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Smart Food Packaging Films Based on a Poly(lactic acid), Nanomaterials, and a pH Sensitive Dye

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Abstract: Smart packaging materials (SPMs) combine the properties of intelligent and active packaging into a single system, enabling for the monitoring of the packaged product while enhancing its desired conditions. In this study, poly(lactic acid) (PLA) was used as the base polymer and functionalized with in situ synthesized gold nanoparticles (AuNPs) and methyl red (MR) as a pH-sensitive dye. Various additives, including poly(amic) acid (PAA), bromothymol blue (BB), 5-aminosalicylic acid (5AS), glutaraldehyde (GA), and silver and gold nanoparticles (AgNPs, Au NPs), were tested to optimize the SPMs. To evaluate their performance, the synthesized SPMs were characterized using UV-Vis spectroscopy, IR spectroscopy, SEM, microbiological assays, and mechanical tests. Our results revealed that PLA films containing AuNPs and MR exhibited excellent mechanical, chemical, and antimicrobial properties, making them highly suitable for smart packaging applications. In contrast, the addition of PAA disrupted film formation, while AgNPs and blueberry extracts increased the brittleness of the films, thereby limiting their practical use. Furthermore, BB was found to inhibit the in situ synthesis of AuNPs. A real-world application study demonstrated that cheddar cheese wrapped in the optimized PLA films remained unspoiled after 12 months of refrigeration. IR spectroscopy confirmed that no film components migrated into the cheese during the storage period. GA was identified as a critical component for maintaining the structural integrity of the films over the 12-month storage period. This is the first study to report on the development of PLA-based SPMs that incorporate AuNPs, MR, and GA, offering a promising solution for sustainable and intelligent food packaging.

Keywords: smart food packaging; poly(lactic) acid; methyl red; gold nanoparticle; cheddar cheese

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1. Introduction

The rapid and continuous growth of the global population, which is expected to reach approximately 10 billion by 2050, presents significant challenges in ensuring global food security and supply [1,2]. One of the most critical concerns in this context is the increasing amount of food loss, which accounts for over 30% of food produced annually [3]. Food loss and waste occur at various stages of the food supply chain, from production to consumption, and are driven by factors such as improper storage, microbial contamination, and inadequate packaging technologies [4]. These losses threaten food security and contribute to environmental degradation through excessive resource consumption and greenhouse gas emissions, making food preservation a key priority in achieving sustainable development. To address these challenges, there is an urgent need for innovative food packaging solutions

that can extend shelf life, prevent contamination, and minimize food waste. Such solutions align with the United Nations Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger), SDG 3 (Good Health and Well-being), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action).

In this regard, smart food packaging technologies have gained significant attention as a promising approach to improving food quality and safety while reducing food loss [5,6]. Smart packaging integrates two main functionalities: active packaging and intelligent packaging. Active packaging interacts with the packaged food and its environment by incorporating antimicrobial, antioxidant, or moisture-controlling agents, thereby extending shelf life [3]. In contrast, intelligent packaging enables real-time food quality monitoring through colorimetric sensors, freshness indicators, and time-temperature indicators, providing critical information on food spoilage [7]. Combining these active and intelligent functionalities into a single system represents a cutting-edge strategy for sustainable food packaging, enhancing food safety while reducing waste generation [8]. Among the various materials explored for smart food packaging, biodegradable polymer-based nanocomposites have emerged as the most promising candidates due to their sustainability, improved mechanical properties, and functional versatility [2]. Poly(lactic acid) (PLA), in particular, has been extensively studied as an eco-friendly alternative to conventional petroleum-based plastics [9]. PLA is derived from renewable agricultural sources, such as maize and sugarcane, making it a biodegradable and sustainable polymer that aligns with global efforts to reduce plastic pollution and carbon emissions [9,10]. PLA's life cycle assessment has demonstrated its ability to contribute to CO_2 reduction in supporting climate action goals (SDG 13). Despite its environmental benefits, however, PLA alone has limitations regarding its gas barrier performance, thermal stability, and antimicrobial activity, which restrict its direct application in food packaging [11]. To overcome these limitations, PLA-based composite materials are often designed by incorporating other biopolymers, bioactive compounds (e.g., lycopene [12] and nisin [13]), or nanostructured materials, such as metal nanoparticles, to enhance its functional properties [14].

This study developed an advanced PLA-based smart food packaging material by functionalizing a PLA matrix with methyl red (MR) as a pH-sensitive indicator and in situ synthesized gold and silver nanoparticles as antimicrobial agents. The incorporation of MR enables real-time colorimetric detection of food spoilage, providing a clear and rapid visual indication of microbial contamination and food decomposition. Meanwhile, gold and silver nanoparticles contribute to the active antimicrobial protection of the packaging system by inhibiting the proliferation of spoilage-causing microorganisms. The PLA composite film also enhances the barrier properties against moisture and oxygen, extending food shelf life. By integrating these active and intelligent packaging functions into a single biodegradable PLA-based system, this study aims to advance sustainable food packaging technology, offering a practical solution for enhancing food safety, extending shelf lives, and reducing food loss while promoting eco-friendly alternatives to conventional plastic packaging.

