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Enhancing the Functional Properties of LDPE for Active Packaging: The Role of Cloisite 30B-Ascorbyl Palmitate Hybrids in Inducing Antimicrobial and Antioxidant Activities

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Abstract
This study presents a novel strategy for fabricating active food packaging by endowing low-density polyethylene (LDPE) films with antimicrobial and antioxidant properties. To achieve this, hybrid materials were synthesized by intercalating ascorbyl palmitate (AP) molecules into the galleries of Cloisite 30B nanoclays via an ultrasonication method. These Cloisite 30B-AP hybrids were subsequently incorporated into an LDPE matrix through melt-mixing to produce LDPE/(Cloisite 30B-AP) nanocomposite films. The structural properties of the synthesized hybrids and the resulting nanocomposite films were thoroughly characterized. Wide-angle X-ray diffraction (WAXD) analysis of the Cloisite 30B-AP hybrids revealed a significant increase in the d-spacing of the nanoclay compared to pristine Cloisite 30B. This expansion confirms the successful intercalation of AP molecules between the silicate layers. Furthermore, the WAXD pattern of the nanocomposite film indicated a high degree of dispersion of the Cloisite 30B-AP hybrids within the LDPE matrix. The optimized nanocomposite films demonstrated potent antimicrobial efficacy against both Gram-positive and Gram-negative bacterial strains. Concurrently, they exhibited substantial antioxidant activity, displaying a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity of up to 85%. Consequently, this work introduces a new, single active material—the Cloisite 30B-AP hybrid—that is highly effective at imparting dual-functionality, combining both antioxidant and antimicrobial characteristics, to polymeric food packaging materials.
Keywords: Nanocomposite, Ascorbyl Palmitate, Active Packaging, Antioxidant, Antimicrobial Films.

1. Introduction
Food packaging serves as a primary barrier, protecting contents from environmental factors that cause spoilage, including microbial contamination, oxygen, moisture, and radiation [1]. Among polymeric materials, polyolefins are extensively used in food packaging due to their low cost, favorable mechanical and thermal properties, and high chemical resistance. However, evolving socio-economic demands for enhanced food safety, hygiene, and cost-effectiveness have driven significant innovation in packaging technologies [2].

The field of packaging has undergone considerable transformation, with a growing recognition of the need for active food packaging systems. These advanced materials are designed to incorporate active agents, such as antioxidants or antimicrobials, to improve food preservation [2, 3]. Unlike conventional packaging, which acts as a passive barrier, active packaging plays a dynamic role in extending food shelf life. This study focuses on integrating such antimicrobial and antioxidant functionalities into low-density polyethylene (LDPE), one of the most prevalent food packaging polymers.

A major challenge in food safety is preventing microbial contamination and growth within packaged products. Pathogenic microorganisms can contaminate food and water supplies, leading to illness upon ingestion [4, 5]. Antimicrobial food packaging presents a viable solution to this problem by inhibiting or retarding the growth of microorganisms present on the food surface or within the package. Consequently, the development of packaging materials that actively reduce bacterial impact is a key objective of this work.

Conventional methods for creating antimicrobial polymers include chemically anchoring biocides to the polymer backbone [6, 7], using intrinsically antimicrobial polymers [8], or loading antimicrobial agents like quaternary ammonium salts, zinc oxide, or silver into the polymer matrix [9-11]. While effective, these approaches often face limitations, such as the leaching of active agents into food and potential toxicity concerns. To overcome these drawbacks and provide safer, non-toxic antimicrobial activity, research has shifted towards incorporating natural biopreservatives [12, 13]. In line with this, the present study explores the use of organically modified clays. Certain modified clays, owing to their cationic constituents, have demonstrated effective broad-spectrum antimicrobial activity [14], making them a promising candidate for integration into LDPE films.

Oxidative rancidity, driven by free radical reactions, is another leading cause of food deterioration [15]. Incorporating antioxidant activity into packaging materials is an effective strategy to mitigate this issue. Antioxidant active packaging functions by quenching free radicals, thereby halting the chain reaction of lipid and protein oxidation and ultimately increasing food shelf life [15, 16]. The predominant method for producing such packaging is the incorporation of antioxidant fillers during melt compounding. This requires the antioxidant to be stable under the high temperatures and aggressive processing conditions of polymer manufacturing. While researchers have incorporated low-molecular-weight antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) or essential oils [17, 18], significant losses can occur due to volatility, degradation, or premature radical scavenging during processing [19]. Therefore, the selection of a stable antioxidant agent is critical.

