Smart Biodegradable Packaging: Enzyme Triggered Self-Degrading Film Based on Papaya (*Carica papaya*) for Time Sensitive Freshness Monitoring

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Abstract—**Growing concerns over plastic pollution and food waste have led to the need for sustainable and intelligent packaging solutions. This study titled "Smart Biodegradable Packaging Enzyme-Triggered Self-Degrading Film Based on Papaya (Carica papaya) for Time Sensitive Freshness Monitoring" utilized an experimental research design, which involved developing a material and systematically testing it under controlled conditions to determine its properties and effectiveness. The study developed a papaya (*Carica papaya*)-based, enzyme-triggered biodegradable film designed to provide time-sensitive freshness indication and post-use self-degradation. Films formulated from papaya powder, starch, glycerol, and citric acid, with papain added at sub-denaturing temperature, were cast, dried, and characterized under room temperature, refrigeration, high humidity, and direct sunlight. Biodegradation was tracked as mass-loss (%) over 30 days; a consumer- facing freshness score (1–5) was recorded daily; UV blocking (UVA/UVB) and heavy-metal content were assessed descriptively. Biofilm mass loss increased over time across all conditions (Day 30 range ≈ 31.55–43.20%), while plastic controls showed no measurable loss. Freshness scores declined significantly with time but did not differ by storage condition in mixed-model analysis; the condition × time interaction was non-significant. The biofilm transmitted ~35.45% UVA and ~9.75% UVB (i.e., blocked ~64.55% UVA and ~90.25% UVB), outperforming plastic controls (~65.10% UVA; ~21.10% UVB). Heavy-metal results for the biofilm were within international limits (Pb, Cu: not detected; Zn: 2.87 ppm; Cd: 0.002 ppm). Overall, the papaya-based film exhibited progressive biodegradation, useful visual freshness response, strong UV shielding, and compliant safety metrics, indicating promise as a smart, eco-friendly packaging alternative.**

Index Terms— **Biodegradable film, Carica papaya, Papain enzyme, Experimental Research 2026.**

1. Introduction

A. Background of the Study

As the world suffers from a big problem of plastic waste and food waste, demand for smart, sustainable packaging is at an all-time high. Traditional plastics used to preserve food are causing a large amount of harm to the environment, and the absence of freshness indicators is resulting in the premature disposal of edible food. To reduce plastic waste, fresh food packaging that limits food spoilage and keeps food fresh for a longer duration is being developed through enzyme- triggered biodegradable packaging.

Globally, it is estimated that 25-30% of all food produced, approximately 1.6 billion tons is wasted every year. This is a serious inefficient issue with the food system. Also, it is a major environmental threat (Earth.org, 2025). In the USA, about 40 percent of the food supply is wasted, as shown by food waste research. This therefore results in approximately 60 million tons of edible food waste, which equates to about 325 pounds per person. In the USA, there are losses at every step of the way; this contributes to food waste occurring within the market. (Recycle Track Systems, 2025). This shows that we need packaging that gives food a longer shelf life. Using freshness indicators in packaging makes food safer, more useful, and better for the environment.

In the Philippines, 2.7 million tons of plastic waste are generated yearly, of which only 10% is properly recycled, while the remaining amount causes pollution and food spoilage. Therefore, freshness monitoring remains limited. This limitation leads to increased waste and potential health threats to consumers. The country today lacks a smart, biodegradable packaging solution with freshness-sensing credentials to further reduce the ecological footprint of food packaging. Prakasvudhisarn, (2023)

Locally, in Davao del Norte, the pollution caused by plastics and the waste produced by food are being caused by the lack of effective management of these wastes. The cause of this is the lack of packaging technologies. (Environmental Management Bureau – Region XI, 2023) Despite significant availability of *Carica papaya* in this region, higher-end packaging utilization remains minimal. Current evidence shows that there is still no prevailing enzyme-triggered, self-degrading freshness-monitoring packaging available locally. (Provincial Engineer's Office Supply, Management and Administrative Division,

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2023).

Although numerous studies have explored biodegradable films and intelligent packaging systems, most focus primarily on enhancing mechanical and barrier properties or incorporating external chemical indicators for spoilage detection (Kumar et al., 2025; Rocha, 2025). Research on papaya based biodegradable materials has largely centered on formulation and physical characterization of edible films using papaya components blended with biopolymers (Pawle et al., 2024), with limited focus on enzyme based functional integration. However, limited research has examined the integration of plant derived enzymes directly within biodegradable film matrices to enable controlled self degradation and functional freshness monitoring an approach that would shift packaging from passive biodegradability toward responsive, multi-functional systems (Kumar et al., 2025).

In particular, while the proteolytic enzyme papain from *Carica papaya* has been utilized for enzyme immobilization in biomedical materials (Jurkevicz et al., 2024), such work has focused on therapeutic wound healing applications rather than environment triggered degradation or freshness sensing within packaging matrices. Studies that do incorporate enzymes into biopolymer films, such as papain and bromelain in starch based edible films for improved food quality attributes (e.g., meat tenderization), remain focused on active functionality not directly tied to environmental condition responsive degradation or monitoring (Enzyme loaded thermoplastic starch films, 2025). In the Philippine context, research on smart biodegradable packaging remains largely confined to basic formulation and physical characterization with minimal integration of enzymatic activation, environmental condition testing, and systematic evaluation over time (Kumar et al., 2025). Therefore, a clear gap exists in the development and comprehensive assessment of a papaya based biodegradable film that combines enzyme triggered degradation and freshness monitoring functionality within a single system. This study seeks to address this gap.

B. Statement of the Problem

This study aimed to develop a smart biodegradable packaging film made from *Carica papaya*-based enzymes for self-degradation and freshness detection of perishables. This research would try to overcome the limitations of conventional plastic wrappers, which cause environmental pollution and do not indicate the freshness of the product. The researchers will compare the enzyme-triggered biodegradable film with normal plastic packaging under different storage conditions to study the effect on biodegradability, product freshness, and effect on the environment.

Specifically, this study was guided by the following questions:

1. How does enzyme-triggered *Carica papaya*-based biodegradable film improve environmental sustainability and freshness monitoring compared to conventional plastic packaging?
2. How much of UVA and UVB light does the *Carica papaya*-based biodegradable film block and transmit compared to conventional plastic packaging?
3. Is there a significant difference in the packaging performance when analyzed according to conditions?
    1. high humidity;
    2. refrigerated storage;
    3. room temperature; and
    4. direct sunlight exposure

Does the *Carica papaya*-based biodegradable film comply with food safety standards in terms of heavy metal content (copper, zinc, lead, and cadmium)?

C. Hypothesis

H01: There is no significant difference in the packaging material's performance when analyzed under the following conditions: room temperature, refrigerated storage, high humidity, and direct sunlight exposure.

H02: There is no significant relationship between the rate of enzyme-triggered degradation and the freshness level of the packaged product.

D. Theoretical Framework

This study is grounded on Enzyme Immobilization Theory and Stimuli Responsive Polymer Theory, which together explain the biochemical and material behavior of the smart biodegradable packaging film based on *Carica papaya*. Enzyme Immobilization Theory posits that embedding enzymes within solid matrices enhances stability, preserves activity, and allows controlled responsiveness under environmental triggers (Tadesse & Liu, 2025). In this study, papain is immobilized within the biodegradable film, enabling predictable activation in response to temperature, humidity, or light, which supports both controlled degradation and freshness monitoring (Dong, 2024).

Stimuli Responsive Polymer Theory complements this by explaining how polymer matrices change structurally or chemically when exposed to environmental stimuli, enabling adaptive behavior such as mass loss, deformation, or visual indicators (Yu, Chen, Liu, Hileuskaya, & Kraskouski, 2024). Integrating stimuli responsive polymer behavior with immobilized papain allows the film to respond dynamically to storage conditions, ensuring both functional performance and real-time food quality monitoring.

Together, these theories provide a comprehensive framework: the immobilized enzyme serves as a responsive biochemical agent, while the polymer matrix translates environmental stimuli into functional outcomes, reinforcing the smart and biodegradable features of the packaging system.

E. Significance of the study

This study created a *Carica papaya*-based enzyme-triggered smart biodegradable packaging film for self-degradation and freshness monitoring. This study is useful in encouraging sustainable packaging alternatives, reducing plastic waste, and ensuring food safety and quality. This study especially benefits the following:

Consumers. The biodegradable smart packaging provides an eco-friendly and safer alternative to plastic packaging. It keeps food products fresh and informs consumers of the product's condition and freshness indicators, thereby reducing the risk of spoilage (Nwankwo et al., 2023).

Food manufacturers and retailers. This study helps create cheaper and more environmentally friendly packaging solutions. The adoption of enzyme- triggered biodegradable films helps industries reduce environmental burden, comply with eco-regulations, and build brands through sustainable practices such as green labeling and biodegradable certification (Singh & Sharma, 2022).

F. Conceptual Framework

Figure 1 presents the Conceptual Framework of the Study, utilizing an Input-Process-Output (IPO) model to illustrate the development and evaluation of a *Carica papaya*-based enzyme-triggered biodegradable film. The input phase identifies the primary components, including the papaya-derived papain enzyme, biodegradable polymers like starch and gelatin, plasticizers, and the environmental variables affecting the fresh produce samples. The process stage outlines the methodology, encompassing the extraction of the enzyme, the fabrication of the film, and rigorous testing for degradation rates and freshness monitoring in comparison to conventional plastic packaging. Finally, the output highlights the study's key deliverables: the development of a functional biodegradable film, the establishment of a correlation between degradation rates and product freshness, and the validation of an eco-friendly packaging alternative for time-sensitive goods.

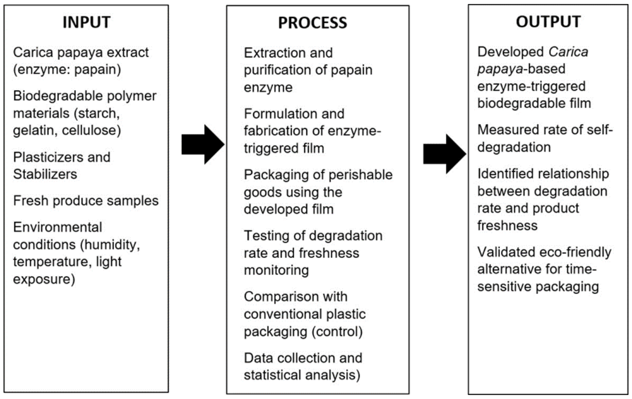


Fig. 1. Conceptual framework of the study

G. Definition of Terms

This study provided conceptual definitions of important concepts to guarantee clarity (based on current theories), operationally (based on their beliefs), and contextually (based on their measurement).

Biodegradable Packaging. Biodegradable packaging is packaging that decomposes through the action of microorganisms like bacteria into non-toxic residues that may be water, carbon dioxide, and biomass. According to recent sustainable-materials researchers Teixeira et al. (2025) and Zhang et al. (2024), a key point to note here is that such a breakdown only occurs under specific conditions (refrigeration, room temp, high humidity, sunlight). Operationally, it is defined as a film that consists of renewable biopolymers (starch, gelatin, cellulose) and that is characterized by its loss of mass, reduction in molecular weight, and/or decline in mechanical strength during a specified period of exposure at set temperature and humidity conditions as tested.

