CHEMICAL ENGINEERING TRANSACTIONS
VOL. 112, 2024
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Selena Sironi, Laura Capelli

ISBN 979-12-81206-13-7; **ISSN** 2283-9216
DOI: 10.3303/CET24112040

Antibacterial and Antioxidant Performance of Thermoplastic Starch/Polyethylene Active Film Packaging through the Incorporation of Aloe Vera Gel

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The thermoplastic starch-polyethylene (TPS/PE) film was introduced many years ago as biodegradable packaging. However, the lack of intrinsic antibacterial and antioxidant capabilities is an issue since these properties are essential for preventing foodborne pathogens and oxidation. In this paper, aloe vera (AV) gel was utilized as an active component to enhance the functionality of TPS/PE film. This paper aimed to investigate the antibacterial and antioxidant properties of TPS/PE film upon adding AV gel. Four different samples (TPS, TPS/AV, TPS/PE, and TPS/PE/AV) were prepared via melt-blending and hot-pressing techniques focusing on visual appearance, functional group, transparency, antibacterial, and antioxidant properties. A melt-mixer was used to prepare the resin, which was then hot-pressed to form a film. The TPS appeared colourless, but after the addition of AV gel, the film turned yellowish caused by crosslinking in the polymer matrix between TPS and AV gel. The TPS/PE/AV film was the most transparent, while the TPS film was the opaquest. The growth of gram-negative bacteria (Escherichia coli) was inhibited with a 1 mm inhibition zone around the films that contained AV gel. After adding AV gel, the TPS/PE film demonstrated 2.2 % DPPH scavenging activity in a 95 % ethanolic solution. In summary, this paper demonstrated that adding AV gel improved the TPS/PE functionality, enabling it to be utilized as an active biodegradable food packaging film.

1. Introduction

Food spoilage can occur during preparation, distribution, or while on the market, posing a significant problem for the food industry. Two major issues in guaranteeing food safety are contamination by foodborne pathogens and deterioration caused by food oxidation. Foodborne diseases are a global issue, affecting nearly one-tenth of the population and resulting in 420 000 deaths each year (World Health Organization, 2022). These diseases are caused by consuming food contaminated with pathogens such as *Escherichia coli* (*E. coli*). Harvested vegetables may become contaminated due to mishandling during storage and transportation. Meanwhile, oxidation deteriorates food, leading to unpleasant flavors and odors. This process can occur when foods are exposed to air, commonly affecting the lipid content of the food.
In this context, active and environmentally friendly food packaging has caught attention as a way of protecting foods against bacterial growth and oxidation while simultaneously reducing packaging waste. Starch is most often utilized biopolymer as a raw material for producing films due to its high amylose content, which provides strength to the film (Mohd Nizam *et al.*, 2021). Unfortunately, starch alone has bad mechanical properties, which are fragile and have brittle characteristics (Yusof, Jai and Hamzah, 2020). Although starch-based films are brittle, they can be improved by adding plasticizers such as glycerol to form thermoplastic starch (TPS). Plasticizing agent are generally essential to overcome the weakness of film, mainly brittleness (Manshor *et al.*, 2018). However, compared to synthetic-based polymers, TPS's poor mechanical properties make it unsuitable

Paper Received: 4 May 2024; Revised: 16 June 2024; Accepted: 30 July 2024
Please cite this article as: Abd Karim S.F., Azmi M.N.A., Abd Razak N., Adnan S.A., Mulana F., 2024, Antibacterial and Antioxidant Performance of Thermoplastic Starch/Polyethylene Active Film Packaging through the Incorporation of Aloe Vera Gel, Chemical Engineering Transactions, 112, 235-240 DOI:10.3303/CET24112040

for food packaging applications. Tanjung et al. (2023) reported that adding polyethylene (PE) to TPS improved the film's mechanical properties. However, TPS/PE-based film does not possess intrinsic antibacterial and antioxidant capabilities.

