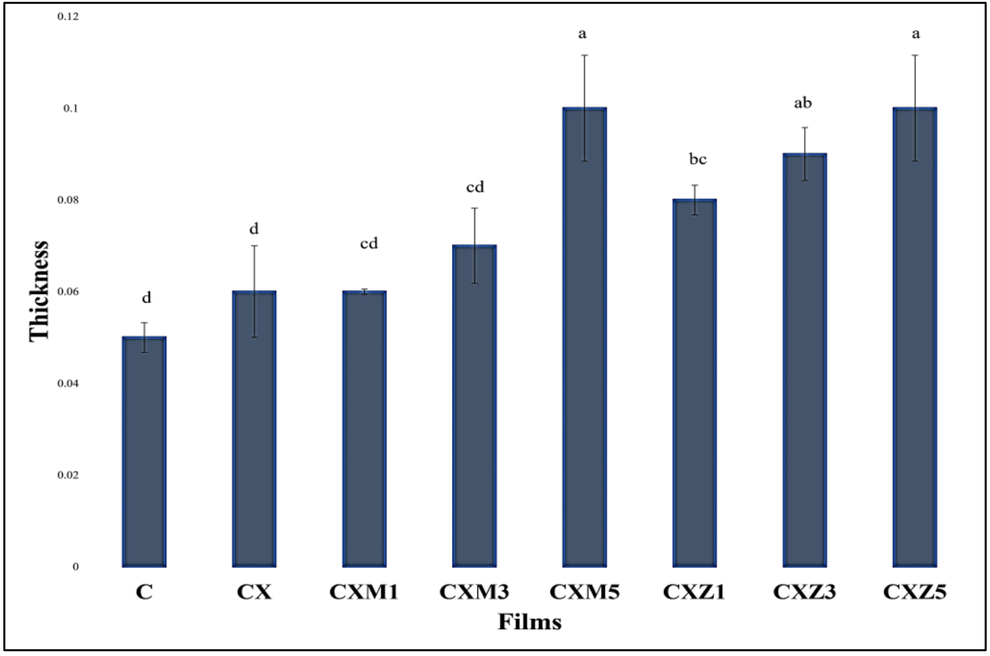


Figure 4.2: Measured thickness of all the films (Values are tabulated as Mean±Standard Deviation, n=3; Significantly different values are represented by different letters, at p≤0.05 using Tukey test)

Transparency/Opacity

Transparency in food packaging is for appealing among consumers as well as visual surveillance of goods during storage. The opacity values are summarized in the table 4.4 and the figure 4.3 represents the pattern. Visual evaluation, however, indicated that all of the films were transparent. The least opaque (most transparent) film discovered to be C film. The amount of transparency started reducing with the usage of xanthan gum and nanoparticles. The opacity of the nanocomposites intensifies along with the concentration of nano fillers, suggesting that the nanofillers (both MMT nanoclay and nano ZnO) distributed in them was the reason for the decrease in transparency. Other scientists have also reported similar observations (Indumathi et al., 2019; Saral et al., 2019). Nevertheless, upon closer examination, it became clear that none of the films appeared completely opaque and were rather transparent.

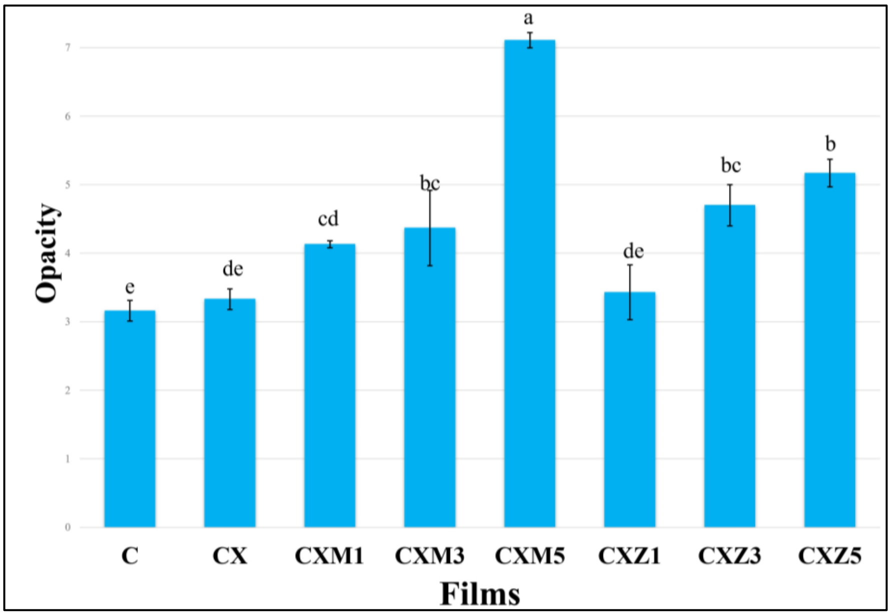


Figure 4.3: Opacity values of all the films (Values are tabulated as Mean±Standard Deviation, n=3; Significantly different values are represented by different letters, at p≤0.05 using Tukey test)

Table 4.4: Opacity of all the films (Values are tabulated as Mean±Standard Deviation, n=3; Distinct letters within a column represent significantly varied values, at p≤0.05 using Tukey test)

S. No.	Film Code.	Opacity
1.	C	3.16 ± 0.15^{e}
2.	CX	3.33 ± 0.15^{de}
3.	CXM1	4.13 ± 0.05^{cd}
4.	CXM3	4.36 ± 0.55^{bc}
5.	CXM5	7.11 ± 0.11^{a}
6.	CXZ1	3.43 ± 0.4^{de}
7.	CXZ3	4.7 ± 0.3^{bc}
8.	CXZ5	5.17 ± 0.2^{b}

Surface Morphology Analysis through FE-SEM

Field Emission Scanning Electron Microscopy was carried out to assess the surface appearance of the produced films, and the resulting microscopic images are shown in Figure 4.4 and figure 4.5. A uniform, smooth surface with tiny clusters that are either agglomeration of chitosan molecules or undissolved chitosan particles, may be seen in the SEM picture of pure chitosan. Numerous articles reported similar results (Hiremani et al., 2022; Rodrigues et al., 2020; Yadav et al., 2021). Surface of the film became smoother and brittleness got reduced due to addition of xanthan gum to the chitosan (figure 4.4).

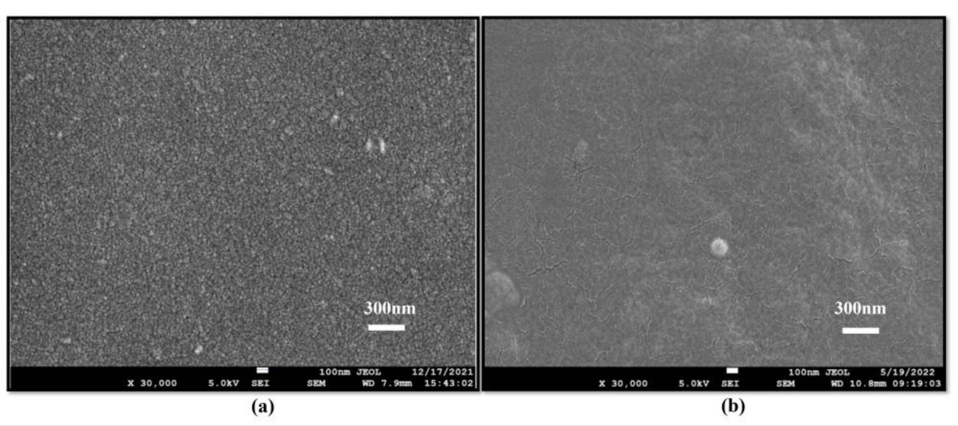


Figure 4.4: Surface appearance of C and CX [(a) chitosan and (b) chitosan-xanthan gum blend]

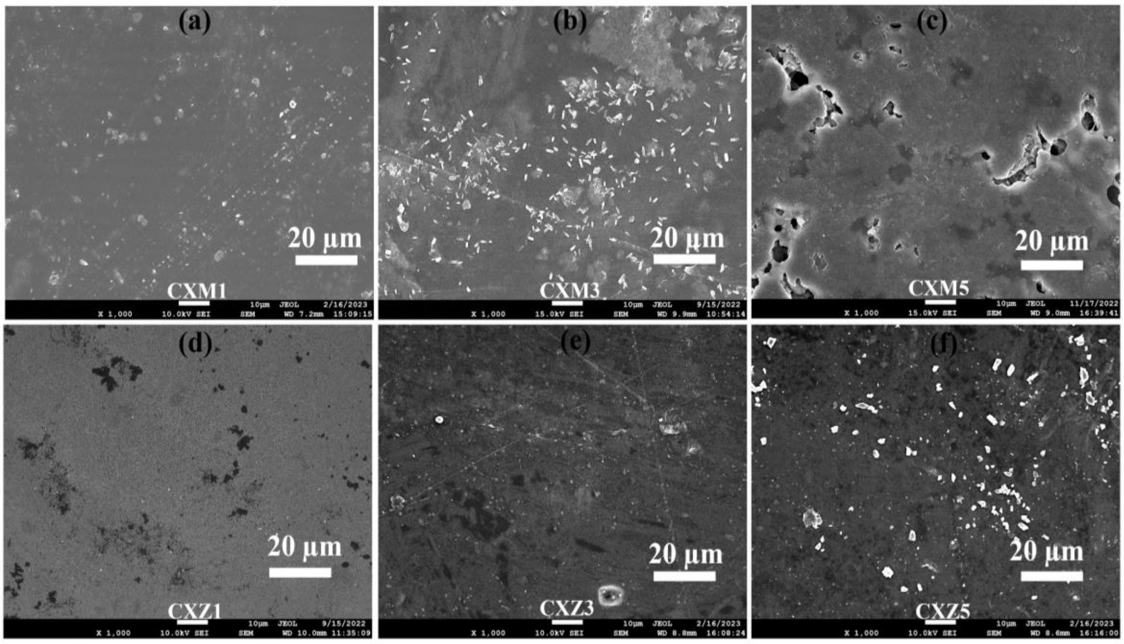


Figure 4.5: SEM micrographs of the nanocomposite (CXM1: 1% Montmorillonite nanoclay reinforced chitosan & xanthan gum blend, CXM3:3% Montmorillonite nanoclay reinforced chitosan & xanthan gum blend, CXM5: 5% Montmorillonite nanoclay reinforced chitosan & xanthan gum blend, CXZ1: 1% ZnO nanoparticles reinforced chitosan & xanthan gum composite, CXZ3: 3% ZnO nanoparticles reinforced chitosan & xanthan gum composite, CXZ5: 5% ZnO nanoparticles reinforced chitosan & xanthan gum composite)

The surface of all the bio-nanocomposites clearly displayed dispersed fillers. In the surface of CXM1 (figure 4.5 a) and CXZ1 (figure 4.5 d) lesser amount of nanoparticles was observed. When nanoparticles are provided, the surface roughness of the nanocomposites rises, in accordance to their ratio. According to Figure 4.5 b and e, the CXM3 and CXZ3 film surfaces appear to have the most uniform distribution containing nanoclay and nanoparticles, respectively. This is appropriate to establish compatibility among the materials that could ultimately improve film properties. Surface of CXM5 seems to have pore formation (figure 4.5 c), probably created by aggregates of fillers. The stiffness of the film (lowest flexibility compared to other films) is also responsible for its breakage. When the ZnO level was increased to 5 %, the nanoparticles began to agglomerate (Figure 4.5 f). This is consistent with other scientists' observations that nanofillers begin to aggregate after a particular ratio (Goudar et al., 2020; Saral et al., 2019).

ImageJ software was utilized for quantitative analysis to validate the qualitative findings. The software was used to process scanning electron microscopy images, enabling the investigation of particle sizes and distribution patterns of nanofillers. This was achieved by setting the scale using a known parameter like the scale bar and then analyzing the measurements. As previously demonstrated and discussed, no particles were observed on the surfaces of pristine chitosan (C) and chitosan-xanthan gum blend (CX). Nanoparticles were observed to have sizes ranging from less than 11 nm to 100 nm, with CXM5 showing a pore size of approximately 256 nm. On average, nanoparticles in CXZ3 were around 30 nm or less, but some agglomerations were noted, typically measuring between 95 nm to 108 nm. The CXZ5 film exhibited the highest agglomeration, with dimensions ranging from around 350 nm to 600 nm. The distribution of nanofillers was quantified by counting particles within specific areas, revealing an increase in nanofiller content with higher percentages utilized in the nanocomposite. These results, obtained using ImageJ software, corroborate the qualitative analysis mentioned earlier.

EDX

Energy-dispersive X-ray spectroscopy or analysis is an analytical method used for the elemental analysis or chemical characterization of a substance. Here, EDX spectra of two best ratio films i.e., CXM3 among clay nanocomposites and CXZ3 among clay nanocomposites are provided (figure 4.6 and 4.7).

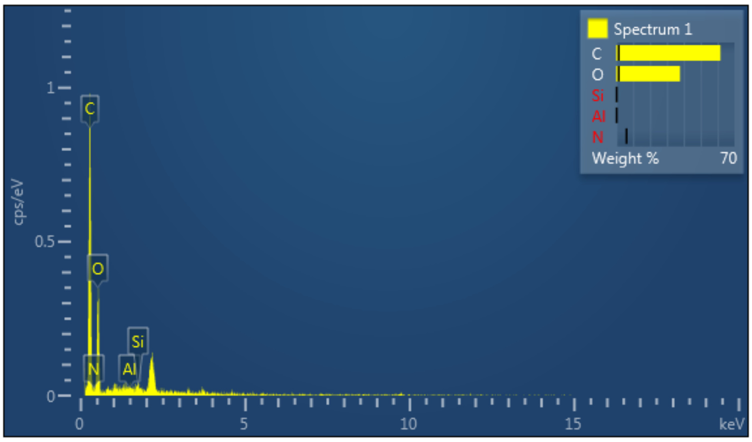


Figure 4.6: EDX of CXM3 nanocomposite

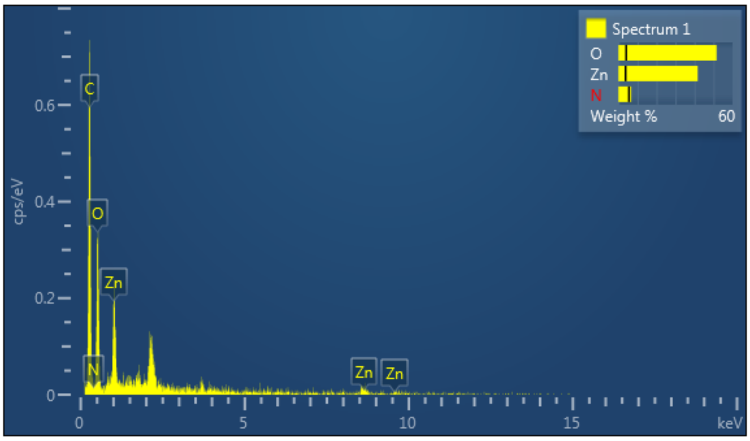


Figure 4.7: EDX of CXZ3 nanocomposite

The EDS spectra of clay nanocomposite (CXM3) shows the existence of Si, Al, O, that correspond to the elements found in montmorillonite structure. The occurrence of Zn and O, which are related to ZnO nanoparticles observed in the EDX spectrum of the nanocomposite (CXZ3) film.

Mapping

Mapping of CXM3 and CXZ3 film showed in figure 4.8 and 4.9, respectively, which portray the homogenous distribution of the elements (Rodrigues et al., 2020).