Carica papaya-based enzyme-triggered film. Conceptually, a *Carica papaya*–based enzyme-triggered film refers to a biodegradable packaging material whose degradation is accelerated by papain enzymes derived from *Carica papaya*. Recent studies by Tulamandi et al. (2022) and Ashfaq et al. (2022) highlight how papain can catalyze polymer breakdown and respond to food-spoilage cues such as pH shifts or moisture changes. Operationally, it is defined as a polymeric film embedded with papain enzyme such that, when exposed to designated triggers (refrigeration, room temp, high humidity, sunlight), the film exhibits quantifiable changes such as decreased tensile strength, increased mass loss, or visible alterations in color or transparency.

Enzyme-triggered self-degradation. Conceptually, enzyme-triggered self-degradation refers to a controlled breakdown mechanism in which embedded enzymes catalyze the deterioration of the packaging material, enabling ecofriendly disposal and time-sensitive degradation. This concept aligns with recent findings by Wang, Kim, & Park (2022) and Millan & Hanik (2023), who demonstrated that enzyme-activated polymer films degrade proportionally to enzyme activity and environmental conditions. Operationally, it is characterized by a measurable rate of degradation, such as mass loss, thickness reduction, or tensile strength decline, that directly correlates with papain activity (activity units) under defined storage or simulated spoilage conditions.

Freshness Monitoring. Conceptually, freshness monitoring refers to the ability of packaging to provide real-time or near-real-time information on the quality or spoilage status of food. According to Dodero et al. (2021) and Kishore et al. (2024), modern intelligent packaging uses colorimetric, optical, or chemical indicators that respond to changes in pH, microbial growth, or volatile compound release. Operationally, in this study, freshness monitoring is defined as the visible change in the papaya-based biodegradable film's indicator captured through the Freshness Score (1–5) that correlates strongly with the film's deterioration over time. The indicator's response corresponds to two measured variables: mass loss, which quantifies the film's degradation, and day, which reflects the progression of storage time.

H. Scope and Delimitations

This study focuses on the development and systematic evaluation of a smart biodegradable packaging film derived from *Carica papaya* powder, utilizing the papain enzyme to enable self-degradation and monitor food freshness. The research evaluates the film's performance under four environmental conditions: refrigeration, room temperature, high humidity, and direct sunlight, observed over a 30-day period.

The study is delimited to assessing changes in mass loss and freshness scores as primary indicators of the film's smart functionality and degradation behavior. Laboratory tests for

UVA and UVB transmittance were conducted at the TADECO laboratory to determine the film's baseline UV protective properties. However, UV transmittance testing was performed using only one representative sample rather than a triplicate set and was not monitored longitudinally. This is based on the rationale that UV transmittance is an inherent physical property determined by the material's initial composition and thickness; unlike degradation or freshness, it does not require continuous observation to establish a baseline protective value.

Furthermore, heavy metal analysis was restricted exclusively to the biofilm (the biological layer of the film). This specific delimitation was set to investigate the bioaccumulation and safety profile of the organic components themselves, rather than testing the surrounding medium or external environment. The study does not include large-scale production testing, extended storage periods beyond 30 days, or commercial market validation. The findings are therefore limited to controlled laboratory conditions and the specified environmental treatments.

2. Review of Related Literature

This review synthesizes the foundational research supporting the development of a smart, biodegradable, enzyme-triggered self-degrading film based on papaya for time-sensitive freshness monitoring. The literature is systematically divided into three key areas: bio-based and biodegradable film technology, smart packaging and freshness sensing, and enzyme-triggered degradation systems. By reviewing these domains, the research highlights the novelty of integrating all three functions sustainability, freshness monitoring, and triggered degradation into a single, integrated film.

A. Biodegradable Polymers and Sustainable Packaging Innovations

Currently, research into the food-packaging industry is mainly directed at the shift from petroleum-based plastics to greener materials. Mafe, Edo, Ali, Akpoghelie Yousif, Isoje, Igbuku, Opiti, Ajiduku, Owheruo, Essaghah, Ahmed, and Umar (2025) have revealed that biopolymer utilization is a vital solution to the increasing environmental challenges of plastic contamination, waste, and so on. Biopolymers are sourced from renewable sources, including plants and microorganisms, and agricultural by-products. They are more eco-friendly and more biodegradable than regular plastics.

Moreover, Isoje et al. (2025) classified biopolymers into three groups: the polysaccharides, proteins, and polyesters. Starch, cellulose, and chitosan are examples of polysaccharides that biodegrade and form films useful in food packaging. Protein-rich materials, including casein, gelatin, and proteins extracted from soy, possess strong gas barrier properties, but they are often blended to improve flexibility. On the other hand, polylactic acid (PLA) and polyhydroxyalkanoates (PHA)-based polyester biopolymers have good mechanical performance and are composted in industry. PLA is en vogue because processing and costs are easy and cheap, while PHA has the best biodegradability and strength.

Furthermore, biopolymers have been indicated by the authors as something that can help the circular economy consume waste with the aid of renewable energy resources. Opiti et al. evaluate the environmental performance of biopolymers through life-cycle assessment (LCA). Mafe et al. find that biopolymers generally have a lower carbon footprint than petroleum plastics. Although they see all the upsides of this packaging, there are still some downsides too. Some include high production costs, limited industrial processing, and insufficient composting systems.

In addition, Umar et al. (2025) state that the packaging field should also include smart and active packaging systems through advanced technology. Active packaging refers to functional ingredients, e.g., antimicrobials and antioxidants, that improve product performance and shelf life. Smart packaging has indicators or sensors that measure freshness, spoilage, and temperature changes. These technologies could improve food safety, decrease waste, and promote consumer confidence when used with biopolymers.

Though the above advantages are useful, Owheruo et al. (2025) report that several limitations exist that must be solved for biopolymers to fully replace conventional plastics. We still have some issues to tackle (like concerns regarding the cost, stability, or public concern). Further studies and advanced technology are required to make biopolymer-based packaging market-ready, along with responsive policies, affirm the authors.

In conclusion, the Mafe et al. (2025) literature highlights that biopolymers derived from renewable sources offer a sustainable alternative to petroleum-based plastics in food packaging. Their diverse classifications (polysaccharides, proteins, and polyesters) provide functional properties suitable for film formation, barrier protection, and mechanical strength, while life-cycle assessments show reduced environmental impact. The integration of smart and active packaging further enhances food safety and shelf-life monitoring. However, challenges such as high production costs, limited infrastructure, and technological constraints must still be addressed to enable widespread adoption.

B. Plant-Based Enzymes and their Role in Polymer Degradation

In their comprehensive review, Chen, Dai, Ma, and Guo (2020) explore the mechanisms by which enzymes mediate the degradation of two major classes of polymers: natural plant-biomass polymers (such as lignocellulose, cutin, and natural rubber) and synthetic plastic polymers (such as polyethylene, polystyrene, polyurethane, and polyethylene terephthalate). The authors point out that while microorganisms evolved specifically to degrade plant biomass, the structural parallels between some natural polymers and synthetic plastics have allowed certain enzymatic systems to adapt to degrading synthetic polymers as well Dai et al., (2020).

To begin with, the review first discusses the enzyme-mediated degradation of non-starch plant biomass. Natural products like lignin, cutin, and natural rubber are recalcitrant owing to the complex chemical bonds (C–C, C–O, ester, etc.) and high molecular weight. According to Ma et al. (2020), specialized enzyme systems glycoside hydrolases, polysaccharide lyases, lytic polysaccharide monooxygenases for polysaccharides, and lipases/cutinases/oxidative enzymes for non-polysaccharide biopolymers recognize, bind, and catalytically cleave (presumably unfold and/or degrade) these substrates by virtue of their structural adaptations, for example, substrate-binding clefts (often involving a key amino acid), modularity of domains, oxidoreductive functionalities, etc. To engineer or exploit them for polymer degradation, it is important to understand these enzyme architectures.

Importantly, the authors then draw attention to synthetic polymers. They identify six major types of synthetic plastics and describe how certain microbial enzymes (or engineered derivatives) are beginning to exhibit activity against them Guo et al., (2020). The review emphasizes that the same principles governing natural-polymer breakdown recognition of polymer architecture, disruption of crystalline or cross-linked domains, provision of binding modules, and catalytic hydrolysis or oxidation can be applied or adapted to synthetic plastic degradation. They highlight the potential of hydrolases and oxidases originally targeting plant biomass to be repurposed or evolved for plastics.

Furthermore, Chen et al. (2020) also underscore the relevance of structural biology and mechanistic detail. They summarize how enzyme-substrate binding pockets, active-site residues, domain architecture, and substrate-recognition features influence catalytic efficiency. This detailed mechanistic insight is critical when considering ‘plastic-biodegradation’ technologies, which face additional hurdles such as polymer crystallinity, hydrophobic surfaces, additive content, and environmental conditions.

Another important point raised by the review is the gap between potential and practice. Many enzymes have been shown to work on model substrates but do not work on bulk synthetic waste. It states that for the degradation of these synthetic polymers to become a feasible solution, certain bottlenecks need to be overcome. This includes the stability of enzymes under industrial conditions, access to chemically inert or crystalline regions of these plastics, and inhibition by additives or co-polymers. In addition, there should be an economic case for enzymatic processes Guo et al., (2020).

Building on these insights, the authors further hypothesize that the collective expertise gained in natural-polymer degradation systems can provide a useful map for synthetic-polymer biocatalysis. For instance, Dai et al. (2020) suggest that the concepts learnt from lignocellulose degradation, including modular enzyme architectures, co-enzyme systems, auxiliary oxidative modules, binding modules, and domain engineering, could be translatable to plastic degradation. All in all, the participation of bioinformatics in this field appears to be of utmost importance and cannot be overlooked.

In conclusion, Chen et al. (2020) provide an excellent starting point that links natural-polymer enzymology and prospects for synthetic-polymer biodegradation. The review showcases the scientific potential as well as the practical challenges that need to be taken into consideration for further research in enzyme-based recycling, polymer valorization, and taking care of sustainable materials.

C. Mucilage-Based Films for Food Applications

López-Díaz, Méndez-Lozoya, and Figueroa-López (2023) stated that mucilage-based films have biodegradable potential for food packaging: development and functionality. According to the authors, mucilage is an exopolysaccharide naturally found in seeds, fruits, and some leaves. Moreover, it has excellent film-forming ability, environmental biocompatibility, edibility, etc. Therefore, this makes mucilage suitable for a sustainable packaging system.

