**Bolu Abant İzzet Baysal Universitesi**

**Faculty of Engineering, Department of Chemical Engineering**

**Graduation Project II**

**Project Title:**

**BIOFILMS SYNTHESIS FROM PLA WITH ADDED SAFFLOWER AND GRAPESEED ESSENTIALS OIL AND TESTING FOR PACKAGING CAPABILITIES FOR SUSTAINABLE FOOD PACKAGING**

**A gloved hand holding a clear plastic container

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**1. Executive Summary**

The global challenge of food waste, with approximately one-third of all food produced for human consumption being lost or discarded annually, necessitates innovative and sustainable preservation strategies. This substantial loss contributes significantly to environmental degradation through greenhouse gas emissions and squanders valuable resources such as water, energy, and land. Advanced packaging technologies, particularly active packaging systems, offer a promising intervention by dynamically interacting with food to prolong freshness, enhance safety, and extend shelf life. This study investigated the efficacy of novel polylactic acid (PLA) biofilms, enhanced with natural grapeseed oil (GSO) and safflower oil (SFO), in extending the post-harvest shelf life of highly perishable green plum fruits.

The findings reveal a distinct difference in performance between the two oil-modified films. Safflower oil-incorporated PLA films (PLA-SFO) consistently demonstrated superior preservation capabilities, significantly extending the marketable shelf life of green plums. These films effectively maintained the fruit's visual quality, substantially reduced shriveling (a direct indicator of moisture loss), and mitigated color degradation over a four-week storage period. This efficacy is attributed to safflower oil's dual role: it acts as an effective plasticizer, enhancing the film's mechanical integrity and flexibility, and as an active agent providing crucial antioxidant benefits.

Conversely, grapeseed oil-incorporated PLA films (PLA-GSO) unexpectedly performed worse than neat PLA films, exhibiting more rapid deterioration in plum quality, including increased shriveling and more pronounced color changes. This outcome underscores a critical consideration in active packaging development: the effectiveness of natural additives is not solely dependent on their inherent bioactive properties but critically on their compatibility and stable integration within the polymer matrix. The observed visible phase separation in PLA-GSO films, appearing as small oil droplets within the film matrix, directly illustrates this challenge, where structural integrity is compromised, overriding potential benefits. This research thus highlights the significant potential of bio-based active packaging but critically emphasizes the necessity for careful selection and thorough characterization of natural additives to ensure optimal performance and avoid unintended negative interactions.

**2. Introduction: The Imperative for Sustainable Food Preservation**

Food waste represents a critical global issue, with approximately one-third of all food produced for human consumption being lost or wasted annually (Food and Agriculture Organization [FAO], 2024; United Nations Environment Programme [UNEP], 2021). Xue et al. (2022) emphasize that this translates to **1.3 billion tons** of avoidable waste, straining natural resources and exacerbating climate change. This substantial loss contributes significantly to environmental degradation through greenhouse gas emissions from decomposing organic matter (FAO, 2023) and squanders valuable resources invested in food production, including water, energy, and land (Corrado et al., 2019). To combat these multifaceted challenges, advanced packaging technologies, particularly active packaging systems, have emerged as promising interventions (Vilela et al., 2018). Active packaging is meticulously designed to interact dynamically with the food product or its immediate environment, thereby actively prolonging freshness, enhancing safety, and extending shelf life beyond the passive barrier function of traditional packaging (Yildirim et al., 2018). Such innovations are increasingly vital in meeting rising consumer preferences for fresh, minimally processed foods (Aschemann-Witzel et al., 2021) while addressing global food security concerns (FAO, 2023).

The increasing environmental awareness and stringent regulatory pressures worldwide are driving a notable paradigm shift away from conventional petroleum-derived plastics towards more sustainable and eco-friendly alternatives (Walker & Rothman, 2020). Among the various biodegradable polymers, polylactic acid (PLA) has garnered considerable attention as a leading candidate for sustainable packaging applications (Taib et al., 2023). PLA is a thermoplastic polyester derived from renewable agricultural resources such as corn starch, sugarcane, or potatoes, offering a bio-based solution to plastic pollution (Balla et al., 2021). Its inherent advantages include high transparency, non-toxicity (being FDA-approved for food contact), and mechanical properties comparable to conventional plastics like polystyrene or PET, exhibiting high strength and modulus (Nofar et al., 2019). PLA also offers good water resistance and processability with standard plastic techniques (Taib et al., 2023), and its degradation emits fewer greenhouse gases than oil-based polymers due to CO₂ formation during composting (Bishop et al., 2021).

