

Physicochemical properties and antibacterial activity of cornstarch-based films incorporated with lemongrass (*Cymbopogon citratus*) essential oil

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Abstract

Bioplastic films prepared from cornstarch incorporated with lemongrass essential oil (LEO) were evaluated in terms of their physicochemical and antibacterial properties. The films were prepared via solution casting to contain different amounts of LEO (0%, 0.5%, 1%, and 2% w/w). The films were characterized for thickness, color properties, moisture content, tensile strength, and elongation at break. Antibacterial activity against *Staphylococcus aureus* (BIOTECH 1582) and *Salmonella typhimurium* (BIOTECH 1826) was determined via disk diffusion using 6 mm film discs on Mueller–Hinton agar. Significant improvement in elongation at break ($25.06 \pm 7.39\%$) at 0.5% LEO, and increase in film thickness and moisture content with LEO addition were observed. All films containing LEO displayed strong antibacterial activity. Interestingly, the control film (0% LEO) exhibited notable baseline antibacterial activity (inhibition zones >29 mm) probably due to the vinegar and glycerol in its formulation. Considerable increase in antimicrobial activity occurs with 2% LEO against *S. aureus*. Overall, films prepared with 0.5% LEO showed the best possible balance between mechanical flexibility and antimicrobial activity, suggesting that this formulation may be used in preparing active food packaging materials.

Keywords: antibacterial packaging, elongation at break, microbial zone of inhibition, tensile strength

Introduction

Food packaging plays a crucial role as it protects food ingredients and products from potential damage and deterioration (Petkoska et al., 2021; Shanbhag et al., 2023). Conventional food packaging includes paper, glass, plastics, and metals. Although over 20% of paper and paperboard are recycled, various plastics are recycled at a very low rate (< 20%) (Jeevahan & Chandrasekaran, 2019). Thus, the common practice of discarding packaging materials

especially conventional plastics poses a serious environmental problem, as these are widely used and are non-biodegradable. Moreover, conventional plastics are derived from non-renewable petroleum reserves and generate carbon dioxide and toxic substances when incinerated (Petkoska et al., 2021).

Due to pressing issues surrounding the use of conventional plastics, the food industry now considers the development of biodegradable, active, and edible packaging materials among its top priorities. These alternative packaging

materials include films that are prepared from edible materials such as lipids, proteins, and polysaccharides. These bioplastic films may be formed into wraps, pouches, bags, capsules, and casings (Jeevahan & Chandrasekaran, 2019).

In recent years, starch-based films have attracted considerable attention due to their inherent biodegradability, high availability, and low cost (Dorado et al., 2017). Starch is a natural polymer that may be formed into colorless, odorless films with low oxygen permeability (Cano et al., 2014; Garcia et al., 2020). However, starch-based films suffer from high water vapor permeability because of the abundance of hydroxyl groups in starch, and likewise exhibit poor mechanical properties (Dorado et al., 2017; Garcia et al., 2020). To overcome these drawbacks, a plasticizer is added to the film matrix to improve flexibility and mechanical properties (Youssef & El-Sayed, 2018). Low-molecular weight polyols are often used as plasticizers, with glycerol being the most studied polyol. Molecular weight is associated with the efficacy of a plasticizer: the smaller the molecule, the greater the plasticizing effect. Aside from plasticizers, crosslinking agents such as vinegar can be added to improve the film's strength. Vinegar is an acetic acid solution that releases acetate and hydrogen ions that, in turn, react with starch polymers so that they become disordered. This disorder arising from the ionization of water and acetic acid renders the film more homogenous (Shanbhag et al., 2023).

To obtain unique functionality, additives such as antimicrobial agents, colors, and flavors are incorporated into the starch matrix depending on its intended use (Bilal, Zhao & Iqbal, 2020). For example, to prevent microbial growth, starch-based films can be infused with antimicrobial agents such as essential oils (EOs). When incorporated into the film matrix, EOs can be released into the food upon contact with the film surface (do Evangelo, 2019). Furthermore, EOs serve to reduce the water vapor permeability of starch-based films (Garcia et al., 2020). EOs have an oily and volatile nature which influence the structural characteristics and degree of hydrophobicity of polymeric films, thus altering their mechanical and barrier properties (do Evangelo et al., 2019; Atares & Chiralt, 2016).

