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Improving the Printability of PLA-Based Active Packaging Films Using Exopolysaccharide Obtained by *Ligilactobacillus Salivarius* KC66L

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ABSTRACT

Polylactic acid (PLA) has been identified as a promising bio-based polymer for sustainable food packaging applications. Nevertheless, its limited functionality and surface characteristics may constrain its performance in advanced packaging and printing applications. In this study, exopolysaccharide produced by Ligilactobacillus salivarius KC66L (EPS-KC66L) was incorporated into PLA films at 1, 2.5, and 5 wt% to investigate its effects on the chemical, thermal, optical, surface, printability, and antibacterial properties of the resulting films. The PLA/EPS films were prepared via solvent casting and characterized by ATR-FTIR, thermogravimetric analysis, UV–Vis spectroscopy, contact angle and instrument-derived surface energy measurements, colour and gloss analysis, and printing tests. The analysis of ATR-FTIR spectra indicated that the physical incorporation of EPS into the PLA matrix did not result in significant alterations to the characteristic structure of the PLA. The study found that there was no adverse effect of EPS incorporation on thermal stability, and that the maximum degradation temperature increased slightly, from 384.5 °C for neat PLA to 387.4 °C for PLA/5-EPS. The incorporation of EPS resulted in a modification of the optical and surface characteristics of the films. The 5 wt% formulation exhibited reduced visible transparency, a lower water contact angle (61.2°) and a higher instrument-derived surface energy (42.9 mJ m^{-2}). Following the printing process, PLA/5-EPS demonstrated the lowest color difference (ΔE_{00} = 1.82) in relation to the printed reference sample. This finding suggests that PLA/5-EPS exhibits enhanced color reproduction capabilities when compared with unprinted PLA (ΔE_{00} = 4.71). However, no detectable inhibition zones against Escherichia coli or Staphylococcus aureus were observed in the agar diffusion assay. The investigation established that EPS-KC66L modified the surface and printing-related properties of PLA films without compromising their thermal stability. Of the formulations examined, the 5 wt% formulation demonstrated the most favourable surface wettability and print colour reproduction. These findings demonstrate the potential of microbial EPS as a functional modifier for the development of sustainable and printable PLA-based packaging materials.

KEY WORDS
PLA, Exopolysaccharide (EPS), Printability, Surface free energy, Ligilactobacillus salivarius

1. Introduction

Packaging plays a critical role in maintaining the quality and safety of food products throughout the supply chain, from production to consumption, while also extending shelf life and reducing food losses. However, a substantial proportion of conventional food packaging materials are derived from petroleum-based polymers that are resistant to biodegradation, contributing to the accumulation of plastic waste and posing significant challenges to environmental sustainability. The packaging sector represents one of the major areas of plastic consumption, and the widespread use of single-use packaging has further intensified the need for more sustainable alternatives. Consequently, in recent years, increasing research attention has been directed toward the development and application of biodegradable polymers derived from renewable resources, which offer lower environmental impact and considerable potential for sustainable food packaging (Hassan, 2026).

Polylactic acid (PLA) has emerged as a promising alternative to conventional petroleum-based polymers due to its bio-based origin, biocompatibility, processability, and favorable physicochemical properties for packaging applications. Despite the significant potential of neat PLA in the packaging sector, it possesses only limited intrinsic biological functionality. This restricts its ability to provide active protection beyond the conventional barrier and containment functions of packaging. This limitation is particularly relevant to the development of next-generation active food packaging systems designed to directly contribute to food preservation and shelf-life extension. Consequently, there has been significant research interest in enhancing the functional performance of PLA through incorporation of natural or biologically derived active components. Recent studies have demonstrated that the integration of antimicrobial and other functional agents into PLA-based materials can impart additional bioactive properties. This represents a promising strategy for the development of active, more sustainable food packaging systems (Zeng et al., 2026).

It is evident that microbial biopolymers have attracted increased attention among the functional components incorporated into active packaging systems. This is due to their potential to combine sustainability with the desired functional properties. Exopolysaccharides (EPS) are a prominent class of microbial biopolymers, defined as high-molecular-weight polysaccharides secreted extracellularly by microorganisms. In addition to their biocompatibility, low toxicity, and film-forming ability, certain EPS have been reported to exhibit antioxidant and antimicrobial activities, making them promising candidates for food packaging applications. A notable increase in research interest has been observed focusing on EPS produced by lactic acid bacteria and their potential utilization as structural or functional components in food packaging materials. Recent studies have demonstrated that EPS derived from various microorganisms, including species of the *Lactobacillus* and *Ligilactobacillus* genera, may exhibit favorable film-forming and rheological characteristics, along with biological activities that could be exploited in the development of functional and active food packaging systems (Mazumder et al., 2025). *L. salivarius* is a promising probiotic bacterium capable of producing a variety of biologically active metabolites (Yuksekdag et al., 2025). Moreover, microbial postbiotics comprise a diverse range of bioactive molecules, including exopolysaccharides, which may contribute to their functional properties (Çelik et al., 2024; Yuksekdag et al., 2025). The incorporation of EPS into a polymer matrix has been demonstrated to have the potential to impart biological functionality to the resulting material. Furthermore, molecular and interfacial interactions between the polymer chains and EPS result in modification of the material's structural and surface characteristics. Such interactions have the capacity to influence several key properties of the resulting films, including surface morphology, hydrophilicity, surface free energy, and barrier performance. Indeed, a recent study demonstrated that incorporating exopolysaccharides (EPS) from *Bacillus licheniformis* into alginate-based films resulted in strong hydrogen-bonding interactions between the polymer matrix and the EPS. As a result, significant changes in the films' morphology, mechanical properties, and barrier properties were observed (Bayrak & Omeroglu, 2026). In a biopolymer-based packaging system, it is essential to establish relationships between additive-polymer interfacial interactions, additive dispersion and aggregation behaviour, and the resulting measurable surface and barrier properties, in order to understand the practical performance of the developed materials (Hassan, 2026).