Aloe vera (AV) gel is a biopolymer with functional components that can be utilized as a film material for active packaging. The presence of salicylic acid, cinnamic acid, sulfur, and lupeol in AV gel is expected to give it antimicrobial properties. Whereas its antioxidative potential comes from phenolic and polysaccharide (e.g., mannan, acemannan, glucomannan) components (Dagmara, Katarzyna and Krzysztof, 2020). Previous studies by Gürler (2023) and Bajer et al. (2020) incorporated AV gel into starch-based film to improve its microbial resistance and extend the shelf-life of packaged foods. However, a few studies have investigated AV gel's effect on the characteristics of TPS/PE-based film for active food packaging applications. Additionally, most studies utilized the film-forming solution method to prepare the film, which does not accurately reflect real-world industrial practices.

Therefore, this paper aimed to investigate the changes in the antimicrobial performance of the TPS/PE-based film after adding AV gel. In support of the antimicrobial performance, the presence of antioxidant activity should validate the antibacterial performance. On top of that, the film characterization, like thickness, transparency, and functional group, should also be monitored to prove that AV was entrapped/crosslinked in the prepared polymer matrix based on TPS/PE. This study proved AV sustained in the TPS/PE polymer matrix and inhibited the growth of *E-Coli*.

2. Methodology

2.1 Materials

Powdered soluble potato starch brand Bendosen (Laupik Chemical, Malaysia), glycerol with a molecular weight of 92.09 g/mol (Chemiz (M) Sdn. Bhd., Malaysia), low-density polyethylene with a density of 0.915 g/cm^3 (Vistec Technology Services, Malaysia), and aloe vera gel (Chemieconnex, Malaysia).

2.2 Preparation of the film

The moisture content of the starch was removed by drying process in a universal oven (UFP800, Memmert) at 70 ℃ for 24 h. Then, 21 g of starch was mixed manually at room temperature with 9 g of glycerol to form thermoplastic starch (TPS). The formula and procedure to prepare the film were based on a previously established method (Siti Fatma *et al.*, 2022). Then, the TPS was sealed and stored in a desiccator for 24 h. TPS was melt-blended with polyethylene (PE) and aloe vera (AV) gel in a melt-mixer (HAAKE PolyLab OS RheoDrive 7, Thermo Scientific) at 170 ℃, 60 rpm, 30 min to produce a solid resin. The samples were produced with different formulations, as listed in Table 1 . Then, remove the solid resin and crushed using a compact crusher (HMRV50-19, Rexmac) to reduce its bulk structure to a smaller size. Then, the crushed resin was subjected to a hot-pressing technique using a hot-press machine (QC-602A, Cometech) to form a film. Three steps were involved during the hot-pressing process: 1) pre-heating at 130 ℃, 10 min to ensure uniform heat transfer distribution across the sprinkled resin on the plate; 2) hot-pressing at 130 ℃, 700 psi, 10 min; and 3) cooling the film to 40 ℃ while tap water was running. Finally, the film was peeled off the plate and placed in a desiccator.

Table 1: The film's formulation

Samples	Starch (g)	Glycerol (g)	PE (g)	AV gel (g)
TPS	21	9	-	-
TPS/AV	21	9	-	9
TPS/PE	21	9	3	-
TPS/PE/AV	21	9	3	9

*21 g of starch and 9 g of glycerol, representing a 70:30 ratio
*3 of g PE, representing 10% of the total amount of starch and glycerol
*9 g of AV gel, representing 30% of the total amount of starch and glycerol

2.3 Thickness, Visual Appearance and Transparency of the Film

The thickness of the rectangular films (400 mm × 300 mm) was measured at three positions (bottom, middle, and top) using a digital micrometer (0-25 mm Mitutoyo 293-230-30). Triplet data were obtained and the average value was recorded. Additionally, the visual appearance of the films was photographed using a smartphone camera (Apple iPhone 11 Pro). The transparency (in terms of opacity) of the films was determined using a UV-vis spectrophotometer (Lambda 750, PerkinElmer) at a wavelength of 600 nm, following the methods used by another researcher. The opacity of the film samples (400 mm × 300 mm) was calculated according to Eq (1) (Domene-López *et al.*, 2019):

$$Opacity = \frac{Abs_{600}}{x} \tag{1}$$

where: Abs_{600} is the absorbance measured at 600 nm, and x is the thickness (mm) of the films. Triplet data were obtained.

2.4 Functional Group

The functional groups in the film were identified using a Fourier transform infrared (FTIR) spectrometer (Spectrum One, PerkinElmer). The spectrum ranged between 4000-500 cm^{-1} with a resolution of 4 cm^{-1} and 64 scan times.