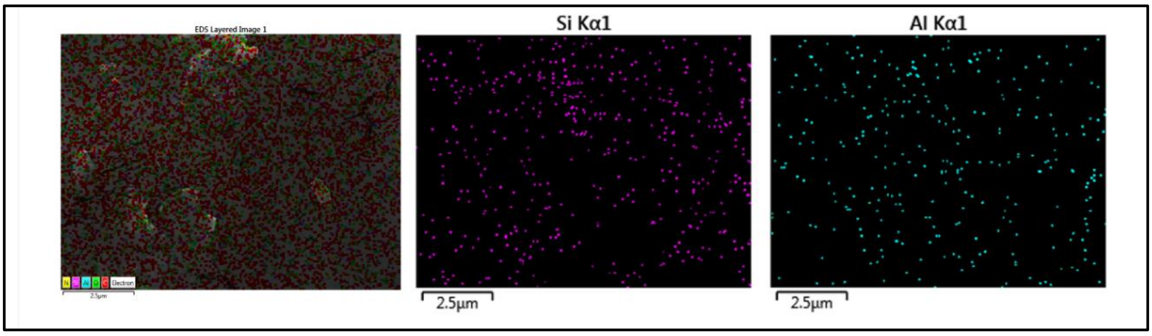


Figure 4.8: Mapping of CXM3 nanocomposite

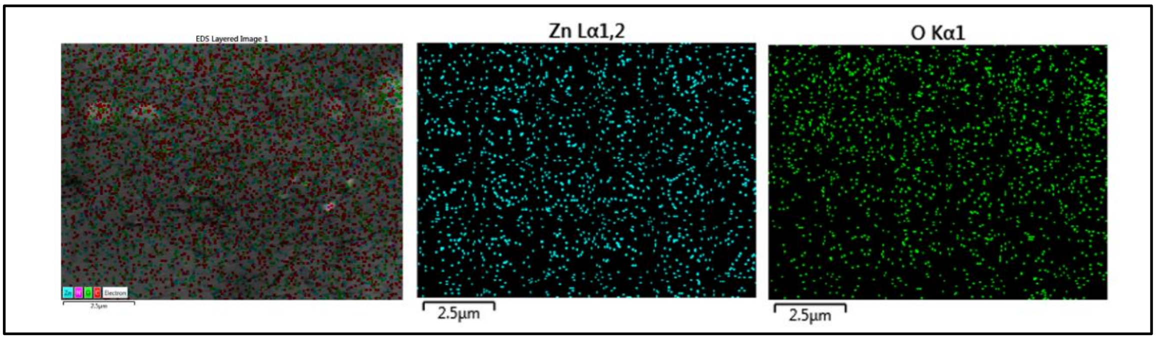


Figure 4.9: Mapping of CXZ3 nanocomposite

Fourier Transform Infrared spectroscopy

To ascertain the film's structural integrity FTIR was conducted. Figure 4.10 shows that film C has the band at 3231 cm^{-1} which is related to the stretching vibration of free hydroxyl (–OH). In addition, the amino groups present in chitosan molecules undergo both symmetric and asymmetric stretching of their N–H bonds (Saral et al., 2019 Siripatrawan & Harte, 2010). 2882cm^{-1} suggest existence of C–H bond. The amide I band, located at 1635 cm^{-1}, and the amide II band, located at 1539 cm^{-1}, were caused by C=O stretch and N-H bending, respectively (Brugnerotto et al., 2001; Indumathi et al., 2019). Symmetric deformation of the CH_2 group's vibrational modes is indicated by the peaks at 1405 cm^{-1} (Sanuja et al., 2015). 1151 cm^{-1} is associated with the asymmetric CO vibrations in oxygen bridge caused by chitosan deacetylation (Roy et al., 2018). The vibrational modes are connected to the peaks at 1066 cm^{-1} and 1022 cm^{-1} through stretching of the groups –OH, 3′–OH and 5′–OH (LH. Li et al., 2010) in chitosan molecules. Moreover, C–O is seen at the peak of 1022 cm^{-1} (Rodrigues et al., 2020). The presence of each of these previously listed functional groups is anticipated in the chitosan (de Morais Lima et al., 2017).

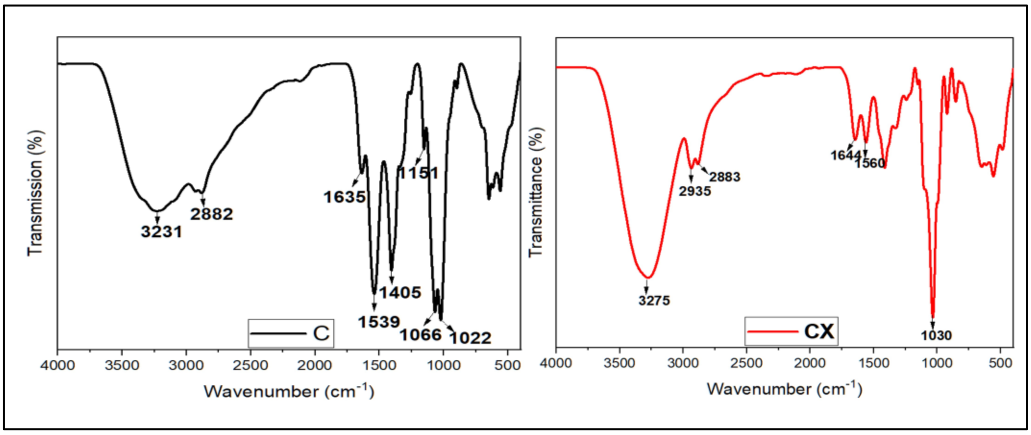


Figure 4.10: FTIR graphs of films C and CX (C: pristine chitosan, CX: chitosan & xanthan gum composite)

In blend film CX, the presence of peak at 3275 cm^{-1} signifies O-H stretching and hydrogen bonding. This peak moved slightly upwards compared to naive chitosan, indicating intermolecular hydrogen bonding between the biopolymers. This finding aligns with the research of Indumathi and colleagues in 2019 (Indumathi et al., 2019). The peaks at 2883 cm^{-1} and 2935 cm^{-1} indicates strong N–H stretching, while the peak at 1560cm^{-1} signifies presence of COO^{-} of xanthan gum and 1644 cm^{-1} corresponds to N-H bending of chitosan. Aforementioned observations validate the formation of complex system involving oppositely charged polyelectrolyte biopolymers, resulting in a well-structured blend. Comparable observation was studied by Lima and colleagues (de Morais Lima et al., 2017). Notable, the absence of peaks at 1066 cm^{-1} and 1022 cm^{-1} in the composites indicates bond formation involving the functional group (OH).

As shown in figure 4.11, in 1 & 2% clay nanocomposite films, FTIR peaks confirm strong O-H stretch and strong intermolecular H bond forming and also weak intermolecular bonding. In 5% clay nanocomposite film, the peak of O-H stretching and strong intermolecular bonding became less intense compared to previous two clay composites (1 and 3%). This means 5% clay nanocomposite film have lower and weak intermolecular bonding. As expected from nanoclay, the addition of MMT in the chitosan framework produces a peak shift linked with NH and OH groups, suggesting that –NH_2 and –OH groups of the chitosan probably are building H-bonds with –OH of clay (Wang et al., 2006). Strong and broad peak around 3271 cm^{-1} is associated with O-H stretch and intermolecular bonds. 2924 cm^{-1} states weak intermolecular bond formation. Additional peak at 2851 cm^{-1} corresponds to weak intermolecular bonding, which appeared in CXM3 specifically.

Broad band near 1012 cm^{-1} indicates Si–O vibrations originating from montmorillonite addition, confirms the presence of nanoclay in the composite, as seen in FTIR of MMT nanoclay (Paluszkiewicz et al., 2011). Excess of nanoclay displayed comparatively bigger peak here.

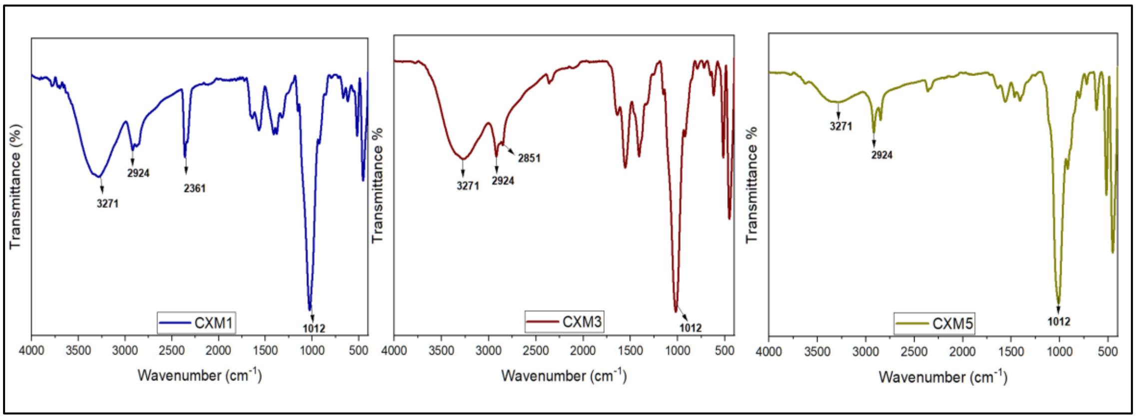


Figure 4.11: FTIR graphs of films CXM1, CXM3, CXM5 (CXM1: chitosan, xanthan gum, 1% MMT nanoclay composite, CXM3: chitosan, xanthan gum, 3%

MMT nanoclay composite, CXM5: chitosan, xanthan gum, 5% MMT nanoclay composite)

In the ZnO nanocomposite films (Figure 4.12), the employment of nano ZnO particles in the combined biopolymer matrix demonstrates that, as expected from ZnO nanoparticles, the peak corelating to -OH group is moved to a higher wavenumber (3256 cm^{-1}, 3293 cm^{-1}, 3299 cm^{-1}). These phenomena may be attributed to the formation of strong hydrogen interactions between nanomaterials and the biopolymers (Rodrigues et al., 2020). All the nanocomposites displayed comparable peaks, however, intensity in peaks associated with CXZ5 were lower, which could possibly be linked with the aggregation of nanoparticles, which usually lessen its interaction with polymer matrix (Lee et al., 2021). The peak located at 2929 cm^{-1} signifies intermolecular bonding and O-H stretch. 2361 cm^{-1}, which is equivalent to the potential for C–H, C=O, and –OH stretches. The band at 1404 cm^{-1} indicates O–H bending, whereas the peak at 1560 cm^{-1} confirms the existence of xanthan gum (de Morais Lima et al., 2017). The composite film's peak at 853 cm^{-1} implies that it contains nano ZnO. ZnO's stretching mode, which was measured at 489 cm^{-1}, indicates that ZnO nanoparticles are present in the mixture (Nirmala et al., 2013). FTIR investigation on ZnO nano-powder revealed comparable peaks.

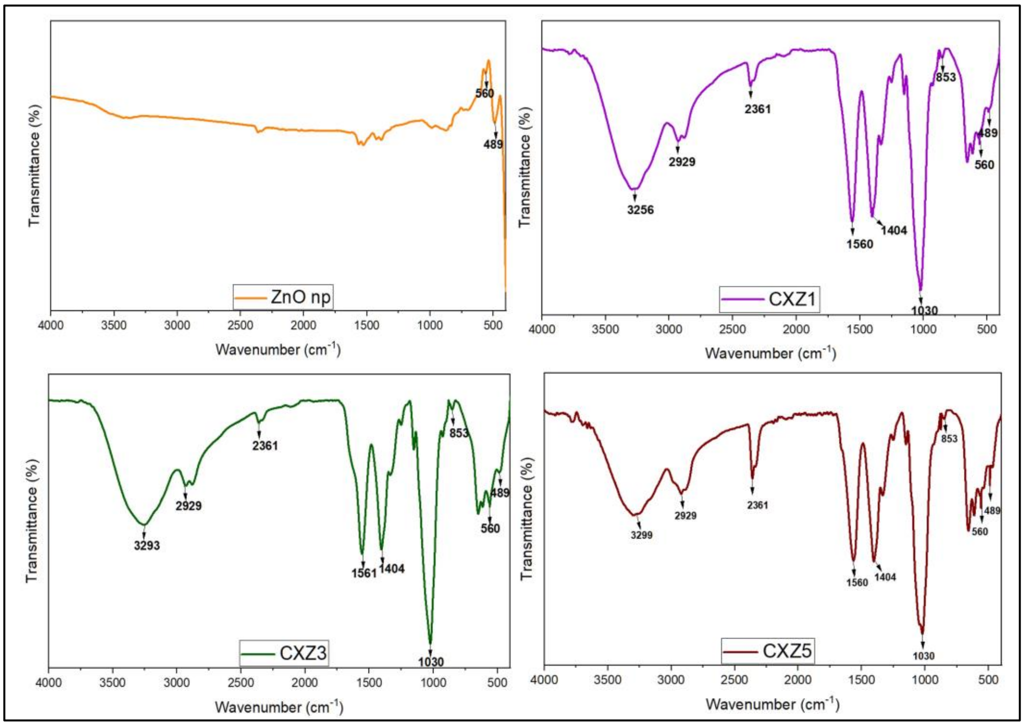


Figure 4.12: FTIR analysis of ZnO nanoparticles and ZnO nanoparticles reinforced bionanocomposites (ZnO np: ZnO nanoparticles, CXZ1: Chitosan-xanthan gum-1% nano ZnO film, CXZ3: Chitosan-xanthan gum-3% nano ZnO film, CXZ5: Chitosan-xanthan gum-5% nano ZnO film)

Hence, every nanocomposite film through analysis confirms that it, contains fillers, xanthan gum, and chitosan. Strong intermolecular bonding between the components is another finding of the FTIR research that points to fine compatibility. Chitosan and xanthan gum have a high probability of forming polyelectrolyte complexes with functional groups that are opposing to each other.

Thermogravimetric Analysis

Considering that temperature might have an impact on a film's stability, the thermal behavior of the synthesized package was examined. The amount of mass change that occurs when a substance is put to a program for regulated temperature is determined by thermogravimetric analysis. Therefore, thermogravimetric analysis allows for the determination of details like, to measure the temperature range at which the sample obtains a stable chemical make up as well as the rate at which other process like dehydration, oxidation, combustion takes place. For every sample examined in a nitrogen atmosphere shown in figure 4.13, 4.14, and 4.15 displays the TGA/DTG curve.

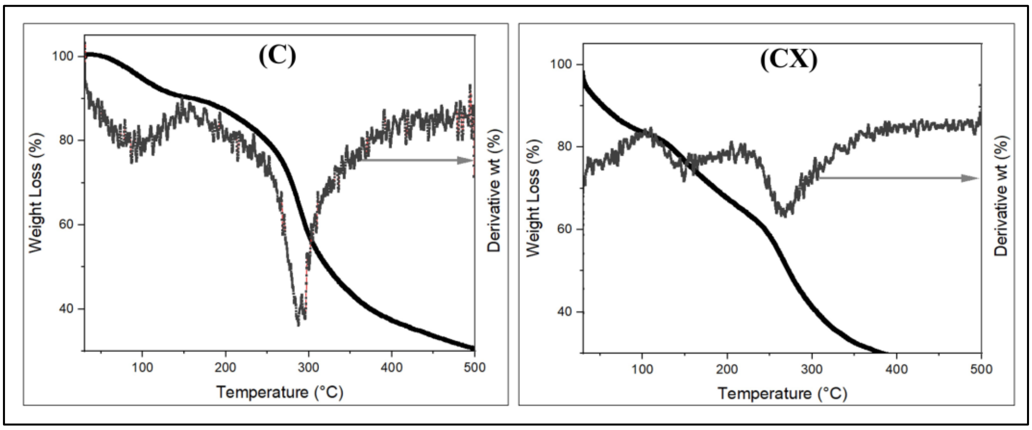


Figure 4.13: Thermal analysis of films C and CX (C: pristine chitosan, CX: chitosan-xanthan gum combined)

As indicated by the graph, the discharge of H_2O molecules that are connected to the functional groups i.e., amino and hydroxyl groups of the chitosan is represented by the initial thermal stage in the thermogram of the C film. The release process initiates at 31.01 °C and increases to 170 °C, making up 9.957% of the mass of the chitosan in total. The loss of organic material and the heat degradation of chitosan molecules are represented by the second thermal stage. This stage begins at 170 °C and ends at 425 °C, resulting in a mass loss of 54.075%. Chitosan was finally left with 30.406 % at 498.99°C. When compared to control film C, all other manufactured films have demonstrated equivalent level of heat stability. Within those, the CX film had a higher rate of degradation, and the CXZ3 film had a marginally better record of thermal stability. At the conclusion, it still had 45.74 weight percent at 499.28°C. This slight improvement (Yadav et al., 2019) is probably the result of improved matrix and filler interaction. Bhawna and colleagues also discovered that the TGA-DTG graph of all the films they had produced showed no variation (Soni et al., 2016).