EOs are the best replacement to petroleum-derived additives in food packaging materials

because they are naturally abundant, eco-friendly, and possess superior antimicrobial and antioxidant attributes (Zubair et al., 2022). However, although the antimicrobial properties of several EOs in biodegradable films have been widely studied, there are very few reports on their effects on film properties (do Evangelo et al., 2019). Some such reports include works on starch/chitosan films infused with lemongrass essential oil (LEO) wherein antimicrobial activity was evaluated along with water vapor permeability, water solubility, and mechanical properties (Perdana et al. 2021); on cassava starch/chitosan films incorporated with LEO where antimicrobial activity and mechanical properties were evaluated against storage time (Perdana et al., 2022); and, on cornstarch films containing *Zanthoxylum bungeanum* essential oil (ZYO) wherein antimicrobial activity and physical characteristics (morphology, optics, mechanical and barrier properties) were reported (Wang et al., 2020). In this study, we will report a combination of microbiological (efficacy against Gram-positive and Gram-negative bacteria) and physicochemical (surface color, transparency, moisture content, tensile strength, elongation) evaluations of LEO-infused films prepared from cornstarch, glycerol, and vinegar, with focus on the effects of varying LEO concentrations (0.5–2% w/w LEO). As such, this study centers on the potential of cornstarch matrices combined with commonly available plasticizer and acid to help address the dual challenge of environmental pollution from synthetic packaging and food spoilage due to microbial contamination. Furthermore, this study provides insights into optimizing film composition for mechanical performance and antimicrobial efficacy.

Methodology

Preparation of films

Commercial cornstarch (Cream pure cornstarch), distilled water (Absolute), and 5% acidity vinegar (Heinz distilled vinegar) were purchased from a local supermarket. Laboratory-grade glycerol (Scharlau A.R.) and LEO were procured from Chemline Scientific Corporation and Young Living Philippines, respectively.

The cornstarch-based films were prepared via solution casting according to the methods of Resianingrum et al. (2016), do Evangelo et al. (2019) and Wang et al. (2020) with some modifications. The starch-based mixture consisted of distilled water, cornstarch, vinegar, and glycerol to which LEO was added at different amounts. Films were prepared by mixing cornstarch (7.67% w/w), glycerol (7.67% w/w), and vinegar (7.67% w/w) in water (77% w/w) and heated at 80–85 °C using a covered double-boiler steamer for 15 min to prevent volatilization of oil. LEO was added directly to the solution at 0%, 0.5%, 1%, and 2% w/w of total solution weight (Table 1) and stirred thoroughly. The mixture (Figure 1a) was cast onto wax-paper-lined trays (380 × 280 mm) and dried at 40 °C for 72 h. Dried films were stored in desiccators until analysis. The preparation of packaging films was done in triplicates for each treatment.

Film thickness

The thickness of the films was measured as the average of ten measurements taken at the center and periphery of the film using a micrometer caliper.

Tensile strength and elongation at break

The tensile strength (TS) and percent elongation at break (%E) of the sheets were determined according to the ASTM D882 (Standard Test Method for Tensile Properties of Thin Plastic Sheeting) using an Instron Universal Testing Machine (Model 5585H). All

film samples were pre-conditioned at $23 \pm 2^\circ\text{C}$ and $50 \pm 5\%$ relative humidity for at least 48 h prior to mechanical and color testing in accordance with ASTM D618 (Standard Practice for Conditioning Plastics for Testing). For every formulation, ten replicates were tested. To account for batch-to-batch variation, these replicates were taken from each of the three independently prepared film sheets for each treatment. The TS was calculated using Eq. 1 where L_p is the peak load (N) and a is the cross-sectional area of the strips (m^2) while the %E was determined using Eq. 2 where Δl is the change in length at breaking point (mm) and l is the original length (mm) of the film strip.

$$TS = \frac{L_p}{a} \quad \text{Eq. 1}$$

$$\%E = \left(\frac{\Delta l}{l} \right) \times 100 \quad \text{Eq. 2}$$

Color properties

The color of the cornstarch-based films was measured using the CIELAB coordinates (L^* , a^* , and b^*) obtained from Chroma Meter CR-400 (Minolta, Tokyo, Japan). Prior to measurements, the instrument was calibrated using the conventional two-point method – zero calibration (black standard) and white calibration using the white calibration tile provided by the manufacturer. Films from each formulation was tested in three runs, with three replicates for

Table 1
Formulation of the cornstarch-based films.