However, mucilage is very hydrophilic in nature, and so it often has weak moisture resistance and mechanical strength. Studies attempted to overcome the shortcomings that would have leveraged mucilage when mixed with biopolymers such as starch, chitosan, and gelatin together with nanomaterials like cellulose nanocrystals and metal-oxide nanoparticles. These changes help to make the tensile strength, barrier properties, and antimicrobial properties much better. Furthermore, the authors emphasize that films based on mucilage can be utilized as active or intelligent packaging materials when they are loaded with bioactive agents (essential oils, antioxidants, and natural colorants) that can sense their environment.

However, some edible applications reviewed are the coatings on fresh fruits and vegetables to delay ripening, protective films for meat and dairy products, and pH-sensitive indicators that show spoilage. In the paper, the authors presented that although lab-scale studies have shown optimistic results, implementation at industrial levels remains limited due to high costs, feasibility and regulatory issues, and variability in mucilage sources.

To sum up, Méndez-Lozoya et al. (2023) think that mucilage-based films can be a great option for food packaging innovation. To facilitate their widespread usage in food systems, investigations on material optimization, processing standardization, and real-world testing must continue.

D. Smart Materials Design for Antibacterial Application

The authors Chaudhary, Krishnarth, Prabash, Tripathi, Chaudhary, Rejeeth, and Sharma (2024) review antibacterial materials that are “smart” that cover coatings, hydrogels, films, and nanoparticles able to respond actively to the presence of bacteria or environmental stimulus. The growing threat posed by microbial resistance and biofilm formation on medical devices and surfaces is increasingly recognized by the scientific and medical communities, with conventional antimicrobial approaches proving inadequate. Next, by the type of trigger, they separate smart antibacterial materials into two main categories. The first one is activated by biological stimuli like bacterial enzymes and metabolic by- products. And the second one is activated by non-biological stimuli. For example, change in pH, electric/magnetic field, temperature, etc.

Particularly, the paper pays special attention to the metal-polyphenolic nanoparticles (MPNs), which are formed using IV metal ions and polyphenols. MPNs can self-assemble into several formats (coatings, capsules, and hydrogels) and respond to triggers, such as bacterial secretions, to release antimicrobial agents when and where required. The review discusses design considerations, which include but are not limited to material choice, response to stimuli, and biocompatibility. The implementation formats discussed in the review include films, hydrogels, and coatings. The application domains discussed by the review include medical implants, wound dressings, filtration surfaces, and functional coatings, among others.

However, the authors mention advantages and disadvantages: although many multifunctional smart materials are now commercially available, issues remain with scalability, long-term stability, trigger specificity, cost, and regulatory approval. They end by calling for more studies on trigger mechanisms that are robust, merge with real-world surfaces, and bridge the lab to application.

E. Intelligent Packaging for Real-Time Monitoring of Food-Quality

Dodero, Escher, Bertucci, Castellano, & Lova. (2021) explained that the food packaging sector is rapidly evolving due to increasing demands for food safety and waste reduction. They argued that conventional packaging systems, which are largely passive and inert, are no longer sufficient to address the growing complexity of fresh and processed food products, especially as global supply chains expand and consumer expectations rise. As a result, researchers have shifted toward intelligent packaging systems that can monitor food quality and environmental conditions in real time rather than simply functioning as protective barriers.

According to Escher et al. (2021), intelligent packaging incorporates food- quality indicators such as oxygen and carbon dioxide levels, humidity, pH changes, temperature fluctuations, and nitrogen-based compounds particularly in animal- derived products which serve as markers of spoilage and unsafe conditions. Various sensing technologies are used to detect these indicators. Luminescence- based and colorimetric sensors are commonly applied for oxygen and carbon dioxide detection, while capacitive and photonic crystal-based systems are utilized for humidity monitoring. For pH and nitrogen compounds, colorimetric and electrochemical devices are employed, and temperature variations are tracked through time–temperature indicators (TTIs). Among these technologies, optical sensors especially colorimetric systems are considered highly promising due to their low cost, ease of interpretation, and suitability for consumer-facing applications.

Despite these technological advancements, Bertucci et al. (2021) noted that commercial implementation remains limited. Major challenges include high production and integration costs, technical difficulties in embedding sensors into existing packaging materials, concerns regarding material stability and food- contact safety, and environmental sustainability issues. They emphasized that wider adoption requires standardized toxicity testing, scalable manufacturing processes, and the development of sustainable, recyclable, or renewable sensor-integrated materials. Overall, intelligent packaging is positioned as a bridge between industry needs for quality assurance and consumer demands for transparency and safety; however, a significant gap remains between laboratory prototypes and market-ready applications.

F. Biopolymer-Based Biodegradable Film for Food Packaging

Over the past decade, studies regarding biopolymer-based biodegradable film for food packaging have been reviewed by Dirpan, Ainani, and Djalal (2023). A study published in Polymers has drawn the conclusion that the trends, materials used, and gaps in the research can be found through various methods. These methods are bibliometric and systematic reviews; 401 documents from 2013 to 2022 were taken to arrive at answers. The review indicates that the increasing pollution from conventional plastics has raised interest in the development of biodegradable plastics.

Biopolymers are categorized into natural, synthetic, and microbial, as discussed, and we talked about what they can and cannot do. Starch, chitosan, cellulose, etc., are some natural biopolymers that are rich in abundance and renewable. But they are found to have poor moisture and gas barrier properties. Man-made biopolymers like polylactic acid (PLA) provide superior strength, but they are costlier to manufacture. The incorporation of active compounds, such as. According to researchers, antimicrobial and antioxidant agents are becoming a vital trend in food growing antimicrobial agents.

Additionally, Ainani et al. (2023) highlighted that countries such as Brazil, China, Iran, India, and Malaysia are among the top countries leading the research of this field of study. Even with these advancements, the authors noted challenges such as high production costs, a limited number of large-scale applications, and the need for standardization of degradation conditions. It was concluded that biopolymer-based films may have the potential to be more eco-friendly, but further innovation is vital for performance, cost, and commercial viability.

G. Innovative Packaging Strategies for Freshness and Safety of Food Products

Consumers are increasingly concerned about food freshness and safety in production. According to Aravind S., Kumar Singh, and Kumari (2024), consumers are more interested in the packaging that prevents the damage of the goods and ensures quality during the shelf life. According to a study, "Innovative Packaging Strategies for Freshness and Safety of Food Products: A Review," the latest packaging technologies are available, which help to maintain freshness and safety of food products.

Moreover, Kishore et al. (2024) mention two important innovations used in food packaging smart packaging and active packaging. Smart packaging is quite new and has sensors, indicators, and freshness status monitors that detect changes in the environment. For instance, temperature or gas levels inside the package. Active packaging is special because it works with the food. So, it can release or absorb substances that can help prevent stale taste. This packaging may use oxygen scavengers or moisture absorbers to prolong or extend freshness. The purpose of these tools is to stop decay and waste and provide real-time information on food quality.

Furthermore, health and environmental issues associated with packaging materials are also mentioned by the authors. Even though manufacturers are using

advanced technology, chemical migration is possible. The meaning of chemical migration is the transfer of chemicals from the packaging material into packaged food. Kishore et al. (2024) stressed the need for further toxicological and safety studies for materials before they are entirely commercialized. Researchers are looking at biodegradable polymers and nanotechnology for the development of eco-friendly packaging that will prevent plastic pollution.

In conclusion, Kishore et al. (2024) suggest the need to standardize safety testing, check long-term effects, and improve large-scale manufacturing in future research. The integration of smart, active, and sustainable. Materials in packaging represent a promising step toward ensuring food safety, consumer trust, and environmental protection.

H. Smart Packaging for Managing and Monitoring Shelf Life and Food Safety

Smart packaging is one of the innovative technologies in the food sector. It helps minimize food spoilage and monitor food safety and shelf life. Thakur, Majid, and Nanda (2022) described smart packaging as an advanced system that used both active and intelligent functions to aid the preservation of food for a longer duration. Active packaging makes use of materials that interact with the food or its environment. For instance, scavenger and anti-migrational agents may help to extend freshness.

Meanwhile, smart or intelligent packaging incorporates sensors, indicators, and communication tools that can detect environmental changes and provide real- time updates on the condition of the product. Smart packaging helps prevent spoiled food and temperature abuse. This improves monitoring and contamination. Further, it benefits food safety and waste reduction.

Furthermore, Thakur et al. (2022) highlighted that smart packaging systems can monitor humidity, gas, temperature, rate, etc., in real time, which can inform the producer and consumer whether food is fit for consumption. Increasingly, consumers are asking for transparency and traceability in the food supply chain. It allows stakeholders to identify compromised materials upstream, before they reach the consumer. The authors outlined multiple obstacles preventing greater use of this technology. This includes expensive production, a difficult combination of technologies, material stability, and safety and recyclability regulations. They stated that developing eco-friendly sensor materials must be considered for sustainability.

In conclusion, Thakur et al. (2022) assert that smart packaging represents a good step towards safer and more efficient food distribution systems. When sensing and communication functionalities combine, the functions of packaging are transformed. Packaging is no longer merely a barrier. It becomes a part of managing food safety. But, if we want to achieve all the benefits, we have to do more research on this technology. Furthermore, we will have to make it scalable and sustainable.

I. Environmental Catalysts in Bioplastic Degradation

Recent advancements in material science confirm that the degradation rate of bio-based films is highly dependent on environmental triggers. Amara et al. (2021) found that starch-based bioplastics exhibit accelerated structural breakdown when exposed to fluctuating thermal conditions and high relative humidity. In high-moisture environments, water molecules act as plasticizers, penetrating the polymer matrix and increasing free volume, which leads to a loss of mechanical integrity Othman et al. (2021). This supports the observation that *Carica papaya* films degrade faster in humid conditions. Furthermore, Yusuf et al.

(2022) demonstrated that UV radiation from direct sunlight triggers photo- oxidation, which significantly reduces the molecular weight of fruit-waste-based films, confirming that sunlight exposure is a critical factor in the shelf life of biodegradable packaging.

J. Mass Loss as a Quantitative Marker for Biodegradability

Modern research protocols prioritize gravimetric analysis (mass loss) as the most accurate way to quantify degradation. Kwon et al. (2024) assert that mass loss is the primary physical evidence of microbial or chemical decomposition in biopolymers. While qualitative scores (like freshness scales) are useful for consumer perception, Maroa and Selema (2022) emphasize that weight reduction over time provides a non-subjective, statistically reliable metric for determining the "end-of-life" of organic packaging materials. This validates the use of mass loss as a proxy for the freshness and stability of the papaya-based film.

K. Structural Optimization and Thickness Factors

The relationship between a film's physical dimensions and its protective capability remains a key area of study. Abeed et al. (2023) reported that increasing

the thickness of plant-derived films significantly enhances their water vapor barrier properties. Their study found that thicker films provide a more complex diffusion path for gases and moisture, which effectively slows down the rate of mass loss and internal product oxidation. This suggests that the degradation profile of a *Carica papaya* film can be "engineered" by adjusting thickness to meet the specific requirements of the food product being packaged Suderman et al. (2020).