2.5 Antibacterial Activity

The antibacterial activity of the films was assessed using the Kirby-Bauer test against *Escherichia coli* (*E. coli*), a gram-negative bacterium. Initially, circular film samples (diameter of 6 mm) were sterilized. A petri plate containing Mueller-Hinton agar was prepared and inoculated with *E. coli*. Then, sterilized films were placed on the inoculated agar and incubated at 37 ℃ for 24 h. Finally, the diameter of the inhibition zone around each film was measured.

2.6 Antioxidant Activity

The antioxidant activity of the films was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, following a previously reported method (Janani *et al.*, 2020). DPPH, a free radical, was utilized to determine the capacity of antioxidants to neutralize it. As antioxidants interacted with the DPPH radical, the color of the reaction mixture changed from purple to yellow, leading to a decrease in absorbance value. This method served to assess the antioxidant properties of the films. The films (30 mm × 30 mm) were immersed in 100 mL of 95 % ethanol for 24 h. The ethanolic solution represented as a simulant for fatty foods (Janani *et al.*, 2020). Then, 1 mL of the extracted solution of the films was mixed with 2 mL of 0.17 mM DPPH in methanol. The mixture was left in a dark place for 30 min. Then, the absorbance of the mixture was measured at 517 nm using a UV-vis spectrophotometer (Lambda 750, PerkinElmer). The antioxidant activity of the films was quantified in terms of DPPH inhibition, calculated using Eq (2):

$$DPPH\ inhibition\ (\%) = \frac{A_c - A_s}{A_c} \times 100 \tag{2}$$

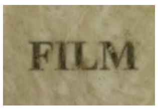
where: A_c is the absorbance of the control solution (1 mL distilled water + 2 mL DPPH), and A_s is the absorbance of the sample solution (1 mL sample + 2 mL DPPH).

3. Results

3.1 Thickness and Visual Appearance

Table 2 shows the thickness and visual appearance of the film after hot-pressing. Upon the addition of AV and PE into TPS, the thickness of the TPS film increased. TPS film was the thinnest, whereas TPS/PE/AV film was the thickest. Adding PE and AV gel to TPS increased the film's total solid content. Pinzon et al. (2018) reported that adding polyphenolic compounds in aloe vera gel to starch-chitosan blend films has a crosslinking effect that increases thickness.

Table 2: The thickness and visual appearance of the film after hot-pressing

Samples	TPS	TPS/AV	TPS/PE	TPS/AV/PE
Thickness	0.874 ± 0.014	0.953 ± 0.014	0.912 ± 0.006	0.964 ± 0.005
Film after hot-pressing				
Opacity	1.312 ± 0.4602	0.444 ± 0.4206	1.153 ± 0.4333	0.401 ± 0.4110

Based on Table 2, the addition of PE into TPS did not significantly change the film's color. However, the addition of AV gel caused significant changes in the color of the films, turning them yellow. During the crosslinking between TPS and AV at 170 °C operating conditions, the increasing concentration of solids, including the organic acids and small dispersed bioactive polymers in AV gel, leads to this yellowness. This is in agreement with the previous study that found the yellowness index of starch film incorporated with citric acid increased at 165 °C (Reddy and Yang, 2010). Therefore, it was suggested that the organic acids in AV gel contributed to the yellowness of TPS/AV and TPS/PE/AV films due to exposure to high temperatures during the melt-blending process. The findings align with those of Abd Karim, Idris, et al. (2022), who observed increased film yellowness upon adding aloe vera gel to thermoplastic starch-based blends.

3.2 Transparency

High transparency in films for food packaging applications is crucial, as it facilitates visual inspection of food freshness and appearance before purchase. Opacity data was tabulated in Table 2. The data showed that the opacity values of TPS/AV and TPS/PE/AV were significantly lower than those of TPS and TPS/PE, indicating that the addition of AV gel increased film transparency. These results were consistent with studies by Ortega-Toro et al. (2017), who had observed that films made from starch with a higher aloe vera ratio increased film transparency due to aloe vera's impact on the film's microstructure (starch crystallization and polymer chain arrangement), influencing light scattering by particulate matter. Additionally, Gutiérrez & González (2017) produced plantain flour-based films with higher aloe vera gel content exhibited smoother surfaces, as the aloe vera gel helped to reduce surface roughness. This decrease in roughness led to greater transparency in the films because the smoother surfaces minimized light scattering. Therefore, this paper proposed that TPS and TPS/PE, with lower transparency, could be employed when shielding food from light is necessary. On the other hand, TPS/AV and TPS/PE/AV, with higher transparency, may ease visual inspection of the food.