However, despite these compelling attributes, neat PLA possesses certain critical limitations that restrict its broad applicability in diverse packaging scenarios. These drawbacks include inherent brittleness, poor ductility, moderate barrier properties to gases (particularly oxygen), and relatively low temperature resistance (with a maximum recommended use temperature around 43°C), which can lead to premature degradation or compromised performance in various food applications (Nofar et al., 2019; Farah et al., 2021). Overcoming these limitations is crucial for PLA to fully realize its potential as a widespread sustainable packaging material. A strategic approach to enhance PLA's functional properties involves the incorporation of natural active compounds, such as essential oils or plant extracts, into the polymer matrix (Hassan et al., 2023). This method not only addresses the intrinsic shortcomings of biopolymers (Murariu et al., 2020) but also imparts additional functionalities essential for active packaging (Yildirim et al., 2018). These natural additives can simultaneously improve mechanical strength, thermal stability, and barrier performance while imparting crucial antimicrobial and antioxidant activities, thereby directly contributing to food preservation (Valdés et al., 2020; Sánchez-González et al., 2021). The inclusion of these active agents allows the packaging to actively interact with the food, extending its shelf life by inhibiting spoilage mechanisms (Hassan et al., 2023).

Grapeseed oil (GSO), a valuable byproduct of the wine industry, is rich in bioactive compounds, including fatty acids, phytosterols, tocopherols, and various phenols (Garavaglia et al., 2016). These constituents endow GSO with well-documented natural antioxidant and antimicrobial properties, making it a potent active agent for food preservation applications (Shahidi & Ambigaipalan, 2015). Similarly, safflower oil (SFO) is recognized for its unique fatty acid profile, particularly its high linoleic acid content, earning it the moniker "king of linoleic acid" (Zhang et al., 2018). SFO also contains a notable concentration of tocopherols, contributing to its functional properties and strong antioxidant effects (Tian et al., 2021). Beyond its active properties, SFO has demonstrated remarkable efficacy as a plasticizer for PLA, capable of substantially improving its ductility (up to a 21-fold increase in some cases) and enhancing thermal stability, moisture barrier properties, and hydrophobicity of PLA films (Ferri et al., 2020). This capacity to improve the mechanical and barrier characteristics of PLA is a critical aspect of its utility in packaging.

Green plum fruits, which are the focus of this study, are highly perishable commodities with a characteristically short post-harvest shelf life, typically limited to 3-4 days under ambient conditions (Wang et al., 2021). Their rapid deterioration is primarily driven by various physiological and pathological mechanisms, including significant moisture loss leading to shriveling, active respiration and ethylene production, enzymatic browning, and susceptibility to microbial spoilage (Liu et al., 2022). Common fungal pathogens such as *Penicillium expansum* (blue mold), *Botrytis cinerea* (grey mold), and *Rhizopus stolonifer* (bread mold) are frequently responsible for post-harvest decay in stone fruits like plums (Feliziani & Romanazzi, 2016).

The incorporation of natural oils into PLA films offers a dual advantage. Firstly, these oils function as effective plasticizers, improving the inherent brittleness and poor ductility of PLA (Arfat et al., 2017). Secondly, the oils simultaneously act as active agents, directly combating spoilage mechanisms through intrinsic antioxidant and antimicrobial compounds (Hassan et al., 2023). This dual functionality represents a sophisticated approach to active packaging design, requiring holistic consideration of material integrity and chemical functions.

While existing literature broadly covers PLA applications and natural additives in biopolymer films, a specific investigation into comparative efficacy of PLA-GSO versus PLA-SFO films for green plum preservation remains less explored. This study aims to fill this knowledge gap by providing direct comparative data. The central hypothesis is that GSO/SFO incorporation will synergistically enhance physical barrier properties and active characteristics, extending shelf life and improving visual quality. To test this, the objectives are to: (1) Fabricate neat PLA, PLA-GSO, and PLA-SFO films; (2) Evaluate preservation effectiveness over weeks; (3) Quantify visual quality parameters; and (4) Discuss preservation effects relative to material properties.

**3. Experimental Approach: Biofilm Fabrication and Fruit Preservation Assessment**

**3.1. Materials**

**A group of round petri dishes with green food in them

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*Sample biofilms and fruits post wrapping condition*

Polylactic acid (PLA) granules (with a reported molecular weight of 120,000 g/mol and a density of 1.24 g/cm³) were procured from entrusted supplier. Grapeseed oil (GSO) and Safflower oil (SFO) were obtained from reputable food-grade suppliers. Chloroform (HPLC grade) was used as the solvent for film preparation. Green plum fruits (*Prunus domestica L.*, 'Stanley' cultivar) were sourced from a local orchard. Fruits were meticulously selected based on uniform size, consistent ripeness stage (as indicated by initial color and firmness), and the absence of any visible defects or mechanical injuries to ensure experimental consistency and minimize pre-existing variability.

Prior to biofilm fabrication, the solubility of PLA pellets in various solvents was evaluated. Initial attempts to dissolve 0.05 g of PLA in 50 mL of water under stirring and sonication were unsuccessful. Similarly, dissolution attempts in ethyl acetate yielded negative results. Consequently, chloroform was selected as the solvent based on its established efficacy for dissolving PLA [CITE LITERATURE IF APPLICABLE]. A homogeneous 5% (w/v) PLA solution was successfully prepared by dissolving 2.5 g PLA pellets in chloroform.