However, the industrial applicability of food packaging materials is not determined solely by their biological activity, mechanical performance, or barrier properties. The packaging surface must also provide a suitable substrate for the printing of product information, branding elements, traceability data, and other graphical content. Therefore, printability represents an important technological requirement that should be considered alongside the functional performance of sustainable packaging materials. This aspect is particularly important for non-absorbent polymeric substrates such as PLA, where adequate wetting of the surface by the printing ink and strong adhesion after drying are critical for achieving satisfactory print quality. Previous studies on PLA films have demonstrated that modifications to surface characteristics can significantly influence wettability and ink adhesion, so directly affecting printing performance (Izdebska-Podsiadły, 2021).

Surface free energy (SFE) is one of the key parameters leading the interactions between a polymeric substrate and printing ink. The ability of an ink to wet and spread

over the substrate and then form a stable and uniform ink layer largely depends on the relationship between the surface free energy of the solid substrate and the surface tension of the ink. However, printing performance is not governed by surface energy alone; surface polarity and topography (roughness) also play important roles in determining ink wetting, spreading, and adhesion. In PLA-based systems, changes in both the polar and dispersive components of surface free energy have been shown to influence ink-substrate interactions and, consequently, overall printing performance (Izdebska-Podsiadły, 2021).

The importance of these relationships has become increasingly evident as research interest in sustainable printed packaging grows. For instance, studies on PLA/PCL/SiO2-based biopolymer films have demonstrated that variations in film composition can significantly alter surface morphology, contact angle, and surface free energy. These factors are closely associated with ink adhesion and overall print quality. These findings indicate that incorporating functional components into a biopolymer matrix can influence not only the structural and physicochemical characteristics of the resulting material but also its surface-ink interactions and, consequently, its printability (Poljaček et al., 2026). Therefore, when evaluating the effects of functional components incorporated into active packaging systems, it is important to consider not only conventional mechanical and barrier properties but also the relationship between preprint surface characteristics and post-printing performance.

Recent studies have increasingly focused on the potential of microbial exopolysaccharides (EPS) in sustainable and active food packaging systems, with research primarily concentrating on their film-forming capacity and the impact on the barrier, mechanical, and biological properties of packaging materials (Mazumder et al., 2025). On the other hand, several studies have investigated the relationships among surface free energy, wettability, and ink adhesion in PLA-based packaging materials (Poljaček et al., 2026). However, studies directly linking the surface modifications induced by incorporating microbial-derived EPS as a functional additive into PLA-based active packaging films to their printability characteristics remain limited. A thorough evaluation of the effects of EPS incorporation on the physicochemical properties of PLA surfaces and its influence on printing performance is therefore essential to comprehending the practical and industrial applicability of sustainable active packaging materials.

Accordingly, in the present study, EPS derived from *Ligilactobacillus salivarius* KC66L (EPS-KC66L) was incorporated into PLA films at different concentrations. The effects of EPS incorporation on the chemical structure, thermal stability, optical properties, surface wettability, surface energy, and printing performance of the resulting films were systematically investigated. Particular emphasis was placed on establishing the relationship between EPS-induced changes in surface characteristics and the color reproduction and gloss properties of the printed films. In addition, the antibacterial activity of the films against Escherichia coli and Staphylococcus aureus was evaluated.

2. Methods

2.1. Materials

Polylactic acid (PLA, Mw ≈ 60,000 g mol^{-1}) was purchased from Sigma-Aldrich and used as the polymer matrix. Trichloroacetic acid (TCA) and Tween 80 were also obtained from Sigma-Aldrich. All solvents and other chemicals used in the experiments were of analytical grade and were used as received without further purification.

2.2. Isolation of Exopolysaccharide (EPS)

EPS was isolated from *Ligilactobacillus salivarius* KC66L using a modified procedure based on previously reported methods (Kanmani et al., 2011; Shi et al., 2014). The activated bacterial culture, adjusted to a McFarland standard of 0.5, was inoculated into de Man, Rogosa, and Sharpe (MRS) medium at 2% (v/v) and incubated at 37 °C for 24 h. The resulting active culture was subsequently inoculated into 800 mL of MRS medium at 2% (v/v) and incubated at 37 °C for an additional 48 h.