2. Materials and Methods

2.1. Materials

The polylactic acid (PLA) was a gift from Klöckner Pentaplast (İzmir, Turkiye). The methyl red (MR) (ACS reagent, crystalline), Bromothymol Blue (BTB) (95%), Borane dimethylamine (DMAB) (97%), anhydrous acetone (99.5%), chloroform (99%), 25% Glutaraldehyde in water (grade II), pyromellitic dianhydride (97%), pyromellitic dianhydride (97%), N, N′-dimethylacetamide, $AgNO_3$ (≥99.0%), $HAuCl_3 \cdot H_2O$ (99.995%), and 5-aminosalicylic acid (5AS) were purchased from Sigma–Aldrich (Ankara, Turkiye). The

Mannose p-aminobenzoic acid (sugar ligand, SL) was previously synthesized in our laboratory and published elsewhere [15]

2.2. Preparation of Smart PLA Films

Different PLA concentrations, ranging between 6–10 g/100 mL in chloroform, were used to develop standalone film materials. PLA alone was cast on films by solvent casting or casting machine. An amount of 6 g/100 mL (6%) of PLA gave an even surface in all cases, so 6% was chosen as the appropriate PLA concentration throughout the study. To enhance the physicochemical and microbiological properties of the PLA films, pH-sensitive dyes and glutaraldehyde were added to the PLA solution along with silver (I) and gold (III) ions to form in situ synthesized nanoparticles to advance the antimicrobial potential of the packaging films regarding the preliminary experimental founding ratios [15,16]. In addition to the PLA films, poly(amic acid) (PAA) polymers (synthesized from pyromellitic dianhydride and pyromellitic dianhydride in DMAC) were mixed with PLA to increase the hydrophobicity of the smart food packaging material. The solution was cast onto a 10 cm × 24 cm glass substrate with an automatic film applicator (Krosgen Biyoteknoloji Ltd., İstanbul, Turkiye) with 100 mm/s. The thickness of the cast film was adjusted by a four-sided applicator with a gap size of 200 µm. After casting, the film was dried in a fume hood under atmospheric conditions at 25 °C and between 30–50% humidity conditions for one hour and kept in a desiccator until they were used.

2.3. Optical Characterization

The PLA films were (3 × 1 cm) directly placed in a UV-Vis spectrophotometer (PG Instruments T60 Visible Spectrophotometer) for absorbance and surface plasmon resonance peak characterization.

ATR-FTIR (Bruker Platinum ATR, Ankara, Turkiye) was used for a functional group characterization of the PLA films (1 × 1 cm).

2.4. Solvent Resistance of the PLA Films

Various buffer solutions at different pH values were prepared to evaluate the solvent resistance performance of the PLA films. Considering raw milk's pH of 6.8 and a range of 5.0 to 5.5 during cheese ripening [17], acetate buffers were used at pHs of 4.5, 5.0, and 5.5 at 50 mM, while phosphate buffers were at pHs of 6.0, 6.5, 7.0, 7.5, and 8.0 at 50 mM. Besides the buffers, a 5% ammonia solution was used to test swelling and color changes in the films.

2.5. Physical Characterization

Contact angles of PLA films were tested using 18.2 MΩ pure water with Attension Theta Goniometer (Biolin Scientific). A drop of pure water (10 µL) was placed on the film, which was followed by measurement taking at the end of the 5 s incubation period. Scanning Electron Microscopy (SEM, FEI Quanta FEG 250) was used to characterize the surface morphology of the PLA films.

Resistance to water vapor transfer was measured according to the ASTM method (1995) [18]. The films were cut into 90 × 90 mm pieces, and each piece was placed in a 10 mL glass container previously filled with fused silica instead of calcium chloride. The cup was then sealed with a cover and placed into a humidity chamber set at 25 °C with a relative humidity (RH) of 75% for 72 h (Figure 1A). During the experiment, the sealed cup was weighed periodically to an accuracy of ±0.0001 g. The weight gain of the cup determined the water vapor that was transported into the cup. Once the relationship between weight gain (Δw) and time (Δt) became linear, the slope of the plot was used to

calculate the water vapor transmission rate (WVTR). Similarly, an oil penetration test was performed by turning the oil-containing glass tube upside down for 12 h (Figure 1B).

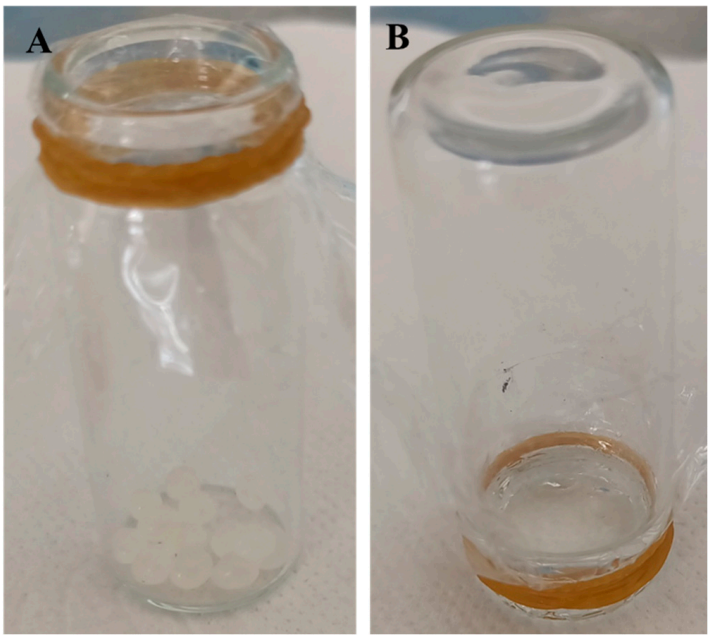

Figure 1. (**A**) Water-vapor transfer and (**B**) oil leakage test of the PLA films.