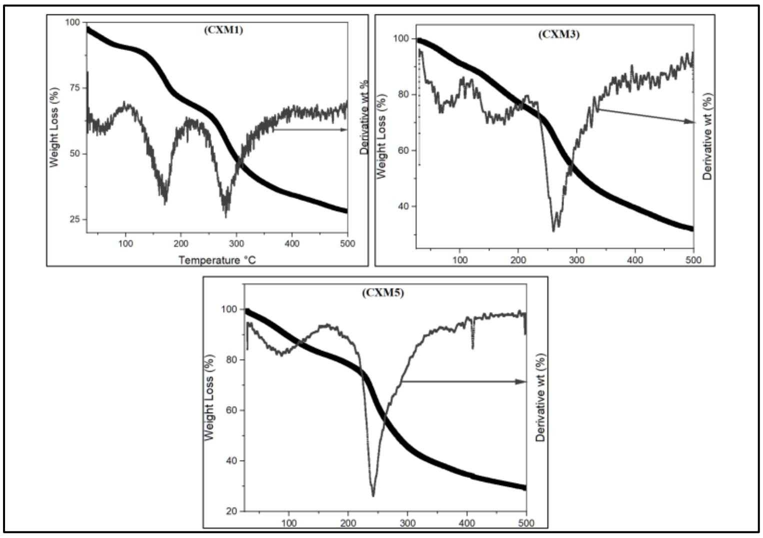


Figure 4.14: Thermal analysis of MMT reinforced bionanocomposites (CXM1: Chitosan-xanthan gum-1% MMT film, CXM3: Chitosan-xanthan gum-3% MMT film, CXM5: Chitosan-xanthan gum-5% MMT film)

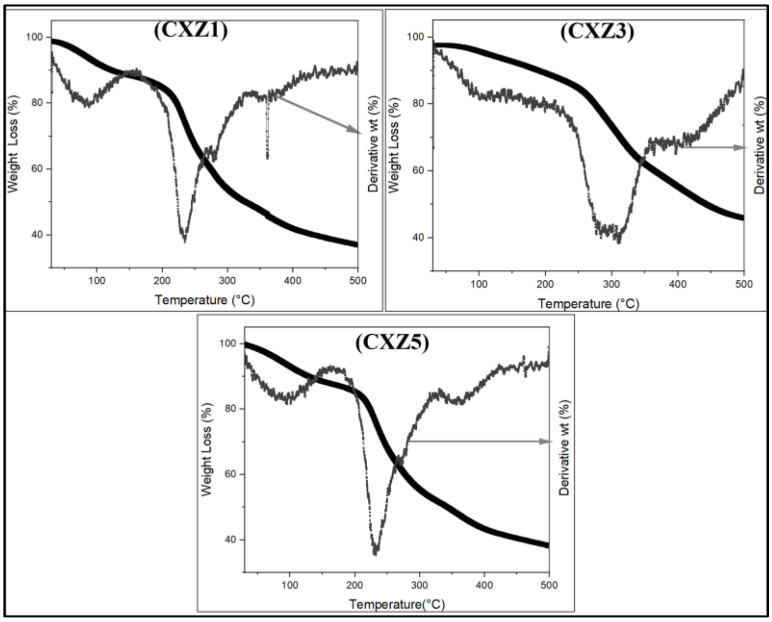


Figure 4.15: Thermal analysis of ZnO reinforced bionanocomposites (CXZ1: Chitosan-xanthan gum-1% nano ZnO film, CXZ3: Chitosan-xanthan gum-3% nano ZnO film, CXZ5: Chitosan-xanthan gum-5% nano ZnO film)

Mechanical Capability Analysis

Since polymer films should withstand the usual stresses experienced in their practical application and should be capable to safeguard food items throughout shipment, transit, and storage. Elevated elongation typically signifies great material, when accompanied with strong tensile properties. Mechanical property of all the prepared films in the current study were tabulated in Table 4.5 that displays the strengths and elongations at break of bionanocomposite films that are untreated biopolymer (C), combined biopolymers (CX), nanocomposites with different weight percentages (CXM1, CXM3, CXM5, CXZ1, CXZ3, and CXZ5). Tensile strength values lower than 10 MPa are considered to imply inferior mechanical qualities in films, according to Krochta and De-Mulder Johnston's categorization. As observed in this study, the control film (C) has the least strength. Chitosan's poor mechanical strength, according to researchers, are caused by its natural hydrophilicity (Indumathi et al., 2019). Its strength was shown to somewhat increase (from 3.67 MPa to 5.68 MPa) when xanthan gum and chitosan were combined. These results suggest that the presence of xanthan gum can improve interactions between molecules and cohesive force of the films. Their opposing functional groupings collaborate to provide a rise in strength and the potential for the creation of polyelectrolyte complexes, which led to a partial reduction in flexibility (de Morais Lima et al., 2017). Other researchers also discovered that greater cooperation among the components is attributed for boosting tensile strength, and decreased flexibility (Butnaru et al., 2019; Lee et al., 2021). However, nanofillers incorporation played great role in significant improvement of tensile strength, as well as elongation %.

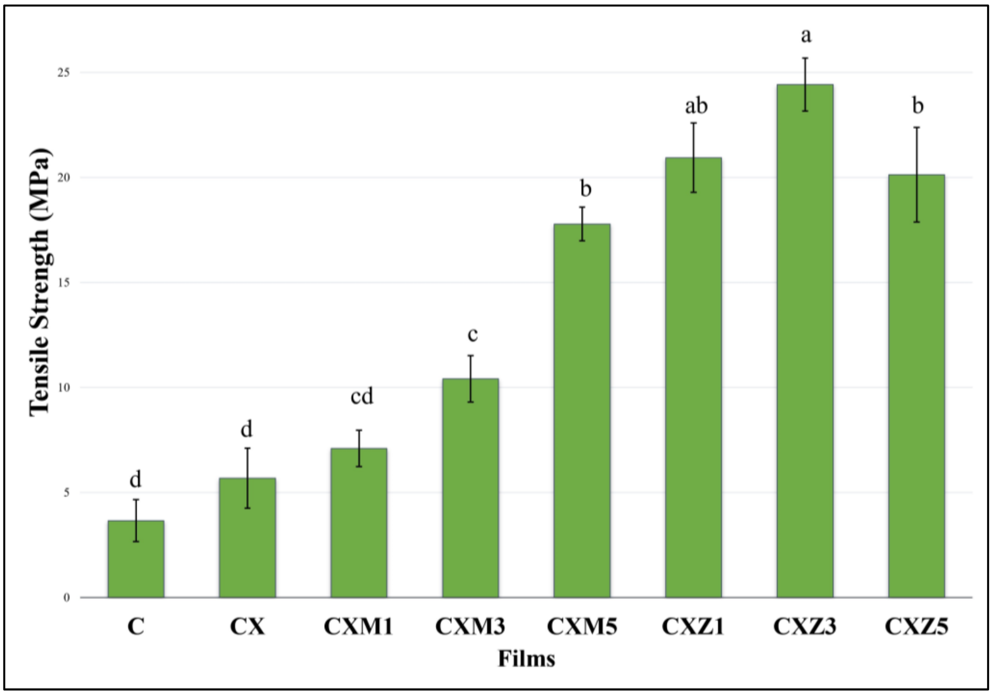


Figure 4.16: Tensile Strength of all the films (Values are tabulated as Mean±Standard Deviation, n=3; Significantly different values are represented by different letters, at p≤0.05 using Tukey test)

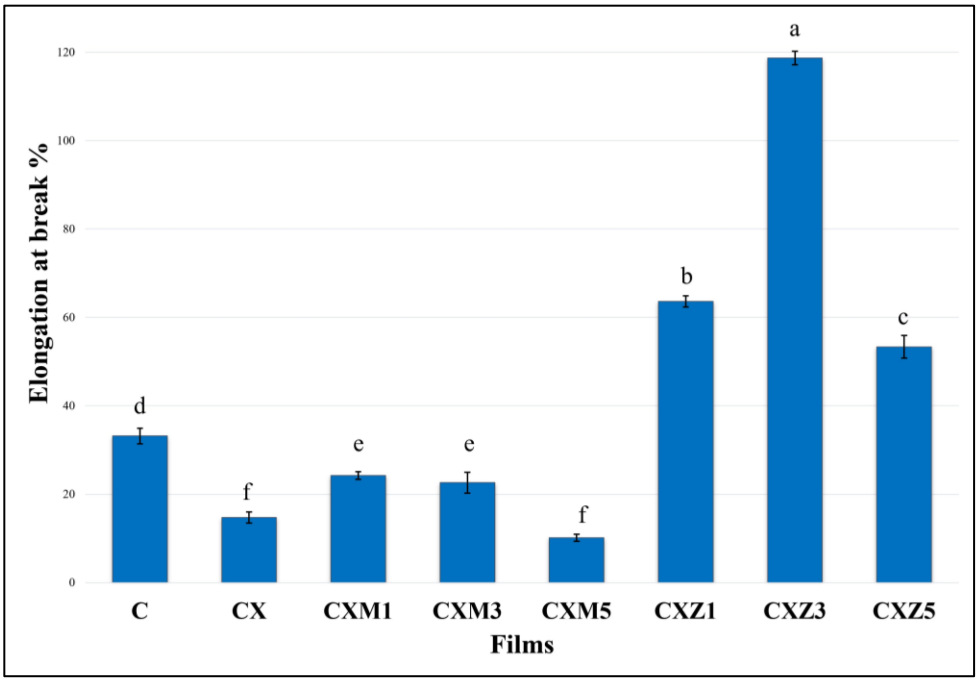


Figure 4.17: Elongation at break% (Values are tabulated as Mean±Standard Deviation, n=3; Significantly different values are represented by different letters, at p≤0.05 using Tukey test)

Moreover, 1% of montmorillonite nanoclay reinforcement resulted into more increase in tensile strength and good amount of flexibility. In 3 and 5% nanoclay incorporation also, gradual improvement in tensile strength was observed, but elongation started decreasing. Higher amount of nanoclay made the film stronger, but reduced flexibility, and made stiff in nature. In case of ZnO-based nanocomposites, all the films displayed improved mechanical performance in contrast to the control film C; revealing greater cooperation among matrices and fillers. The result aligns with past investigations (Amini et al., 2023; Hoque et al., 2022; Suyatma et al., 2023). According to reports, composite films produced with different nanomaterial strengthen the final product (Bumbudsanpharoke & Ko, 2019; de Oliveira et al., 2022; Dharini et al., 2022; Wu et al., 2022).

Table 4.5: Mechanical property of all the films (Values are tabulated as Mean±Standard Deviation, n=3; Distinct letters within a column represent significantly varied value, at $p \leq 0.05$ using Tukey test)

S. No.	**Films**	**Tensile Strength (MPa)**	**Elongation at break (%)**
1.	C	3.66 ± 1.00^{d}	33.16 ± 1.75^{d}
2.	CX	5.68 ± 1.43^{d}	14.72 ± 1.29^{f}
3.	CXM1	7.1 ± 0.86^{cd}	24.21 ± 0.88^{e}
4.	CXM3	10.41 ± 1.1^{c}	22.6 ± 2.34^{e}
5.	CXM5	17.78 ± 0.8^{b}	10.15 ± 0.8^{f}
6.	CXZ1	20.94 ± 1.65^{ab}	63.6 ± 1.25^{b}
7.	CXZ3	24.42 ± 1.26^{a}	118.66 ± 1.52^{a}
8.	CXZ5	20.13 ± 2.25^{b}	53.33 ± 2.56^{c}

Larger surface area of the nano-filler promoted its interactions, facilitating the process of stronger cross-linking with the polymer chains, and leading to the formation of more robust and durable films (Lee et al., 2021). With ZnO nanoparticle incorporation ratio, the tensile strength gradually increased, reaching 20.94 MPa in CXZ1 and 24.42 MPa in CXZ3. These results surpassed the tensile strength of LDPE (8–10 MPa), Ethylene vinyl alcohol (6–19 MPa), Polycaprolactone (4 MPa), and are comparable to High-density polyethylene (19–31 MPa) (Yadav et al., 2019, 2020). However, after being loaded with an additional 5 % ZnO, the film's tensile strength began to decline. This can be attributed to the excessive ZnO adhering to the combined biopolymer matrix at this specific loading concentration. This observation is in accordance with the findings of Lomate and coresearchers, who reported this in case of LDPE/Cu nanocomposite. In this current study, 3% ZnO reinforced composite gained the highest (118.66%) elongation at break, around 3.57 times higher than the control. That implies ZnO nanoparticles and the enhancement of tensile strength exhibit no influence with the mobility of polymer chains and even improve the stretchy mechanism, as stated by other researchers (Amini et al., 2023; Dehghani et al., 2019). Therefore, this concentration (CXZ3) may be considered optimum for achieving the necessary enhancement in mechanical characteristics. Based on the outcomes of FTIR assessments, this ratio composition represented the greatest proportion of hydrogen bonding between molecules. Microscopy images from SEM proved advancements in homogeneity. The nanocomposite film displayed fewer interface flaws, because of the uniform distribution of ZnO nanomaterials in the combined biopolymer matrix, which prevents molecular coagulation and maximizes the strength (Silvano et al., 2018). The clustering of nanofillers is to blame for the fact that, in both situations, tensile strength and elongation percentage first increase before they start to decrease gradually (Biswas et al., 2023; Yadav et al., 2019).

Water absorption capacity (WAC) %

For further practical uses, a film's water sensitivity must be assessed in order to determine its water absorption capacity. The outcomes of the studies are displayed in Table 4.6 below. Pristine chitosan film C possessed greatest WAC of 75.68% owing to its inherent limitation i.e., hydrophilicity. With a blend of MMT/ZnO nanomaterials and xanthan gum, its affinity for water sensitivity was significantly reduced. Similar patterns were seen in the findings that other researchers had also reported (Hiremani et al., 2022). Chitosan's hydrophilic functional groups (OH and NH_2) are what primarily account for its strong water absorption. As seen in two different earlier studies (Yadav et al., 2019, 2020), Yadav and colleagues states that lack of -OH groups owing to H-bonding with nano fillers are is charge of the reduced absorption of moisture. However, Ni and colleagues claim that ZnO nanoparticles can increase hydrophobicity. Their constructed starch film confirmed this claim by reducing the film's hydrophilicity through the addition of 2% nano ZnO (Ni et al., 2018). MMT-based nanocomposite also showed hydrophobicity (Dai et al., 2022).