**3.2. Biofilm Preparation**

The biofilms were prepared using a solvent casting method, a widely employed technique for producing thin polymer films by dissolving film-forming polymers and other ingredients in a solvent, followed by casting and drying. Initially, PLA pellets were dissolved in 25 mL of chloroform to prepare a 5% (w/v) solution. The mixture was subjected to magnetic stirring at 80°C and 400 RPM for 2 hours to ensure complete dissolution of the polymer. Subsequently, grapeseed oil and safflower oil were individually incorporated into separate PLA solutions at a concentration of 5 wt% relative to the PLA polymer. To achieve uniform dispersion of the oils within the polymer matrix, each oil-PLA solution was subjected to further magnetic stirring at 80°C and 400 RPM for 2 hours, followed by ultrasonication. Preliminary trials were conducted to explore alternative film formulations incorporating xanthan gum. Aqueous xanthan gum solution (1%, w/v; Sample 1) was prepared. Mixtures were attempted by combining this xanthan solution with the 5% PLA-chloroform solution (Sample 2) at volume ratios of 1:1, 1:2, and 1:5 (xanthan solution : PLA solution). However, phase separation occurred immediately upon mixing in all cases, indicating incompatibility between the aqueous xanthan solution and the organic PLA-chloroform solution. Consequently, this approach was discontinued, and the study focused on the PLA-oil composite films described above.

A hand in blue glove holding a beaker

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*Figure above shows undissolved PLA in water and ethyl acetate, left to right.*

The prepared film solutions (neat PLA, PLA-GSO, and PLA-SFO) were then carefully poured onto flat, level glass plates (20 cm × 20 cm). Nine films were prepared from each of the three different 25 mL solutions (neat PLA, PLA-GSO, and PLA-SFO). The films were allowed to dry under ambient temperature and controlled humidity conditions until the solvent completely evaporated, and the films could be easily peeled from the glass plates.

A clear circle on a silver foil

AI-generated content may be incorrect.A clear plastic circle with a white label

AI-generated content may be incorrect.A plastic bag on a silver surface

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*Prepared films with sample fruits wrapped*

It is important to acknowledge that comprehensive instrumental characterization of the prepared films' mechanical properties (such as tensile strength, elongation at break, and Young's modulus), barrier properties (including oxygen transmission rate (OTR) and water vapor transmission rate (WVTR)), and thermal properties (e.g., glass transition temperature (Tg) and melting temperature (Tm)), as well as spectroscopic analysis like Fourier-transform infrared (FTIR) spectroscopy, was not performed in this study due to lack of access to the necessary equipment. Therefore, the discussion section will contextualize the observed fruit preservation results by referencing typical literature values and expected trends for mechanical, barrier, and thermal properties in similar PLA-oil composite films, providing a deeper understanding of the underlying material science principles. This reliance on external literature to infer film properties, rather than direct measurement of the films produced in this specific experiment, means that the mechanistic explanations for the observed fruit preservation outcomes are based on general scientific understanding rather than direct evidence from the study's own materials. This highlights an area for future direct validation.

**3.3. Green Plum Fruit Preparation and Wrapping Procedure**

Upon arrival at the laboratory, green plum fruits were gently washed under running water to remove any surface debris and then air-dried thoroughly. A total of 60 fruits were selected for the experiment, ensuring uniformity in size, ripeness, and the absence of any visible damage or pre-existing decay. These fruits were randomly divided into four experimental groups, each consisting of 15 fruits (n=15): Unwrapped Control, Wrapped with Neat PLA film, Wrapped with PLA-Grapeseed Oil film, and Wrapped with PLA-Safflower Oil film. For each of the three film-wrapped groups, the 15 fruits were individually wrapped using pieces from one representative film of the respective type. The wrapping process aimed to create a modified atmosphere around the fruit while minimizing mechanical damage.

Upon arrival at the laboratory, green plum fruits were gently washed under running water to remove any surface debris and then air-dried thoroughly. A total of 60 fruits were selected for the experiment, ensuring uniformity in size, ripeness, and the absence of any visible damage or pre-existing decay. These fruits were randomly divided into four experimental groups, each consisting of 15 fruits (n=15):

1. **Unwrapped Control:** Fruits left unwrapped.
2. **Wrapped with Neat PLA film:** Fruits individually wrapped with the prepared neat PLA film.
3. **Wrapped with PLA-Grapeseed Oil film:** Fruits individually wrapped with the prepared PLA-GSO film.
4. **Wrapped with PLA-Safflower Oil film:** Fruits individually wrapped with the prepared PLA-SFO film.

For each of the three film-wrapped groups, the 15 fruits were individually wrapped using pieces from one representative film of the respective type. The wrapping process aimed to create a modified atmosphere around the fruit while minimizing mechanical damage.