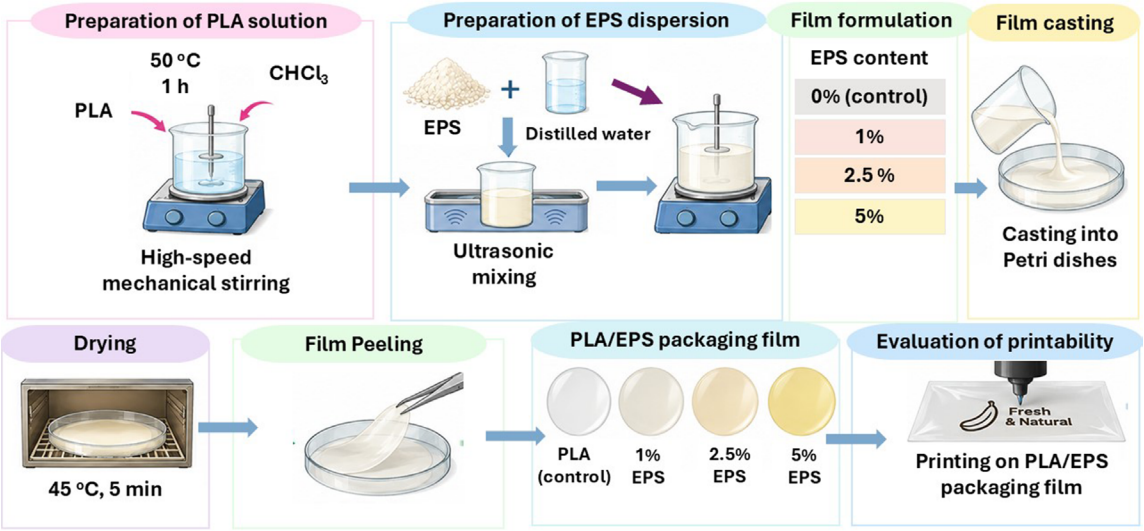
Following incubation, the culture was heat-treated at 95 °C for 10 min and allowed to cool to room temperature. An 80% (v/v) TCA solution was added, and the mixture was stirred for 2 h. The culture was then centrifuged at 9000 rpm for 15 min at 4 °C, and the resulting supernatant was mixed with three volumes of cold 96% ethanol (1:3, v/v) to precipitate the EPS. After incubation at 4 °C for 24 h, the precipitated EPS was recovered by centrifugation at 9000 rpm for 15 min. The recovered EPS was dissolved in sterile distilled water, lyophilized, and stored at 4 °C until further use.

2.3. Preparation of PLA/EPS Packaging Films

In the present study, PLA-based composite packaging films were prepared using a solvent casting approach. This approach has been widely employed in the fabrication of biopolymer and exopolysaccharide-based films. However, the present study introduces several modifications based on previously reported methods (Ferreira et al., 2014; Guerrero et al., 2024). Polylactic acid (PLA) was used as the primary film-forming polymer, while the previously isolated EPS-KC66L was incorporated into the film formulations as a func-

tional additive. Chloroform was used as the solvent, and Tween 80 was employed as a surfactant.

A 10% (w/v) PLA solution was prepared in chloroform and mechanically stirred at high speed at 50 °C for 1 h to obtain a homogeneous polymer solution. Subsequently, Tween 80 was added at a concentration of 3%. To prepare the EPS-KC66L phase, 0.5 g of EPS-KC66L was dissolved in 5 mL of distilled water. The resulting EPS-KC66L dispersion was incorporated into the PLA/Tween 80 solution to obtain films containing 1, 2.5, and 5 wt% EPS-KC66L relative to the PLA content. To promote homogeneous dispersion of EPS-KC66L within the PLA matrix, the resulting mixtures were ultrasonicated for 4 min. The film-forming mixtures were then poured into Petri dishes and cast using the solvent-casting method. The films were dried at 45 °C for 5 min to facilitate solvent removal and film formation. After drying, the films were carefully peeled from the Petri dishes and subsequently used for further characterization. A PLA/Tween 80 film without EPS-KC66L (0% EPS-KC66L) was prepared under the same conditions and used as the control. A schematic representation of the preparation procedure for the PLA/EPS packaging films is presented in Figure 1.



» **Figure 1:** *Schematic representation of the preparation and printing of PLA/EPS packaging films*

2.4. Characterization

The chemical structures of the prepared packaging films were characterized by attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy using a Bruker IFS 66/S spectrometer. The spectra were recorded over the wavenumber range of 400–4000 cm^{-1} using 32 scans to identify the characteristic functional groups of PLA and EPS and to investigate possible interactions between the film components.

Thermogravimetric analysis (TGA) was performed using a PerkinElmer STA6000 instrument. The samples were heated from 30 to 750 °C under a nitrogen atmosphere at a heating rate of 10 °C min^{-1}. The thermogravimetric profiles were analyzed to determine the temperatures corresponding to 5% and 10% mass loss (T_5 and T_{10}, respectively) and the maximum degradation temperature (T_{max}), and to evaluate the effect of EPS incorporation on the thermal stability and degradation behavior of the PLA-based films.

The optical properties of the films were evaluated using a Shimadzu UV-2450 UV–Vis spectrophotometer (Kyoto, Japan). Light transmittance measurements were performed to evaluate the optical transparency of the films. In addition, the visual appearance and transparency of the prepared films were documented photographically as they were placed in Petri dishes.

The surface wettability of the films prior to printing was evaluated using a Pocket Goniometer PG-X, version 3.4 (FIBRO System AB, Sweden). Water contact angles were measured using the integrated CCD camera and determined by the instrument software. Surface energy values were obtained directly from the PG-X software using the measured contact angle data and its built-in calculation procedure. Measurements were performed in triplicate, and the results are expressed as mean ± standard deviation. The contact angle and instrument-derived surface energy values were used to comparatively evaluate changes in the surface characteristics of the PLA/EPS films resulting from EPS incorporation.

The color characteristics of the packaging films were measured using an X-Rite eXact portable spectrophotometer in accordance with ISO 13655:2017. Measurements were performed using 0°/45° geometry with a polarization filter, a 2° standard observer, and D50 illumination over the wavelength range of 400-700 nm. Five measurements were performed for each sample, and the mean values were used for further

analysis. Color values were expressed in the CIELAB color space as L*, a*, and b*, where L* represents lightness from black to white, a* represents the green (-a*) to red (+a*) axis, and b* represents the blue (-b*) to yellow (+b*) axis. Color differences (ΔE_{00}) were calculated according to the CIEΔE_{2000} color-difference formula in accordance with ISO 11664-6:2014.