2.6. Microbiological Characterization

The PLA films' antimicrobial activity was evaluated using Mueller Hinton Broth and Mueller Hinton Agar Sigma Aldrich (Ankara, Turkiye). An amount of 10^6 CFU/mL of *Staphylococcus epidermidis* (gram +) and *Escherichia coli* (gram −) were inoculated in 2 mL of Mueller Hinton Broth containing 0.5 × 1.0 cm PLA film. Alongside this, 2 × 2 cm films were placed on top of the freshly prepared agar, followed by 1×10^8 CFU/mL of bacteria, which were placed on two sides of the film. When the films were placed on the freshly prepared agar, the agar covered the surface, allowing for the visualization of the antibacterial activity coming from the films.

2.7. Real Sample Application

Cheddar (Burger) Cheese, one of the most consumed cheeses in the world and known for its high-fat content, was used in the film experiments. A total of 12 months of incubation inside refrigeration (+4 °C) was performed to monitor the migration of the film components into the film-wrapped cheese sample, where ATR-FTIR (Bruker Platinum ATR) was used for the monitoring.

3. Results and Discussions

3.1. Smart PLA Film Synthesis

Hand-casting was selected for its simplicity and speed, allowing for an evaluation of how altering the composition affected color changes and physical strength. This uneven nature is related to the thickness difference within the film (Figure 2). Additionally, the composition influenced the flexibility of the films. The introduction of silver ion and blueberry extracts made the films brittle. The film's color showed dependence on the pH indicator and the amount of the metal ion.

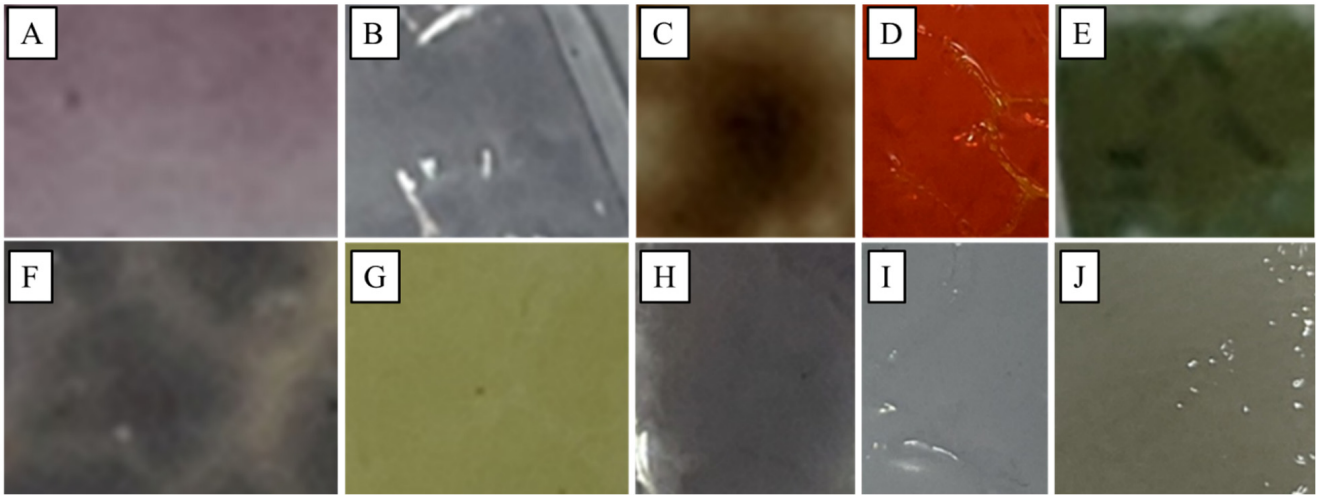

Figure 2. Digital images of some of the synthesized PLA films cast by hand. (**A**) PLA with 0.04 mg/mL of $HAuCl_4 \cdot H_2O$ and 0.4 mg/mL of DMAB; (**B**) PLA with 0.08 mg/mL of $HAuCl_4 \cdot H_2O$ and 0.4 mg/mL of DMAB; (**C**) PLA with 0.08 mg/mL of $AgNO_3$ and 0.4 mg/mL of DMAB; (**D**) PLA with 0.04 mg/mL of Methyl Red; (**E**) PLA with 0.04 mg/mL of BTB and 0.08 mg/mL of $HAuCl_4 \cdot H_2O$ and 0.4 mg/mL of DMAB; (**F**) PLA with 0.04 mg/mL of MR and 0.08 mg/mL of $HAuCl_4 \cdot H_2O$; (**G**) PLA with 0.04 mg/mL of BTB; (**H**) PLA with 0.04 mg/mL of MR and 0.08 mg/mL of $HAuCl_4 \cdot H_2O$; (**I**) PLA and (**J**) PLA with 0.4 mg/mL of blueberries extract. In all cases, the solutions contained 1 mg/mL of GA. All contained 0.08 mg/mL of 5AS, except (**I**,**J**).

UV-vis characterization of PLA films allows for the determination of both transparencies of the films, the formation of AuNPs and AgNPs, and the introduction of small molecules that absorb light. Films with a 1 cm width and a 3 cm length were cut and placed into the UV-vis spectrometer (PG Instruments T60) cell, where absorbance was measured between 190 and 1100 nm. As shown in Figure 3, PLA does not give any characteristic peaks, and the absorbance spectra reveal characteristic peaks associated with the formation of both AuNPs and AgNPs.