Components	% Lemongrass essential oil (LEO)			
	0	0.5	1.0	2.0
Cornstarch (g)	10	10	10	10
Distilled water (g)	100	99.35	98.7	97.4
Glycerol (g)	10	10	10	10
Vinegar(g)	10	10	10	10
LEO (g)	0	0.65	1.3	2.6
Total (g)	130	130	130	130

each run. The whiteness index (WI) that acts as an indirect indicator of changes in the visual characteristics of the film (Garcia et al., 2020; Wang et al., 2020) was computed using Eq. 3.

$$WI = 100 - \sqrt{(100 - L^*) + a^{*2} + b^{*2}} \quad \text{Eq. 3}$$

Moisture Content

The moisture content of the films was obtained according to the methods of Wang et al. (2020) with some modifications. The samples were first conditioned at $60 \pm 5\%$ RH for 48 h. Approximately 1 g of a film sample was cut into tiny pieces and placed in a tared crucible. The crucible containing the sample was placed in an oven at $100 \pm 5^\circ\text{C}$ for 24 h, and then allowed to equilibrate at room temperature in a desiccator before re-weighing. Thereafter, the crucible was returned to the oven for 30 min, and then cooled and reweighed. These re-drying and reweighing steps were repeated until the weight of the two previous readings did not differ by more than 0.001 g. The moisture content (MC) of the film was calculated using Eq. 4.

$$MC = \frac{\text{conditioned weight} - \text{final weight}}{\text{conditioned weight}} \times 100 \quad \text{Eq. 4}$$

Microbial activity of the films

Prior to the conduct of the microbial activity test, Gram-positive *Staphylococcus aureus* (BIOTECH 1582) and Gram-negative *Salmonella typhimurium* (BIOTECH 1826) obtained from the University of the Philippines Los Baños - National Institute of Biology and Biotechnology (UPLB-BIOTECH), were grown at room temperature of around 30°C generating 9.63×10^8 CFU mL⁻¹ and 3.3×10^9 CFU mL⁻¹ of *S. aureus* and *S. typhimurium*, respectively.

The antimicrobial activity of the films were evaluated by disk diffusion assay according to the Kirby-Bauer method (Christenson et al., 2017) with modifications. The films were cut into 6-mm diameter discs and placed on the surface of the Mueller-Hinton agar (MHA) previously inoculated with 0.1 mL of the bacteria culture

through spread plating (Wang et al., 2020). The turbidity of the inoculant was adjusted with sterile broth prior to being spread onto the agar plates to an approximate cell density of 1.5×10^8 CFU mL⁻¹. The antimicrobial effects of cornstarch-based films containing different concentrations of LEO against *S. aureus* and *S. typhimurium* were carried out using a zone of inhibition assay on MHA. The plates were then incubated at 37°C for 24 h. After incubation, the inhibition zone was measured on the film disks (Manab et al., 2011). The film with no EO (0% LEO) served as the untreated control. Three replicates from each of the three runs of each treatment were tested.

Statistical Analysis

All means and standard deviations were calculated and analyzed statistically using Microsoft Excel. Significant differences between the samples were determined for all characteristics measured using analysis of variance (ANOVA) at a 95% confidence level and Tukey pairwise comparison.

Results

All films were macroscopically homogeneous without visible oil droplets or exudates. Figure 1a shows a sample of heated film solution on tray while Figure 1b shows samples of dried films.

Tables 2 and 3 list the physiochemical characteristics of the films. Films with 0.5% LEO exhibited the highest tensile strength (0.966 ± 0.252 MPa) and elongation at break ($25.06 \pm 7.39\%$), outperforming both untreated films and films treated with higher amounts of LEO. It appears that disruption of the polymer network occurred at higher amounts of LEO, resulting in weaker mechanical performance. Loss of transparency with incorporation of LEO suggests its entrapment in microdroplets, consistent with literature reports on EO-polymer films. Also, incorporation of LEO significantly increased film thickness, consistent with literature reports.

The films exhibit antibacterial activity as indicated by the presence of circular zones of inhibition in disk diffusion assays for *S.*

Figure 1

(a) a tray of film solution, and (b) samples of dried LEO-treated cornstarch films.



Table 2

Thickness, tensile strength, and elongation at break of cornstarch-based films incorporated with lemongrass essential oil (LEO).