Ascorbyl palmitate (AP), a lipid-soluble derivative of ascorbic acid, is a highly effective antioxidant widely used in food, pharmaceutical, and cosmetic products [20, 21]. Despite its efficiency, its low chemical stability can limit its application in plastic processing [20, 22]. Thus, developing a method to incorporate AP into plastics while preserving its antioxidant activity during melt processing is essential for creating effective antioxidant packaging.

Encapsulation is a well-established technique for protecting active molecules from degradation under harsh chemical or thermal conditions, thereby minimizing activity loss [20]. In this study, we propose the use of layered clay as a protective carrier for AP. It is anticipated that incorporating a clay-AP hybrid into LDPE will yield an active packaging material with combined antimicrobial and antioxidant properties.

This work details the preparation of LDPE composites with Cloisite 30B-AP hybrids via melt compounding. The resulting blends were characterized using spectroscopic and thermal analytical techniques. Furthermore, their antimicrobial efficacy and free radical scavenging capacity (DPPH assay) were evaluated.

2. Materials and Methods

2.1. Materials

A commercial low-density polyethylene (LDPE, grade LH0075) with a melt flow index of 0.85 g/10 min and a density of 0.919 g/cm³ was supplied by Iran Petrochemical in granular form. The organically modified montmorillonite clay, Cloisite® 30B (modified with methyl, tallow, bis-2-hydroxyethyl, quaternary ammonium chloride), was obtained from Southern Clay Products. Ascorbyl palmitate (AP) was purchased from Sigma-Aldrich. All chemicals were of analytical grade and used without further purification. The microbial strains *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 25922) were acquired from the Iranian Research Organization for Science and Technology (IROST).

2.2. Preparation of Cloisite 30B-AP Hybrids

Cloisite 30B and ascorbyl palmitate (AP) were combined at a 1:1 weight ratio and dispersed using an ultrasonicator at room temperature for 30 minutes to form uniform Cloisite 30B-AP hybrid dispersions.

2.3. Preparation of LDPE Nanocomposite Films

LDPE nanocomposite films with varying compositions (Table 1) were prepared via melt compounding using a twin-screw extruder (L/D ratio of 25:1) at a screw speed of 150 rpm. The temperature profile was maintained at 160 °C from the feed zone to the die. The compounded material was pelletized after extrusion through a multistrand die.

Subsequently, films with a thickness of 60–70 μm were produced using a blown-film extrusion line. The barrel and die zones were set at 160 °C, and the screw speed was maintained at 150 rpm. All films were processed under identical conditions.

The Cloisite 30B content was fixed at 5 wt% for all nanocomposites to ensure consistent antimicrobial properties, based on previous literature and preliminary studies for this system [23, 24]. The detailed formulations are presented in Table 1.

Table 1. Formulations of the prepared LDPE nanocomposite films.

Composition	LD	LD-AP	LD-CB	LD-CBAP
LDPE	100	95	95	90
AP	-	5	-	-
Cloisite 30B	-	-	5	5
Cloisite 30B-AP	-	-	-	5

2.4. Measurement of Antioxidant Efficiency

The antioxidant efficiency of the films was evaluated by measuring their 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, following established methods for polymeric samples [25, 26]. Film specimens (1 cm × 1 cm) were immersed in 20 mL of a 100 μM methanolic DPPH solution and incubated in the dark at room temperature for 24 hours. The absorbance of the solution was then measured using a UV-Vis spectrophotometer. A control experiment, containing only the DPPH solution without a film sample, was conducted in parallel.

The DPPH scavenging activity (%) was calculated using the following equation:
Scavenging Activity (%) = $[(A_B - A_S) / A_B] \times 100$
where A_B is the absorbance of the blank control and A_S is the absorbance of the sample.