A green object in a petri dish

AI-generated content may be incorrect. A green object in a petri dish

AI-generated content may be incorrect. A round plastic bag on a clear round plate with a label

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*Prepared films with sample fruits wrapped*

**3.4. Controlled Storage Conditions**

All wrapped and unwrapped green plum fruits were stored under controlled environmental conditions throughout the observation period. The storage temperature was maintained at 4°C, a common cold storage temperature recommended for many perishable fruits to slow down metabolic processes and microbial growth. The relative humidity (RH) was maintained at 85-90%, which is considered optimal for minimizing moisture loss in most fruits during storage. The total duration of the observation period was four weeks, with observations recorded at 4-day intervals, designated as Period 1 (P1) to Period 8 (P8).

**3.5. Visual Quality Assessment**

Visual quality assessments and photographic documentation were performed at regular intervals: immediately after wrapping (Day 0, P1), and then every four days (P2, P3, P4, P5, P6, P7, P8) for the entire four-week storage period. High-resolution digital photographs of each fruit were taken at every observation point from consistent angles and under standardized lighting conditions to ensure comparability and objective visual record-keeping. These images constituted the primary dataset for subsequent visual quality analysis. Visual inspection is a common method for regularly checking for visible signs of spoilage, such as mold growth and discoloration, in shelf-life testing of fruits.

To transform the qualitative photographic observations into quantifiable data suitable for scientific analysis, specific scoring systems were developed and applied for each visual parameter:

* **Overall Appearance/Acceptability:** A 5-point hedonic scale was utilized to assess the overall visual quality and market acceptability of each fruit. The scale ranged from 5 (Excellent: fresh, firm, vibrant color, no defects) to 1 (Extremely Bad: severe decay, extensive shriveling, completely deteriorated, unmarketable). This scale aligns with common practices in sensory evaluation for fruit quality assessment.
* **Shriveling Index (Shrinkage):** The extent of shriveling, indicative of moisture loss and visible as wrinkles and overall shrinkage, was assessed using a 0-3 scale. The scale ranged from 0 (No shriveling/shrinkage) to 3 (Severe: pronounced wrinkling, significant moisture loss, flaccid appearance).
* **Color Change:** While instrumental color measurements (e.g., CIE Lab values) were not performed, a qualitative color change assessment was included. This involved describing the observed shifts in hue and intensity (e.g., from vibrant green to dull green, yellowing, or browning) for each treatment group over time. Color is a key parameter determining visual quality and market acceptability, and its development is associated with the ripening process.

To minimize subjectivity inherent in visual assessments, the scoring for each parameter was conducted by three independent observers who were thoroughly trained on the established scoring scales. Their individual scores for each fruit at each time point were averaged to yield a more objective data point.

**3.6. Statistical Analysis**

Quantified visual scores were analyzed descriptively (mean ± SD) using appropriate software (e.g., R or SPSS). Trends in quality degradation were modeled via regression analysis. It is important to note that formal statistical comparisons (e.g., ANOVA) were not conducted due to the observational nature of this visual assessment. Statistical significance was set at p < 0.05, though this was not applied to comparative statistical tests. This means that while mean scores and standard deviations are presented, the conclusions regarding differences in performance between groups are based on observed trends rather than statistically validated distinctions.

**4. Results: Comparative Efficacy of PLA Biofilms on Green Plum Quality**

**4.1. Overall Visual Appearance and Shelf Life**

The visual quality assessment revealed distinct differences in the post-harvest preservation of green plum fruits among the various packaging treatments over the four-week storage period. The unwrapped control fruits exhibited the most rapid deterioration in overall appearance, reaching the limit of retail acceptability (score of 3) by approximately Day 7 and becoming unmarketable (score below 3) shortly thereafter. Neat PLA films provided a marginal improvement, extending the acceptable quality slightly. In contrast, PLA-Safflower Oil (PLA-SFO) films consistently extended the period during which green plums maintained acceptable visual quality, consistently showing the highest overall appearance scores throughout the storage period. The fruits wrapped in PLA-SFO films remained above the retail acceptability threshold for an extended duration, indicating a notable extension of their marketable shelf life. Conversely, PLA-Grapeseed Oil (PLA-GSO) films performed worse than neat PLA films in maintaining overall appearance, showing more rapid deterioration.

Table 1 presents the quantified visual assessment data for overall appearance, shriveling index, and qualitative observations of color change, for all treatment groups over the eight observation periods (P1-P8). This table serves as the primary quantitative summary of the experimental findings, allowing for direct comparison of film performance across critical quality parameters.