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T \frac{\Delta C'}{k_C S_C}\frac{\Delta H'}{k_H S_H}} \quad (1)$$

where $\Delta L'$, $\Delta C'$, and $\Delta H'$ represent the differences in lightness, chroma, and hue between the reference and sample, respectively. S_L, S_C, and S_H are the weighting functions for the lightness, chroma, and hue components, respectively, while k_L, k_C, and k_H are parametric factors accounting for variations in experimental and viewing conditions. R_T is the rotation term that accounts for the interaction between chroma and hue differences in the blue region of the CIEL*a*b* color space.

2.5. Printing of PLA/EPS Packaging Films

The prepared packaging films were printed using a red solvent-based printing ink supplied by Siegwerk. Printing trials were performed using an IGT F1 printability tester at a printing speed of 1 m min^{-1} and a printing pressure of 300 N. Identical printing conditions were applied to all film formulations to enable direct comparison of their printing performance. The adhesion of the printed ink layer to the film surface was evaluated using a cross-cut tape test with transparent tesa® 4124 PVC tape. Following the formation of the cross-cut pattern, the tape was firmly applied to the printed surface and subsequently peeled off at an angle of 180°. The degree of ink adhesion was evaluated using a five-point visual scale. The scale ranged from 1, representing very poor adhesion with substantial ink removal, to 5, representing very good adhesion with no ink visible upon removal.

2.6. Antibacterial Activity of PLA/EPS Packaging Films

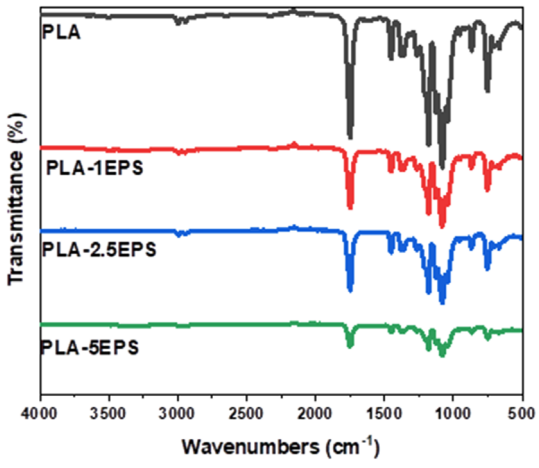
The antibacterial activity of the PLA/EPS films was qualitatively evaluated against *Escherichia coli* ATCC 11229 and *Staphylococcus aureus* ATCC 25923 using a film agar diffusion assay adapted from Ozdemir et al. (2023). Fresh bacterial colonies were transferred into Mueller-Hinton (MH) broth and incubated at 37 °C for 1-2 h. The turbidity of the bacterial suspensions was adjusted to 0.5 McFarland standard (~1.5×10^8 CFU mL^{-1}), and the suspensions were uniformly inoculated onto Mueller-Hinton agar plates using a sterile swab. Film samples corresponding to control PLA and PLA films containing 1, 2.5, and 5 wt% EPS-KC66L were aseptically placed directly onto the inoculated agar surface using sterile forceps. The plates were incubated at 35-37 °C for 24 h. Following incubation, antibacterial activity was qualitatively evaluated based on the presence or absence of a visible inhibition zone around the film samples. The PLA film without EPS was used as the negative control.

3. Results and discussion

3.1. Structural Characterization of PLA/EPS Packaging Films

ATR-FTIR analysis was performed to investigate the chemical structure of the PLA/EPS packaging films (Figure 2) and possible interactions between PLA and EPS-KC66L. Neat PLA exhibited its characteristic absorption bands at 2995 and 2945 cm^{-1}, corresponding to C-H stretching vibrations, together with a strong ester carbonyl (C=O) stretching band at 1749 cm^{-1}. The bands detected at approximately 1452 and 1382 cm^{-1} were attributed to CH_3 bending vibrations, while the bands at 1181, 1128, 1081, and 1043 cm^{-1} were associated with C-O-C and C-O stretching vibrations of the PLA ester backbone (Enumo et al., 2020).

It was demonstrated that the incorporation of EPS-KC66L resulted in no alteration to the characteristic absorption bands of PLA in the composite films. The carbonyl band remained unchanged at 1748-1749 cm^{-1}, while the major C-O/C-O-C bands were observed at approximately 1180-1181, 1127-1128, and 1080-1081 cm^{-1}. No distinct new absorption bands or substantial shifts in the major PLA bands were observed as EPS content increased. These findings indicate that the incorporation of EPS-KC66L did not substantially alter the chemical structure of the PLA matrix and provide no evidence for the formation of new covalent bonds between the components (Wang et al., 2020). Overall, the spectral results suggest that EPS-KC66L was physically incorporated into the PLA matrix without evidence of substantial chemical modification of the PLA structure.



» **Figure 2:** *FTIR spectra of PLA/EPS packaging films*

3.2. Thermal Characterization of PLA/EPS Packaging Films

Thermogravimetric analysis revealed a low-temperature mass-loss region followed by the thermal degradation of the PLA-rich matrix. The TGA results are summarized in Table 1. A noticeable mass loss occurred below approximately 160 °C in all formulations, including the PLA control (Figure 3). Therefore, this event was not attributed exclusively to EPS-KC66L incorporation and may instead be associated with the removal of absorbed moisture and/or low-molecular-weight or volatile constituents present in the film formulations. The extent of this initial mass loss did not show a systematic dependence on EPS-KC66L concentration, with the PLA/2.5-EPS film exhibiting a comparatively greater mass loss in this region.