2.5. Antimicrobial Activity Assessment

The antimicrobial activity of the nanocomposite films was determined using the colony-forming count method according to ASTM E2180-07. Tests were conducted using *S. aureus* and *E. coli* with an initial inoculum concentration of 1.5×10^8 CFU/mL and film specimens sized 1 cm × 1 cm.
Overnight cultures of the microorganisms were adjusted to a concentration of 1.5×10^6 cells/mL. One milliliter of this adjusted culture was inoculated into 100 mL of a molten agar slurry (0.3% Agar-agar, 0.85% NaCl). A 100 µL aliquot of the inoculated slurry was pipetted onto the surface of each test film and an untreated control. After a 24-hour contact period, surviving microorganisms were recovered by eluting the agar slurry from the film into a neutralizing broth, followed by vortexing to ensure complete removal.
Serial dilutions of the eluent were prepared, spread on agar plates, and incubated at 37 °C for 48 ± 2 hours. The resulting colonies were counted, and the percentage reduction of microorganisms was calculated using the formula:
Reduction (%) = $[(a - b) / a] \times 100$
where a is the number of organisms recovered from the untreated control and b is the number of organisms recovered from the treated test sample.

2.6. Characterization

X-ray Diffraction (XRD): The intercalation of ascorbyl palmitate into the clay and the dispersion of nanoparticles within the polymer matrix were analyzed using a Philips X'Pert X-ray diffractometer with Cu Kα radiation (λ = 1.54 Å), operating at 40 kV and 40 mA. Scans were performed at ambient temperature over a 2θ range of 2°–10°.
Thermogravimetric Analysis (TGA): Thermal stability was assessed using a Polymer Laboratories TGA-1500 instrument. Samples were heated from room temperature to 600 °C at a rate of 10 °C/min under a nitrogen atmosphere.
Fourier Transform Infrared (FTIR) Spectroscopy: Chemical structures were analyzed with a Bruker IFS 48 spectrometer. Spectra were recorded in the range of 500–4000 cm^{-1} at a resolution of 4 cm^{-1}, averaging 16 scans per sample.
Transmission Electron Microscopy (TEM): The microstructure of the nanocomposites and the dispersion of clay layers were examined using a Philips EM2085 microscope operated at an accelerating voltage of 100 kV.

3. Results and Discussion

The incorporation of nanolayered silicates is an established strategy for modifying the properties of polymeric materials. Prior research has demonstrated that adding small quantities of modified nanoclays to produce polymer-clay nanocomposites can enhance mechanical and thermal properties [27]. Furthermore, nanocomposites containing exfoliated organoclay platelets exhibit superior barrier properties, as the dispersed clay layers create a tortuous path that impedes the diffusion of gases and vapors [28, 29].

As noted previously, certain organically modified clays can also impart antimicrobial activity to polymers [23, 24]. The biological efficacy of the clay is highly dependent on the chemical nature of its organic modifier. Cloisite 30B, which is modified with a quaternary ammonium salt (methyl, tallow, bis-2-hydroxyethyl, quaternary ammonium chloride), has been shown to possess significant intrinsic antimicrobial activity [23]. For this reason, it was selected for the present study.

Beyond its antimicrobial function, the organically modified clay in this work also serves as a carrier for ascorbyl palmitate (AP) molecules. The layered structure of Cloisite 30B was utilized to encapsulate and protect AP. In addition to its excellent antioxidant properties, AP features a long hydrophobic alkyl chain. This chain is expected to improve compatibility with the hydrophobic LDPE matrix through van der Waals interactions and potential entanglement with polymer chains, thereby promoting the durability of the antioxidant film.

3.1. Characterization of Cloisite 30B–AP Hybrids

The structure of the prepared Cloisite 30B-AP hybrid was first investigated using X-ray diffraction (XRD) and compared to pristine Cloisite 30B. As shown in Figure 1, the XRD pattern of pure Cloisite 30B exhibits a characteristic peak at $2\theta = 4.84°$, corresponding to a basal d-spacing of 18.34 Å. For the Cloisite 30B-AP hybrid, this peak shifted to a lower angle of $2\theta = 4.11°$, which corresponds to an increased d-spacing of 21.12 Å. The peak also broadened and experienced a slight reduction in intensity. This increase in d-spacing is indicative of the intercalation of AP molecules between the silicate layers, expanding the clay gallery height. This result confirms that Cloisite 30B can effectively function as a nanocarrier for AP.