In the case of the Bromothymol Blue (BTB) addition, a characteristic peak intensity at 410 nm arose. In comparison, the further combination of 0.04 mg/mL of Au^{3+} and 0.4 mg/mL of borane dimethylamine increased the height and sharpness of the peak at 410 nm. Initially, the peak was thought to belong to BTB, but the PLA–AuNP films exhibited a similar peak of ~420 nm. Similarly, PLA–AgNP films showed a sharp peak at ~410 nm. These observations suggest that the polymer strongly influences the surface plasmon resonance (SPR) spectra of both AgNPs and AuNPs. For the PLA–BTH–AuNP films, a broad peak from 530 nm to 680 nm refers to the surface plasmon resonance (SPR) peak of the formed AuNP. The broad SPR peak refers to non-spherical AuNP formation [15]. In the case of the introduction of Methyl Red (MR) to PLA films, a peak of ~450 nm arose, which disappeared upon adding the Au^{3+} solution. This suggests that the MR triggered AuNP formation, as PLA–MR–Au films exhibited a characteristic SPR peak in the ~550 nm region. The addition of a reducing agent to the PLA–MR–Au medium further directed AuNP synthesis and growth, as evidenced by the SPR peak shifting from 550 nm to a broader range (520–660 nm). These results indicate that the observed color changes were related to the formation of AuNPs of varying sizes and shapes, depending on the concentration of metal ions.

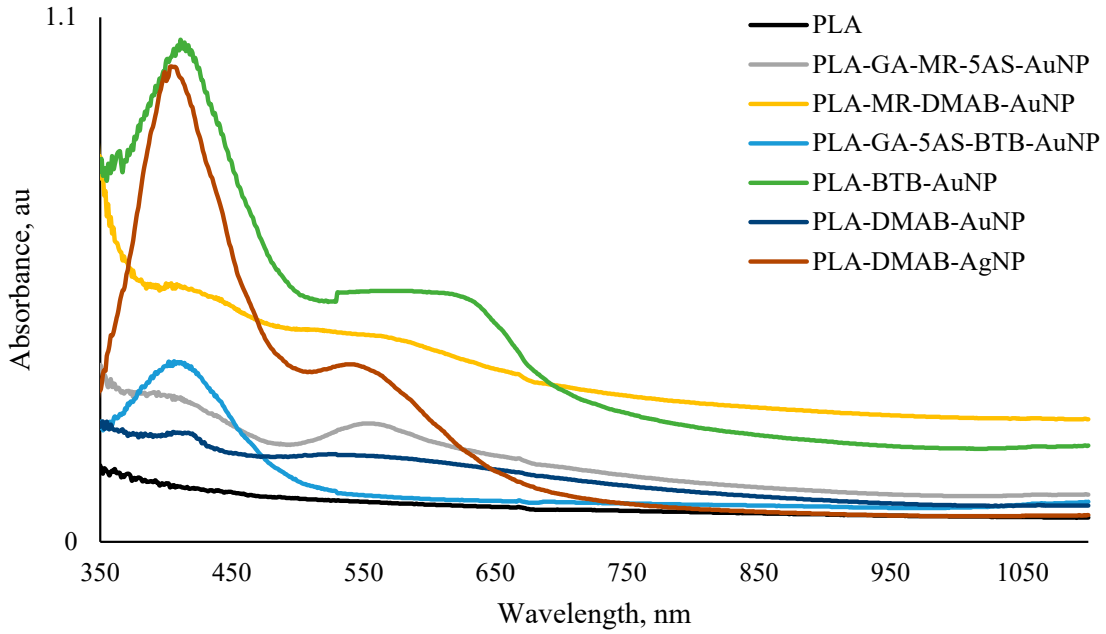

Figure 3. UV-vis spectra of some of the synthesized PLA films. PLA: Poly(lactic)acid; MR: Methyl Red; BTB: Bromothymol blue; DMAB: Dimethylamine Borane.

PAA has free functional carboxyl and secondary amino groups, so it was added to PLA to increase available free functional groups for further characterization and hydrophobicity [15]. However, as seen in Figure 4, mixing PLA and PAA did not provide proper food packaging material. DMAC results in brittle and highly amorphous PLA, so PLA–PAA did not form a continuous film. Even though increasing the PLA ratio (Figure 4B) allowed integrity protection, the formed structure was highly amorphous and brittle and showed no flexibility.

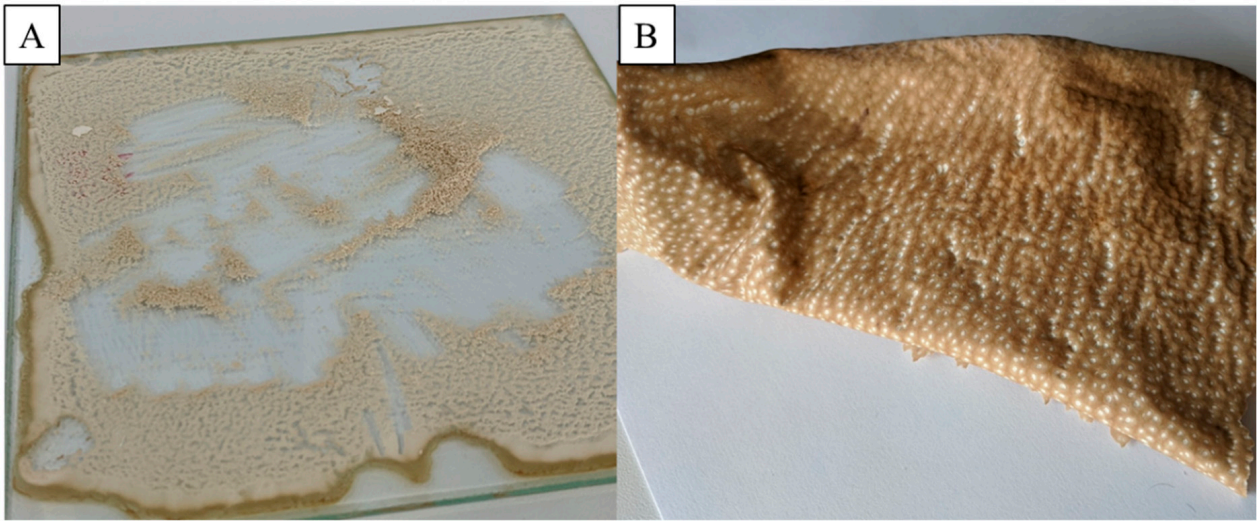

Figure 4. Digital camera images of PAA–PLA films. (**A**) 3:1 PAA/PLA and (**B**) 1:3 PAA/PLA films.