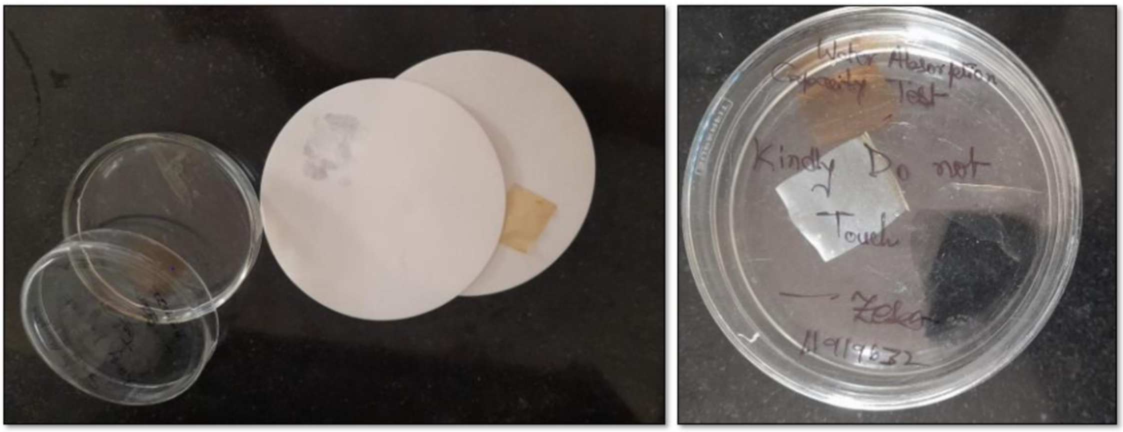


Figure 4.18: Water absorption capacity (WAC) test

Table 4.6: Water Absorption Capacity % (Values are tabulated as Mean±Standard Deviation, n=3; Distinct letters within a column represent significantly varied values, at $p \leq 0.05$ using Tukey test)

S. No.	Films	WAC %
1.	C	75.68 ± 1.84^{a}
2.	CX	68.60 ± 0.38^{b}
3.	CXM1	39.76 ± 0.41^{e}
4.	CXM3	33.74 ± 1.35^{f}
5.	CXM5	51.37 ± 0.95^{d}
6.	CXZ1	31.07 ± 1.56^{f}
7.	CXZ3	30.36 ± 1.2^{f}
8.	CXZ5	55.11 ± 1.75^{c}

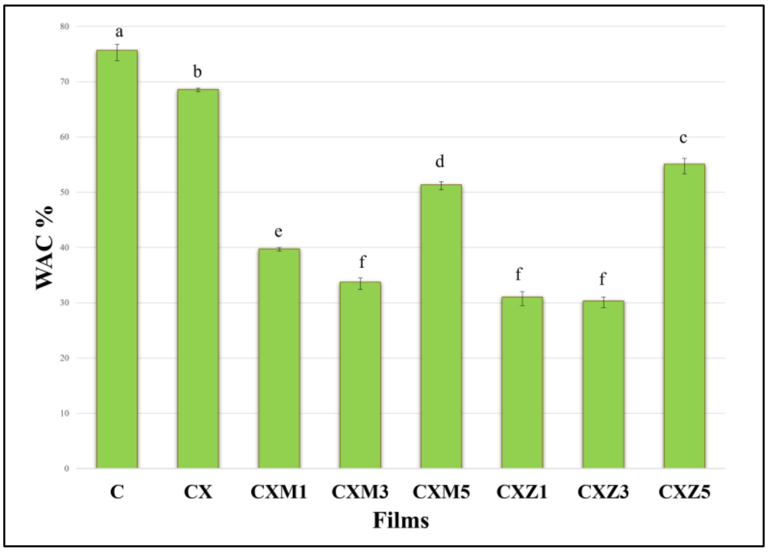


Figure 4.19: Water Absorption Capacity % (Values are tabulated as Mean±Standard Deviation, n=3; Significantly different values are represented by different letters, at $p \leq 0.05$ using Tukey test)

4.3 Barrier Properties

Water Vapor Transmission Rate (WVTR)

The shelf life of packaged foods is significantly influenced by the transmission of water vapor to a certain rate. Foods that have been packaged are easily damaged by moisture absorbed from the environment, hence the packaging film needs to have an extremely strong moisture barrier. WVTR is responsible in determining the film's stability and ability to delay the microbiological deterioration of stored food (Saral et al., 2019). Table 4.7 summarizes and Figure 4.21 represents the water vapor transmission rate data for the manufactured films. The permeability of water vapor was determined to be g/m^2 /day.

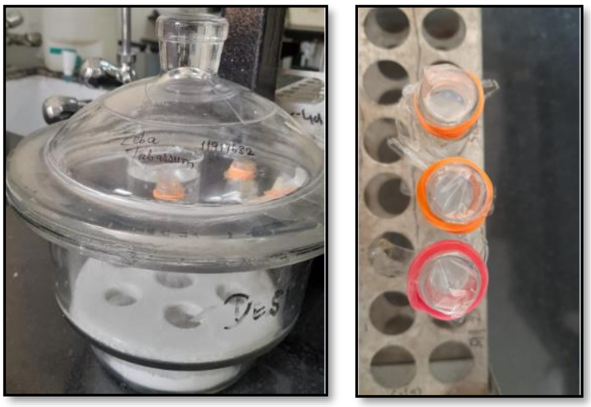


Figure 4.20: Moisture transmission test

The pristine chitosan film (C) had the greatest Water Vapor Transfer Rate (WVTR) measuring 560.93 g/m^2/day, because of its inherent hydrophilic qualities. This finding aligns with the results of other researchers (Sanuja S et al., 2015; Saral et al., 2019). Chitosan and xanthan gum were combined to create a film (CX) that had moderate hydrophilicity and roughly 11.79% less permeation of water vapor than the unmodified chitosan (C). The interactions and hydrogen bond creation between the biopolymers are accountable for this reduction. The results reported here are consistent with observation by other researchers working in this area (Hiremani et al., 2022; Indumathi et al., 2019; Saral et al., 2019; Soni et al., 2016; Uthaya Kumar et al., 2020; W. Zhang et al., 2023). FTIR spectroscopy also suggest the same. The addition of nanofillers lowered the WVTR value (CXZ3<CXZ1<CXM3<CXZ5<CXM1<CXM5), indicating that the nanofillers successfully filled the vacant space in the film structure, giving the bioplastic a significant improvement against moisture resistance. CXZ3, CXZ1 and CXM3 displayed the best result by decreasing WVTR 54.96%, 51.77%, and 47.61%, respectively. Similar claims were put forward by numerous other studies that nanomaterial utilization in biopolymer or blends is accountable for the decrease in vapor diffusion. And one of the main variables that contributes to enhanced water barrier behavior is the less or impermeable characteristics of the nanofiller (Indumathi et al., 2019; Mujeeb Rahman et al., 2018; Noshirvani et al., 2017; Saral et al., 2019; Yadav et al., 2019).

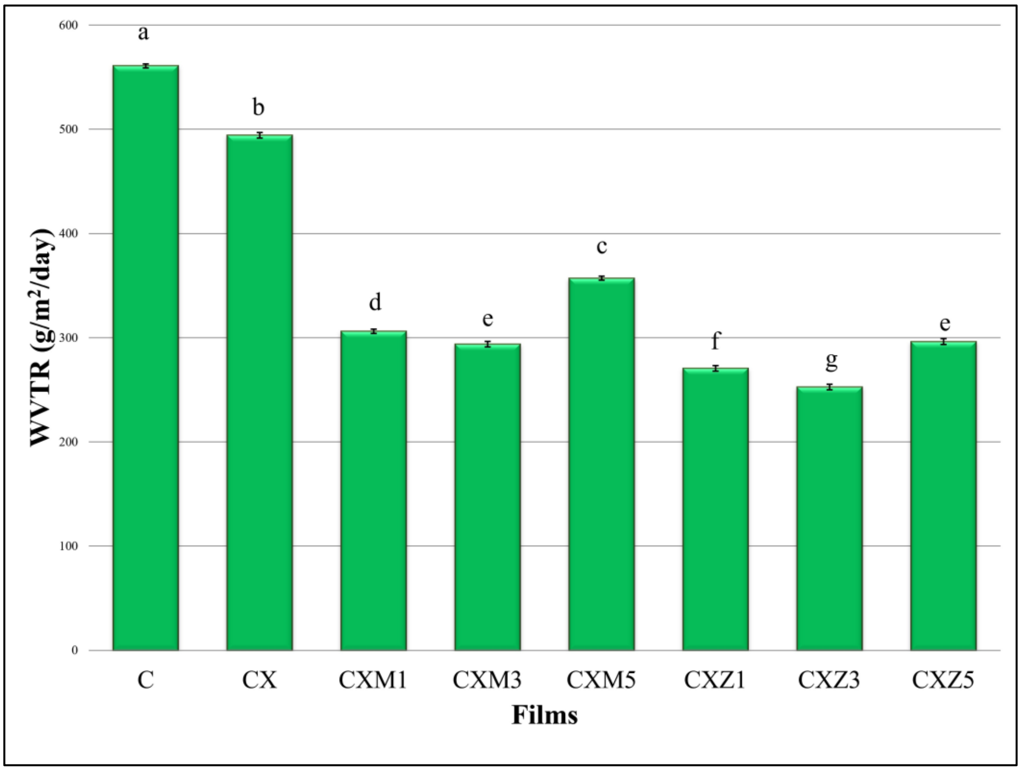


Figure 4.21: Water Vapor Transmission Rate (Values are tabulated as Mean±Standard Deviation, n=3; Significantly different values are represented by different letters, at $p \leq 0.05$ using Tukey test)

Table 4.7: Water Vapor Transmission Rate (Values are tabulated as Mean±Standard Deviation, n=3; Distinct letters within a column represent significantly varied values, at $p \leq 0.05$ using Tukey test)

S. No.	**Films**	**WVTR (g/m^2/day)**
1.	C	560.92 ± 1.94^{a}
2.	CX	494.26 ± 2.55^{b}
3.	CXM1	306.15 ± 1.94^{d}
4.	CXM3	293.83 ± 2.65^{e}
5.	CXM5	357.1 ± 1.94^{c}
6.	CXZ1	270.48 ± 2.65^{f}
7.	CXZ3	252.62 ± 2.68^{g}
8.	CXZ5	296.29 ± 2.76^{e}

Oxygen Transmission Rate and Permeability

Although, with regard to fresh fruit and vegetables undergoing respiration, entirely suppressing gas permeation is not desirable (Indumathi et al., 2019; Robertson, 2005) However, excessive oxygen inside the packaging might reduce the shelf life of food by creating an environment that is conducive to microbial growth (Ariyarathna et al., 2017). Transport and movement of oxygen can lead to food spoilage due to oxidation that can degrade sensory and nutritional qualities, able to promote number of changes in its features to food properties, i.e., alteration of taste, color, texture and other aspects. Using packaging made of nanocomposite materials with a sufficient oxygen barrier helps to improve food quality and expand its lifespan. The ability of oxygen gas to permeate through packaging films depends on its microstructure and structural configuration, as well as vacant space in structural network etc. Thus, the oxygen transmission rate and permeability value (shown in Table 4.8) can be decreased by strong connections and systematically arranged hydrogen bonding.

Figure 4.22: Gas barrier test

The introduction of the xanthan gum to the chitosan (CX) resulted in 3.34% decrease in OTR, in comparison to the untreated chitosan C. It clearly implies that alteration in the interchain arrangements is due to interaction between the both biopolymers. Various researchers came to similar conclusions, emphasizing the effectiveness of combining biopolymers to elevate the gas barrier, as demonstrated in different studies (Hiremani et al., 2022; Indumathi et al., 2019). The research findings indicate that compared to single (C) and combined (CX) biopolymer film, the inclusion of both types of nanofillers substantially lowered transmission of oxygen gas. Studies have shown that the capacity of the nanostructures to plug holes and improved molecular interaction, which produced compact structures, were likely responsible for this improvement (Saral et al., 2019; Soni et al., 2016). The oxygen barrier qualities of commercial food packaging composed of LDPE and LLDPE are restricted, allowing substantial permeation of oxygen (4000 cm^3/m^2/day). Excessive oxygen circulation rates through the packing film are because of its insufficient ability to withstand oxygen diffusion, which can quickly contaminate packed food with microbes. According to the current investigation's findings, CXM3, CXZ1, and CXZ3 showed promising potential as packaging materials that can effectively protect food from germs and stop oxidation. Other investigations utilizing bio-based composite films have presented similar outcomes (Indumathi et al., 2019; Saral et al., 2019).

It's crucial to remember that the ability of oxygen penetration through the film can change depending on elements like its surrounding environment and the thickness of the packaging material. All of the fabricated films used in this investigation show gradual rise in oxygen permeability with film thickness when 0.02308 atm pressure at 25 °C was taken into account.

Table 4.8: Oxygen Transmission Rate and Oxygen Permeability (Values are tabulated as Mean±Standard Deviation, n=3; Distinct letters within a column represent significantly varied values, at $p \leq 0.05$ using Tukey test)

S. No.	Film	Oxygen Transmission Rate (cm^3/m^2.day)	Oxygen Permeability (cc/m·24 h·atm)
1.	C	1910.64 ± 1.15^a	4.12 ± 0.01^b
2.	CX	1846.92 ± 0.57^b	4.78 ± 0.02^{ab}
3.	CXM1	1783.4 ± 1.15^e	4.63 ± 0.77^{ab}
4.	CXM3	1719.96 ± 1.5^f	5.21 ± 0.33^{ab}
5.	CXM5	1821.43 ± 1.6^c	7.89 ± 0.1^a
6.	CXZ1	1630.57 ± 0.44^g	5.62 ± 0.02^{ab}
7.	CXZ3	1579.66 ± 0.28^h	6.15 ± 0.02^b
8.	CXZ5	1809.03 ± 0.96^d	7.86 ± 0.03^a

UV Barrier Property

When packaged goods are exposed to UV and visible light, they can readily decay, change colour, and lose nutrients. Therefore, the capacity of packaging to screen out UV light is essential. Most of the commercial packaging have limited ability to absorb UV light (Ramos et al., 2013). In figure 4.24, 4.25, 4.26 the ultra violet-visible absorption spectrum of the manufactured films is between 200-800 nm wavelength.

Figure 4.23: UV absorption test through spectroscopy

The chitosan film in its pure form exhibited minimal absorption, whereas, the engineered nanocomposite film showed increased absorption, particularly in the UV spectrum. This highlights the films effectiveness as a robust UV barrier, indicating its great potential for UV protection (Hiremani et al., 2022).

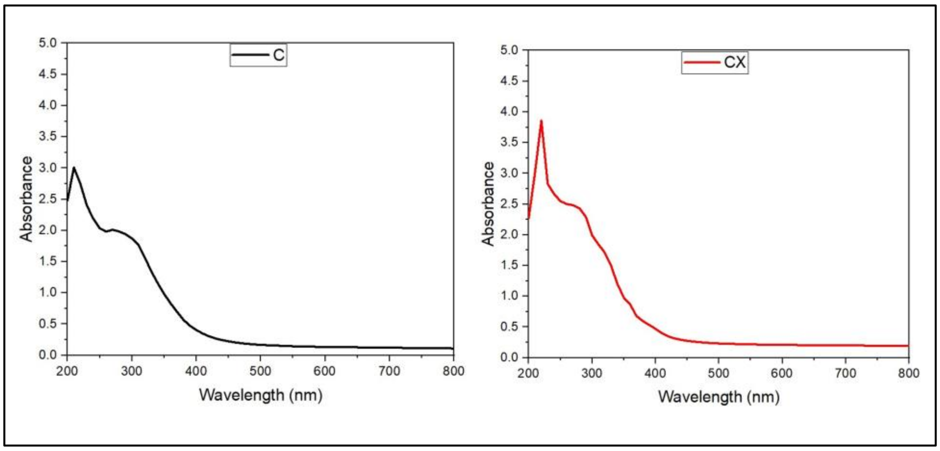


Figure 4.24: UV absorption of film C and CX (C: pure chitosan film, CX: chitosan-xanthan gum)

The UV-blocking characteristics of the film had been significantly impacted by the addition of ZnO and MMT nanoparticles. Other researchers have made a similar claim, noting that nanocomposites could potentially be able to shield food from UV rays (Indumathi et al., 2019; Nath et al., 2022; Palak et al., 2022; Saral et al., 2019).