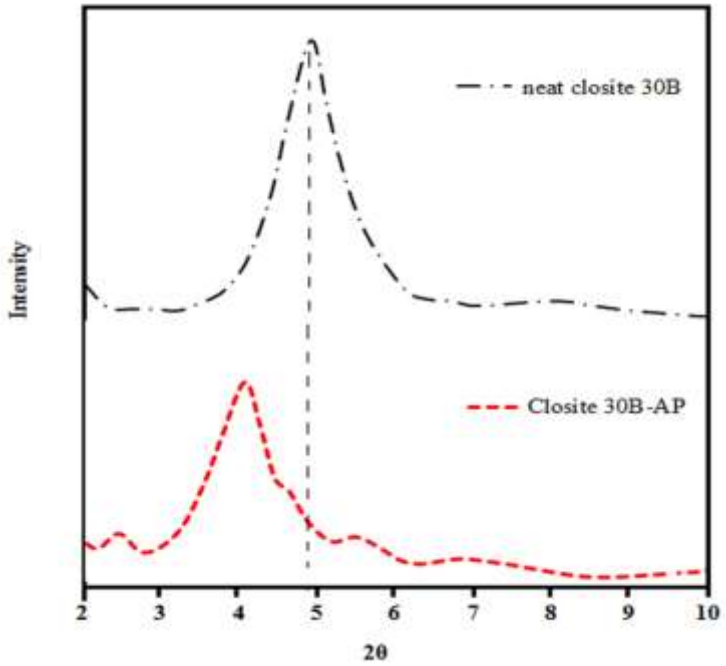

Figure 1. Wide-angle X-ray diffraction (XRD) patterns of pristine Cloisite 30B and the synthesized Cloisite 30B-Ascorbyl Palmitate (CB-AP) hybrid.

The formation of the hybrid was further confirmed by Fourier Transform Infrared (FTIR) spectroscopy (Figure 2). The spectrum of neat Cloisite 30B shows characteristic peaks: a broad

band at ~3400 cm^{-1} (O-H and N-H stretching vibrations), a strong band between 950–1100 cm^{-1} (Si-O stretching), and peaks at 1685 cm^{-1} and 1472 cm^{-1} (attributed to C=O stretching and C-N/N-H vibrations, respectively, from the tallow amide modifier).

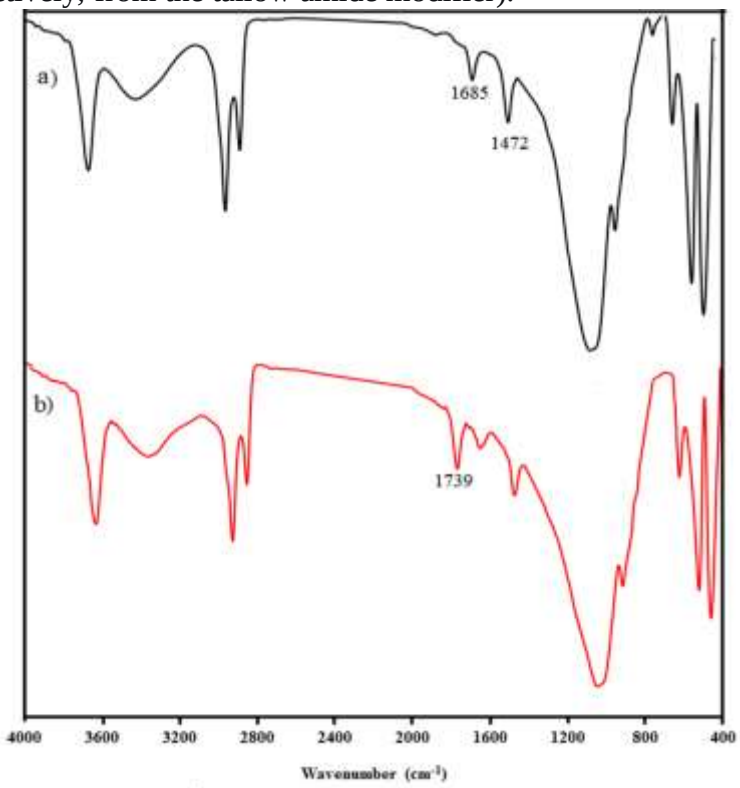

Figure 2. Fourier-transform infrared (FTIR) spectra of (a) neat Cloisite 30B and (b) the Cloisite 30B-Ascorbyl Palmitate (CB-AP) hybrid.