PLA gives C-H stretching at ~2998 cm^{-1}, C = O absorption at ~1750 cm^{-1}, and C-O-C vibrations at ~1190 and ~1130 cm^{-1} IR peaks (Figure 5). Since PLA was the major component of the films, a clear representation of new peaks was not observed.

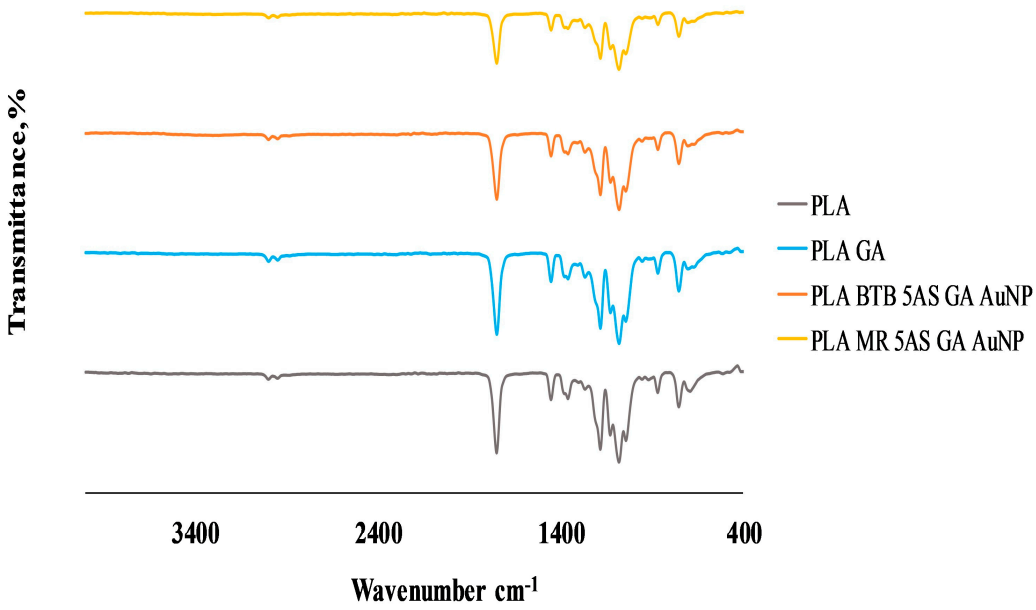

Figure 5. Infrared spectra of some of the synthesized PLA films.

3.2. Physical and Chemical Characterization of the PLA Films

3.2.1. Contact Angle

The contact angles of the PLA films are summarized in Table 1. If the contact angle exceeds 90°, the surface is considered hydrophobic. The addition of GA and nanoparticles enhanced the hydrophilicity of the films (Table 1). Conversely, other formulations increased hydrophobicity, which is a critical property for creating impermeable films. Therefore, it can be said that nanoparticles will improve their antibacterial capability and physical properties. The improvements in hydrophobicity can be attributed to interactions between the nanoparticles and the PLA surface, which likely modified the surface energy. These interactions may have roughened the surface, reducing its affinity for water and resulting in higher contact angles. This is consistent with the observed increase in hydrophobicity, which is essential for enhancing the antibacterial and physical properties of the films.

Table 1. The contact angle of some of the PLA films.

PLA Film	Average Contact Angle (°) (n = 3)
PLA	75.89 ± 0.67
PLA-GA (0.5 mg/mL)	81.10 ± 0.88
PLA-GA (1 mg/mL)	62.30 ± 0.89
PLA-GA (1 mg/mL)-MR-5AS-AuNP	84.10 ± 0.78
PLA-GA (1 mg/mL)-5AS-DMAB-AuNP	83.10 ± 0.76
PLA-GA (1 mg/mL)-BTB-5AS-AuNP	85.20 ± 0.18
PLA-GA (1 mg/mL)-MR-5AS-AgNP	83.70 ± 0.77
PLA-GA (1 mg/mL)-MR-DMAB-AgNP	88.70 ± 0.27

MR and BTB concentrations were 1 µg/mL, while the DMAB concentration was 10 µg/mL in all conditions. Concentrations of 5AS, $HAuCl_4 \cdot H_2O$, and $AgNO_3$ were at 0.08 mg/mL.

3.2.2. Tensile Strength

Tensile strength is a crucial parameter for food packaging materials, and the produced ones' is given in Figure 6. The addition of GA improved the mechanical properties of the films, while the incorporation of gold nanoparticles further enhanced these properties. The difference observed between DMAB-mediated and SL-mediated gold nanoparticle synthesis may have resulted in nanoparticles of varying sizes, which influenced the mechanical properties of the films. In comparison, commercial food packaging materials such as polyethylene (PE) and polypropylene (PP) typically have tensile strengths ranging from 20 to 40 MPa [19]. For instance, PLA films generally exhibit tensile strengths of around 20 MPa, while PP films can have tensile strengths of up to 35 MPa [20]. The modified PLA films with incorporated nanoparticles demonstrated comparable or superior tensile strength to some conventional food packaging materials. This enhancement suggests that the modified PLA films could provide more durable and effective food packaging solutions.