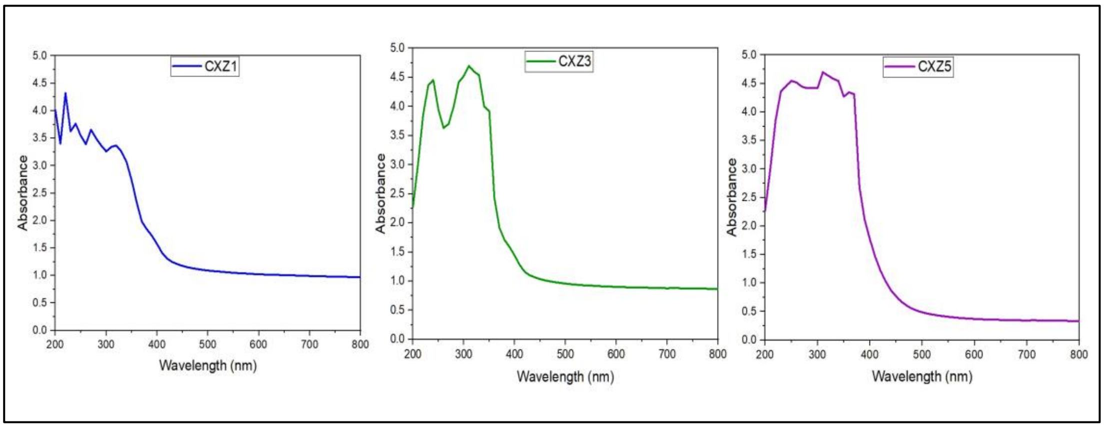


Figure 4.25: UV absorption of ZnO bionanocomposites (CXZ1: Chitosan-xanthan gum-1% nano ZnO film, CXZ3: Chitosan-xanthan gum-3% nano ZnO film, CXZ5: Chitosan-xanthan gum-5% nano ZnO film)

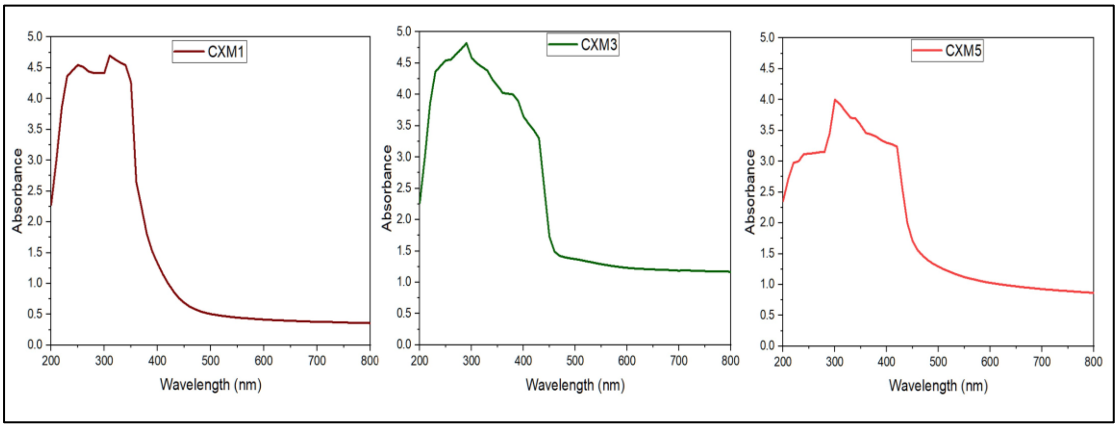


Figure 4.26: UV absorbance of MMT nanoclay bionanocomposite films (CXM1: Chitosan-xanthan gum-1% MMT nanoclay, CXM3: Chitosan-xanthan gum-3% MMT nanoclay, CXM5: Chitosan-xanthan gum-5% MMT nanoclay)

4.4 Biodegradability

Biodegradable films don’t pollute, thereby doesn't turn to environmental hazard. The degradation process is determined by the nature, chemical composition/modification, crystallinity, content of the fillers used etc. (Vasile et al., 2018). Fillers can limit the degradation process and cause improvement in its durability. It can be achieved through establishment of dense network or because of their antimicrobial function (Glaskova-Kuzmina et al., 2021; Neibolts et al., 2020; Platnieks et al., 2021). Environmental elements such as microbes, temperature, humidity, the pH of the medium (soil) etc. have a substantial impact on the structure, morphology, and characteristics of polymeric materials (Vasile et al., 2018). Alteration in weight and structure of the film is beneficial to determine the biodegradation of the tested film. In this work, to check the weight loss% of the film, they are soil buried in laboratory condition, as shown in figure 4.27.

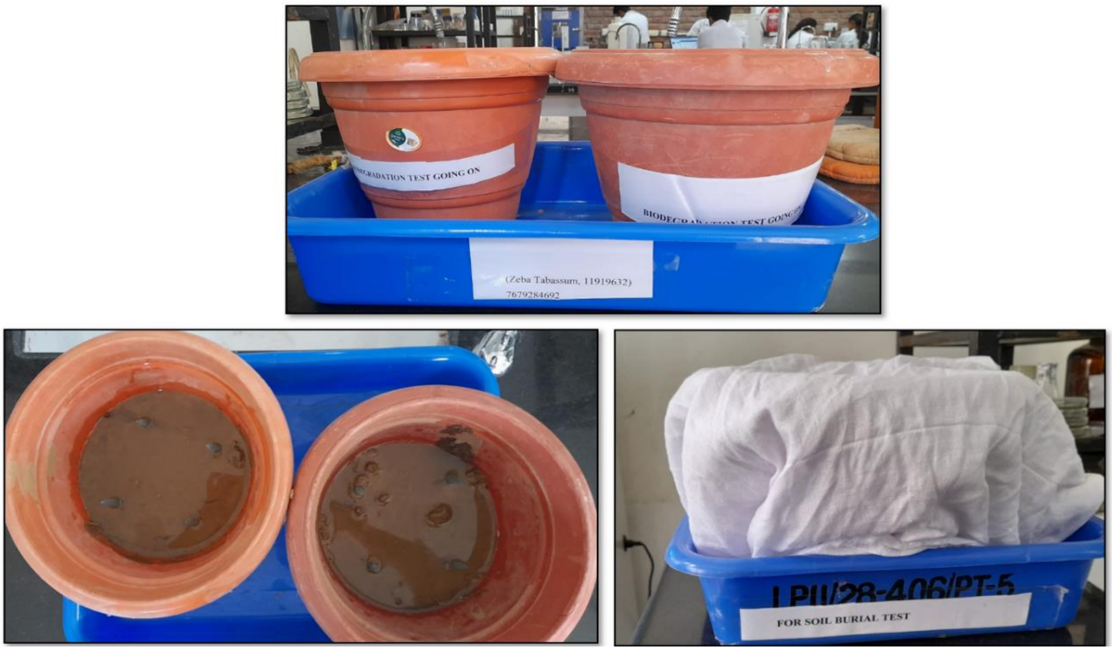


Figure 4.27: Soil burial biodegradation in laboratory experiment

In general, experiments with soil burial reveal a two-stage deterioration, commencing with the film's internal water diffusion, which causes them to swell and promotes the development of bacteria on them, enzymatic and other secreted degradation occurs in the second stage, causing mass loss and film sample disintegration (Hiremani et al., 2022). All of the produced films that were analyzed for biodegradation lost weight, had alterations to their structural integrity, and underwent bulk erosion type degradation (Kabir et al., 2020). The control pure chitosan film (C) degraded completely in 1st month, while the CX films degraded about 70% of its initial weight. Polymeric films that are hydrophilic tend to degrade more rapidly and experience higher weight loss rate overall. The presence of moisture in the soil weakens the polymer structure by entering it and rendering the glycosidic connections undergo hydrolysis. Indumathi and team observed that chitosan film they created completely broke down within first month (Indumathi MP et al., 2019). Whereas, Hiremani et al. found to have degradation rate of 78.34 ± 2.00% in pure chitosan film within first 15 days (Hiremani et al., 2022). With respect to water absorption, cassava flour-base plastic also demonstrated quicker breakdown (Wahyuningtyas & Suryanto, 2017).

CX lost its 100% weight in 1.5 months. The MMT (1, 2 & 3%) and ZnO (1, 2 & 3%) bionanocomposite films are more durable and had lower weight loss% in soil burial degradation compared to untreated one. That means, combining chitosan with xanthan gum and introducing nanomaterial strengthened the film's endurance by virtue of their intermolecular connections. CXM1, CXM5 and CXZ5 degraded completely of its initial weight within 2 months. In case of CXZ5 and CXM5, agglomeration of fillers i.e., ZnO nanoparticles and montmorillonite nanoclay and pore formation lead to easy penetration of water which eventually causes higher rate of degradation rate. Other researchers also claimed that in the event that nanofillers are not sufficiently dispersed within the biopolymer matrix, they may contribute to improve degradability (Glaskova-Kuzmina et al., 2021). CXM3, CXZ1 and CXZ3 exhibited 34.46 & 93.48%, 31.41 & 88.36% and 29.37 & 88.61% wight loss in 1^{st} and 2^{nd} month, respectively.

Black patches started to appear on the bionanocomposite films about a week after they were buried in the soil. The same conclusions were reached by Krishnamurthy and Amritkumar in PolyLacticAcid based bioplastic (Krishnamurthy & Amritkumar, 2019). Moreover, the entire film’s color progressively turned darker and transparency started reducing. Figure 4.28 depicts color change and hole formation in the film as a sign of degradation process. This event is supported by the work of other researchers (Vasile et al., 2018). As time passes, and the film degrades, the brown color of the soil also shifted to blackish type. This is a clear indication of humus accumulation and ability of microorganisms to degrade these materials (Rachmawati et al., 2015). Kabir and his colleague researchers also asserted the same thing, that microbial deterioration impacts the durability and appearance of polymeric material (Kabir et al., 2020).

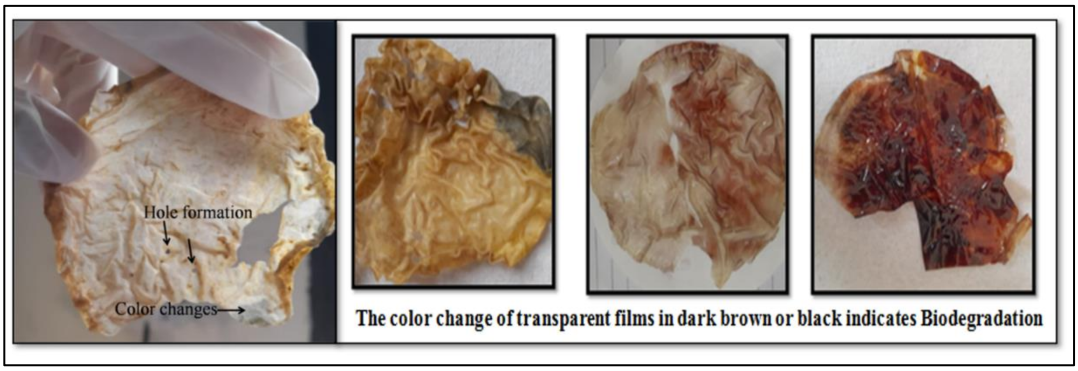


Figure 4.28: Degradation sign through color changes and hole formations

Pure Chitosan film

1st week

2nd week

3rd week

Figure 4.29: Fastest degradation process of pure chitosan film

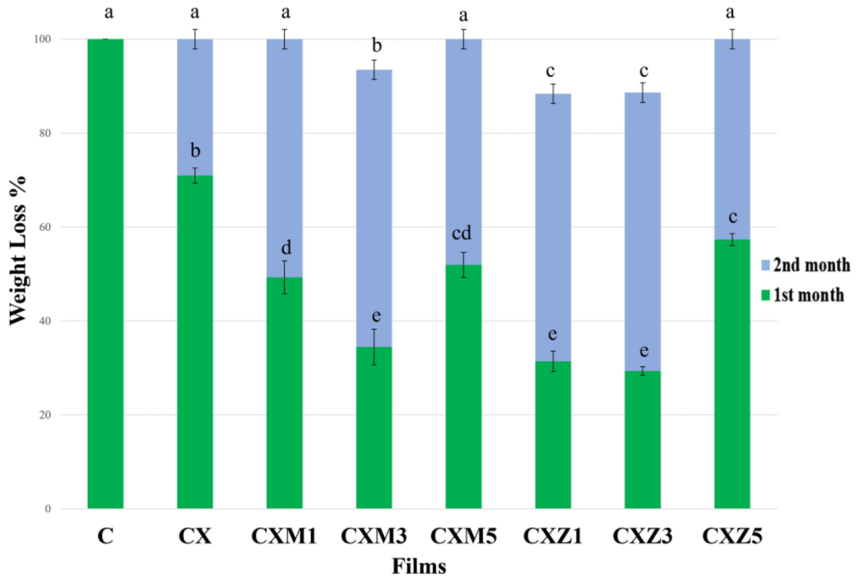


Figure 4.30: The Bar Graph represents biodegradation in terms of weight loss% of each film in 1st two months (Values are tabulated as Mean±Standard Deviation, n=3; Significantly different values are represented by different letters, at $p \leq 0.05$ using Tukey test)

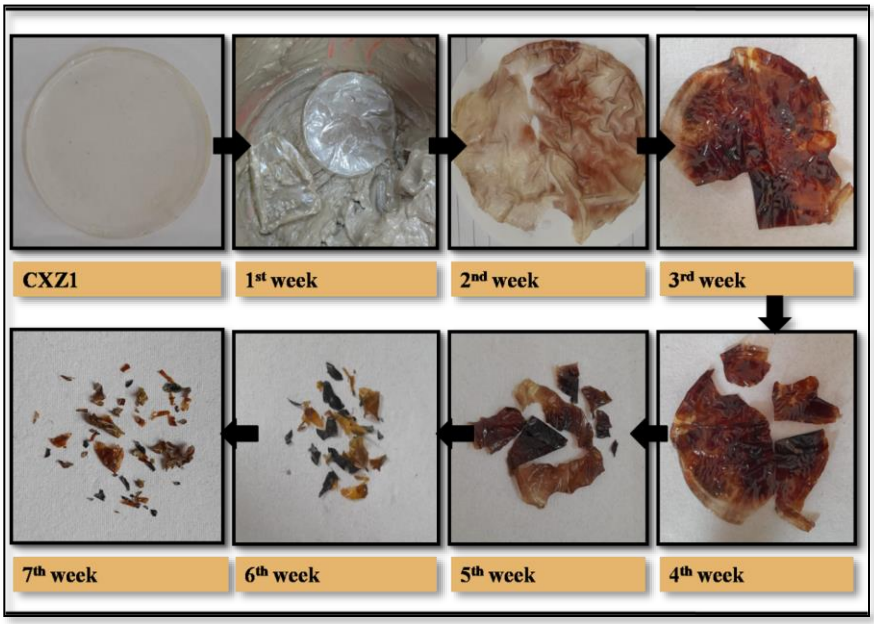


Figure 4.31: Lowest degradation rate in ZnO nanocomposite film (CXZ1)

Table 4.9: Weight loss% of all the films (Values are tabulated as Mean±Standard Deviation, n=3; Distinct letters within a column represent significantly varied values, at $p \leq 0.05$ using Tukey test)

S. No.	Film	Weight Loss in 1st month	Weight Loss in 2nd month
1.	C	100±0% ^{a}	NA
2.	CX	70.96±1.593% ^{b}	100±0% ^{a}
3.	CXM1	49.27±1.977% ^{d}	100±0% ^{a}
4.	CXM3	34.46±3.53% ^{e}	93.48±0.5% ^{b}
5.	CXM5	51.92±2.687% ^{cd}	100±0% ^{a}
6.	CXZ1	31.41±2.19% ^{e}	88.36±0.69% ^{c}
7.	CXZ3	29.37±0.923% ^{e}	88.61±1.46% ^{c}

8.	CXZ5	57.32±1.284% ^{c}	100±0% ^{a}

Soil utilized in biodegradation test were submitted for its physiochemical analysis and results are summarized in table below

Table 4.10: Soil Analysis (Values are tabulated as Mean±Standard Deviation)

S. No.	**Parameter**	**Result**
1.	pH	8.5±0.7
2.	N (nitrogen available)	633±2.64 kg/ha
3.	P (Phosphorus available)	266±0.4 kg/ha
4.	K (Potassium available)	0.19±0.05 %

4.5 Antimicrobial Efficacy

The most common types of microorganisms that attack perishables including ginger and corn were shown in table 4.11. The test microorganisms were chosen according to previous researches done by other researchers in same field.