In the spectrum of the Cloisite 30B-AP hybrid, a new peak appears at approximately 1739 cm^{-1}. This peak can be assigned to the C=O stretching vibration of the ester group in the palmitate moiety of AP. Additionally, the broad O-H/N-H band around 3400 cm^{-1} is widened and shifted, suggesting the formation of hydrogen bonds between the hydroxyl groups of AP and the OH/NH groups on the clay or its modifier. These findings provide strong evidence for the successful incorporation of AP into the Cloisite 30B matrix.

3.1.1. Thermal Stability of Cloisite 30B-AP Hybrids

The thermal stability of the pure components and the hybrid was evaluated by thermogravimetric analysis (TGA), as shown in Figure 3. Pure AP undergoes a single, distinct degradation step beginning at approximately 220 °C, associated with the breakdown of its thermally labile structure. Neat Cloisite 30B begins to degrade around 275 °C due to the decomposition of its organic modifier.

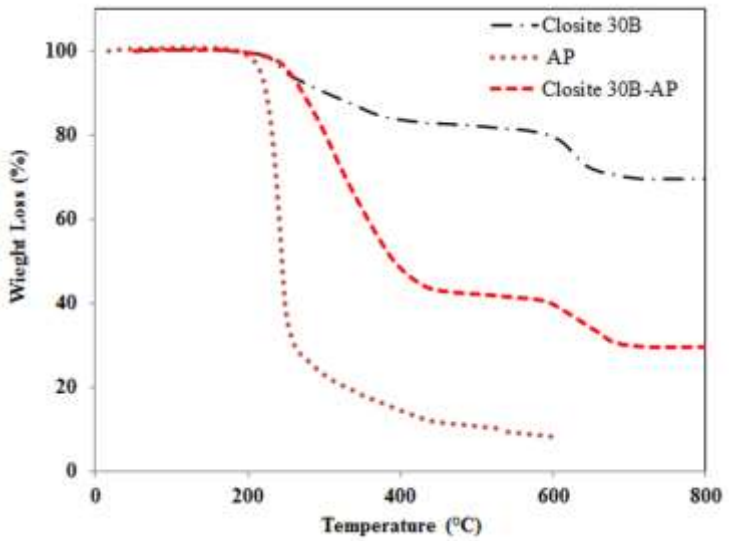

Figure 3. Thermogravimetric analysis (TGA) curves of pure ascorbyl palmitate (AP), neat Cloisite 30B, and the Cloisite 30B-AP hybrid.

Notably, the initial decomposition temperature of the Cloisite 30B-AP hybrid is higher than that of pure AP. This enhanced thermal stability is likely due to the barrier effect of the clay platelets, which act as an insulator and mass transfer barrier, protecting the intercalated AP molecules. The TGA results thus demonstrate that the clay significantly improves the thermal stability of AP, making the hybrid suitable for high-temperature melt processing.

To the best of our knowledge, this is the first report demonstrating the successful intercalation of AP into an organoclay to develop a protective carrier system. This approach is designed to shield the antioxidant molecules from thermal and oxidative degradation during processing. It is therefore anticipated that the antioxidant efficacy of AP will be preserved after its incorporation into LDPE via melt compounding.

3.2. Characterization of LDPE Nanocomposite Films

Nanocomposite films with different formulations (Table 1) were prepared by melt-compounding LDPE with a constant loading (5 wt%) of either ascorbyl palmitate (AP), Cloisite 30B (CB), or the pre-formed Cloisite 30B-AP (CB-AP) hybrid at 160 °C using a twin-screw extruder.

To investigate the dispersion of the CB-AP hybrid within the polymer, the X-ray diffraction (XRD) pattern of the LDPE/CB-AP (LD-CBAP) nanocomposite was compared to that of the neat hybrid (Figure 4a). The characteristic diffraction peak of the CB-AP hybrid at $2\theta = 4.11°$ disappeared in the LD-CBAP composite. This loss of peak definition suggests that the ordered layered structure of the clay was disrupted, leading to either intercalation or exfoliation of the silicate layers within the LDPE matrix. This enhanced dispersion is likely due to the expanded interlayer spacing of the hybrid and improved compatibility with the hydrophobic polymer.