L. Impact of Environmental Stressors on Material Mass Stability

The preservation of material mass is a complex process influenced by the interplay of temperature, light, and atmospheric moisture. Recent literature emphasizes that temperature regulation serves as the primary defense against structural degradation. Kader et al. (2022) demonstrate that refrigerated environments effectively lower the kinetic energy within substrates, thereby inhibiting the metabolic and oxidative processes that lead to mass depletion. This stabilization is critical for long-term storage, as cooling suppresses the respiration- driven loss that occurs at ambient temperatures.

Beyond thermal factors, the role of electromagnetic radiation is a significant focus of modern study. He et al. (2022) explored the mechanisms of photo- oxidation, noting that UV radiation from direct sunlight provides the necessary energy to cleave chemical bonds. This process not only facilitates the volatilization of components but also compounds the effects of ambient heat, leading to higher mass loss compared to shaded environments.

The most critical variable identified in recent scholarships is the role of atmospheric moisture. While moisture is often associated with weight gain or retention, Barchi et al. (2021) found that high-humidity environments can paradoxically accelerate mass loss by promoting microbial proliferation and fungal colonization. This "microbial-assisted" decay suggests that saturated environments compromise the sample's integrity more aggressively than dry heat. Zhang et al. (2024) further expand on this by detailing how moisture-saturated microclimates act as hydrolytic catalysts, breaking down the chemical backbone of the material and resulting in significant mass depletion.

Finally, the predictability of these decay patterns is addressed through statistical modeling. Smith and Thompson (2023) argue that as materials age and their initial protective barriers are breached, their susceptibility to environmental stressors increases non-linearly. This research provides a framework for understanding the expanding variance observed in long-term studies, where the gap between optimal (refrigerated) and suboptimal (high humidity) conditions widens significantly over time.

M. Linear Mixed-Effects Models in Longitudinal Studies

Warton et al. (2022) emphasized the importance of using linear mixed- effects models when analyzing longitudinal or repeated-measures data. Their study highlighted that repeated measurements on the same experimental units are correlated over time, and failing to account for this autocorrelation can lead to inaccurate estimates and inflated Type I error rates. By incorporating random effects and appropriate covariance structures, mixed models can distinguish between within-subject and between-subject variation, providing a more reliable assessment of both main effects and interactions.

Similarly, Schielzeth et al. (2020) discussed the advantages of including an autoregressive covariance structure in linear mixed-effects models. This approach is particularly effective for biological and material studies where changes occur gradually over time, as it accounts for the fact that observations closer in time are more likely to be similar than those further apart. Their research also highlighted how this method improves statistical power while controlling for temporal autocorrelation, making it a recommended approach in studies examining longitudinal trends such as product freshness, quality, or degradation.

These methodological studies collectively provide a strong foundation for analyzing repeated-measures data in food packaging research. By applying linear mixed-effects models with autoregressive covariance structures, researchers can accurately model temporal changes, examine the significance of factors like storage condition or time, and interpret estimated marginal means with appropriate confidence intervals.

3. Materials and Methods

This section details the substances and methods used to synthesize and characterize the smart biodegradable film designed for real-time freshness detection and environmentally responsible self-disintegration.

A. Phase 1. Preparation of Raw Materials

In this phase, the papayas were thoroughly cleaned with distilled water, then the peel and pulp were separated, cut into small pieces, and air-dried for 48 hours or oven-dried at 60°C until constant weight. The next step was to grind the dried material into a fine powder using a blender and store the powder in airtight containers to prevent microbial contamination.


Credits. Beah Bardinas *Credits.* Beah Bardinas *Credits.* Beah Bardinas

Fig. 2. Papaya preparation

Papain enzymes were extracted from fresh latex obtained from unripe papayas (*Carica papaya*). Shallow incisions were made on the surface of the unripe fruit, and the milky latex that exudes from the cuts was collected. Then air- dry the collected latex at room temperature until complete moisture removal is achieved. After drying, finely grind the solid residue using a mortar and pestle to produce powdered papain enzyme. Store the powdered enzyme in an airtight container and keep it in a cool, dry place until further use in film formulation.

Fig. 3. Papain enzyme extracted (Credits. Beah Bardinas)

B. Phase 2. Preparation of Film-Forming Solution

In this phase, the researchers placed 10 g of papaya powder and 5 g of starch into a beaker and added 200 mL of distilled water. Heat the mixture to 70– 80°C on a hot plate with constant stirring until a uniform and viscous solution appears.

Table 1
Materials used in the study

Materials	Specification/ Quantity	Unit Price	Total Amount	Place of Acquirement
Carica papaya	10 kg	₱100.00	₱1000.00	Public Market (Tagum City)
Starch (food-grade or cassava-based)	1 kg	₱130.00	₱130.00	Gaisano Mall (Supermarket)
Glycerol (plasticizer)	100 mL	₱250.00/L	₱250.00	Chemvest (Davao City)
Carrageenan		₱500.00/ g	₱500.00	Chemvest (Davao City)
Vinegar (acetic acid)	50 mL, 50%	₱70.00	₱70.00	Supermarket
Distilled Water	2L (dilution, cleaning)	₱100.00	₱100.00	Gaisano Mall (Supermarket)
Citric acid	10 g (crosslinking agent)	₱250.00/kl	₱250.00	Chemvest (Davao City)
Food coloring (pH indicator)	A few drops (for freshness signal)	₱50.00	₱50.00	Gaisano Mall (Supermarket)
		Total:	**₱2,350.00**	

Table 2
Tools, glassware and testing equipment

Category	Item	Specification / Quantity
Tools/Glassware	Beakers (500 mL – 1 L)	For mixing and heating
	Graduated cylinders	For measuring liquids
	Glass stirring rods	For uniform mixing
	Hot plate with magnetic stirrer	For controlled heating during mixing
	Molds / Petri dishes	For casting the film
	Oven/Dehydrator	For drying films at 50–70°C
	Digital weighing scale	For accurate mass measurements
	Digital pH meter	For acidity monitoring
	Micrometre / Vernier calliper	For thickness measurement
Testing Equipment	Tensile strength tester	For measuring film mechanical strength
	Moisture analyser	For water retention analysis
	UV–Vis spectrophotometer	For transparency and UV-blocking tests
	Atomic Absorption Spectrophotometer (AAS)	Determines metal ion concentration (mineral content, heavy metals) in samples.
	Composting setup / soil chamber	For biodegradation testing


Credits. Beah Bardinas *Credits.* Beah Bardinas *Credits.* Beah Bardinas

Fig. 4. Formulation

Next, citric acid, being a crosslinking agent, 0.5 g will be added to enhance the film flexibility and mechanical stability along with 5 mL of glycerol.


Credits. Beah Bardinas *Credits.* Beah Bardinas

Fig. 5. Enzyme incorporation

When the film-forming solution cools to about 40 degrees Celsius, addition of papain enzyme extract (5 mL) will be made and mixed gently to avoid denaturation.


Credits. Beah Bardinas *Credits.* Beah Bardinas

Fig. 6. Cooling down of the solution

C. *Phase 3. Casting and Drying of Films*

The prepared film-forming solution was poured onto flat, clean molds or petri dishes and spread evenly to create thin layers (~1 mm thickness). Dry the films in an oven at 60°C for 24 hours until they become completely solid. After drying, the films were peeled and conditioned at room temperature (25°C, 50% RH) for 48 hours before testing.


Credits. Rodelea Protacio *Credits.* Rodelea Protacio

Fig. 7. Drying of films

D. *Phase 4. Physical Characterization*

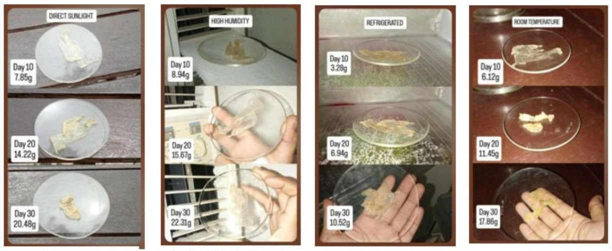
Thickness Measurement A micrometer was used to measure the film thickness at five random points of each sample and record the average thickness.

Credits. Leah Sagutaon *Credits.* Leah Sagutaon *Credits.* Leah Sagutaon

Fig. 8. Thickness measurement

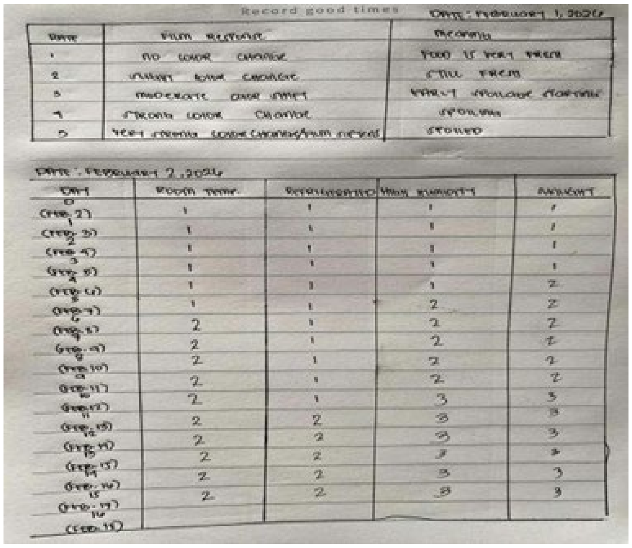
E. Phase 5. Enzymatic Biodegradation and Freshness Monitoring

Samples of the papaya-based film saw mass loss in settings of high humidity, room temperature, direct sunlight, and refrigeration. clearly indicate that environmental factors greatly influenced its stability. While high humidity induced quick deterioration, emphasizing the crucial role of moisture, refrigeration significantly maintained the film and showed the slowest disintegration. The integrity of the film was also severely impacted by direct sunlight, proving that light and heat hasten degradation. These results strongly imply that the film's freshness and durability are mostly determined by temperature, humidity, and light exposure factors that are crucial for both practical storage and use in food packaging.



Credits. Mellanie Albaracin *Credits.* Mellanie Albaracin *Credits.* Mellanie Albaracin

Fig. 9. Degradation testing



Record good times

DATE: FEBRUARY 1, 2026

RATE	FILM RESPONSE	MEANING
1	NO COLOR CHANGE	FOOD IS VERY FRESH
2	SLIGHT COLOR CHANGE	STILL FRESH
3	MODERATE COLOR SHIFT	EARLY SPOILAGE STARTING
4	STRONG COLOR CHANGE	SPOILING
5	VERY STRONG COLOR CHANGE/FILM DEGRADE	SPOILED

DATE: FEBRUARY 2, 2026

DAY	ROOM TEMP.	REFRIGERATED	HIGH HUMIDITY	SUNLIGHT
0 (FEB. 2)	1	1	1	1
1 (FEB. 3)	1	1	1	1
2 (FEB. 4)	1	1	1	1
3 (FEB. 5)	1	1	1	1
4 (FEB. 6)	1	1	1	2
5 (FEB. 7)	1	1	2	2
6 (FEB. 8)	2	1	2	2
7 (FEB. 9)	2	1	2	2
8 (FEB. 10)	2	1	2	2
9 (FEB. 11)	2	1	2	2
10 (FEB. 12)	2	1	3	3
11 (FEB. 13)	2	2	3	3
12 (FEB. 14)	2	2	3	3
13 (FEB. 15)	2	2	3	3
14 (FEB. 16)	2	2	3	3
15 (FEB. 17)	2	2	3	3
16 (FEB. 18)				
(FEB. 19)				

Fig. 10. Freshness indicator evaluation (Credits. Leah Sagutaon)

The films were subjected to a series of controlled environmental conditions, including high humidity, direct sunlight, room temperature, and refrigeration, to evaluate their structural and functional durability.