3.3 Functional Group

Figure 1 shows the FTIR spectra obtained for the TPS-based films. The peaks were then tabulated as listed in Table 3. The primary raw material was TPS, which exhibited peaks at 3305 cm^{-1}, 2928 cm^{-1}, and 1015 cm^{-1}. The addition of AV gel did not have a significant impact on peak presence since all peaks for TPS/AV and TPS/PE/AV resembled the previously mentioned TPS peaks. TPS and AV gel exhibited O-H functional groups, representing water in the material. The combination of TPS and AV gel widened and sharpened the peak of the O-H functional group in TPS/AV and TPS/PE/AV. Bajer et al. (2020) also attributed this peak to O-H stretching groups of aloe vera gel constituents (e.g., uronic acid, mannose, galacturonic acid, aloin, or emodin). Adding PE to TPS sharpened the 2928 cm^{-1} peak and formed a new peak at 2849 cm^{-1}. These peaks were attributed to the presence of C-H functional groups in PE at 2915 cm^{-1} and 2848 cm^{-1} (Panrong, Karbowiak and Harnkarnsujarit, 2020). The spectrum between 1600-500 cm^{-1} was identified as a fingerprint region for polymer blends. The combination of TPS and AV gel resulted in stronger peaks at 1015 cm^{-1} and 1017 cm^{-1} for TPS/AV and TPS/PE/AV, respectively. Bajer et al. (2020) stated that these intense peaks at 1200-900 cm^{-1} are caused by the C-O-C stretching vibration of polysaccharides in both aloe vera gel and starch.

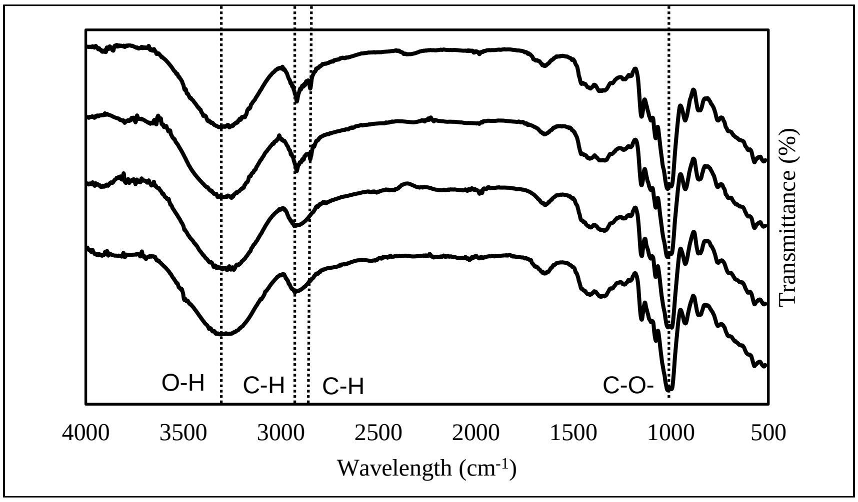


Figure 1: FTIR spectra for TPS-based films

Table 3: Signals of the FTIR spectra

Samples	O-H	C-H (methyl & methylene group)	C-H (methoxy group)	C-O-C
TPS	3305	2928	-	1015
TPS/AV	3274	2930	-	1015
TPS/PE	3253	2918	2849	1016
TPS/PE/AV	3264	2918	2849	1017

3.4 Antibacterial Activity

Films with antibacterial properties could hinder microbial growth on the surface of food. Figure 2 shows visual images of the inhibition zone around the films against gram-negative bacteria (*E. coli*). The inhibitory zone was measured and recorded in Figure 2. The TPS and TPS/PE did not exhibit growth inhibition zones against *E. coli*. Fortunately, the addition of AV gel to TPS and TPS/PE showed slight antibacterial efficacy against bacterial growth. The inhibition diameters for TPS/AV and TPS/PE/AV were 1 mm, respectively. This revealed that the active components like aloin, aloe-emodin, and cinnamic acid in AV gel appeared to impart antibacterial properties to TPS-based films. Similarly, Gürler (2023) had reported that an increment of the inhibition zone around 2 mm upon the addition of aloe vera to the chitosan-gelatin-starch composite film inhibited the growth of *E. coli*. Therefore, this paper presented TPS/AV and TPS/PE/AV films as potential options for preventing foodborne pathogens.