Treatment (% LEO)	Thickness (mm)	Tensile Strength (MPa)	Percentage Elongation at Break
0% (Control)	0.120 ± 0.034^b	0.860 ± 0.303^{ab}	14.072 ± 3.101^b
0.5%	0.177 ± 0.051^a	0.966 ± 0.252^a	25.060 ± 7.390^a
1%	0.161 ± 0.041^{ab}	0.594 ± 0.171^{bc}	14.500 ± 2.866^b
2%	0.186 ± 0.029^a	0.475 ± 0.166^c	12.930 ± 3.880^b

Each data point represents the mean $\pm$ standard deviation of 10 replicates obtained from each formulation. The multiple comparisons were determined by Tukey at $p < 0.05$. Means in the same column with different letters are significantly different.

Table 3

Color values, whiteness index(WI), and moisture content of cornstarch-based films incorporated with lemongrass essential oil (LEO).

Treatment (% LEO)	L*	a*	b*	WI	Moisture Content (%)
0% (Control)	92.249 ± 0.610^a	-0.049 ± 0.043^a	3.690 ± 0.218^b	91.412 ± 0.597^a	22.195 ± 0.331^c
0.5%	91.456 ± 0.490^a	-0.303 ± 0.068^b	4.131 ± 0.311^b	90.496 ± 0.409^a	24.920 ± 0.364^b
1%	91.949 ± 0.963^a	-0.421 ± 0.086^c	4.950 ± 0.566^a	90.531 ± 1.040^a	28.839 ± 0.778^a
2%	92.082 ± 1.253^a	-0.410 ± 0.103^c	4.919 ± 0.530^a	90.661 ± 1.297^a	24.145 ± 0.634^b

Each data point represents the mean $\pm$ standard deviation of triplicates obtained from triplicate preparations of films. The multiple comparisons were determined by Tukey at $p < 0.05$. Means in the same column with different letters are significantly different. L*, a*, b* are the coordinates of the CIELAB color space diagram. L* (0 to 50 is considered dark while 51 to 100 is considered light), a* (positive values indicate redness while negative values indicate greenness), b* (positive values indicate yellowness while negative values indicate blueness).

typhimurium (Figure 2a) and *S. aureus* (Figure 2b). The diameter of these zones (Figure 2c) was measured and summarized in Table 4. While Tukey's HSD test identifies differences between treatments, the biological significance

was determined based on the classification of inhibition zone diameters. All samples demonstrated very strong inhibition (>20 mm), indicating effective antibacterial activity.

Figure 2

(a) disk diffusion assays for *S. typhimurium* (L-R: 0%, 0.5%, 1%, 2%), (b) *S. aureus* (L-R: 0%, 0.5%, 1%, 2%), and (c) the method for estimating diameters of microbial inhibition zones.