Transmission electron microscopy (TEM) was employed to gain further insight into the nanoscale structure of the LD-CBAP film (Figure 4b). The TEM micrograph confirms a mixed morphology of intercalated and exfoliated clay layers, demonstrating the successful and homogeneous distribution of the CB-AP hybrid throughout the LDPE matrix.

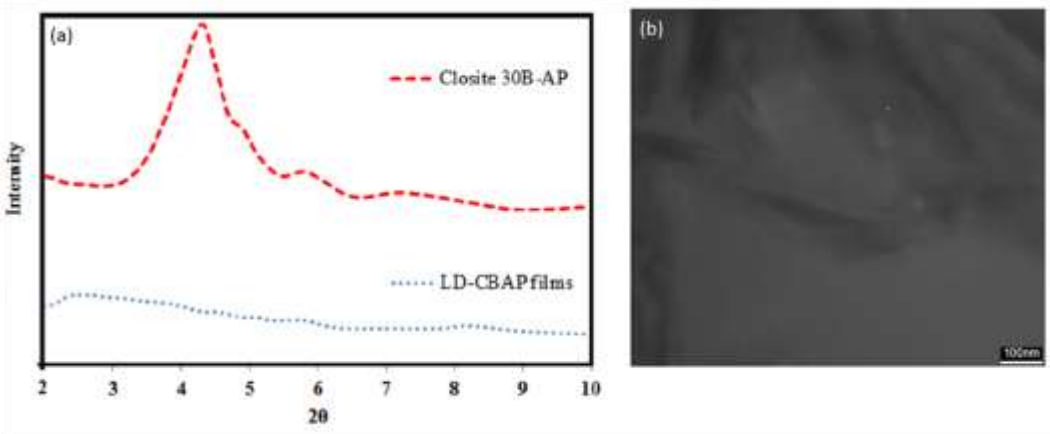

Figure 4. Structural characterization of the LD-CBAP nanocomposite: (a) XRD patterns of the Cloisite 30B-AP hybrid and the final LDPE/CB-AP (LD-CBAP) film; (b) Transmission electron microscopy (TEM) image showing the dispersion of clay layers within the LDPE matrix.

3.2.1. Antimicrobial Activity of Prepared Films

A key objective of this work was to develop packaging materials with effective antimicrobial properties. To this end, a constant amount (5 wt%) of Cloisite 30B was incorporated into the LDPE films as an antimicrobial agent. The antimicrobial efficacy of the resulting nanocomposites was quantitatively evaluated using the colony-forming count method, with results summarized in Figure 5 and Table 2.