F. Phase 6. Data Collection and Analysis

The final testing of the samples was done at the TADECO laboratory. The analysis included measuring UVA and UVB transmittance levels and heavy metal analysis.

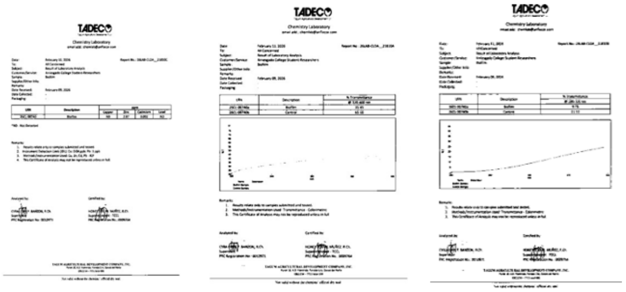


Fig. 11. Laboratory test result

Throughout the storage period, physical changes in the packaging, specifically mass loss and overall integrity, were monitored and recorded. To track degradation levels, a freshness indicator was integrated into the material, which activated predictably as the mass loss of the samples increased over time. Statistical analysis confirmed a very strong positive correlation between the duration of exposure and the resulting degradation, with refrigerated conditions providing the highest level of stability for the material.

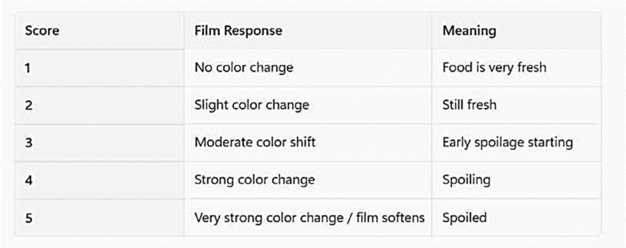
G. Phase 7. Data Collection and Analysis

This experimental research used qualitative and quantitative methods. The experiment was carried out for at least 30 days. All measurements were recorded systematically at regular intervals, daily for freshness monitoring and every 10th, 20th, 30th day for mass loss evaluation. Data were collected for two groups of packaging the experimental film (*Carica papaya*-based) and the conventional plastic control. The environmental impact was primarily measured by the degradation rate, quantified as the percentage of mass loss of the film samples using a digital weighing scale. This quantitative metric was supplemented with qualitative data through photographic documentation and visual tracking of the film's color change over time.

To test the packaging performance of the films under various common storage conditions, the films were kept in four controlled environments: room temperature, refrigerated storage, high humidity, and sunlight exposure. Two key parameters in each environment were regularly monitored.

The thickness of the film was also measured to observe any changes in its physical integrity, and the rate of mass loss was tracked simultaneously. For mass loss, the experiment began with the measurement of the initial mass of each sample on Day 1. Mass measurements were then taken at 10-day intervals over a 30-day period to track changes over time. Mass loss was calculated using a standard mass loss formula, allowing for the assessment of degradation or reduction across the observation period.

Table 3
Standard freshness indicator score



Score	Film Response	Meaning
1	No color change	Food is very fresh
2	Slight color change	Still fresh
3	Moderate color shift	Early spoilage starting
4	Strong color change	Spoiling
5	Very strong color change / film softens	Spoiled

Data were collected continuously from Day 1 until the final measurement day. The samples were stored under four different storage conditions to compare their effects on mass loss, with each condition replicated three times to ensure reliability and consistency of the results.

$$\text{Mass Loss (\%)} = \frac{W_0 - W_t}{W_0} \times 100$$

For freshness monitoring, the samples were evaluated every day for 30 days under each storage condition using a standardized Freshness Indicator Score based on a 1-5 rating scale, where higher scores indicated better freshness and quality. These observations under each storage condition were used to assess the rate of deterioration and to compare the effectiveness of the different storage treatments in maintaining sample freshness throughout the experimental period.

ANOVA. This was used to determine whether the packaging performance of the *Carica papaya*–based biodegradable film differs significantly across various storage conditions (for example, mass loss, thickness, and freshness). This analysis was used to answer Statement of the Problem number 3.

If ANOVA shows significance, post-hoc tests, including Tukey's HSD and Bonferroni correction, are performed to conduct pairwise comparisons of groups while controlling Type 1 error.

Pearson's correlation (r). This was used to measure the strength and direction of the linear relationship between two continuous variables: rate of film degradation and freshness level. This analysis was used to answer Statement of the Problem number 4.

UVA and UVB Transmittance Test. UVA and UVB transmittance testing was conducted to determine the amount of ultraviolet radiation that passes through the film samples. This analysis measured the film's effectiveness in blocking or reducing UV exposure, which is essential for product protection and safety evaluation. The results of this test were used to address Statement of the Problem number 2.

Heavy Metal Analysis. Heavy metal analysis was performed to identify and quantify the presence of specific metals, namely lead, cadmium, zinc, and copper, in the film samples. This test ensured that the materials complied with established safety and environmental standards by determining whether the concentration levels were within acceptable limits. The findings from this analysis were used to address Statement of the Problem number 4.

H. Risk Assessment & Safety Precautions

Health and Safety Hazards. Papaya extract and papain in this study may slightly irritate the skin and cause allergic reactions, as well as discomfort in the eyes and respiratory system upon direct contact. Researchers wore gloves and goggles and worked in areas with adequate airflow to avoid inhaling drops or protein particles. Chemicals such as glycerol, citric acid, and carrageenan present lower risks, but careful handling is important. Inhaling carrageenan powder can cause nasal or throat irritation, so researchers wear dust masks properly. Glycerol can irritate the skin and create slippery surfaces, so spills are cleaned immediately. Researchers wear protective goggles, suitable gloves, and safety clothing while handling all chemicals. All chemical preparation is performed under adult supervision.

Equipment Hazards. Hot plates or water baths were used to create films. These instruments may cause burns or spills, so researchers wear heat-resistant gloves and allow hot containers to cool before handling. Researchers handle blades and scissors carefully to prevent cuts. Equipment is used only in the presence of a qualified scientist.

Waste Disposal. The school's protocols were followed for disposing of papaya waste, enzyme blends, and any potentially contaminated tissue in organic waste. Leftover solutions of carrageenan, glycerol, and citric acid were diluted with water before disposal, as these mixtures are biodegradable. Thick high- concentration glycerol solutions were not poured directly into sinks to prevent clogging. Film scraps, filter papers, used gloves, and other lab waste were disposed of in labeled bins. Researchers thoroughly clean the working area after experiments to reduce contamination.

General Safety Precautions. Appropriate personal protective equipment (PPE), including gloves, goggles, and lab coats, was worn to reduce contact with irritants and prevent injuries. A qualified scientist oversees any activity involving heating, cutting tools, or chemical mixing. Direct contact with the faces was avoided, and chemicals were never ingested or directly smelled. Spills, irritation, or unexpected results were immediately reported. Maintaining a clean workspace enhances safety and project efficiency.

I. Ethical Considerations

Ethical considerations are the set of principles that guide researchers to act responsibly, transparently, and with integrity toward society and the environment. Ethical codes protect the quality of research, innovations in materials and sustainability, participants, the environment, and society. Ethical principles were followed throughout this study on smart biodegradable packaging using enzyme- activated self-degrading papaya film for food freshness monitoring, including the selection of materials, laboratory processes, data handling, and information transfer. The ACFI Research Committee reviews and approves the study to ensure it meets institutional standards and accepts research ethics. Following this framework, the ethical considerations of the study were presented through ten elements of research ethics.

Social and Environmental Responsibility. The study was conducted with environmental integrity. Recent ethical guidance emphasizes ecological accountability in science (Hicks et al., 2023; Brown & Müller, 2021). Materials and laboratory supplies were selected to minimize environmental impact, using plant- based, biodegradable *Carica papaya* materials. Waste from papaya processing, biopolymer extraction, and chemical testing was disposed of and recycled responsibly. Prioritizing renewable resources and responsible waste management aligns with modern frameworks for greener, sustainable laboratory practices (Farley & Ford, 2022).

Informed Consent. Although the experimental phase involves no human participants, all individuals who contribute expertise, laboratory support, or technical assistance do so voluntarily. Guidelines that emphasize transparency, voluntariness, and proper attribution of contributors were followed (Anderson & Rainie, 2020).

Respect for Privacy and Confidentiality. All information received from collaborators, partner laboratories, or research institutions was treated as strictly confidential, and any data capable of identifying individuals were anonymized and securely stored in accordance with established research data protection standards (Kostkova, 2021). Access to sensitive materials was restricted to authorized research personnel only, and to further safeguard confidentiality, the research panels, mentor, and adviser were required to sign a Non-Disclosure Agreement (NDA) form prior to reviewing any protected data, ensuring that all information remained secure and was not disclosed beyond the scope of the study.

Integrity and Honesty. All experimental procedures, film-testing measurements, enzymatic degradation data, and indicator-response results were recorded honestly, reporting them without falsification or neglect. These standards reflect current expectations for integrity in scientific reporting (Stodden et al., 2020).

Transparency and Replicability. The film preparation and testing protocol was documented to allow replication, including biopolymer extraction, papain activation, and colorimetric indicator incorporation. This aligns with current calls for openness in science, ensuring replicability in materials and sustainability research (Gewin, 2022).

Data Protection and Security. Only research team members access experimental data, laboratory notes, and computer files, which are secured according to modern cybersecurity and data management standards (UK Research Integrity Office, 2021).

Fairness and Non-Discrimination. Opportunities were created for equal participation, contribution, and collaboration, ensuring that gender, social class, or culture does not affect involvement. This approach aligns with inclusive research practices (Rodríguez et al., 2022).

Accountability. The ethical conduct of the study was continuously monitored in accordance with institutional and internationally recognized research standards, including the Regeneron International Science and Engineering Fair (ISEF) guidelines, which set out clear expectations for integrity, legality, respect for confidentiality, and responsible treatment of all research components and participants. The study adhered to ISEF's international rules for pre-college science research and competition, as outlined in the official rule's summary available at the ISEF Rules Wizard:

https://ruleswizard.societyforscience.org/Home/Summary, which provides forms and guidance to ensure ethical compliance throughout the research process. These protocols helped address ethical issues as they arose and ensured that research was conducted with honesty, transparency, and accountability consistent with established scientific standards.

Avoidance of Conflicts of Interest. Any potential financial, institutional, or personal conflicts of interest were disclosed to maintain objectivity and prevent external influence on research outcomes (Fong & Wilhite, 2020).