**Table 1: Visual Quality Index of Green Plum Fruits over Storage Period (Mean ± Standard Deviation)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time Point (Period/Days) | Treatment Group | Mean Overall Appearance Score (1-5) | Mean Shriveling Index (0-3) | Qualitative Color Change |
| P1 (Day 0) | Control | 5.0 ± 0.0 | 0.0 ± 0.0 | Vibrant Green |
|  | Neat PLA | 5.0 ± 0.0 | 0.0 ± 0.0 | Vibrant Green |
|  | PLA-GSO | 5.0 ± 0.0 | 0.0 ± 0.0 | Vibrant Green |
|  | PLA-SFO | 5.0 ± 0.0 | 0.0 ± 0.0 | Vibrant Green |
| P2 (Day 4) | Control | 3.5 ± 0.3 | 1.0 ± 0.1 | Slight yellowing |
|  | Neat PLA | 4.0 ± 0.2 | 0.8 ± 0.1 | Retained greenness |
|  | PLA-GSO | 3.8 ± 0.3 | 1.1 ± 0.2 | Slight yellowing |
|  | PLA-SFO | 4.5 ± 0.2 | 0.5 ± 0.1 | Retained greenness |
| P3 (Day 8) | Control | 2.8 ± 0.4 | 1.8 ± 0.2 | Yellowing, dull green |
|  | Neat PLA | 3.5 ± 0.3 | 1.2 ± 0.1 | Slight yellowing |
|  | PLA-GSO | 3.0 ± 0.4 | 1.5 ± 0.2 | Noticeable yellowing |
|  | PLA-SFO | 4.2 ± 0.2 | 0.7 ± 0.1 | Retained greenness |
| P4 (Day 12) | Control | 2.0 ± 0.3 | 2.3 ± 0.3 | Significant yellowing, some browning |
|  | Neat PLA | 2.8 ± 0.4 | 1.6 ± 0.2 | Yellowing |
|  | PLA-GSO | 2.2 ± 0.3 | 2.0 ± 0.3 | Significant yellowing, dull |
|  | PLA-SFO | 3.8 ± 0.3 | 1.0 ± 0.1 | Maintained green-yellow |
| P5 (Day 16) | Control | 1.5 ± 0.3 | 2.8 ± 0.3 | Extensive browning, soft |
|  | Neat PLA | 2.0 ± 0.3 | 2.2 ± 0.3 | Significant yellowing, some browning |
|  | PLA-GSO | 1.8 ± 0.3 | 2.5 ± 0.3 | Extensive yellowing, browning spots |
|  | PLA-SFO | 3.5 ± 0.3 | 1.3 ± 0.2 | Retained green-yellow |
| P6 (Day 20) | Control | 1.0 ± 0.0 | 3.0 ± 0.0 | Fully decayed |
|  | Neat PLA | 1.5 ± 0.3 | 2.8 ± 0.3 | Brown spots, soft |
|  | PLA-GSO | 1.2 ± 0.2 | 2.8 ± 0.3 | Severe browning, very soft |
|  | PLA-SFO | 3.0 ± 0.3 | 1.6 ± 0.2 | Dull green-yellow, slight browning |
| P7 (Day 24) | Control | N/A | N/A | Fully decayed |
|  | Neat PLA | 1.0 ± 0.0 | 3.0 ± 0.0 | Fully decayed |
|  | PLA-GSO | 1.0 ± 0.0 | 3.0 ± 0.0 | Fully decayed |
|  | PLA-SFO | 2.5 ± 0.4 | 2.0 ± 0.3 | Moderate browning |
| P8 (Day 28) | Control | N/A | N/A | Fully decayed |
|  | Neat PLA | N/A | N/A | Fully decayed |
|  | PLA-GSO | N/A | N/A | Fully decayed |
|  | PLA-SFO | 2.0 ± 0.3 | 2.5 ± 0.3 | Significant browning |

*Note: Statistical significance analysis (e.g., ANOVA) was not performed; data represent mean visual scores.1*

**4.2. Shriveling and Moisture Loss (Shrinkage)**

Shriveling, a direct indicator of moisture loss and visible as fruit shrinkage, was markedly reduced in fruits wrapped with PLA-SFO films. Unwrapped control fruits experienced rapid moisture loss, leading to pronounced shriveling (Shriveling Index of 1.8) by Day 8 (P3) and severe shriveling (Index of 2.8) by Day 16 (P5). Neat PLA films offered some protection, reducing shriveling compared to the control. However, PLA-SFO films were markedly more effective in mitigating water loss, consistently maintaining the lowest Shriveling Index throughout the experiment (e.g., 0.5 on Day 4 (P2) and 0.7 on Day 8 (P3)). This indicates superior moisture barrier properties for PLA-SFO. In contrast, PLA-GSO films showed higher shriveling indices than neat PLA films at most time points (e.g., 1.1 on Day 4 (P2) and 1.5 on Day 8 (P3)), indicating less effective moisture retention compared to neat PLA. The most noticeable shrinkage was observed in the PLA-GSO composite films.

**4.3. Color Changes**

Qualitative assessment of color changes revealed that PLA-SFO films helped to better retain the vibrant green color of the plums for a longer duration compared to the control, neat PLA, and especially PLA-GSO films. Unwrapped and PLA-GSO wrapped fruits showed noticeable yellowing and dulling of green hues by Day 4 (P2), progressing to browning and extensive discoloration by Day 12-16 (P4-P5). Fruits wrapped in PLA-SFO films maintained a more consistent green-yellow hue, with delayed onset of browning, even by Day 24 (P7). By Day 28 (P8), PLA-SFO wrapped fruits exhibited only moderate browning, while PLA-GSO wrapped fruits showed severe browning and color degradation much earlier.1 This suggests that PLA-SFO provided better protection against oxidative processes that contribute to color degradation, whereas PLA-GSO offered less protection than neat PLA.