Table 4.11: List of the most common microorganisms responsible for perishable products

S. No.	**Microorganisms**			**Test Done**
	Gram positive bacteria	**Gram negative bacteria**	**Fungi**	
1.	*Staphylococcus aureus*			√
2.	*Bacillus subtilis*			√
3.	*Lactobacillus* sp.			✕
4.		*Escherichia coli*		√
5.		*Pseudomonas aeruginosa*		√

6.			*Candida albicans*	√
7.			*Aspergillus niger*	√
8.			*Penicillium* sp.	×

The best film to investigate for food packaging applications would be these 3 films i.e., CXM3, CXZ1, and CXZ3, which have the better mechanical, and barrier qualities among others. Therefore, gram positive and gram negative food pathogenic bacteria *S. aureus* and *E. coli*. and fungi *C. albicans* were used as test subjects for the antimicrobial activity of CXM3, CXZ1 and CXZ3. The obtained results from disc diffusion method shows the impact of the films on microbial growth inhibition. Figure 4.32 illustrates the produced films (best 3) antimicrobial efficacy in terms of zones of inhibition. Table 4.11 lists the dimensions of the zone of inhibition in millimeters (mm) for the manufactured films. The amino group of chitosan, which confers natural antioxidant and antimicrobial action, has a major influence in the functional activity of chitosan. Although it is well known from literature (Divya et al., 2017; Ke, Deng et al., 2021; Kravanja et al., 2019; Verlee et al., 2017) that chitosan poses inherent antimicrobial efficacy, the present study discovered no obvious inhibition zone for the pure chitosan film (C). While examining antimicrobial effect using the disc diffusion approach, several other studies also reported that no clear inhibition zones were detected. To create an inhibitory zone, Li et al. advised that chitosan molecules should be immobilized in the matrix rather than diffused (Li et al., 2019; Zheng et al., 2019).

Clay nanocomposite (CXM3) showed no inhibition zone for gram negative bacteria *Escherichia coli*, whereas, both ZnO-based nanocomposite films (CXZ1 and CXZ3) were not able to present any magnitudes of zone of inhibition against fungi *Candida albicans*. CXM3 film presented significant inhibitory antibacterial efficacy with diameter of 30mm against *S. aureus*. As previously reported in the literature, this may be connected to montmorillonite nanoclay's high specific area, which allows it to absorb the bacteria and adhere them to their surface (Wang et al., 2006). According to Aris Giannakas et al., use of modified montmorillonite with thechitosan-poilyvinyl alcohol blend also proved to upgrade the antibacterial property (Giannakas et al., 2016). However, it didn't demonstrate inhibition zone against *E. coli*. This is most likely caused by the defense provided by *E. coli* cell wall lipopolysaccharides or outer membrane proteins.

Furthermore, in case of ZnO-based nanocomposites. Zn^{2+} ions, which damage bacterial cell walls, are thought to be the cause of antibacterial action (Al-Naamani et al., 2016), as the ZnO nanoparticle content expanded, the zone of inhibition widened. CXZ3 demonstrated higher inhibition zone than CXZ1 against both types of bacteria. Food packaging films must correspond to good physicochemical standards as the major goal This is opposed to the study of Saral and coworkers (Saral et al., 2019), where they found their fabricated ZnO-based composite resulted into more resistant to *E. coli* than to *S. aureus*.

Table 4.12: Zone of inhibition presented by different films against different microbial strains (Values are tabulated as Mean±Standard Deviation, n=3; Distinct letters within a column represent significantly varied values, at p≤0.05 using Tukey test)

S. No.	Sample	Microorganism	MTCC No.	Incubation	Zone of Inhibition (mm)
1.	C	*Escherichia coli*	443	37°C for 24 hours	-
		Staphylococcus aureus	87	37°C for 24 hours	-
		Candida albicans	227	27°C for 48hours	-
2.	CXM3	*Candida albicans*	227	27°C for 48hours	-
		Staphylococcus aureus	87	37°C for 24 hours	30±1.0^{a}
		Candida albicans	227	27°C for 48hours	13±1.4^{b}
3.	CXZ1	*Escherichia coli*	443	37°C for 24 hours	12±0.95^{b}
		Staphylococcus aureus	87	37°C for 24 hours	24±1.8^{b}

		Candida albicans	227	27°C for 48hours	-
4.	CXZ3	*Escherichia coli*	443	37°C for 24 hours	16±1.73^a
		Staphylococcus aureus	87	37°C for 24 hours	25±1.0^b
		Candida albicans	227	27°C for 48hours	-
		Bacillus subtilis	2413	37°C for 24 hours	13.5±0.5^b
		Pseudomonas aeruginosa	741	37°C for 24 hours	15±1.0^b
		Aspergillus niger	872	27°C for 48hours	23.33±1.52^a

As CXZ3 exhibited overall best results among all the characterization results previously mentioned; it additionally, possessed the best results with biggest zone of inhibition among all the 3 tested samples, it is further subjected to investigation regarding antimicrobial efficacy against 3 more microbial strains i.e., *Bacillus subtilis* (gram positive bacteria), *Pseudomonas aeruginosa* (gram negative bacteria), *Aspergillus niger* (fungi), as well as Shelf-life studies and Toxicity test. *Bacillus* sp., *Pseudomonas* sp., *Aspergillus* sp. are very common microorganisms to attack most of the perishables including ginger and corn. According to the result achieved, CXZ3 composite is able to resist microbial growth of *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Aspergillus niger* with inhibition diameter of 13.5, 15 and 23.33 mm, respectively (Table 4.11, figure 4.33).

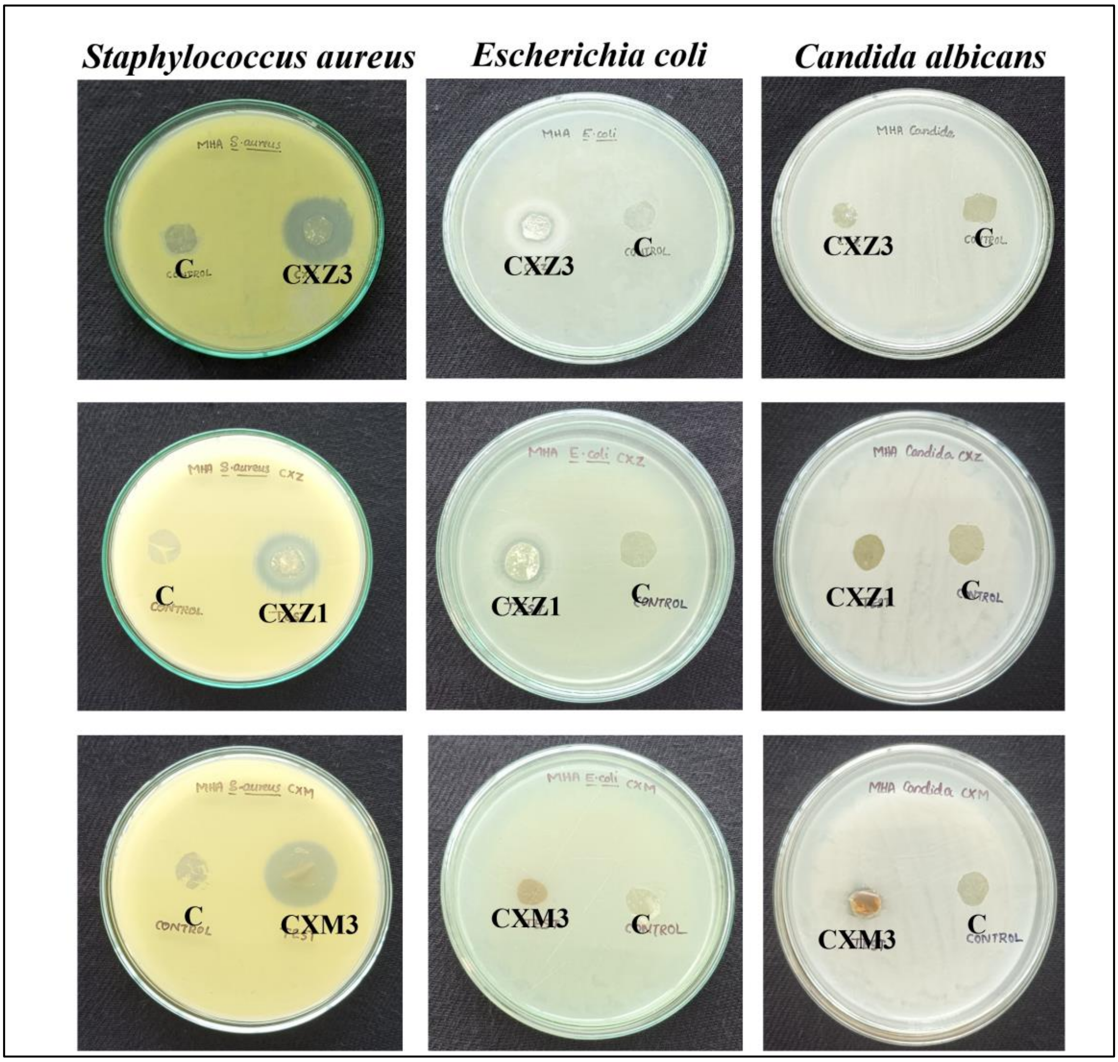


Figure 4.32: Inhibition zone displayed by the best 3 films

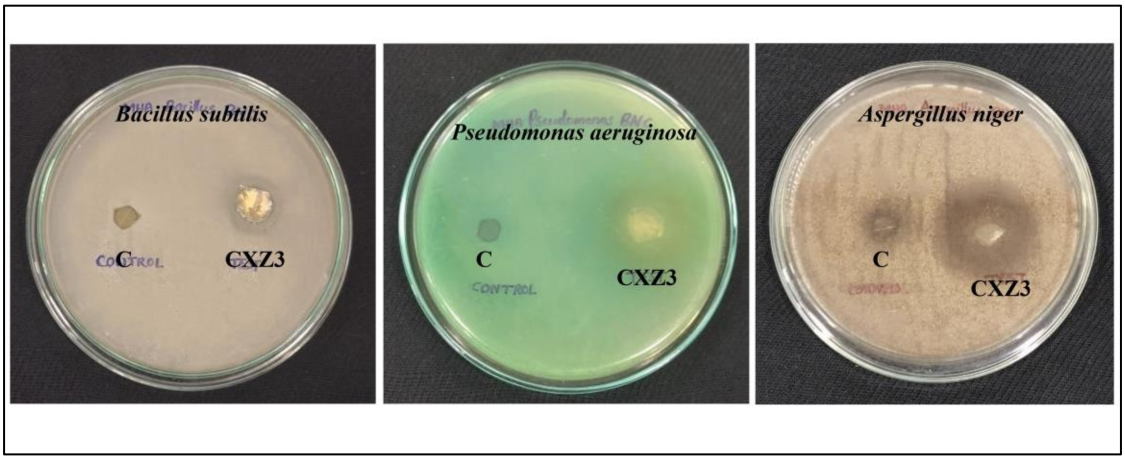


Figure 4.33: Antimicrobial efficacy of CXZ3 against additional 3 microbes

4.6 Shelf-Life Studies

When taking into account, in light of all the prior characterization results, CXZ3 film appeared to be the most effective combination. By utilizing this optimized film as a protective layer for ginger slices and corn seeds, the capacity of CXZ3 to safeguard perishable food items and ability to lengthen its shelf life was assessed. The experimental setup involved the testing ginger and corn wrapped in the nanocomposite, while uncovered plate was used as control. After a 7 days period, visual inspection revealed that the uncovered food items appeared spoiled and inedible, whereas those covered with the nanocomposite exhibited better appearance (Figure 4.34 and 4.35), maintaining color, texture and aroma consistently. Corn seeds covered with the nanocomposite was in consumable condition till 5 days in room temperature. Despite a minor moisture loss, no sign of microbial spoilage was detected. However, control group exhibited high moisture loss on 3rd day and microbial spoilage was observed from 5th day onwards. In case of ginger slices, after 1 week of observation on packed ginger slice seemed in good condition, while the ginger kept as control displayed microbial spoilage. These results imply that the CXZ3 film might be the best material, potentially replacing traditional plastic food packages.

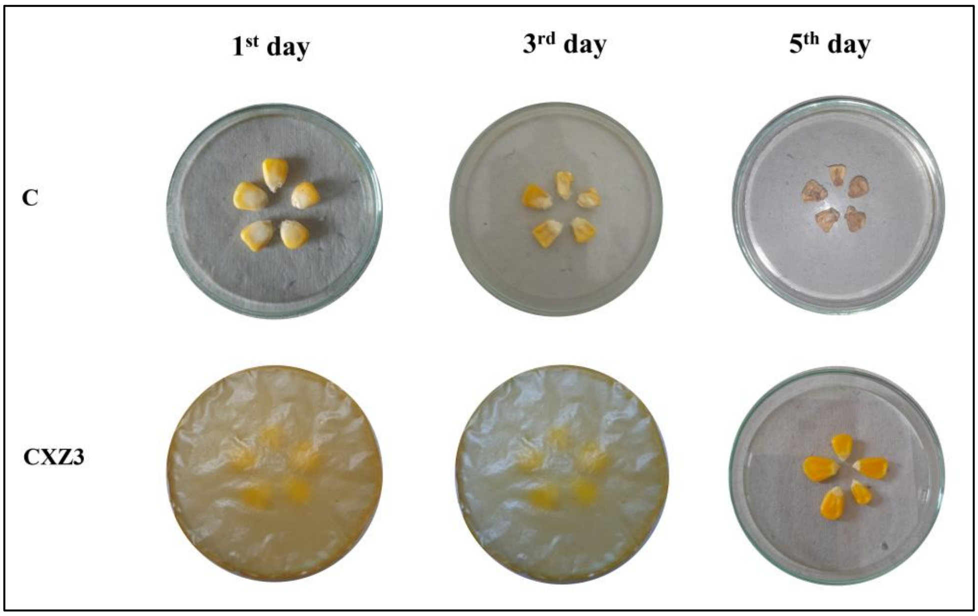


Figure 4.34: Shelf-life studies of corn

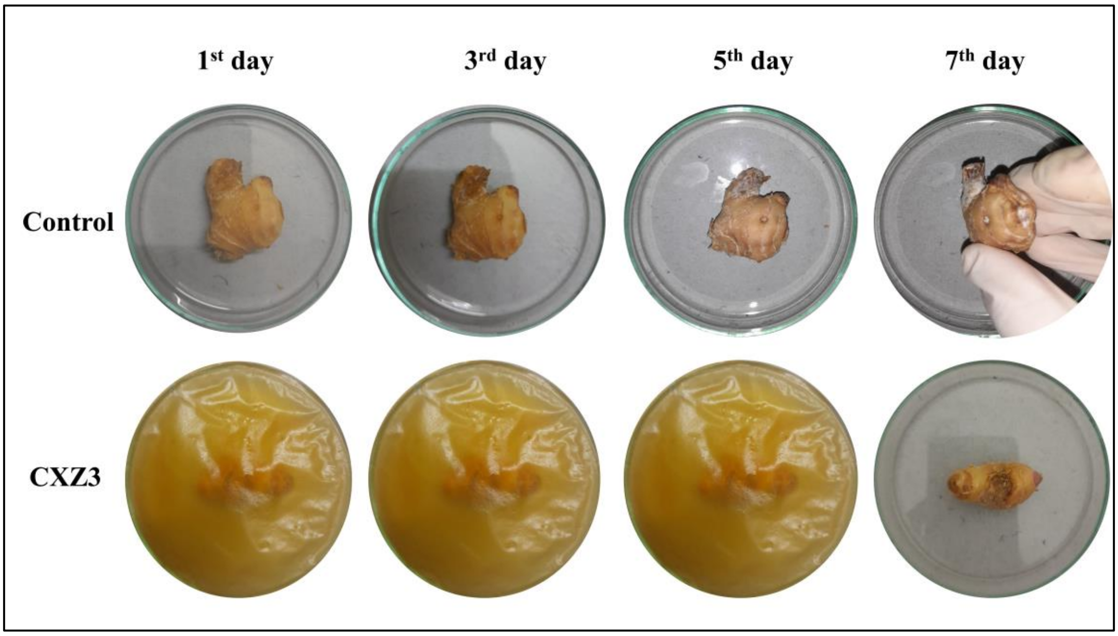


Figure 4.35: Shelf-life studies on ginger

4.7 Study of toxicity

When taking into account all of the preceding characterization results, CXZ3 appeared to be the optimal ratio film. The toxicity of CXZ3 film was examined using the MTT assay, which was carried out in accordance with ISO 10993:5 standard technique. This test assessed the film's impact on L929, mouse fibroblast cell lines, and the result illustrated in Figure 4.36 that represents cell viability and cytotoxicity. According to GB/T 16886.5-2003 (ISO 10993-5: 1999) guidelines, substances are non-cytotoxic if the cell viability exceeds 75%. The CXZ3 identified as the most promising film among all the fabricated package, exhibited exceptional cytocompatibility, showing 87.56% cell viability and 12.44% cytotoxicity. Therefore, 3% ZnO nanoparticles reinforced chitosan and xanthan gum blend film can be confidently deemed biocompatible.