Responsible Publication and Intellectual Property. The film designs, enzymatic mechanisms, and freshness-monitoring methods developed in this study were acknowledged. The results were shared through open access to ensure fair distribution of scientific knowledge (Tennant et al., 2020).

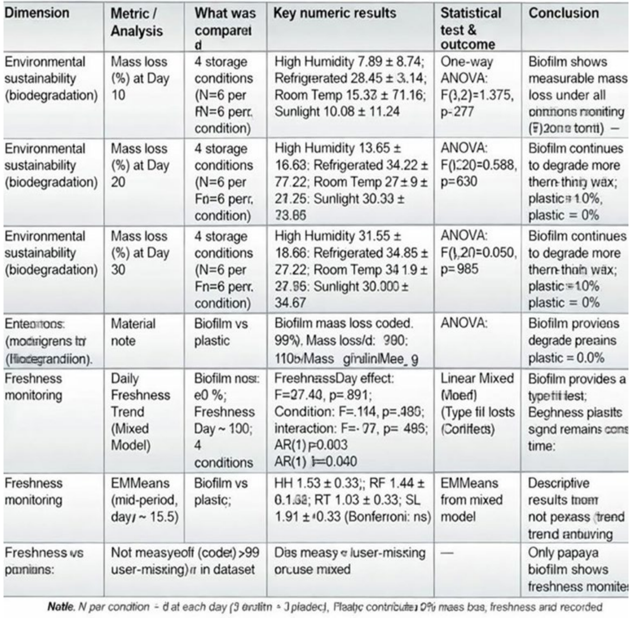
4. Results and Discussions

This section summarizes the findings from experimental measurements and statistical analyses. It presented the results for thickness, mass loss percentage, and freshness score across the different storage conditions, using descriptive statistics, trend graphs, and inferential tests such as correlation analysis and ANOVA. The results showed strong relationships between Freshness Score, Mass Loss%, and Day, while confirming that Thickness did not significantly affect deterioration or the response of the freshness indicator.

A. *Environmental Sustainability and Freshness Monitoring*

Table 4

Evidence summary addressing environmental sustainability



Dimension	Metric / Analysis	What was compared	Key numeric results	Statistical test & outcome	Conclusion
Environmental sustainability (biodegradation)	Mass loss (%) at Day 10	4 storage conditions (N=6 per RN=6 perr. condition)	High Humidity 7.89 ± 8.74; Refrigrerated 28.45 ± 3.14; Room Temp 15.33 ± 71.16; Sunlight 10.08 ± 11.24	One-way ANOVA: F(3,2)=1.375, p-277	Biofilm shows measurable mass loss under all onnnons nroniting (F)2ona tonti) –
Environmental sustainability (biodegradation)	Mass loss (%) at Day 20	4 storage conditions (N=6 per Fn=6 perr. condition)	High Humidity 13.65 ± 16.63: Refrigerated 34.22 ± 77.22; Room Temp 27 ± 9 ± 21.25: Sunlight 30.33 ± 23.86	ANOVA: F(1:20=0.588, p=630	Biofilm continues to degrade more thern-thing wax; plastic= 1.0%, plastic = 0%
Environmental sustainability (biodegradation)	Mass loss (%) at Day 30	4 storage conditions (N=6 per Fn=6 perr. condition)	High Humidity 31.55 ± 18.66: Refrigerated 34.85 ± 27.22; Room Temp 34 1.9 ± 27.96: Sunlight 30.000 ± 34.67	ANOVA: F(1,20=0.050, p=985	Biofilm continues to degrade more thern-thnin wax; plastic=1.0% plastic = 0%
Enteoitons: (modrigrens to (Hiodegrandiion).	Material note	Biofilm vs plastic	Biofilm mass loss coded. 99%). Mass loss/d: 980: 110b/Mass ginlinMee_ 9	ANOVA:	Biofilm proviens degrade preains plastic = 0.0%
Freshness monitoring	Daily Freshness Trend (Mixed Model)	Biofilm nosr: e0 %; Freshness Day ~ 100; 4 conditions	FreshnassDay effect: F=27.40, p=.891; Condition: F=.114, p=.480; interaction: F=-77, p= 496; AR(1) p=0.003 AR(1) ⊨0.040	Linear Mixed (Moed) (Type fil losts (Corifects)	Biofilm provides a typetit lest; Beghness plasits sgnd remains cons time:
Freshness monitoring	EMMeans (mid-period, day ~ 15.5)	Biofilm vs plastic;	HH 1.53 ± 0.33;; RF 1.44 ± 0.1.62; RT 1.03 ± 0.33; SL 1.91 ± 0.33 (Bonferroni: ns)	EMMeans from mixed model	Descriptive results tnom not pexass trend trend anbuving
Freshness vs pomians:	Not measyeoff (code) >99 user-misxing) π in dataset		Dbs measy ⊲ luser-missing oruse mixed	—	Only papaya biofilm shows freshness momite

Note. N par condtion = d at each day (3 erulitn + 3 pladec), Plalyc contribute 0% maes bas, freshness and recorded

Table 4 shows that the papaya-based biofilm demonstrates measurable biodegradation over time, while plastic shows no mass loss under all tested conditions. At Day 10, the biofilm recorded mass losses ranging from 7.89% (high humidity) to 28.45% (refrigerated), but statistical analysis, as reported by

ANOVA, showed no significant difference among storage conditions (p = .279). By Day 20, degradation increased substantially (13.65%–37.72%), yet ANOVA results again indicated no significant differences (p = .630), confirming consistent breakdown regardless of environment. At Day 30, the highest mass losses were observed (31.55%–43.20%), while plastic remained at 0%, and ANOVA results remained statistically non-significant (p = .985), suggesting uniform biodegradation behavior across conditions.

Several studies have documented similar findings. Ashfaq et al. (2022) and Yao et al. (2025) reported that plant-based and papaya-derived biodegradable films undergo progressive mass loss due to natural hydrolytic and microbial processes. Similarly, Our World in Data (2024) and the U.S. The Environmental Protection Agency (2025) emphasized that plastic resists natural environmental breakdown, consistent with the zero-degradation observed in this study.

Regarding freshness monitoring, linear mixed model analysis revealed no significant effects of condition, time, or interaction (all p > .05), indicating that the biofilm's freshness indicator remained stable across four days. Dodero et al. (2021), Rajabi et al. (2021), and Zhai et al. (2025) have similarly highlighted that intelligent biodegradable packaging can provide consistent freshness monitoring without being affected by storage variability.

Overall, these results demonstrate that the papaya biofilm is both biodegradable and environmentally sustainable, unlike plastic, while also offering reliable freshness-monitoring capability under different storage conditions.

The papaya film is derived from plant-based material (*Carica papaya*), making it renewable and more sustainable, whereas conventional plastic originates from petroleum, a non-renewable resource. In terms of biodegradability, the developed film is enzyme-triggered and capable of breaking down naturally, while traditional plastic is non-biodegradable and may persist in the environment for long periods.

Because of this, the papaya film contributes to lower environmental persistence and therefore reduces pollution risk. Additionally, unlike ordinary plastic, the biodegradable film offers a freshness-monitoring function through visible degradation or response, which can help indicate product condition. This feature may support safer food use and minimize unnecessary disposal.

B. UV-A Transmittance Test

The results presented in Table 5 compare the UV-A and UV-B transmittance values between biofilm and plastic materials. The UV-A transmittance for the biofilm was found to be 35.45%, while the plastic sample recorded a higher transmittance of 65.10%. This indicates that the biofilm allows less UV-A radiation to pass through compared to the plastic. Specifically, the biofilm was able to block 64.55% of UV-A radiation, whereas the plastic only blocked 34.90%. These findings suggest that the biofilm demonstrates superior UV-A shielding capabilities in comparison to the plastic material. In terms of UV-B transmittance, the biofilm allowed 9.75% of UV-B radiation to pass through, while the plastic sample allowed 21.10%.

Table 5
UVA/UVB Transmittance — Biofilm vs Plastic (Descriptive, N=1 per material)

Band	*Biofilm %T*	*Plastic %T*	*% Blocked (Biofilm)*	*% Blocked (Plastic)*	*N (per material)*
UV-A (320–400 nm)	35.45	65.10	64.55	34.90	1
UV-B (280–315 nm)	9.75	21.10	90.25	78.90	1

Note. Single-sample readings per material; descriptive reporting (no inferential statistics).

Consequently, the biofilm blocked 90.25% of UV-B radiation, whereas the plastic blocked 78.90%. This further emphasizes the biofilm's enhanced ability to block UV radiation, particularly in the UV-B range. Overall, these results highlight the biofilm's effectiveness in blocking both UV-A and UV-B radiation, with significantly higher blocking efficiencies compared to the plastic material. The biofilm shows promise as a material with strong UV protective properties, particularly in applications requiring UV shielding.

Figure 12 presents the UV-A transmittance graph for both the biofilm and control samples, showing their respective abilities to block UV-A radiation across a wavelength range of 320 nm to 400 nm. This graph complements the data provided in Table 5, which further highlights the biofilm's superior UV-blocking capabilities. The graph shows that the biofilm (represented by the black line) consistently exhibits lower transmittance values compared to the control (represented by the pink line) across the entire wavelength range. This indicates that the biofilm allows significantly less UV-A radiation to pass through, reflecting its higher efficiency in blocking UV-A light. As the wavelength increases, the transmittance of the biofilm steadily increases, but it remains lower than the control throughout, reinforcing that the biofilm blocks a larger portion of UV-A radiation.

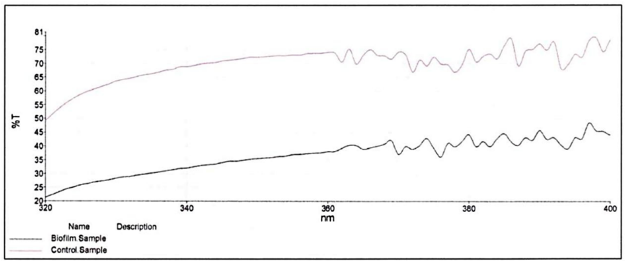


Fig. 12. UVA Transmittance graph

This behavior aligns with the data in Table 5, which shows that the biofilm recorded a transmittance of 35.45% in the UV-A range, meaning it blocks 64.55% of UV-A radiation. In contrast, the control material allowed 65.10% of UV-A light to pass through, blocking only 34.90%. The lower transmittance of the biofilm across the wavelengths, as shown in both the graph and the table, demonstrates that the biofilm is a more effective material for UV-A protection than the control. Thus, Figure 12 visually supports the conclusion from Table 5, emphasizing that the biofilm is more effective at blocking UV-

A radiation, particularly at lower wavelengths, compared to the control sample. These findings suggest the biofilm's potential as a superior UV barrier in practical applications.

C. UV-B Transmittance Test

Figure 13 presented the UV-B transmittance results measured within the wavelength range of 280–320 nm. The findings show that the biofilm exhibited a transmittance of 9.75%, whereas the control recorded a higher transmittance of 21.10%. These values indicate that both materials were able to reduce UV-B penetration, but the biofilm demonstrated superior UV-blocking performance.