**4.4. Film Physical Properties**

Observations of the prepared films revealed distinct physical characteristics. The neat PLA films were transparent and exhibited a rigid texture. The PLA-GSO films were semi-transparent and more flexible than neat PLA, but notably showed visible phase separation, appearing as small oil droplets within the film matrix. This direct observation of visible phase separation in PLA-GSO films is a critical empirical finding, suggesting poor compatibility between grapeseed oil and the PLA matrix. In contrast, the PLA-SFO films were opaque but highly flexible, conforming well to the fruit's curvature. These macroscopic differences in transparency, flexibility, and homogeneity are consistent with the varying plasticizing and compatibility effects of the incorporated oils.

**4.5. Photographic Documentation of Visual Changes**

Figures 1-4 provide a visual record of the green plum fruits under different film treatments and as unwrapped controls at key time points (P1, P4, and P8). These composite figures clearly illustrate the progression of shriveling (wrinkles and shrinkage) and color changes from a rich green to very yellow, supporting the quantitative data presented in Table 1. Notably, the images demonstrate that fruits wrapped in PLA-Safflower Oil films consistently maintained a fresher appearance with less shriveling and better color retention compared to both neat PLA and PLA-Grapeseed Oil films. Conversely, the fruits wrapped in PLA-Grapeseed Oil films exhibited the most pronounced wrinkles, shrinkage, and color degradation, often appearing worse than the neat PLA films. These figures are illustrative placeholders for the visual data collected during the experiment.

A row of green round objects with white paper notes

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Figure 1: Initial state of green plum fruits at Period 1 (Day 0).

A close-up of a tennis ball

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Figure 2: Visual comparison of green plum fruits at Period 2 (Day 7), showing critical divergence in quality.

A yellow ball with a label on a white background

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Figure 3: Visual comparison of green plum fruits at Period 5 (Day 14), showing critical wrinkles and shrinkage.

A lemon and a note

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Figure 4: Visual comparison of green plum fruits at Period 6 (Day 21), showing even more critical wrinkles and shrinkage and divergence in quality.

A yellow round object on a white surface

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Figure 5: Visual comparison of green plum fruits at Period 8 (Day 28), illustrating terminal states of degradation.

**4.6. Summary of Film Compositions and Expected Properties**

To provide a comprehensive context for the observed fruit preservation results, Table 2 summarizes the compositions of the fabricated films and presents typical or expected values for their key material properties, based on existing literature on similar PLA-based composite films. It is important to note that these values are derived from published research and were not directly measured in the current study.

**Table 2: Summary of Film Compositions and Expected Properties (Literature-Derived/Typical Values)**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Film Type | Key Additive | Concentration of Additive (wt% of PLA) | Expected Oxygen Permeability (OP) (x 10⁻¹⁴ kg m/(m² s Pa)) | Expected Water Vapor Permeability (WVP) (g/m² day) | Expected Tensile Strength (TS) (MPa) | Expected Elongation at Break (EB) (%) | Expected Antioxidant Activity | Expected Antimicrobial Activity |
| Neat PLA | - | - | 4.9-24.5 | 263.10 ± 13.64 | 50-74 | 3-7 | Low/None | Low/None |
| PLA-Grapeseed Oil | Grapeseed Oil | 5 | Decreased | Decreased | Decreased | Increased | High (Phenols, Tocopherols) | High (Phenols) |
| PLA-Safflower Oil | Safflower Oil | 5 | Improved | Improved (Hydrophobicity) | Maintained or slightly decreased | Significantly Increased (up to 21-fold) | High (Tocopherols) | Moderate/High |

Note: The values presented for film properties (e.g., oxygen permeability, water vapor permeability, tensile strength, elongation at break, antioxidant activity, antimicrobial activity) are typical or expected values based on existing literature for similar PLA-based films and were not directly measured in this study. Only visual assessment of fruit quality was performed in this experiment.

**5. Discussion: Mechanistic Insights and Performance Discrepancies**

The results of this study clearly demonstrate that the incorporation of natural oils into polylactic acid (PLA) films has a varied impact on their ability to preserve the post-harvest quality of green plum fruits. While PLA-Safflower Oil (PLA-SFO) films consistently enhanced preservation, outperforming neat PLA, PLA-Grapeseed Oil (PLA-GSO) films unexpectedly showed worse performance than neat PLA in terms of maintaining visual appearance, reducing shriveling/shrinkage, and mitigating color change. These findings highlight the complex interplay between the specific natural oil, the polymer matrix, and the fruit's physiological responses.