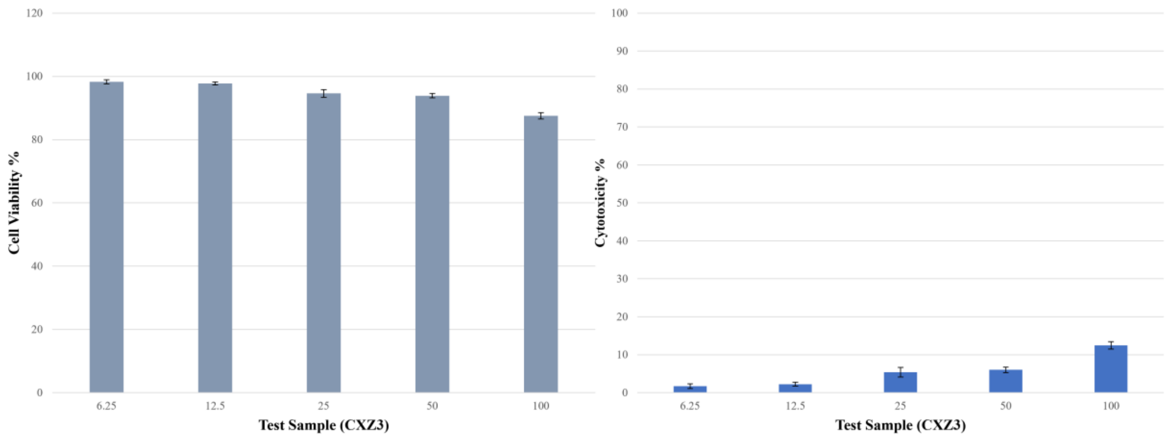


Figure 4.36: Cell viability % and toxicity% of optimized film

CHAPTER V - SUMMERY AND CONCLUSION

5.1 Summery

The issue associated with the non-sustainable packaging waste and shorter shelf life of perishables is addressed in the thesis, "Development of biodegradable nanocomposite films for extending the shelf life of ginger and corn". Here, representative perishable food items are ginger and corn. The research suggests developing a new kind of packaging film with superior barrier qualities against moisture, oxygen, UV and microbial development by combining biodegradable polymers with nanomaterials. Materials were selected after a thorough assessment of the literature. Chitosan was chosen as the main matrix, while xanthan gum on the other hand was selected to enhance properties of the chitosan. Blending strategy was planned for both the biopolymers to overcome inherent limitations of chitosan (such as low mechanical capability and water/moisture absorption). Optimization study was carried out for biopolymer blend film (chitosan-xanthan gum) by addition of xanthan gum with different ratio in the chitosan matrix. Chitosan:xanthan gum=2:1 proved to be the best film, which was further subjected to formation of bio-nanocomposite film with incorporation of nanofillers (ZnO nanoparticles and montmorillonite nanoclay) with 1%, 3%, 5% and 7%.

A wide range of characterization was performed for these films, as it is crucial to assess its properties and performances. Fourier Transform Infrared spectroscopy investigated structural alteration, that further indicated new bond formation due to interaction of the materials utilized to fabricate the film. In thermogravimetric analysis, all the films possessed thermal stability almost similar to the control, whereas the optimized film exhibited better outcome. Mechanical testing was carried out to explore tensile strength (in MPa) and elongation at break (%). All the composites resulted in higher strength than the pristine one. Surface appearance of the films was studied through Field Emission-Scanning Electron Microscopy. Most homogenous distribution of nanofillers was found at 3% of ZnO nanoparticles and monmorillonite nanoclay. EDX analysis confirmed elemental analysis and mapping discovered elemental distribution. In terms of barrier performances, the fabricated nanocomposites resulted into decreased water absorbance capacity %, water vapor transmission rate, oxygen transmission rate. Moreover, UV absorption was increased in the nanocomposites. To check ecofriendliness of the synthesized films, biodegradability was evaluated in natural soil for first 2 months. All the synthesized films exhibited great biodegradability nature, however, durability of nanocomposites extended to some extent. Antimicrobial efficacy displayed clear inhibition zone against gram positive and gram-negative bacteria, along with fungal strain. All these improved characteristics can help prolong shelf-life of perishable items, according to the studies carried out on ginger and corn. Finally, toxicity was checked for the best film (3% ZnO reinforced chitosan-xanthan gum blend), which revealed that the film is biocompatible, safe to be utilized in packaging of food materials. The suggested biodegradable nanocomposite film tackles environmental issues raised by conventional plastic packaging in addition to improving perishable’s shelf lives. The film has the benefit of minimizing environmental impact and reducing waste by using biodegradable ingredients.

5.2 Conclusion

In the current work, effective production of the films was achieved through the formation of bio-nanocomposite films were successfully fabricated by ZnO nanoparticles and montmorillonite nanoclay incorporation of xanthan gum in chitosan, by a straightforward and economical solution casting approach. Different ratio was tested to optimize the best ratio. Various methods of instrumentation were allowed for a detailed analysis. Utilization of xanthan gum boosted strength to a certain threshold. The subsequent inclusion of nanofillers, had a beneficial effect and greatly improved the film's mechanical performance. Increasing % of components and their ratio resulted into gradual increase in its strength. Moreover, ZnO nanocomposites displayed better performance in both tensile strength and elongation at break %. All of the nanocomposites have improved UV protection, humidity and oxygen barrier. All of the films exhibited high degradability, demonstrating their sustainability for the sake of the planet. According to the results, CXZ3 (composite of chitosan and xanthan gum containing 3% ZnO nanoparticles) appeared to be the best formulation out of all the created films, revealing outstanding physical and mechanical characteristics as well as degradability in the natural surroundings. Thermal analysis proved that CXZ3 exhibited better stability compared to control and all the other fabricated films. Additionally, it was proven that the newly fabricated material is non-toxic and has great antibacterial efficacy against gram positive (*Staphylococcus aureus* & *Bacillus subtilis*), gram negative bacteria (*Escherichia coli* & *Pseudomonas aeruginosa*) and fungi *Aspergillus niger* which eventually helps to increase the shelf-life perishables like ginger and corn. Given the aforementioned qualities, it is clear that this film has enormous promise as a biodegradable and eco-logically sound packaging option for food products. As a result, it represents a highly practical substitute for traditional petroleum-based packages.

5.3 Future Recommendations

These future suggestions are recommended to the future researchers in this field to improve the efficiency and practicality of the newly fabricated (optimized ratio under different parameters) biodegradable nanocomposite film.

Advanced characterization Methods like Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), X-ray diffraction (XRD), Nuclear magnetic resonance spectroscopy (NMR) etc. can be conducted to acquire a better understanding of the structure and behavior of the nanocomposite film. Further, research can be conducted on the particular demands and vulnerabilities of various perishable products (fruits, vegetables, meat, and dairy), then tailor the nanocomposite film accordingly to meet those needs. For example, with this strategy, shelf-life extension might be maximized. A thorough life cycle assessment (LCA) of the biodegradable nanocomposite film can be conducted to determine the effects they will have on the environment from the time they are produced until they are discarded and degraded. To confirm the film's usefulness in real world situations, research can be extended to include real world testing under a variety of circumstances, including varying temperature and humidity environments, transit scenarios, and storage times. Make sure the nanocomposite films developed adhere to the requirements for safety and environmental effect before manufacturing at industrial scale after scaling up production from the laboratory level. Examine the viability of making these films on a bigger scale while taking cost, scalability, and quality control into account. Studies should be conducted on focusing to learn how consumers perspective and accept biodegradable packaging and whether they are willing to pay more for products that have a longer shelf life. As the nanocomposite didn’t poses significant antifungal activity against all the fungal strain tested, future research may focus on enhancing its antifungal activity to improve its performance. Incorporation of antifungal agent/components in the film during fabrication might help.

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List of Publications

1. Tabassum Z, Tabassum Z, Mohan A, Girdhar M. Insight into recent trends in ZnO nanoparticle reinforced chitosan-based composite films for sustainable packaging: A critical review on its current status, challenges and perspective. Materials Today: Proceedings. 2024 May 13. https://doi.org/10.1016/j.matpr.2024.05.069
2. Tabassum Z, Mohan A, Girdhar M. A comprehensive review on chitosan based bionanocomposites: Enormous potential for biodegradable food packaging applications of future. In AIP Conference Proceedings 2024 Feb 20 (Vol. 2986, No. 1). AIP Publishing. https://doi.org/10.1063/5.0197876
3. Tabassum Z, Girdhar M, Kumar A, Malik T, Mohan A. ZnO Nanoparticles-Reinforced Chitosan–Xanthan Gum Blend Novel Film with Enhanced Properties and Degradability for Application in Food Packaging. ACS Omega. 2023 Aug 17; 8(34):31318-31332. https://doi.org/10.1021/acsomega.3c03763
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9. Saurav S, Sharma P, Kumar A, Tabassum Z, Girdhar M, Mamidi N, Mohan A. Harnessing Natural Polymers for Nano-Scaffolds in Bone Tissue Engineering: A Comprehensive Overview of Bone Disease Treatment. Current Issues in Molecular Biology. 2024 Jan;46(1):585-611. https://doi.org/10.3390/cimb46010038
10. Selwal N, Tabasum Z, Rahayu F, Yulia ND, Sugiono S, Endarto O, Riajaya PD, Djajadi D, Khamidah A, Wani AK. Therapeutic potential and phytoremediation capabilities of the tobacco plant: Advancements through genetic engineering and cultivation techniques. Biocatalysis and Agricultural Biotechnology. 2023 Sep 10:102845. https://doi.org/10.1016/j.bcab.2023.102845
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12. Tabassum Z, Mohan A, Girdhar M. Development of a Reusable and Sustainable Biocatalyst by Immobilization of Catalase Enzyme On to biowaste hen Egg-Shell powder. Think India Journal 22(16); 2019.
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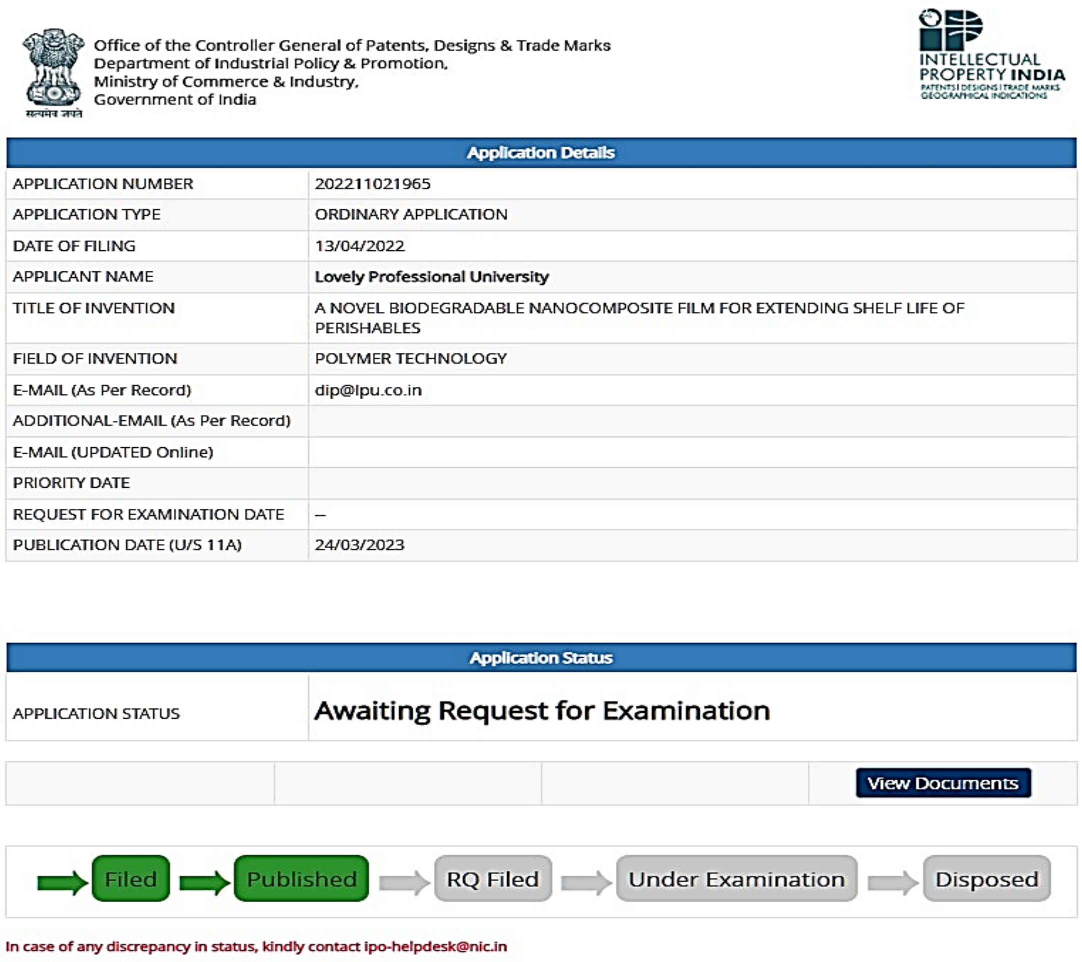
Patent

Patent got published: "A Novel Biodegradable Nanocomposite Film for Extending Shelf Life of Perishables"



Office of the Controller General of Patents, Designs & Trade Marks
Department of Industrial Policy & Promotion,
Ministry of Commerce & Industry,
Government of India

Application Details	
APPLICATION NUMBER	202211021965
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	13/04/2022
APPLICANT NAME	**Lovely Professional University**
TITLE OF INVENTION	A NOVEL BIODEGRADABLE NANOCOMPOSITE FILM FOR EXTENDING SHELF LIFE OF PERISHABLES
FIELD OF INVENTION	POLYMER TECHNOLOGY
E-MAIL (As Per Record)	dip@lpu.co.in
ADDITIONAL-EMAIL (As Per Record)	
E-MAIL (UPDATED Online)	
PRIORITY DATE	
REQUEST FOR EXAMINATION DATE	--
PUBLICATION DATE (U/S 11A)	24/03/2023