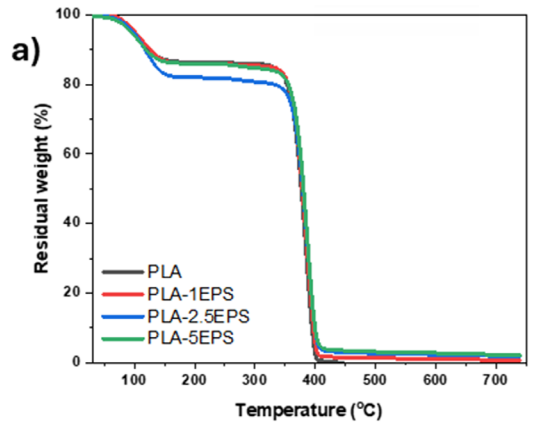
The degradation stage occurred at substantially higher temperatures and was dominated by PLA matrix degradation. The maximum degradation temperature (T_{max}) was approximately 384.5 °C for the PLA control film and 384.9, 387.0, and 387.4 °C for the PLA/1-EPS, PLA/2.5-EPS, and PLA/5-EPS films, respectively. Thus, incorporation of 1 wt% EPS-KC66L had little influence on the degradation temperature, whereas increasing the EPS-KC66L content to 2.5 and 5 wt% resulted in a modest shift of T_{max} toward higher temperatures. These results indicate that EPS-KC66L incorporation did not compromise the thermal stability of the PLA-rich matrix, while the 2.5 and 5 wt% formulations exhibited a slight increase in T_{max}. Overall, the preservation of the characteristic main degradation profile of PLA, together with the relatively small changes in T_{max}, suggests that EPS-KC66L incorporation did not substantially alter the overall thermal degradation behavior of the PLA-rich matrix. This observation is also consistent with the ATR-FTIR results, which showed preservation of the characteristic PLA absorption bands without the appearance of prominent new bands following EPS-KC66L incorporation.

Table 1

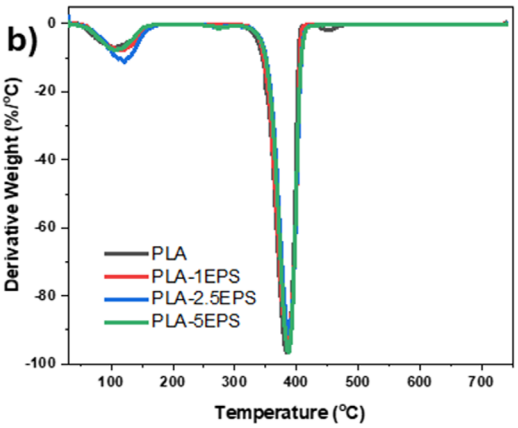
TGA results of PLA/EPS packaging films

	T_5 (°C)*	T_{10} (°C)*	T_{max} (°C)
PLA	95.8	123.3	384.5
PLA/1-EPS	101.6	126.8	384.9
PLA/2.5-EPS	98.6	117.8	387
PLA/5-EPS	94.5	119.4	387.4

*T_5 and T_{10} correspond to temperatures at 5 and 10% total mass loss, respectively, and include the low-temperature loss of moisture and/or volatile constituents.



(a)



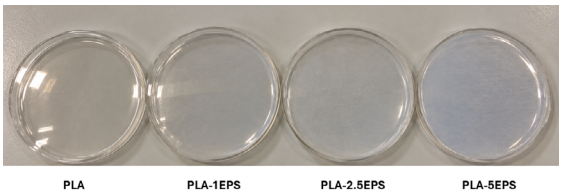
(b)

» **Figure 3:** *a) TGA curves of PLA/EPS packaging films, b) DTG curves of PLA/EPS packaging films*

3.3. Optical Properties of PLA/EPS Packaging Films

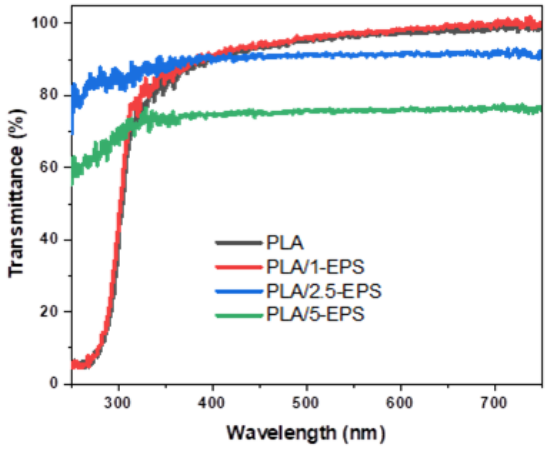
The visual appearance and optical properties of the PLA/EPS packaging films are shown in Figure 4. Control PLA and PLA/1-EPS films appeared transparent and relatively uniform. As the EPS content increased, the films gradually became whiter and more opaque. This change was noticeable in PLA/2.5-EPS and became more pronounced in PLA/5-EPS. Similar decreases in transparency with increasing additive content have been reported for PLA-based composite films and have been attributed to increased light scattering caused by the presence of different phases within the polymer matrix (Sanchez-Garcia et al., 2008; Machado et al., 2021). The whitening observed at higher EPS contents may therefore be related to the distribution of EPS within the PLA matrix.