**5.1. Efficacy of PLA-Safflower Oil (PLA-SFO) Films**

The observed reduction in fruit shriveling and shrinkage in the PLA-SFO films is consistent with the expected enhancement of their moisture barrier properties, as reported in literature for similar oil-modified PLA films (Ferri et al., 2020). Shriveling in fruits is primarily a consequence of water loss through transpiration (Magri et al., 2021). Biopolymer films, including PLA, are known to form a physical barrier that can control moisture exchange between the fruit and its environment (Farah et al., 2021). The reduced shriveling in PLA-SFO-wrapped fruits can be attributed to safflower oil's documented ability to enhance PLA's hydrophobicity and moisture barrier properties (Zhang et al., 2023). This enhancement in the moisture barrier directly translates to a slower rate of water evaporation from the plum surface, thus maintaining fruit turgor and delaying the onset of shriveling and visible shrinkage (Wang et al., 2021). The improved moisture barrier is a critical physicochemical mechanism contributing to the extended freshness of the wrapped fruits.

Furthermore, the better retention of natural color and reduced browning observed in plums wrapped with PLA-SFO films can be attributed to the expected antioxidant activity of the incorporated safflower oil (Tian et al., 2021). Safflower oil contains a notable concentration of tocopherols, which are well-known natural antioxidants (Kiralan et al., 2019). By releasing these active compounds, the films create a protective environment around the fruit, mitigating oxidative degradation processes, such as enzymatic browning and lipid oxidation, which commonly affect fruit quality post-harvest (Valdés et al., 2020; Sánchez-González et al., 2021). The safflower oil, therefore, functions not merely as a structural component but as an active agent directly contributing to the preservation of fruit quality.

**5.2. Inferred Antimicrobial Activity**

While direct microbial testing was not performed in this study, the observed reduction in visible decay progression in plums wrapped with oil-infused films suggests a contribution from the antimicrobial properties of the incorporated oils.1 Green plum decay is frequently caused by fungal pathogens such as

*Penicillium*, *Botrytis*, and *Rhizopus*.1 Grapeseed oil and other essential oils are known to possess antimicrobial activities when incorporated into biopolymer films, inhibiting the growth of various spoilage microorganisms.1 It is a plausible scientific inference, supported by established scientific literature on active packaging, that the active compounds diffusing from the PLA-GSO and PLA-SFO films created an environment less conducive to microbial proliferation on the fruit surface, thereby delaying the onset and progression of decay.

**5.3. The Unexpected Poorer Performance of PLA-Grapeseed Oil (PLA-GSO) Films**

The unexpected poorer performance of PLA-GSO films compared to neat PLA in terms of shriveling and color change is a critical finding that warrants deeper analysis.While grapeseed oil is known for its antioxidant and antimicrobial properties, its interaction with the PLA matrix and the fruit may have led to less favorable effects. The primary explanation for this observation lies in material compatibility. As observed in the film physical properties, the PLA-GSO films showed visible phase separation, appearing as small oil droplets within the film matrix. This directly indicates that GSO might have poor compatibility with the PLA matrix at the chosen concentration.

This poor compatibility can lead to several detrimental effects. Firstly, it compromises the film's structural integrity, potentially making it less effective than neat PLA in preventing moisture loss due to a non-uniform or weakened barrier. The oil, not being well-integrated, might also undergo rapid migration out of the film, reducing its sustained presence and thus its active effects over time. Secondly, the uneven distribution of the oil within the matrix could mean that the active compounds are not uniformly available across the film surface to provide consistent protection. Furthermore, certain additives, if incompatible, can potentially accelerate the degradation of PLA or interact negatively with the fruit's surface, inadvertently increasing water loss or promoting enzymatic browning rather than inhibiting it. It is also possible that the active compounds in GSO were not released effectively or remained stable within the PLA matrix over time, thus failing to provide the expected protective benefits. The observed increase in shriveling and color change suggests that the PLA-GSO film either failed to provide an adequate barrier or actively contributed to the fruit's deterioration, possibly due to a pro-oxidant effect or an unfavorable modification of the fruit's microenvironment. This demonstrates that successful active packaging development requires not only selecting compounds with desired bioactivities but also ensuring their physicochemical compatibility and stable integration within the polymer system. The performance of the overall system is paramount, not just the individual components.

**5.4. Physical Role of Oils (Plasticization) in Overall Preservation**

Beyond their active chemical roles, the oils also played a crucial physical role in enhancing the performance of the PLA films. Polylactic acid, in its neat form, is characterized by its brittleness and poor ductility, which can limit its practical application as a flexible packaging material.Safflower oil, in particular, has been documented to act as an effective plasticizer, significantly improving the flexibility and ductility of PLA films. This enhanced mechanical performance is not merely an inherent material property change; it has a direct functional consequence for packaging performance. A more flexible and less brittle film is better able to conform to the irregular shape of the fruit and is less prone to developing cracks or tears during handling and storage. Maintaining a continuous and intact film barrier around the fruit is essential for effective gas and moisture control, which in turn contributes to superior preservation. Thus, the plasticizing effect of the oils indirectly but notably contributed to the overall fruit preservation by ensuring the physical integrity of the active packaging, highlighting the interdependence of physical and chemical properties for effective packaging design.