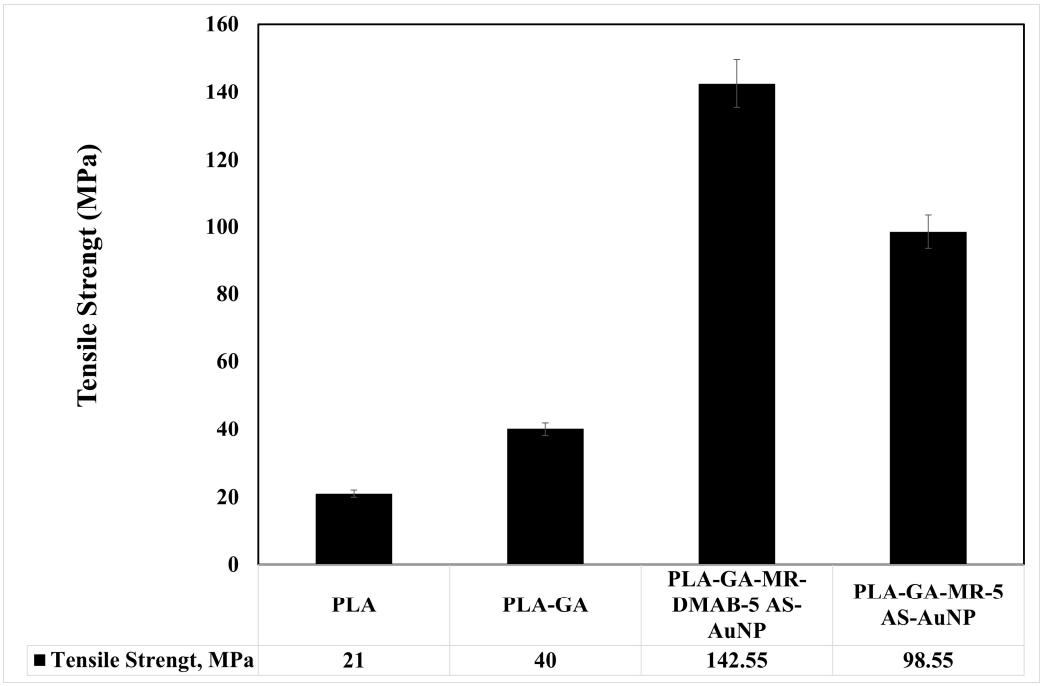

Figure 6. Tensile strength of some of the PLA films. GA: 1 mg/mL, MR: 10 µg/mL, DMAB: 10 µg/mL, 5AS and $HAuCl_4H_2O$: 0.08 mg/mL.

3.2.3. Surface Characterization

Figure 7 shows SEM images of some of the PLA films. Solvent evaporation-mediated phase inversion resulted in featureless surfaces for most films. However, films containing blueberry extracts exhibited pores with an average size of 3.28 ± 0.88 µm [15]. This porosity may be attributed to the strong hydrophilicity of the blueberry extracts, which contrasts with the inherent hydrophobicity of PLA. These differences in hydrophobicity likely influenced the drying kinetics, leading to variations in morphology. Despite their strong antibacterial activity, films containing blueberry extracts were not studied further due to their brittleness.

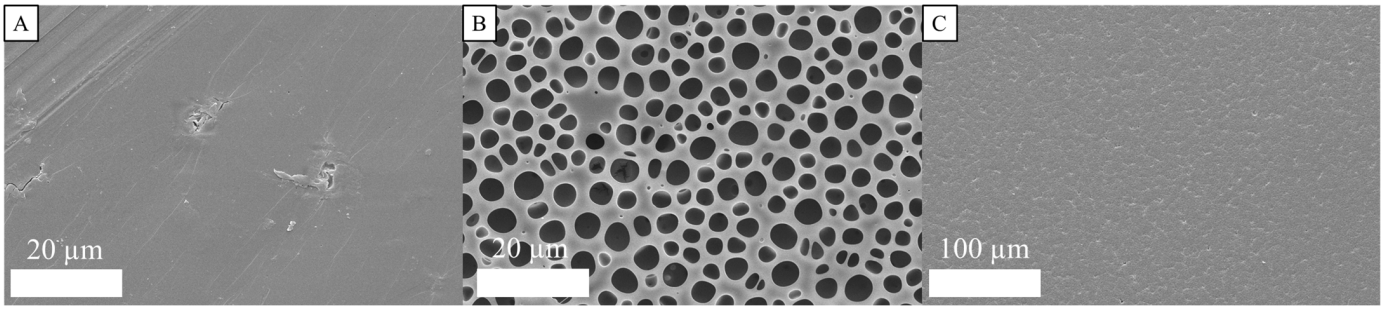

Figure 7. SEM characterization of some of the PLA films. (**A**) PLA film prepared from 6% PLA incubated in 1 mg/mL of GA aqueous solution for 6 h at room temperature; 500× with 20 µm scale (**B**) 1 mg/mL of GA was added into a 6% PLA solution containing 0.4 mg/mL of blueberries extract, which underwent film formation upon casting on the clean glass; 5000× with 20 µm scale (**C**) PLA–AuNP–MR–5AS–GA: 1 mg/mL of GA was added into a 6% PLA solution containing 0.7 mg/mL of Au^{3+} ion, which underwent film formation upon casting on clean glass, 1000× with 100 µm scale.