The UV-Vis transmittance spectra (Figure 5) were consistent with the visual appearance of the films. PLA showed high transmittance in the visible region, as commonly reported for PLA-based packaging films (Boarino et al., 2022; Liu et al., 2024). The addition of



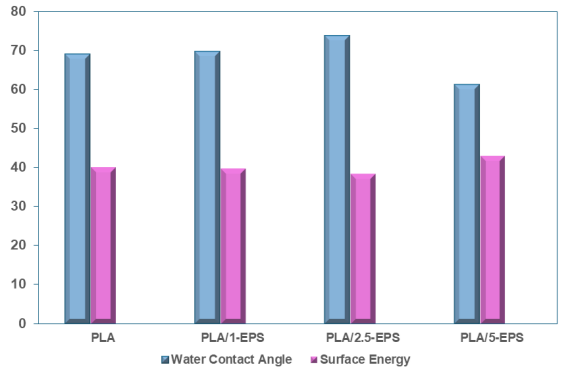
» **Figure 4:** *Visual appearance and transparency of PLA and PLA/EPS packaging films*

1% EPS-KC66L had little effect on the overall transmittance profile, while PLA/2.5-EPS retained a transmittance of around 90% across most of the visible region. A clearer decrease was observed at the highest EPS content, with PLA/5-EPS showing transmittance values of approximately 75-77%. This reduction also agrees with the more opaque appearance of the PLA/5-EPS film observed visually. The differences among the films did not follow a clear trend with increasing EPS-KC66L content, particularly in the UV region. This suggests that the changes in UV transmittance cannot be explained by EPS concentration alone. The optical behavior of the films may also depend on how EPS-KC66L is distributed within the PLA matrix. Previous studies on PLA-based composites have shown that the dispersion and aggregation of the incorporated phase can have a strong influence on light transmission (Boarino et al., 2022). Overall, EPS-KC66L affected the optical properties of the PLA films without causing a complete loss of transparency. The largest change was observed for PLA/5-EPS, which showed the greatest reduction in optical clarity.



» **Figure 5:** *UV-Vis transmittance spectra of PLA and PLA/EPS packaging films*

3.4. Surface Wettability and Surface Energy of PLA/EPS Packaging Films



» **Figure 6:** *Contact Angle and Surface Energy of Produced Films*

The water contact angle and surface energy values of the PLA/EPS films are given in Table 2. PLA showed a water contact angle of 69.0° ± 0.6 and a surface energy of 40.1 ± 0.7 mJ m^{-2}. The contact angle was close to the 70-75° range previously reported for untreated PLA films (Abdulkareem et al., 2021; Muñoz-Shugulí et al., 2023), indicating that the PLA surface was moderately wettable rather than highly hydrophobic. The surface energy was also within the range reported for untreated PLA (33–40 mJ m^{-2}), although these values can vary with film preparation and the method used for surface characterization (Izdebska-Podsiadły & Dörsam, 2017).

Table 2

The water contact angle and surface energy of the PLA/EPS films

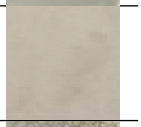
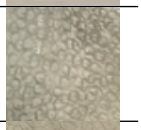
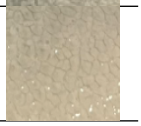
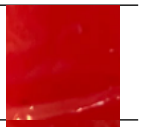
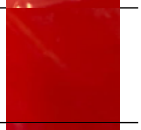
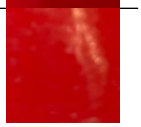
	WCA (°)	SE (mJ m^{-2})
PLA	69.0±0.6	40.1±0.7
PLA/1-EPS	69.7±0.4	39.8±0.4
PLA/2.5-EPS	73.6±0.3	38.4±0.3
PLA/5-EPS	61.2±0.5	42.9±0.6

The addition of 1% EPS-KC66L had little effect on the surface properties of PLA. The contact angle increased slightly from 69.0° to 69.7° ± 0.4, while the surface energy decreased from 40.1 to 39.8 ± 0.4 mJ m^{-2}. These small differences suggest that 1% EPS-KC66L did not markedly change the film surface. At 2.5% EPS-KC66L, the contact angle increased to 73.6° ± 0.3 and the surface energy decreased to 38.4 ± 0.3 mJ m^{-2}, indicating a modest decrease in surface wettability. Similar changes in wettability have been observed in modified and composite PLA films, where the composition and morphology of the surface can affect its interaction with water (Muñoz-Shugulí et al., 2023).

A different trend was observed for PLA/5-EPS. The water contact angle decreased to 61.2° ± 0.5, while

Table 3

Optical properties of printed and unprinted PLA/EPS packaging films

	L^*	a^*	b^*	DE_{00}	Gloss (GU)	Image
Ref. Base Paper	91.85	2.12	-12.81	Ref	6.7	
Unprinted PLA	81.33	2.68	-11.42	7.00	21.5	
Unprinted PLA/1-EPS	83.29	4.38	-10.83	6.62	20.4	
Unprinted PLA/2.5-EPS	81.28	4.10	-6.14	9.26	19.8	
Unprinted PLA/5-EPS	82.96	1.77	-5.28	7.92	18.3	
Ref. Printed Paper	42.35	63.20	24.76	REF	8.9	
Printed PLA	47.55	70.11	28.09	4.71	22.1	
Printed PLA/1-EPS	45.58	67.38	27.25	2.71	20.8	
Printed PLA/2.5-EPS	44.29	66.72	36.03	4.95	19.9	
Printed PLA/5-EPS	42.17	61.78	27.72	1.82	18.7	

the surface energy increased to 42.9 ± 0.6 mJ m^{-2}. The lower contact angle indicates that the film surface became more wettable at the highest EPS-KC66L content. This agrees with the increase in surface energy, as higher surface energy in PLA has been associated with lower water contact angles and improved wettability (Jordá-Vilaplana et al., 2014). The marked change at 5% EPS-KC66L suggests that a higher EPS content had a stronger effect on the film surface.