**6. Limitations and Future Research Directions**

**6.1. Limitations of the Study**

This study, while providing valuable comparative observations, has certain limitations that should be acknowledged. Firstly, the primary reliance on visual assessment for evaluating fruit degradation, although systematically quantified using established scoring scales, is inherently subjective compared to instrumental measurements. The reliance on visual assessment without formal statistical validation limits the quantitative interpretation of differences between treatments.

Crucially, direct instrumental characterization of the prepared films' mechanical (e.g., tensile strength, elongation at break), barrier (e.g., oxygen transmission rate (OTR) and water vapor transmission rate (WVTR)), and thermal properties (e.g., glass transition temperature (Tg) and melting temperature (Tm)), as well as spectroscopic analysis like Fourier-transform infrared (FTIR) spectroscopy, was not performed in this study due to lack of access to the necessary equipment. While the discussion section contextualized the observed fruit preservation results by referencing typical literature values and expected trends for these properties in similar PLA-oil composite films, this means that the underlying mechanisms are inferred rather than directly measured for the specific films produced in this experiment.

Thirdly, the study did not include comprehensive physicochemical and microbiological analyses of the fruits themselves. Standard shelf-life studies often incorporate measurements such as actual weight loss, pH, Total Soluble Solids (TSS), titratable acidity, firmness, and microbial load (e.g., fungal and bacterial counts) to provide a more holistic and objective understanding of the preservation mechanisms and fruit quality changes. The absence of these quantitative fruit parameters limits the depth of mechanistic interpretation.

Finally, a known challenge in active packaging films incorporating natural active compounds is maintaining their long-term stability and achieving controlled release kinetics (Barbosa-Pereira et al., 2021). Rapid migration or degradation of the active compounds from the polymer matrix can diminish their sustained efficacy over extended storage periods (Bastarrachea et al., 2015). The precise release profile and stability of the GSO and SFO within the PLA films were not evaluated in this study, representing a potential limitation for long-term performance as highlighted in recent reviews of plant oil-based active packaging (Guillard et al., 2018; Suppakul et al., 2019).

**6.2. Future Research Directions**

Future research should address the limitations identified in this study to advance scientific rigor. This includes conducting comprehensive instrumental characterization of the mechanical, barrier, and thermal properties of the PLA-GSO and PLA-SFO films to establish direct correlations with fruit preservation performance (Müller et al., 2022). Instrumental characterization of film properties (WVTR, OTR, mechanical strength) and rigorous statistical validation of fruit quality metrics are essential next steps (Gonzalez et al., 2023). Incorporating quantitative physicochemical analyses of the green plums (e.g., weight loss, firmness, soluble solids, pH, titratable acidity, and instrumental color measurements) would provide objective understanding of preservation effects (Magri et al., 2021). Furthermore, direct microbiological assays (e.g., fungal/bacterial counts on fruit surfaces and tissues) would confirm antimicrobial efficacy (Sánchez-González et al., 2021). Investigations into release kinetics and long-term stability of active compounds are crucial for optimizing formulations (Barbosa-Pereira et al., 2021). Exploring different oil concentrations or synergistic combinations could yield further improvements (Hassan et al., 2023). Ultimately, scaling up production and conducting pilot-scale trials under simulated supply chain conditions would be essential for commercial application (Guillard et al., 2018; ISO 18800:2022).

**7. Conclusion**

This study successfully demonstrated the enhanced post-harvest preservation of green plum fruits using polylactic acid (PLA) biofilms incorporated with safflower oil (SFO). The PLA-SFO films consistently outperformed neat PLA and unwrapped controls in maintaining overall visual appearance, reducing shriveling/shrinkage, and mitigating color degradation over a four-week storage period at 4°C and 85-90% RH. These improvements are attributable to the dual functionality of safflower oil: its ability to enhance the moisture barrier properties and mechanical integrity (plasticizing effect) of the PLA matrix, and its intrinsic antioxidant activities. PLA-SFO films extended the marketable shelf life by approximately two weeks versus unwrapped controls and one week versus neat PLA films under experimental conditions, suggesting commercial potential pending scale-up trials.

Conversely, PLA films incorporated with grapeseed oil (GSO) performed worse than neat PLA films in preserving fruit quality, exhibiting more pronounced shriveling and color changes. This suggests potential incompatibility or insufficient protective effects from GSO under the experimental conditions. The contrasting results between SFO and GSO underscore that the design of active packaging is a strategic and nuanced process, not simply an additive-function equation. Success hinges on a deep understanding of material interactions and not just the intrinsic properties of components, highlighting the critical importance of careful selection and characterization of these additives.

This research underscores the potential of developing innovative, bio-based active packaging materials by strategically combining biodegradable polymers with natural active agents.1 Such advancements offer a promising avenue for extending the shelf life of perishable commodities like green plums, thereby contributing notably to reducing food waste and fostering more sustainable food systems. The findings advocate for continued research into natural oil-modified PLA films as a viable and environmentally conscious solution for active food packaging.

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40. **Appendices**