3.2.4. Solvent Resistance

All the PLA film formulations (i.e., plain PLA, PLA–GA, PLA–GA–Extract, PLA–AuNP-GA, and PLA–AgNP–GA) showed strong resistance to the tested buffers. However, they all dissolved in a 5% ammonia solution, which could be related to strong H-bonding between C = O of PLA and N-H groups. Among the tested films, only AuNP-containing PLA films showed partial resistance to the 5% ammonia solution. This concentration is significantly higher than the ammonia levels typically released via microbial metabolism. Another observation was that either SL or DMAB was used to trigger AuNP/AgNP in the presence of MR or BTB, and the formed films showed similar characteristics.

3.2.5. Oil and Water Vapor Transfer Characterization

Resistance to oil and water vapor penetration is essential for maintaining food freshness and preventing flavor loss. Various approaches, such as incorporating nanoparticles, whey proteins, polysaccharides, and lipids, have been used to enhance resistance [15]. All the films gave permeabilities between 0.67–0.72 $\times$ 10^{-12} g m^{-1} s^{-1} Pa^{-1} based on the formula below for water vapor permeability (WVP) [21], while none showed oil leakage:

$$\mathrm{WVP} = \frac{\Delta w}{\Delta t} \times \frac{L}{\Delta P\, A}$$

Here, $\Delta w/\Delta t$ represents the moisture transfer rate (g/s), A is the area exposed to moisture transfer (m^2), L is the film thickness (m), and ΔP is the partial water vapor pressure difference across the film. The value of ΔP was calculated at 2081.325 Pa, assuming the film side's relative humidity (RH) between 75–0% at 25 °C. For each film, at least three replicates were conducted.

3.3. Antimicrobial Activity of the PLA Films

Plain PLA films were used as a control to evaluate the antimicrobial activity of the modified films. The antibacterial mechanism of nanoparticles is primarily attributed to their ability to alter membrane permeability and respiration [22] and significantly reduce ATP levels in bacterial cells [23] as seen in Figure 8. In addition, the presence of sugar ligands and gold and silver nanoparticles resulted in strong antibacterial activity, where the activity was observed as being higher for gram (+) *S. epidermidis* than gram (−) *E. coli*. The introduction of 5AS provided antibacterial activity, which was further enhanced by the presence of AuNPs and MR. Although AgNPs are generally expected to exhibit stronger

antibacterial activity [22], the AgNP-containing films did not demonstrate the same level of physical robustness as the AuNP-containing films.

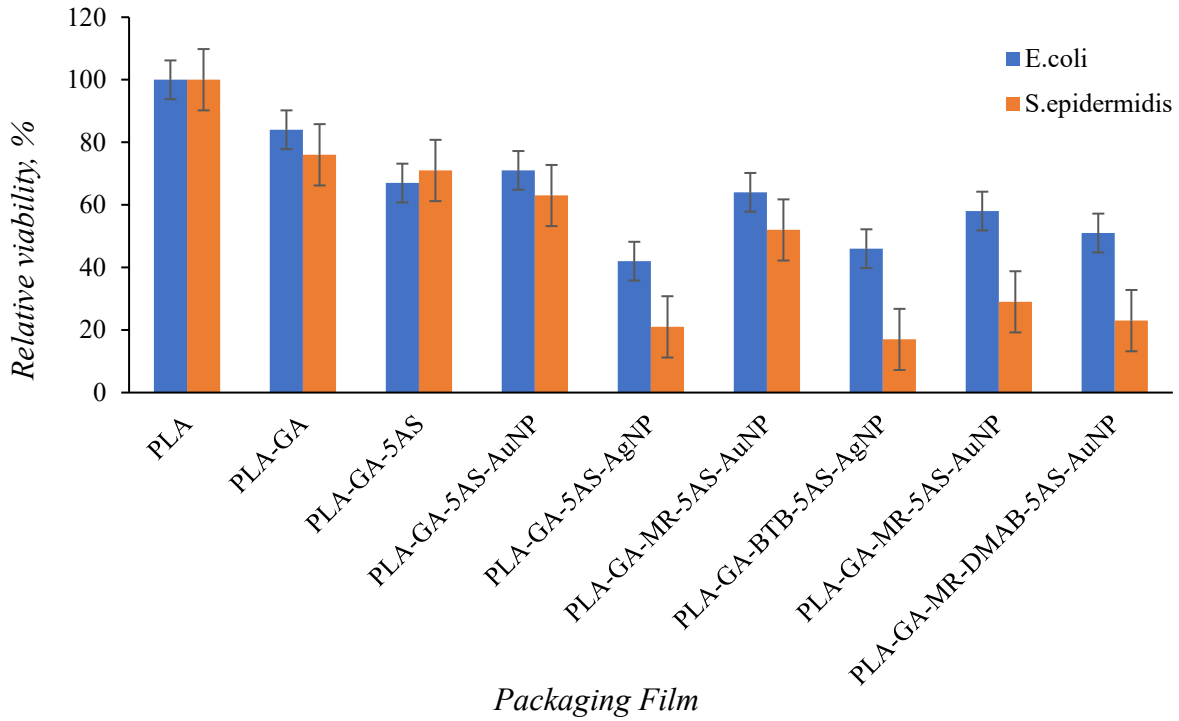

Figure 8. Antibacterial activity of some of the PLA films.

3.4. Validation of Smart Packaging Material

As shown in Figure 9, cheddar cheese wrapped in the selected PLA films remained unchanged in color after 12 months of refrigeration. No bacterial growth was observed. It is important to note that taste alterations were not tested. Figure 10 confirms that no components of the PLA films migrated into the cheese during the incubation period.