From a printing perspective, the changes observed for PLA/5-EPS are particularly important. Surface energy and wettability influence how liquid inks spread and interact with polymer surfaces. In general, a higher surface energy promotes better wetting and contact between the ink and the substrate, although ink adhesion also depends on factors such as ink composition, drying conditions, and interfacial interactions (Jordá-Vilaplana et al., 2014). The higher surface energy measured for PLA/5-EPS may therefore be beneficial for subsequent printing.

3.5. Printability Properties of PLA/EPS Packaging Films

The CIELAB color parameters of the unprinted PLA/EPS films are presented in Table 3. The L* values remained within a relatively narrow range, showing that the addition of EPS-KC66L had little effect on the overall lightness of the films. All PLA-based films had lower L* values than the reference paper, which is expected given the different optical characteristics of polymer films and paper substrates. Previous studies have also shown that the L* values of PLA films can vary depending on film composition, processing conditions, thickness, and measurement conditions (Iglesias Montes et al., 2019; Wang & Rhim, 2016).

The clearest change with increasing EPS-KC66L content was observed in the b* values. Starting from a more negative value for neat PLA, b* gradually shifted toward less negative values as the EPS content increased, particularly at 2.5% and 5%. This shift indicates a decrease

in the blue component and a movement toward the yellow region of the CIELAB color space. In contrast, the a* values did not follow a clear trend, increasing at 1% and 2.5% EPS-KC66L and then decreasing at 5%. Overall, EPS-KC66L affected the film color mainly along the blue-yellow axis, while its effect on lightness and the red–green coordinate was less systematic. Similar changes in CIELAB parameters have been reported for PLA-based composite films following the incorporation of dispersed components (Iglesias Montes et al., 2019; Wang & Rhim, 2016). The changes observed at higher EPS-KC66L contents are also in line with the visual appearance of the films, where more noticeable whitish regions were observed at 2.5% and 5% EPS-KC66L.

The ΔE_{00} values, calculated using the reference base paper, represent the overall color difference between the films and the paper substrate. Among the formulations, PLA/2.5-EPS showed the largest color difference from the base paper. However, the ΔE_{00} values do not directly represent the color change caused by EPS-KC66L, since the base paper rather than neat PLA was used as the reference. Therefore, the ΔE00 values are interpreted as the color difference between each film and the base paper (Luo et al., 2001).

The CIELAB color parameters of the printed PLA/EPS films are presented in Table 3. Printed neat PLA had higher L*, a*, and b* values than the printed reference paper, showing that the color of the printed film differed from that of the paper substrate. The addition of EPS-KC66L mainly affected the a* and b* values. The b* value increased noticeably at 2.5% EPS-KC66L, corresponding to a shift toward yellow. At 5% EPS-KC66L, b* decreased again and was closer to the value measured for neat PLA. A similar concentration-dependent pattern was not observed for the other color coordinates. Thus, the color differences after printing varied with EPS-KC66L content, but did not increase consistently as the EPS concentration increased.

A notable difference was observed in the ΔE00 values of the printed films. The value decreased from 4.71 for neat PLA to 2.71 for PLA/1-EPS and further to 1.82 for PLA/5-EPS. In contrast, PLA/2.5-EPS showed a slightly higher ΔE_{00} value of 4.95. The effect of EPS-KC66L was therefore not concentration-dependent. Among the PLA-based films, PLA/5-EPS showed the smallest color difference from the printed reference paper. The lower ΔE_{00} value of PLA/5-EPS may be related to the surface properties of this film. As discussed above, PLA/5-EPS had the lowest water contact angle and the highest surface energy among the tested formulations, indicating greater surface wettability. Surface wettability and surface energy are known to influence the interaction of printing inks with polymer substrates and can affect ink spreading and the appearance of the printed layer (Aydemir et al., 2021; Poljaček et al., 2026). The improved color match observed for PLA/5-EPS may therefore be associated with its different surface characteristics.

The differences observed after printing may also be linked to the presence of EPS-KC66L at the film surface. Changes in EPS content and its distribution within the PLA matrix can affect surface wettability and, in turn, the interaction of the film with printing ink. This may partly explain why PLA/5-EPS showed a lower ΔE_{00} value than neat PLA. However, ΔE_{00} alone is not sufficient to demonstrate improved ink-film interaction. What can be clearly stated is that PLA/5-EPS showed the closest color match to the printed reference paper, together with the highest surface wettability among the tested films.

The gloss values decreased with increasing EPS-KC66L content in both unprinted and printed films. Before printing, gloss decreased from 21.5 GU for neat PLA to 18.3 GU for PLA/5-EPS. A similar decrease was observed after printing, from 22.1 to 18.7 GU. Changes were also seen in the CIELAB color parameters, particularly in the b* values at higher EPS-KC66L contents. These findings indicate that EPS-KC66L affected both the color and surface gloss of the PLA films.

Gloss gradually decreased as the EPS-KC66L content increased. Similar effects have been reported for biopolymer coatings and polymer films, where surface heterogeneity was found to influence specular gloss (Trezza & Krochta, 2001; Wittmann et al., 2021). The PLA-based films were also noticeably glossier than the reference paper, which is reasonable considering the different surface characteristics of polymer films and paper. Substrate properties have previously been shown to affect gloss, color, and print quality in PLA films (Izdebska-Podsiadły, 2021). PLA/5-EPS is particularly interesting in this respect. It had the lowest gloss after printing, but at the same time showed the lowest ΔE_{00}. In other words, this formulation gave the closest color match to the printed reference paper despite having a less glossy surface.