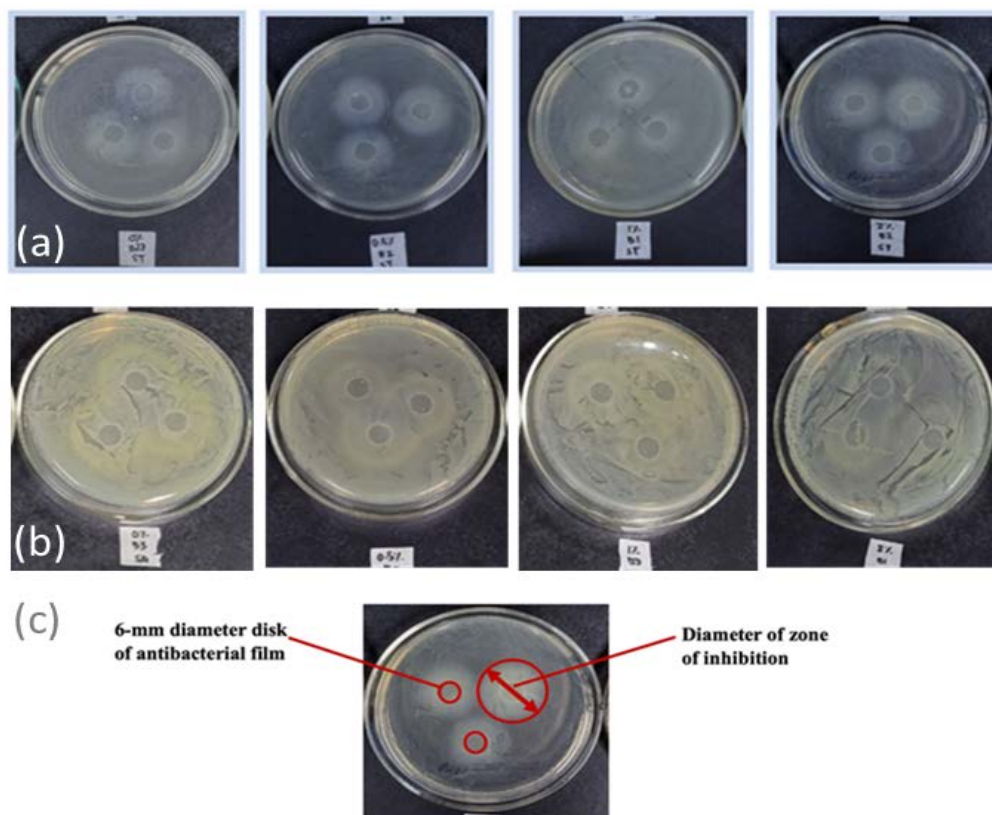


Table 4

Zone of inhibition of antibacterial activity of cornstarch-based films incorporated with lemongrass essential oil (LEO).

Treatment (% LEO)	Diameter of Zone of Inhibition (mm)	
	<i>S. aureus</i>	<i>S. typhimurium</i>
0% (Control)	32.78±4.60 ^{ab}	29.33±1.94 ^{bc}
0.5%	32.22±2.91 ^{abc}	28.44±1.24 ^c
1%	31.89±3.18 ^{abc}	28.33±2.50 ^c
2%	34.22±1.72 ^a	30.67±1.80 ^{abc}

Each data point represents the mean ± standard deviation of triplicates obtained from triplicate preparations of films. The multiple comparisons were determined by Tukey at $p < 0.05$. Means that do not share a letter are significantly different. Inhibition zone diameters ≥ 20 mm are indicative of very strong antibacterial activity (Davis and Stout, 1971).

Discussion

Thickness of films

Incorporating LEO significantly increased the thickness of the cornstarch-based films (Table 2). This result is in agreement with findings from starch-based films incorporated with *Zanthoxylum bungeanum* essential oil (Wang et al., 2020), cornstarch films with orange essential oil (do Evangelho et al., 2019), carboxymethyl xylan films with licorice essential oil (Luis et al., 2019), and cassava starch-based edible films with lemongrass oil (Resianingrum et al., 2016). In their study, Luis et al. (2019) attributed this behavior to the entrapment of microdroplets of licorice essential oil into the film matrix, which gives rise to less dense and thicker films.

In this study, the film with the highest amount of LEO (2% w/w) showed the highest mean thickness. However, the effects of varying amounts of LEO on the thickness of the films are not significantly different from each other. Meanwhile, although the thickness of films prepared with 0%, 0.5% and 2% LEO are significantly different, the thickness of films prepared with 0% and 1% LEO are not significantly different. Nevertheless, the ideal thickness for films which is ≤ 0.25 mm (Hatmi et al., 2020) was achieved in this study.

In the development of packaging materials, the thickness of the film is measured for the evaluation of other properties – structural, mechanical, thermal, and barrier properties. For example, in the case of barrier properties, thicker films are desired since this would mean a longer path for water vapor to traverse to penetrate the film.

Tensile strength (TS)

The mechanical properties of the films were significantly influenced by the addition of LEO (Table 2). The mean TS of the film with 0.5% LEO was the highest (0.966 ± 0.252 MPa); however, it did not differ statistically from that of the control film (0.860 ± 0.303 MPa). In their study on cassava starch-based edible films, Resianingrum et al. (2016) explained that LEO contains solid components that lessen the intercellular gaps in edible films which results in more compact

and firmer film matrices. This may explain the observed increase in TS in films prepared with 0.5% LEO.

At higher amounts of LEO (1% and 2%), the TS decreased significantly relative to the control. Similar results were obtained for cornstarch films containing orange essential oil (do Evangelho et al., 2019); tapioca-based edible films incorporated with cinnamon essential oil (Utami et al., 2019); and chitosan-based films incorporated with thyme, clove, and cinnamon essential oils (Hossenei et al., 2009). The decrease in TS can be attributed to essential oil causing the formation of a heterogeneous film structure (Utami et al., 2019) or the breakup of the polymer film network (Hossenei et al., 2009). However, other factors must be considered when evaluating the TS of films. The concentration and composition of the starch (i.e., amylose and amylopectin) greatly affect the tensile strength (Hatmi et al., 2020).