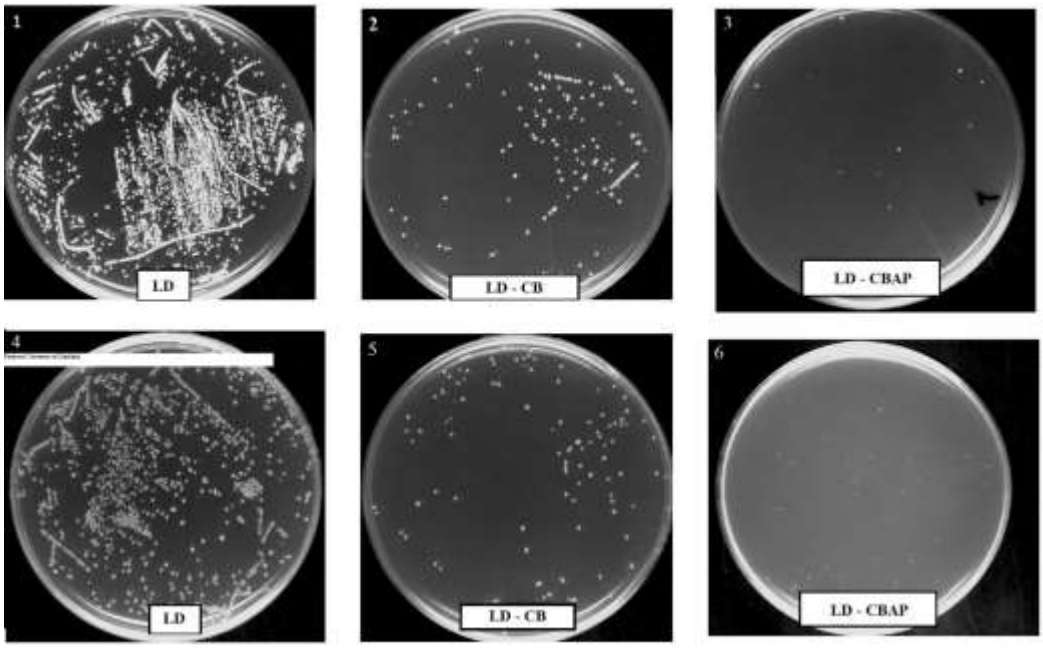

Figure 5. Antimicrobial activity of the prepared films against Escherichia coli (1-3) and Staphylococcus aureus (4-6).

As shown in Figure 5, neat LDPE (LD) exhibited no bactericidal activity. In contrast, the LDPE/Cloisite 30B (LD-CB) composite demonstrated significant antimicrobial activity. The LD-

CB film achieved a bacterial reduction of approximately 79% against the Gram-positive *S. aureus*. However, its efficacy against the Gram-negative *E. coli* was lower, with only a 35% reduction observed. This pronounced activity is attributed to the destructive electrostatic interaction between the positively charged quaternary ammonium groups of the organoclay and the negatively charged bacterial cell walls. The lower activity against Gram-negative bacteria is consistent with literature, as their cell wall structure, featuring an outer lipopolysaccharide membrane, presents a less accessible negatively charged surface, thereby reducing electrostatic interactions with the organoclay [23, 24].

Notably, the LD-CBAP nanocomposite film exhibited significantly enhanced antimicrobial activity against both bacterial strains compared to the LD-CB film. This synergistic effect can be attributed to the combined antimicrobial action of the clay and the incorporated AP molecules, which have been reported to possess intrinsic antibacterial properties [30]. Furthermore, the improved dispersion of the clay layers in the LD-CBAP film, as confirmed by TEM, may provide a greater active surface area, further enhancing antimicrobial efficacy. These results indicate that the LD-CBAP films function as an effective barrier against bacterial growth and are promising for use as active packaging materials.

3.2.2. Antioxidant Activity of Prepared Films

Antioxidant packaging materials can significantly inhibit lipid oxidation, thereby extending the shelf life of food products. Ascorbyl palmitate (AP) is known for its excellent free radical scavenging capacity. It was anticipated that incorporating AP into the packaging material would impart antioxidant functionality. The antioxidant activity of the films was assessed by measuring their ability to scavenge the stable DPPH free radical, which shows a characteristic absorption peak at 517 nm. A decrease in this peak's intensity correlates with the sample's radical scavenging capacity.

As illustrated in Figure 6, neat LDPE (LD) and the LD-CB composite induced only a minor decrease in the DPPH absorption peak. This minimal activity can be attributed to the presence of residual processing antioxidants, such as butylated hydroxytoluene (BHT), commonly added to commercial polyolefins to prevent thermal degradation [31]. In contrast, the AP-containing samples (LD-AP and LD-CBAP) showed a progressive and significant reduction in the DPPH peak intensity. The scavenging mechanism involves the donation of hydrogen atoms from AP molecules to the DPPH radicals, thereby neutralizing them.

Table 2. Antibacterial efficacy of the prepared films, presented as percentage reduction of bacterial colonies.