3.6. Ink Adhesion Behavior of PLA/EPS Packaging Films

The reference paper, neat PLA, and PLA/1-EPS received a score of 5, indicating very good ink adhesion. For PLA/2.5-EPS and PLA/5-EPS, the score decreased slightly to 4, with small amounts of ink observed on the tape after peeling. Despite this decrease, ink adhesion remained good for all PLA/EPS films. The slightly lower scores at 2.5% and 5% EPS-KC66L may be related to changes in the film surface. Ink adhesion to polymer substrates depends on several factors, including surface energy, wettability, and the interactions between the ink and the substrate (Aydemir et al., 2021). Since EPS-KC66L also affected the surface properties of the PLA films, these changes may have influenced their inter-

action with the printing ink. However, the difference was small, and even at the highest EPS-KC66L content, the films maintained good overall ink adhesion.

Adding a second component to PLA does not always have the same effect on ink adhesion. Studies on PLA-based blends have reported different adhesion behavior depending on the type and distribution of the added material and the resulting changes in surface wettability (Poljaček et al., 2026). In our films, the small decrease in adhesion at 2.5% and 5% EPS-KC66L appears to follow the changes observed in the surface properties. Still, the decrease was minor, and good ink adhesion was retained even at the highest EPS-KC66L content.

The frictional behavior of the films was evaluated according to ISO 8295. The reference paper had the lowest COF (0.30), compared with 0.40 for neat PLA. As the EPS-KC66L content increased, the COF rose to 0.41, 0.47, and 0.49 for PLA/1-EPS, PLA/2.5-EPS, and PLA/5-EPS, respectively. The steady increase in COF indicates greater resistance to sliding after the addition of EPS-KC66L.

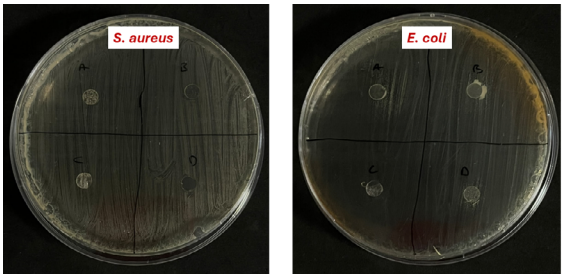
Friction is particularly relevant during film handling and converting, where contact with rollers and other machine surfaces affects web transport (Budinski, 2001). A higher COF can help limit slippage, although too much friction may also make film transport more difficult. In the present films, the increase was gradual, with the highest value measured for PLA/5-EPS.

It is also worth noting that COF, wettability, and ink adhesion did not change in the same way. COF increased steadily with EPS-KC66L content, whereas the contact angle first increased and then dropped at 5% EPS-KC66L. Ink adhesion showed only a small decrease at 2.5% and 5%. These differences are not unexpected, since ink–film interactions depend on several surface properties rather than on a single parameter (Aydemir et al., 2021; Izdebska-Podsiadły, 2021). Despite these changes, all PLA/EPS films retained good-to-very-good ink adhesion.

3.7. Antibacterial Activity of PLA/EPS Packaging Films

The antibacterial activity of the PLA/EPS films against Escherichia coli and Staphylococcus aureus was examined using an agar diffusion assay (Figure 7). No clear inhibition zone was observed around neat PLA (A) or any of the EPS-containing films (B-D). Increasing the EPS-KC66L content did not change this result.

The absence of an inhibition zone alone does not necessarily indicate a lack of antibacterial activity. In agar diffusion tests, the observed response depends on the release and diffusion of antimicrobial components from the material into the surrounding agar. If the active component remains within the polymer matrix



» **Figure 7:** *Agar diffusion assay of PLA/EPS films against E. coli and S. aureus. A: PLA control; B: PLA/1-EPS; C: PLA/2.5-EPS; D: PLA/5-EPS*

or is released only to a limited extent, a clear inhibition zone may not develop even when antibacterial activity occurs through direct contact. In this study, no clear inhibition zone was observed around the films, but no visible bacterial growth was seen on the film surfaces either. Based on this observation, the film surface may have limited bacterial growth to some extent.

4. Conclusions

Microbial EPS from *Ligilactobacillus salivarius* KC66L was successfully incorporated into PLA films without major changes in the chemical structure or thermal behavior of PLA. ATR-FTIR results were consistent with physical incorporation of EPS-KC66L, and only small changes were observed in the main thermal degradation region. The main differences appeared in the optical and surface properties of the films, and they did not always follow the EPS-KC66L concentration. The 5 wt% film was less transparent than neat PLA but had a lower water contact angle and higher surface energy. It also showed the closest color match to the printed reference paper, with ΔE_{00} decreasing from 4.71 for neat PLA to 1.82 for PLA/5-EPS. Increasing the EPS-KC66L content also reduced gloss and increased the coefficient of friction. Ink adhesion, however, remained good to very good for all formulations. No clear inhibition zones were observed against E. coli or S. aureus in the agar diffusion test. At the same time, no visible bacterial growth was seen on the film surfaces. Since this method relies on the release and diffusion of active components from the film, the absence of an inhibition zone does not exclude a possible contact-dependent effect. Of the formulations tested, PLA/5-EPS gave the best combination of surface wettability and color matching after printing, although its optical clarity was lower. Overall, the findings suggest that EPS-KC66L is a promising modifier for adjusting the surface and printing properties of PLA films. Its use in packaging will require a balance between the improved surface characteristics and the loss of transparency at higher EPS contents.

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