Elongation at break

Compared with the other formulations, the film prepared with 0.5% LEO showed a significantly improved elongation at break ($25.060 \pm 7.390\%$), which means that a plasticizing effect occurs at this formulation (Table 2), similar to observations in films with low amounts of essential oil (do Evangelho et al., 2019). Plasticizer molecules disrupt the starch's cohesiveness, decrease intermolecular connections, and increase polymer mobility (Resianingrum et al., 2016). Meanwhile, the decrease in elongation at break at higher amounts of LEO was similarly observed in the study of Hossenei et al. (2009), wherein both the TS and elongation at break decreased in 1% thyme essential oil and 1.5% clove essential oil formulations of chitosan-based films; and in the study of Utami et al. (2019) wherein both the TS and elongation at break decreased when 1% of cinnamon essential oil was added in tapioca-based edible films. According to Utami et al. (2019), essential oils create compact film structures, thereby improving continuity in polysaccharide networks, which leads to a decrease in elongation. Moreover, Hosseini et al. (2009) explains that a cross-linking effect can be induced by strong interactions between polymers and essential oils that reduces the free volume and molecular mobility of polymers, leading to a

decrease in elongation at break.

In this study, it may be proposed that maximum polymer (i.e., starch) mobility occurs at 0.5% LEO incorporation, which resulted in a maximum film elongation of about 25%. Films made from polysaccharides have elongation values from 1 to 80% (Hatmi et al., 2020), which puts the elongation at break of the LEO-infused cornstarch-based film in this study at the lower spectrum of the range of values. This means that the addition of LEO does not significantly influence the elongation at break. The elongation at break might be influenced more remarkably by changes in the amount of plasticizer (i.e., glycerol) relative to cornstarch which unfortunately was not within the scope of this study.

Surface color properties

Table 3 shows the mean of the color parameters L^* (0 to 50 is considered dark while 51 to 100 is considered light), a^* (positive values indicate redness while negative values indicate greenness), b^* (positive values indicate yellowness while negative values indicate blueness), and WI of films prepared with different LEO treatments. There were no significant differences in brightness (L^*) and light transmittance in terms of WI values of the films. The control (0% LEO) films were brighter and more transparent than all the treated films. At the lowest LEO treatment (0.5% LEO), a significant difference in the color of the films with regard to their greenish hue was observed. At 1% LEO, the yellowish hue was significantly different from the control. These changes in color property of LEO-treated films may be attributed to light scattering caused by lipid droplets in the film network (Wang et al., 2020). All in all, compared with the control, and despite the differences in color, the LEO-treated films meet consumer requirements for food packaging materials. Color is a key factor considered in food packaging films, as this can influence consumer perception and acceptance of food products.

Moisture Content

The incorporation of LEO showed a significant increase in the moisture content of LEO-treated films (Table 3). Similar results in EO-treated

films were reported by Wang et al. (2020), Utami et al. (2019), Hossenei et al. (2009), Resianingrum et al. (2016), and do Evangelo et al. (2019). The increase in moisture content of films treated with EO can be attributed to the formation of porous structures that facilitate the entry of water molecules between polymer chains (do Evangelo et al., 2019). This allows water molecules to accumulate and deposit between polymer chains via hydrogen bonding. The wide difference in molecular weight of plasticizer and starch can also cause an increase in moisture content, as greater gaps between molecular weights make for bigger space between molecules that can be occupied by water molecules. The moisture content of films is an important parameter that must be measured and reduced to a minimum, as this influences the growth of microorganisms and the shelf life of food products.