Application Status	
APPLICATION STATUS	**Awaiting Request for Examination**

In case of any discrepancy in status, kindly contact ipo-helpdesk@nic.in

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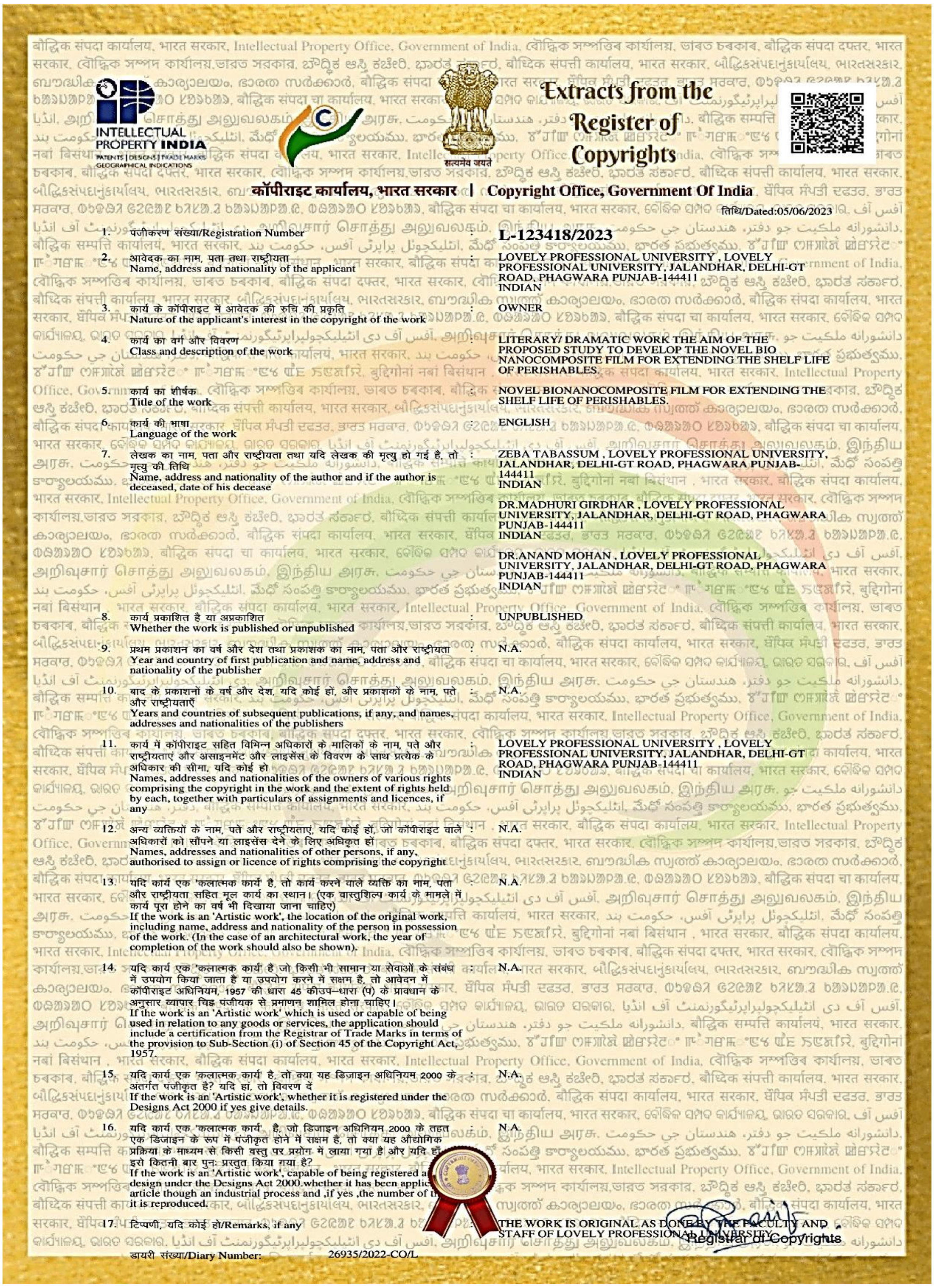
Copyright is granted on “Novel Bionanocomposite Film for Extending the Shelf Life Of Perishables”



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Extracts from the Register of Copyrights

कॉपीराइट कार्यालय, भारत सरकार | Copyright Office, Government Of India

तिथि/Dated:05/06/2023

1.	पंजीकरण संख्या/Registration Number	: **L-123418/2023**
2.	आवेदक का नाम, पता तथा राष्ट्रीयता Name, address and nationality of the applicant	: LOVELY PROFESSIONAL UNIVERSITY , LOVELY PROFESSIONAL UNIVERSITY, JALANDHAR, DELHI-GT ROAD, PHAGWARA PUNJAB-144411 INDIAN
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4.	कार्य का वर्ग और विवरण Class and description of the work	: LITERARY/ DRAMATIC WORK THE AIM OF THE PROPOSED STUDY TO DEVELOP THE NOVEL BIO NANOCOMPOSITE FILM FOR EXTENDING THE SHELF LIFE OF PERISHABLES.
5.	कार्य का शीर्षक Title of the work	: NOVEL BIONANOCOMPOSITE FILM FOR EXTENDING THE SHELF LIFE OF PERISHABLES.
6.	कार्य की भाषा Language of the work	: ENGLISH
7.	लेखक का नाम, पता और राष्ट्रीयता तथा यदि लेखक की मृत्यु हो गई है, तो मृत्यु की तिथि Name, address and nationality of the author and if the author is deceased, date of his decease	: ZEBA TABASSUM , LOVELY PROFESSIONAL UNIVERSITY, JALANDHAR, DELHI-GT ROAD, PHAGWARA PUNJAB-144411 INDIAN DR.MADHURI GIRDHAR , LOVELY PROFESSIONAL UNIVERSITY, JALANDHAR, DELHI-GT ROAD, PHAGWARA PUNJAB-144411 INDIAN DR.ANAND MOHAN , LOVELY PROFESSIONAL UNIVERSITY, JALANDHAR, DELHI-GT ROAD, PHAGWARA PUNJAB-144411 INDIAN
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डायरी संख्या/Diary Number: 26935/2022-CO/L

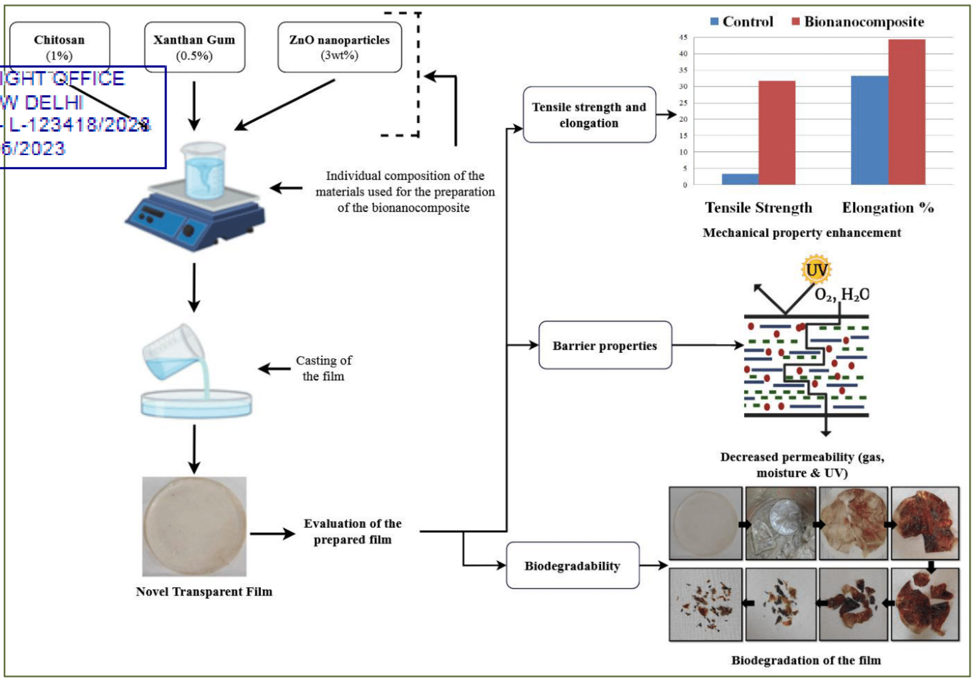
Registrar of Copyrights

Title of the work: Novel bionanocomposite film for extending the shelf life of perishables
Name 0f the authors: Zeba Tabassum, Dr. Madhuri Girdhar, and Dr. Anand Mohan

Summary of Graphical Abstract:

This work is focused to fabricate biodegradable chitosan-xanthan gum blend film incorporated with ZnO nanoparticles or montmorillonite nanoclay. It has been analyzed that recent research in bio-based polymers have proved to be utilized in the field of eco-friendly food packaging industries. Because of their exceptional features, low cost, degradability, non-toxicity, and economic sustainability, as well as their ease of availability and wide range of application, they have gotten a lot of attention. The newly developed bionanocomposites have the capability to replace synthetic-based plastic packaging in order to increase the shelf life of perishables.

Graphical Abstract:



List of Conferences

1. Presented the paper titled “Insight Into Recent Trends in ZnO Nanoparticle Reinforced Chitosan-Based Composite Films for Sustainable Packaging: A Critical Review on Its Current Status, Challenges and Perspective” at 3rd International Conference on Materials Science and Engineering (ICMSE) held on November 23-25, 2023 and organized by the Department of Mechanical Engineering, Dr. B R Ambedkar National Institute of Technology, Jalandhar, Punjab, India.

ICMSE-2023

3rd INTERNATIONAL CONFERENCE ON

MATERIALS SCIENCE AND ENGINEERING

November 23rd - 25th, 2023 (Hybrid Mode)

ICMSE 2023 23 - 25 November, N.I.T. Jalandhar, India

materialstoday: PROCEEDINGS

Registration ID: 2023021306

CERTIFICATE

This is to certify that ***Ms ZEBA TABASSUM*** **of** ***Lovely Professional University, India*** has presented the paper titled ***“Insight Into Recent Trends in ZnO Nanoparticle Reinforced Chitosan-Based Composite Films for Sustainable Packaging: A Critical Review on Its Current Status, Challenges and Perspective”*** at 3rd **International Conference on Materials Science and Engineering (ICMSE)** held on **November 23-25, 2023** and organized by the **Department of Mechanical Engineering, Dr B R Ambedkar National Institute of Technology, Jalandhar, Punjab, India.**

Sharma

Ashok Ku

Chairperson
(Prof. Raman Bedi)

Organizing Secretary
(Dr. Sumit Sharma)

Organizing Secretary
(Dr. Ashok Kumar)

2. Presented a paper on A comprehensive review on Chitosan Based Bionanocomposites: Enormous Potential for Biodegradable Food Packaging Applications of Future in the “**3rd International Conference on Functional Materials, Manufacturing and Performances (ICFMMP-2022)**” held on July29-30th, 2022, organized by Division of Research and Development, Lovely Professional University, Punjab.

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Certificate No.253381

Certificate of Presentation

This is to certify that **Dr./Mr./Ms. Zeba Tabassum** of **School of Bioengineering and Biosciences, Lovely Professional University, Phagwara** has presented a paper on **A comprehensive review on Chitosan Based Bionanocomposites: Enormous Potential for Biodegradable Food Packaging Applications of Future** in the **“3rd International Conference on Functional Materials, Manufacturing and Performances (ICFMMP-2022)”** held on July29-30th, 2022, organized by **Division of Research and Development, Lovely Professional University, Punjab**.

Date of Issue: 30-08-2022
Place: Phagwara (Punjab), India

Prepared by
(Administrative Officer-Records)

Dr. Hitesh Vasudev
Convenor

Dr. Pranav Kumar Prabhakar
Organizing Secretary

Dr. Chander Prakash
Conference Secretary

DSIR
CSIR-CSIO
SERB INDIA
MINISTRY OF SCIENCE AND TECHNOLOGY

3. Presented paper on “A Critical Review on Current Trends and Advancements in Xanthan Gum-Based Films for Sustainable Packaging” in the **International Conference on Synthetic and Pharmaceutical Chemistry** on 15th and 16th September, 2023.

ICSPC International Conference on Synthetic & Pharmaceutical Chemistry

MEDICRATES

Pbri BHOPAL

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NAAC GRADE A++

Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms. ………Zeba……Tabassum………………of………………has participated in ***Poster Presentation/[✓ Oral] Presentation*** *on the topic entitled* A critical review on current trends and advancements in Xanthan Gum based films for Sustainable Packaging *in International Conference on Synthetic and Pharmaceutical Chemistry (ICSPC) held from 15th -16th September 2023 organized by School of Pharmaceutical Sciences, under the aegis of Lovely Professional University, Punjab.*

Dr. Sanjeev Kumar Sahu
(Program Chair)

Dr. Manish Vyas
(General Chair)

Monica Gulati
Dr. Monica Gulati
(Conference Chair)

4. Participated in Poster Presentation on the topic entitled Chitosan based bionanocomposites: enormous potential for biodegradable food packages and coatings of future in **International Conference on Sustainability: Life on Earth 2021 (ICS-LOE 2021)** held on 17-18 December 2021 organized by Department of Botany and Zoology, School of Bio-engineering and Biosciences, and Institute of Forest Productivity, Ranchi, Jharkhand at Lovely Professional University, Punjab.

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वन उत्पादकता संस्थान, रांची
INSTITUTE OF FOREST PRODUCTIVITY

Certificate No. 246712

Certificate of Participation

This is to certify that ***Prof./Dr./Mr./Ms. Zeba Tabassum*** *of* ***Lovely Professional University*** *has participated in* ***Poster Presentation*** *on the topic entitled* ***Chitosan based bionanocomposites: enormous potential for biodegradable food packages and coatings of future*** *in International Conference on* ***Sustainability: Life on Earth 2021 (ICS-LOE 2021)*** *held on 17-18 December 2021 organized by Department of Botany and Zoology, School of Bio-engineering and Biosciences, and Institute of Forest Productivity, Ranchi, Jharkhand at Lovely Professional University, Punjab.*

Date of Issue: 15-06-2022
Place of Issue: Phagwara (India)

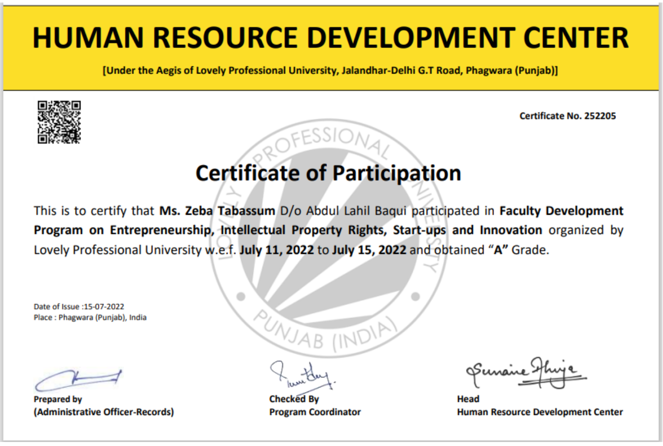
Prepared by
(Administrative Officer-Records)

Dr. Joydeep Dutta
Organizing Secretary, ICS-LOE, 2021

Sharma
Dr. Neeta Raj Sharma
Convener, ICS-LOE, 2021

List of Workshops

1. Participated in Faculty Development Program on Entrepreneurship, Intellectual Property Rights, Start-ups and Innovation organized by Lovely Professional University w.e.f. July 11, 2022 to July 15, 2022 and obtained "A" Grade.



HUMAN RESOURCE DEVELOPMENT CENTER

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Certificate No. 252205

Certificate of Participation

This is to certify that **Ms. Zeba Tabassum** D/o Abdul Lahil Baqui participated in **Faculty Development Program on Entrepreneurship, Intellectual Property Rights, Start-ups and Innovation** organized by Lovely Professional University w.e.f. **July 11, 2022** to **July 15, 2022** and obtained "**A**" Grade.

Date of Issue :15-07-2022
Place : Phagwara (Punjab), India

Prepared by
(Administrative Officer-Records)

Checked By
Program Coordinator

Head
Human Resource Development Center

2. Participated in the AICTE recognized Faculty Development Program on **Smart Materials and Nanotechnology,** conducted by Applied Science Department at NITTTR, Chandigarh.

Certificate No: ICT-1386/23

National Institute of
Technical Teachers Training and Research
Chandigarh

MINISTRY OF EDUCATION, GOVERNMENT OF INDIA

Certificate

This is to certify that

ZEBA TABASSUM

Lovely Professional University, PUNJAB
Punjab

Participated in the AICTE Recognized Faculty Development Programme

on

Smart Materials and Nanotechnology

Conducted by

Applied Science Department

from

15/05/2023 to 19/05/2023 (One Week)

at

NITTTR, Chandigarh

Coordinator

Director