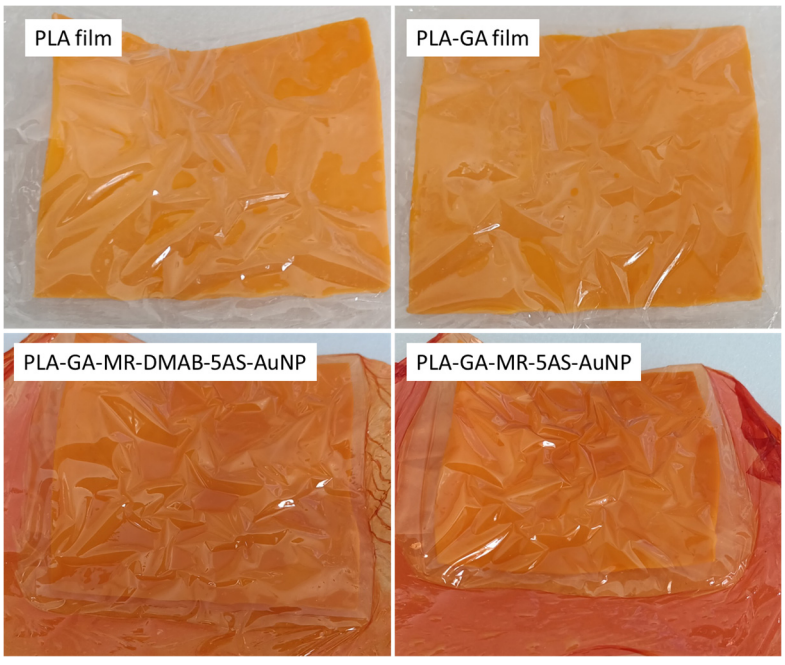

Figure 9. Cheddar cheese in the selected PLA films for 12 months of incubation.

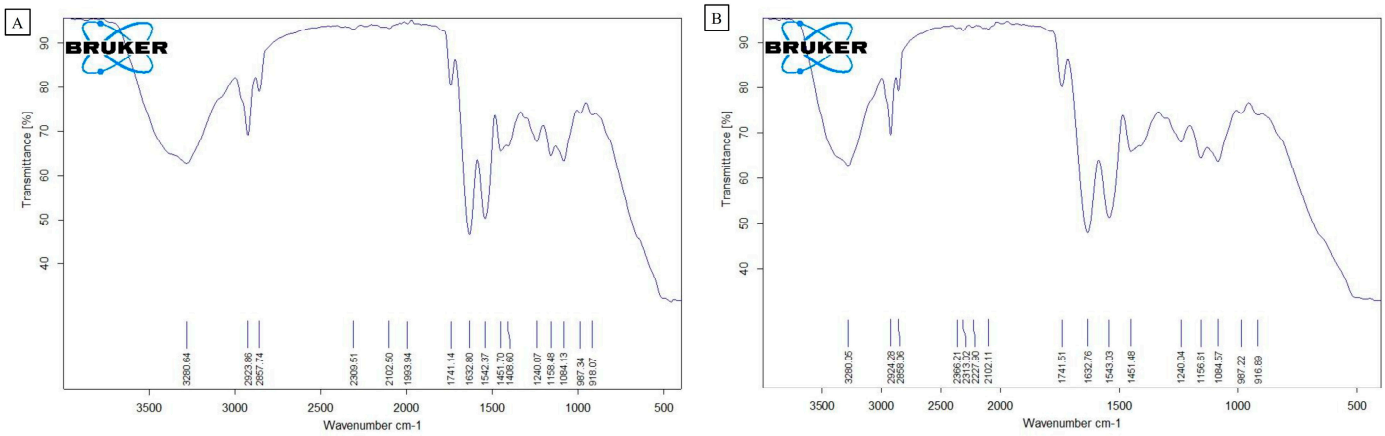

Figure 10. IR spectra of fresh cheese (**A**) and 12-month incubated cheese (**B**).

4. Conclusions

The chemical modification of polylactic acid (PLA) with glutaraldehyde, combined with methyl red as a pH indicator and in situ synthesized gold and silver nanoparticles, significantly enhanced the resulting packaging films' mechanical, chemical, and antimicrobial properties. These modified films exhibited "smart" characteristics, including antimicrobial activity and a color change in response to variations in acidity and ammonia levels, positioning them as promising candidates for advanced food packaging applications. However, despite these promising results, certain limitations, such as the brittleness observed in some films, could hinder their practical application. Future research should optimize the film composition to address these challenges and improve flexibility, durability, and mechanical performance. Scalability challenges for industrial production also need to be carefully considered. These include the cost of nanoparticle incorporation, the ability to maintain consistent quality at larger scales, and the environmental impact of large-scale production processes. Developing cost-effective and scalable nanoparticle synthesis and film fabrication methods will be critical for transitioning these materials from laboratory research to commercial applications. Ultimately, the findings of this study provide a strong foundation for further investigation into the development of PLA-based smart packaging materials. By integrating active and intelligent functionalities into a single biodegradable system, these films offer a sustainable and innovative solution for enhancing food safety, extending shelf lives, and reducing food waste.

Future research should also explore the long-term stability of these films under real-world storage and usage conditions, as well as their potential applications for a broader range of food products. Additionally, the environmental impact of the films' end-of-life disposal should be assessed to ensure their alignment with circular economy principles. Addressing these areas will contribute to advancing sustainable, intelligent food packaging solutions that align with global efforts to reduce plastic waste and promote environmental sustainability.

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Conflicts of Interest: The authors declare no conflicts of interest.

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