Antibacterial activity of films

In this study, the disk diffusion assay was prepared to evaluate the antimicrobial activity of cornstarch-based films incorporated with LEO against *S. aureus* and *S. typhimurium* (Figure 2). The microbial inhibition effect of the films is indicated by the clear zone formed around the films, with a wider clear zone suggesting a stronger antimicrobial activity. Davis and Stout (1971), as cited by Resianingrum et al. (2016), categorized the inhibition power criteria in terms of the diameter of inhibition zone as follows: very strong inhibition (≥ 20 mm), strong (10-20 mm), medium (5-10 mm), and weak (≤ 5 mm). Based on these criteria, the control and all LEO-treated films exhibit very strong antibacterial activity against both *S. aureus* and *S. typhimurium*, where every inhibition zone is more than 20 mm (Table 4). One important finding is that the control film possesses strong inherent antimicrobial property as indicated by sizable inhibition zones (32.78 ± 4.60 mm for *S. aureus*; 29.33 ± 1.94 mm for *S. typhimurium*). This outcome may be attributed to the glycerol and vinegar in the film formulation. Nevertheless, the inhibition zones of the control and LEO-treated films do not differ statistically from each other. It may be noted, however, that the inhibition zone diameter of films with 2% LEO against *S. aureus* is considerably higher than those of the other films.

Both glycerol and vinegar possess antimicrobial properties. In Schlievert and Peterson's (2012) study on the antibacterial activity of glycerol monolaurate in broth and biofilm cultures, it was reported that glycerol monolaurate has potent activity against Gram-positive bacteria including *S. aureus*, and is a bactericidal for a wide range of potential bacterial pathogens. In a study wherein glycerol is a component of the film formulation, Utami et al. (2019) reported antimicrobial activity in tapioca-based edible films when cinnamon essential oil was not incorporated in the film. On the other hand, vinegar is rich in phenolic compounds and is well known to have antioxidant and antimicrobial potential. Bakir et al. (2017) tested the antimicrobial activity of 18 vinegar samples against Gram-negative *S. typhimurium*, *E. coli* and *S. aureus*, and reported that all samples exhibited antibacterial activity against these bacteria with zones of inhibition ranging from 8.58 to 15.81 mm.

The LEO-treated films tended to have a slightly larger zone of inhibition against the Gram-positive bacteria (*S. Aureus*) compared with the Gram-negative bacteria (*S. typhimurium*). A similar outcome was observed by Wang et al. (2020), wherein the inhibitory zones of films incorporated with *Zanthoxylum bungeanum* essential oil against Gram-positive bacteria (*S. aureus* and *L. monocytogenes*) were larger than those against Gram-negative bacteria (*E. coli*). The antibacterial activity of LEO is due to an interaction between the main oil constituents and the bacterial cell membrane. LEO contains lipophilic terpenes that can change the fluidity and permeability of microbial membrane or change the intracellular pH and ATP concentrations, which result in cell rupture that inhibits microbial growth (Majewska et al., 2019). The outer membrane of Gram-negative bacteria contains lipopolysaccharide molecules that limit the diffusion of hydrophobic compounds. In contrast, Gram-positive bacteria have a thick peptidoglycan layer, which can act as preventive barrier to certain essential oils (Burt, 2004, as cited by Wang et al., 2020).

Limitations and future directions

In this study, the transparency of film was indirectly measured by determining the whiteness index (WI). WI was used to measure the color shift and departure from whiteness brought on by the addition of the yellowish LEO. More accurate measurements of transparency can be done by measuring luminous transmittance and haze, typically with ASTM D1003. Other than the determination of film transparency, the following activities are suggested for future study: measurement of barrier properties (i.e., water vapor and oxygen transmission rate); morphological characterization via Scanning Electron Microscopy (SEM) to visualize oil droplet dispersion; quantitative antimicrobial assessment using broth microdilution (MIC) assays; sensory evaluation of the film's impact on packaged food products; and, assessment of the film's biodegradability.

Conclusion

Lemongrass essential oil (LEO) was successfully incorporated into cornstarch-based films. The effect of LEO on the mechanical and physical properties of the films depends on the amount incorporated in the film, e.g., tensile strength and flexibility increased at 0.5% LEO but decreased at higher LEO treatments. The base film exhibits potent inherent antimicrobial activity due to glycerol and vinegar in its formulation. This already strong activity was only slightly enhanced by the addition of LEO. This outcome was probably influenced by the disk diffusion assay's limitations for hydrophobic compounds.