Samples	E. coli	S. aureus
LD	0	0
LD-CB	35	79
LD-AP	10	11
LD-CBAP	48	96

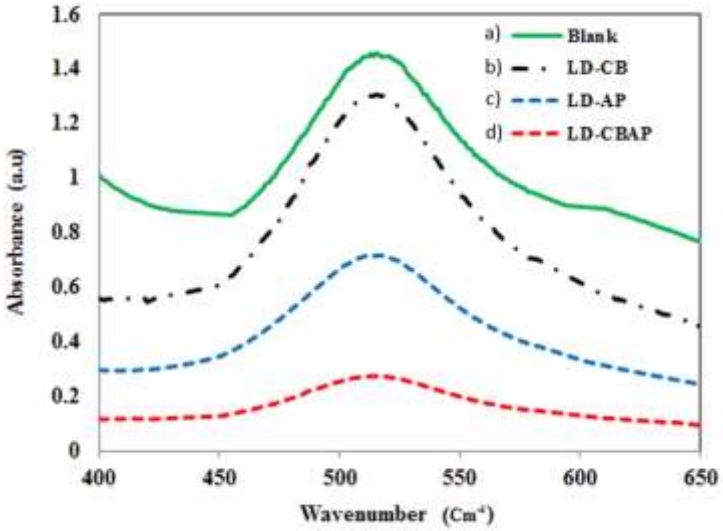

Figure 6. UV-Vis absorption spectra tracking the scavenging of DPPH• radicals by the prepared films: (a) neat LDPE (LD), (b) LDPE/Cloisite 30B (LD-CB), (c) LDPE/Ascorbyl Palmitate (LD-AP), and (d) LDPE/Cloisite 30B-AP hybrid (LD-CBAP).

Critically, the LD-CBAP film demonstrated a more pronounced decrease in the DPPH peak intensity than the LD-AP film at an equivalent AP concentration (Figure 7). This enhanced antioxidant performance is directly linked to the protective role of the clay. The clay layers act as a carrier, shielding the intercalated AP molecules from thermal and oxidative degradation during the aggressive melt-compounding process. Consequently, a greater proportion of AP molecules in the LD-CBAP film retain their active sites, leading to superior radical scavenging activity compared to the LD-AP film, where unprotected AP is more susceptible to deterioration.

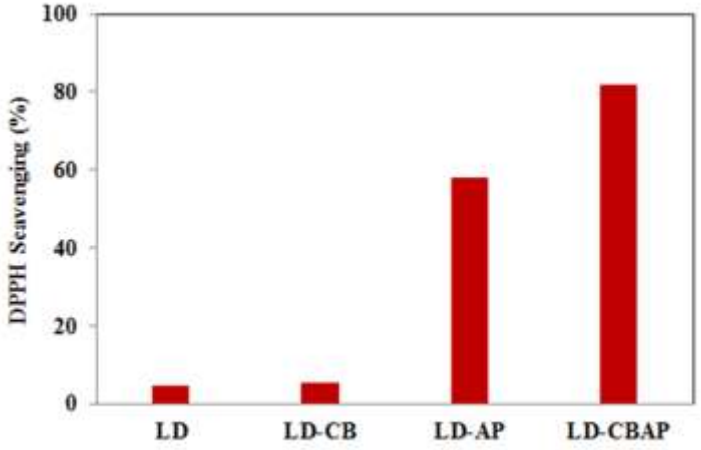

Figure 7. Quantitative assessment of the antioxidant activity for the prepared films, determined by the DPPH radical scavenging assay.

4. Conclusion

Microbial contamination and lipid oxidation are major challenges in food packaging, significantly impacting product quality and shelf life. The development of packaging materials with integrated antioxidant and antimicrobial properties presents a compelling strategy to address these issues.
In this study, a novel single-active material, a Cloisite 30B-ascorbyl palmitate (CB-AP) hybrid, was successfully synthesized and incorporated into LDPE via melt compounding to produce active packaging films. The resulting LDPE/(Cloisite 30B-AP) nanocomposite films exhibited superior performance compared to control materials. They demonstrated:
Enhanced Antimicrobial Activity: Significantly higher efficacy against both Gram-positive and Gram-negative bacteria compared to films containing only Cloisite 30B, indicating a synergistic effect between the clay and AP.
Superior Antioxidant Capacity: A greater DPPH radical scavenging effect than films with directly incorporated AP, confirming that the clay carrier effectively preserves the antioxidant activity of AP during processing.
The successful formation of an intercalated/exfoliated structure, as confirmed by XRD and TEM, was crucial for this performance. These findings demonstrate that the LDPE/CB-AP nanocomposite films possess a high potential for active food packaging applications, offering a dual-functionality of broad-spectrum antimicrobial capability and promising antioxidant activity in a single material.

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