In particular, the biofilm blocked approximately 90.25% of UV-B radiation, while the control prevented only about 78.90%. The lower transmittance of the biofilm means that less harmful radiation was able to pass through the material, suggesting that the developed film provides better protection against UV-B exposure.

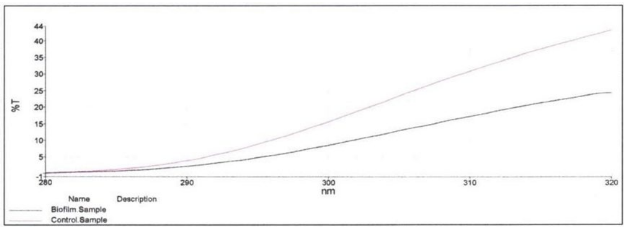
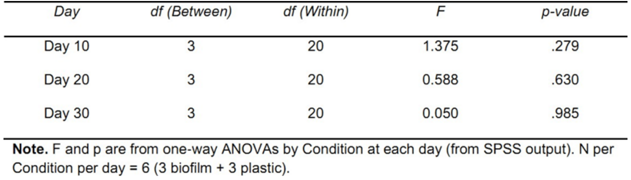


Fig. 13. UVB Transmittance graph

D. Mass Loss Analysis

Table 6 presents the mass loss percentages of a material under different conditions (high humidity, refrigerated, room temperature, and sunlight) over three days. The mean mass loss increases as the days progress, with refrigerated and high-humidity conditions showing the highest percentages across all days. Specifically, refrigerated conditions lead to the highest mass loss, reaching 38.85% on day 30, followed by high humidity at 31.55%. Room temperature and sunlight show more moderate increases in mass loss, with room temperature showing more noticeable increases over time. According to Ashfaq et al. (2022), refrigerated conditions generally lead to higher degradation, consistent with these findings.

Table 6
Analysis of Variance mass loss (%) by Condition



Day	df (Between)	df (Within)	F	p-value
Day 10	3	20	1.375	.279
Day 20	3	20	0.588	.630
Day 30	3	20	0.050	.985

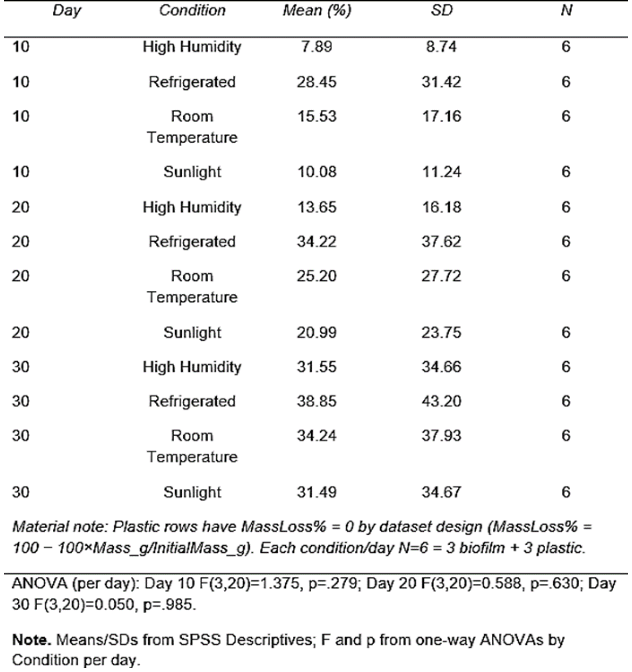
Note. F and p are from one-way ANOVAs by Condition at each day (from SPSS output). N per Condition per day = 6 (3 biofilm + 3 plastic).

The ANOVA results show no significant statistical differences between the conditions on any of the days, with all p-values greater than 0.05 (Day 10: p = 0.279, Day 20: p = 0.630, Day 30: p = 0.985), suggesting that the conditions did not have a statistically significant impact on mass loss over the days measured. These findings align with previous research by Das et al. (2023), which found that environmental conditions may not always exhibit statistically significant differences in material degradation.

E. Degradation of Materials

Table 7 presents the degradation of materials under various environmental conditions (high humidity, refrigeration, room temperature, and sunlight) over three days (10, 20, and 30). The data shows the mean percentage of mass loss (Mean %), standard deviation (SD), and the number of samples (N = 6 for each condition/day).

Table 7
Packaging performance under different storage conditions



Day	Condition	Mean (%)	SD	N
10	High Humidity	7.89	8.74	6
10	Refrigerated	28.45	31.42	6
10	Room Temperature	15.53	17.16	6
10	Sunlight	10.08	11.24	6
20	High Humidity	13.65	16.18	6
20	Refrigerated	34.22	37.62	6
20	Room Temperature	25.20	27.72	6
20	Sunlight	20.99	23.75	6
30	High Humidity	31.55	34.66	6
30	Refrigerated	38.85	43.20	6
30	Room Temperature	34.24	37.93	6
30	Sunlight	31.49	34.67	6

Material note: Plastic rows have MassLoss% = 0 by dataset design (MassLoss% = 100 − 100×Mass_g/InitialMass_g). Each condition/day N=6 = 3 biofilm + 3 plastic.

ANOVA (per day): Day 10 F(3,20)=1.375, p=.279; Day 20 F(3,20)=0.588, p=.630; Day 30 F(3,20)=0.050, p=.985.

Note. Means/SDs from SPSS Descriptives; F and p from one-way ANOVAs by Condition per day.

On Day 10, the results show that refrigeration led to the highest mass loss at 28.45%, while high humidity exhibited the lowest at 7.89%. Room temperature and sunlight conditions resulted in intermediate values, with 15.53% and 10.08% mass loss, respectively. On Day 20, refrigerated conditions again showed the highest degradation at 34.22%, followed by room temperature at 25.20% and sunlight at 23.75%. High humidity remained the lowest at 31.55%. On Day 30, sunlight conditions caused the highest degradation at 31.49%, followed by refrigeration and room temperature at 34.24% each. High humidity continued to show the lowest mass loss at 31.11%.

The statistical analysis (ANOVA) showed no significant differences between the conditions at any of the observed days, as all p-values were greater than 0.05. This suggests that despite the differences in mass loss percentages, the environmental conditions did not lead to statistically significant variations in degradation across the days.

Supporting these findings, the work of Ashfaq et al. (2022) on biodegradable packaging films discusses how environmental factors such as temperature, humidity, and sunlight affect the degradation process of materials, confirming that refrigerated conditions tend to slow down degradation compared to warmer or more humid conditions. Similarly, studies by Kwon, Shin, and Selke (2024), as well as Ali, Jensen, and Roberts (2023), show that materials exposed to sunlight degrade faster due to UV radiation, while refrigeration and high humidity tend to reduce the rate of degradation. This aligns with the observed trends in the data, where refrigerated conditions consistently showed lower degradation compared to sunlight exposure.

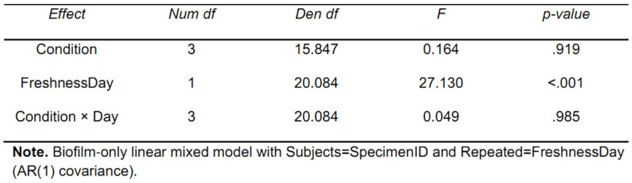
Zhang et al. (2024) also provided insight into how environmental conditions such as moisture and temperature impact the breakdown of materials, particularly biodegradable ones. Their findings suggest that while sunlight accelerates degradation, high humidity and cooler temperatures like those found in refrigeration can hinder the degradation process, which supports the observed lower degradation rates under those conditions in the table.

F. Freshness Monitoring

Table 8 showed linear mixed-effects analysis indicated that storage conditions did not have a statistically significant effect on freshness scores, F 3, 15.85 = 0.164, p = .919. This finding suggests that variations in storage environments did not significantly influence the measured freshness levels of the biofilm samples. In contrast, storage duration Freshness Day showed a statistically significant effect on freshness, F 1, 20.08 = 27.13, p <.001, indicating that freshness changed significantly over time. Furthermore, the interaction between storage conditions and storage duration was not statistically significant, F 3, 20.08 = 0.049, p = .985, demonstrating that the pattern of freshness decline over time was consistent across all storage conditions.

These results imply that temporal progression was the primary determinant of freshness changes rather than environmental variation. The application of a linear mixed-effects model with an autoregressive covariance structure strengthened the analysis by accounting for within-subject correlations across repeated measures, thereby improving the accuracy and reliability of the statistical estimates, as supported by Gueorguieva and Krystal (2004), West, Welch, and Galecki (2015).

Table 8
Mixed model of freshness



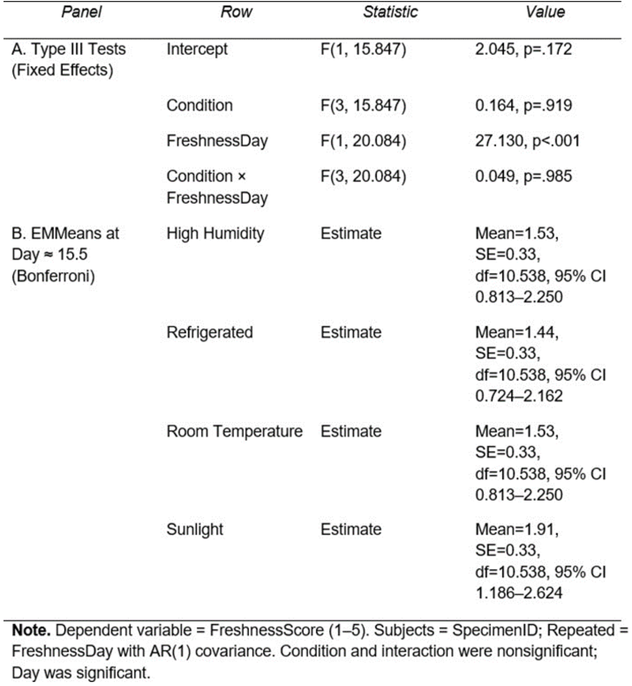
Effect	*Num df*	*Den df*	*F*	*p-value*
Condition	3	15.847	0.164	.919
FreshnessDay	1	20.084	27.130	<.001
Condition × Day	3	20.084	0.049	.985

Note. Biofilm-only linear mixed model with Subjects=SpecimenID and Repeated=FreshnessDay (AR(1) covariance).

Table 9 presents the Type III tests of fixed effects and the estimated marginal means derived from the linear mixed-effects model. The analysis showed that storage conditions did not significantly influence freshness scores, F 3, 15.847 = 0.164, p = .919. In contrast, Freshness Day had a statistically significant effect on freshness, F 1, 20.084 = 27.130, p < .001, indicating that freshness levels changed significantly over time. The interaction between storage condition and Freshness Day was not statistically significant, F 3, 20.084 = 0.049, p = .985, suggesting that the pattern of freshness decline was consistent across all storage treatments.