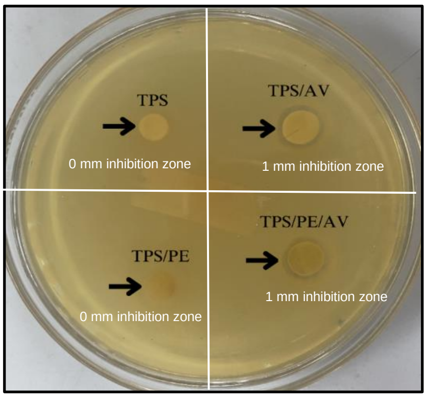


Figure 2: Effect of AV gel on antibacterial activity in TPS-based films against E. coli

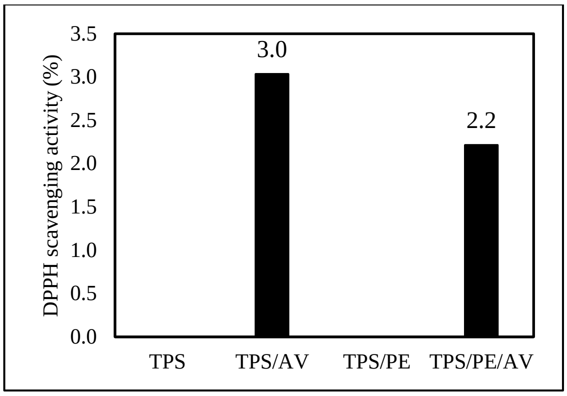


Figure 3: Effect of AV gel on antioxidant activity in TPS-based films

3.5 Antioxidant Activity

Figure 3 shows the antioxidant activity values for TPS-based films. TPS and TPS/PE exhibited no antioxidant activity. TPS/AV and TPS/PE/AV demonstrated 3.0 % and 2.2 % antioxidant activities, respectively. These results were also in agreement with another study conducted by Gürler (2023) and Hadi et al. (2021), who suggested that the antioxidant activity of aloe vera could be attributed to the presence of phenolic compounds. Addition of AV gel into chitosan/gelatin/starch blend increased by 5% DPPH scavenging activity (Gürler, 2023).The presence of an O-H peak between 3253 cm^{-1} and 3305 cm^{-1} in section 3.4 proved the presence of phenolic compounds. These compounds possess redox properties and can absorb and neutralize free radicals. Therefore, this paper demonstrated that TPS/AV and TPS/PE/AV films could prevent lipid oxidation and maintain food quality.

4. Conclusions

In this paper, TPS-based films incorporated AV gel were successfully fabricated using melt-blend and hot-press methods. It was observed that the addition of AV gel increased the yellowness and thickness of TPS-based films, while thicker films provided a more robust physical barrier against microbial penetration. AV gel films were the most transparent compared to films without AV gel. TPS/AV and TPS/PE/AV films exhibited antimicrobial activity against *E. coli* after 24 hours of incubation, which was proved by the presence of a phenolic compound that can disrupt cell membranes. Adding AV gel to TPS and TPS/PE demonstrated antioxidant activity attributed to AV's bioactive compounds, such as polysaccharides and polyphenols. Thus, supported the antimicrobial performance due to the presence of phenolic compounds. Overall, TPS/PE/AV offered significant potential as active packaging materials to help prevent bacterial growth and oxidative damage to fatty foods.

Acknowledgments

Special thanks to the College of Engineering for supporting the process of completing the research activities, which involved a few laboratories. An appreciation is extended to Universiti Teknologi MARA (Shah Alam), particularly the School of Chemical Engineering, for providing the necessary materials and instruments for this work.

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