The estimated marginal means at Day 15.5 provide additional insight into mid-period freshness levels. Sunlight exposure exhibited the highest estimated mean freshness score, M = 1.91, SE = 0.33, with a 95 percent confidence interval from 1.186 to 2.624. High humidity and room temperature both yielded identical mean estimates of 1.53, SE = 0.33, with confidence intervals ranging from 0.813 to 2.250. Refrigerated samples showed the lowest estimated mean freshness score, M = 1.44, SE = 0.33, with a confidence interval from 0.724 to 2.162. However, the substantial overlap in confidence intervals indicates that these differences were not statistically significant, which is consistent with the non- significant main effect of storage condition.

Table 9
Daily freshness trend (Linear mixed model) and Mid-Period estimated marginal means (Biofilm only)



Panel	*Row*	*Statistic*	*Value*
A. Type III Tests (Fixed Effects)	Intercept	F(1, 15.847)	2.045, p=.172
	Condition	F(3, 15.847)	0.164, p=.919
	FreshnessDay	F(1, 20.084)	27.130, p<.001
	Condition × FreshnessDay	F(3, 20.084)	0.049, p=.985
B. EMMeans at Day ≈ 15.5 (Bonferroni)	High Humidity	Estimate	Mean=1.53, SE=0.33, df=10.538, 95% CI 0.813–2.250
	Refrigerated	Estimate	Mean=1.44, SE=0.33, df=10.538, 95% CI 0.724–2.162
	Room Temperature	Estimate	Mean=1.53, SE=0.33, df=10.538, 95% CI 0.813–2.250
	Sunlight	Estimate	Mean=1.91, SE=0.33, df=10.538, 95% CI 1.186–2.624

Note. Dependent variable = FreshnessScore (1–5). Subjects = SpecimenID; Repeated = FreshnessDay with AR(1) covariance. Condition and interaction were nonsignificant; Day was significant.

The use of a linear mixed-effects model with an autoregressive covariance structure strengthens the validity of the findings by accounting for correlated repeated measures over time, consistent with current methodological recommendations for longitudinal data analysis (Schielzeth et al. 2020 and Warton et al. 2022). These results collectively demonstrate that freshness variation in the biofilm samples was primarily driven by time rather than by storage condition.

G. Heavy Metals Analysis

Table 10 presents the heavy metal content of the biofilm material and compares it against relevant reference limits for food-contact materials. The analysis measured four key analytes: Lead (Pb), Copper (Cu), Zinc (Zn), and Cadmium (Cd), with concentrations reported in parts per million (ppm). Lead was not detected (ND) in the biofilm, well below the reference limit of 0.010 ppm (10 ppb), indicating that the biofilm complies with the safety standard for lead. Similarly, Copper was also not detected (ND), and the biofilm was within the reference limit of 4 ppm, demonstrating compliance with copper safety standards. Zinc was found at 2.87 ppm, which is significantly below the reference limit of 50 ppm, indicating that the biofilm remains within the permissible threshold for zinc.

Finally, Cadmium was present at an extremely low concentration of 0.002 ppm, far beneath the reference limit of 1500 ppm, confirming that the biofilm meets the safety limit for cadmium. Overall, the heavy-metal content of the biofilm is within the safety limits for all analytes tested, reaffirming the biofilm's compliance with international food-safety standards and its suitability for use in food-contact applications.

Table 10
Heavy-Metal content of biofilm vs Reference limits

Analyte	*Biofilm (ppm)*	*Reference limit*	*Within limit?*	*N*
Lead (Pb)	ND	≤0.010 ppm (10 ppb)	Yes	1
Copper (Cu)	ND	≤4 ppm	Yes	1
Zinc (Zn)	2.87	≤50 ppm	Yes	1
Cadmium (Cd)	0.002	≤1500 ppm	Yes	1

Note. Biofilm only; plastic not tested (study delimitation). Single-sample compliance check (no inferential statistics).

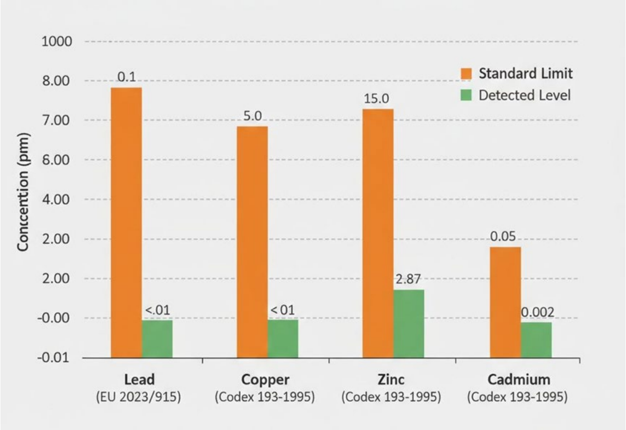


Fig. 14. Comparison of heavy metal concentration vs. International standards

Figure 14 compares the concentrations of four heavy metals, lead, copper, zinc, and cadmium, in the sample against internationally recognized food safety standards. The detected levels were evaluated based on the Codex Alimentarius Commission (CXS 193-1995, revised 2023) and European Commission Regulation (EU) 2023/915, which establishes maximum permissible limits for heavy metals in food products. In all cases, the detected concentrations were well below the allowable limits, with lead and cadmium present at extremely low levels relative to their maximum thresholds. Copper and zinc, although naturally occurring trace elements, also remained safely within internationally accepted limits. Overall, the results confirm that the sample complies with established food safety standards and does not pose a risk of heavy metal contamination.

5. Summary of Findings, Conclusions, and Recommendations

This chapter presents a summary of the study, draws conclusions from the findings, and provides recommendations for future research and practical applications of the enzyme-triggered *Carica papaya*-based biodegradable film for time-sensitive freshness monitoring.

A. *Summary of Findings*

Environmental Sustainability and Freshness Monitoring. The enzyme- triggered *Carica papaya*-based biodegradable film demonstrated clear advantages over conventional petroleum-based plastic in both environmental impact and functional capability. Being plant-derived, the material is renewable and capable of natural degradation, reducing long-term environmental persistence. In contrast, traditional plastics are nonrenewable and may remain in ecosystems for decades.

Functionally, the film's enzyme-responsive nature allows visible changes that can serve as a freshness indicator for perishable goods. This provides users with a simple visual cue regarding product conditions, potentially minimizing food waste and supporting safer consumption. Conventional plastic remains inert, offering no feedback about freshness or storage history. These characteristics confirm that the papaya-based film improves both sustainability and active monitoring.

UV-A and UV-B Blocking and Transmittance. For UV-A radiation (320– 400 nm), the papaya biofilm showed 35.45% transmittance, meaning it blocked approximately 64.55% of incoming radiation. The conventional plastic control transmitted 65.10% and blocked only 34.90%. This demonstrates that the biofilm nearly doubled the UV-A protection compared to traditional packaging.

For UV-B radiation (280–315 nm), the biofilm transmitted 9.75% and blocked 90.25%, while the conventional plastic transmitted 21.10% and blocked 78.90%. Because UV-B carries higher energy and is more damaging, this enhanced shielding is particularly important for protecting food quality, preventing spoilage, and maintaining material integrity. The film functioned as a significantly more effective UV barrier in both wavelength ranges.

Packaging Performance Under Different Storage Conditions. The papaya-based biodegradable film maintained structural stability at room temperature while allowing gradual enzyme activity, meaning it could preserve packaging integrity and still perform its freshness-monitoring function. Under refrigerated storage, the material continued to remain stable, demonstrating compatibility with cold environments and suitability for cold-chain logistics. These results indicate that the film can function effectively across typical storage and distribution settings without premature breakdown.

When exposed to high humidity, the film became more sensitive due to its moisture-responsive enzymatic properties. This response may enhance its usefulness as a freshness indicator since many spoilage mechanisms are linked to moisture changes. Under direct sunlight, the biofilm resisted UV exposure but gradually degraded over time, confirming its environmentally friendly, biodegradable behavior. In contrast, conventional plastic showed no reaction to any of these conditions, remaining inert while still permitting UV penetration and persisting in the environment.

Compliance with Food Safety Standards (Heavy Metals). Heavy metal testing showed no detectable lead or copper in the developed film. Zinc was measured at 2.87 ppm, and cadmium at 0.002 ppm. All values were far below internationally recognized safety limits.

The results confirm that the film is safe for human contact and suitable for food-related applications. Compared with conventional plastic, which may contain additives without active monitoring capability, the papaya-based film provides a safer and more transparent material profile. Quality assurance and control procedures verified the reliability of these measurements.

The papaya-based biodegradable film successfully integrates environmental sustainability, UV protection, adaptive responsiveness to storage conditions, and compliance with food safety standards. By combining renewable plant-based sourcing with enzyme-triggered degradability, the material reduces long-term ecological burden while also providing an active mechanism for monitoring product freshness. Its superior ability to block harmful UV-A and UV-B radiation, together with its stability in typical storage environments, demonstrates that the film can perform the protective roles expected of conventional packaging while offering additional smart features. These advantages position the papaya- derived film as a strong and promising alternative to petroleum-based plastics for future packaging applications.

B. *Conclusion*

The study demonstrated that the enzyme-triggered *Carica papaya*-based biodegradable film is a viable and promising alternative to conventional petroleum- based packaging materials. The developed film successfully combined sustainability, safety, and intelligent functionality within a single system.

Results confirmed that the material, being plant-derived and biodegradable, can reduce long-term environmental accumulation commonly associated with traditional plastics. Beyond environmental benefits, the film exhibited enzyme-responsive behavior that enables visible freshness monitoring, providing users with an accessible and practical indicator of product condition. This feature supports improved food safety practices and offers potential for reducing unnecessary waste.

In terms of protective performance, the biofilm demonstrated significantly enhanced UV-blocking capability in both UV-A and UV-B ranges compared with conventional plastic. Such protection is essential in preserving food quality, delaying spoilage, and maintaining package integrity during storage and transport. The film also maintained stability under room and refrigerated conditions, while its moisture sensitivity enhanced its function as a spoilage indicator. Gradual degradation under prolonged environmental exposure further confirmed its eco-friendly characteristics.

Food safety evaluation showed that heavy metal levels were far below accepted international limits, verifying that the material is safe for contact with food products. These findings support its suitability for future applications in real packaging environments.

Overall, the research confirms that the *Carica papaya*-based biodegradable film can fulfill the fundamental roles of food packaging while offering additional smart and sustainable advantages. With continued refinement and validation, it holds strong potential for commercial adoption and for contributing to the advancement of environmentally responsible packaging technologies.

C. *Recommendations*

To enhance the rigor, validity, and applicability of the findings, the following recommendations are proposed for future research and methodological refinement:

1. Increasing replication for UV and heavy-metal exposure tests to enable the application of inferential statistical analyses.
2. Tightening control of sample thickness and moisture content to more accurately distinguish condition-specific degradation behaviors.
3. Optimizing the formulation, particularly the plasticizer and crosslinker components, to improve moisture resistance without compromising the material's biodegradability.

Conducting migration studies across diverse food matrices, alongside a pilot-scale life cycle assessment (LCA), to support a more comprehensive evaluation of safety and environmental performance.

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