References

- ASTM D882 (Standard Test Method for Tensile Properties of Thin Plastic Sheetings). (2010). ASTM International. West Conshohocken, PA.
- ASTM D618 (Standard Practice for Conditioning Plastics for Testing). (2021). ASTM International. West Conshohocken, PA.
- Atares, L. & Chiralt, A. (2016). Essential oils as

- additives in biodegradable films and coatings for active food packaging. *Trends in Food Science and Technology*, 48, 51-62. <https://doi.org/10.1016/j.tifs.2015.12.001>
- Bakir, S., Devecioglu, D., Kayacan S., Karbancioglu-Guler, F., Capanoglu E. & Toydemir, G. (2017). Investigating the antioxidant and antimicrobial activities of different vinegars. *European Food Research and Technology*, 243, 2083–2094. <https://doi.org/10.1007/s00217-017-2908-0>
- Bilal, M., Zhao, Y., & Iqbal, H. M. (2020). Development and characterization of essential oils incorporated chitosan-based cues with antibacterial and antifungal potentialities. *Journal of Radiation Research and Applied Sciences*, 13(1), 174–179. <https://doi.org/10.1080/16878507.2020.1719336>
- Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods – a review. *International Journal of Food Microbiology*, 94, 223 – 253. <https://doi.org/10.1016/j.ijfoodmicro.2004.03.022>
- Cano, A., Jiménez, A., Cháfer, M., Gonzalez, C., & Chiralt, A. (2014). Effect of amylose:amylopectin ratio and rice bran addition on starch films properties. *Carbohydrates Polymers*, 111, 543–555. <https://doi.org/10.1016/j.carbpol.2014.04.075>
- Davis, W. W. & Stout, T. R. (1971). Disc plate method of microbial antibiotic assay. *Applied Microbiology*, 22(4), 666-670. <https://doi.org/10.1128/am.22.4.659-665.1971>
- do Evangelho, J. A., Dannenberg, G. dS., Biduski, B., el Halal, S. L. M., Dianini Hüttner Kringel, D. H., Marcia Arocha Gularte, M. A., Angela Maria Fiorentini, A. M., & Elessandra da Rosa Zavareze, E. dR. (2019). Antibacterial activity, optical, mechanical, and barrier properties of corn starch films containing orange essential oil. *Carbohydrate Polymers*, 222, 11498. <https://doi.org/10.1016/j.carbpol.2019.11498>
- Dorado, A. A., Peralta, E. K., Carpio, E. V., Lozada, E. P. & Elepaño, A. R. (2017). Biodegradable corn starch/silica nanocomposites for food packaging applications. *Materials Science Forum*, 894, 66-71. <https://doi.org/10.4028/www.scientific.net/MSF.894.66>
- Garcia, A. V., Alvarez-Pérez, O. B., Rojas, R., Aguilar, C. N. & Garrigoss, M. C. (2020). Impact of olive extract addition on corn starch-based active edible film properties for food packaging applications. *Foods*, 9, 1339. <https://doi.org/10.3390/foods90913>
- Hatmi, R. U., Apriyati, E. & Cahyaningrum, N. (2020). Edible coating quality with three types of starch and sorbitol plasticizer. *E3S Web of Conferences* 142. 02003. <https://doi.org/10.1051/e3sconf/202014202003>
- Hosseini, M. H., Razavi, S. H. & Mousavi, M. A. (2009). Antimicrobial, physical, and mechanical properties of chitosan-based films incorporated with thyme, clove, and cinnamon essential oils. *Journal of Food Processing and Preservation*, 33, 727-743. <https://doi.org/10.1111/j.1745-4549.2008.00307.x>
- Jeevahan, J., & Chandrasekaran, M. (2019). Nanoedible films for food packaging: a review. *Journal of Material Science*, 54, 12290–12318. <https://doi.org/10.1007/s10853-019-03742-y>
- Luis, A., Pereira, L., Domingues, F. & Ramos, A. (2019). Development of carboxymethyl xylan film containing licorice essential oil with antioxidant properties to inhibit the growth of foodborne pathogens. *LWT-Food Science and Technology*, 11, 218-225. <https://doi.org/10.1016/j.lwt.2019.05.040>
- Majewska, E., Kozowska, M., Gruczyska-Skowska, E., Kowalska, D. & Tarnowska, K. (2019). Lemongrass (*Cymbopogon citratus*) essential oil: extraction, composition, bioactivity and uses for food preservation: a review. *Polish Journal of Food and Nutrition Science*, 69(4), 327–341. <https://doi.org/10.31883/pifns/113152>
- Manab, A., Sawitri, M. E., Al Awwaly, K. U. & Purnomo, H. (2010). Antimicrobial activity of whey protein-based edible film incorporated with organic acids. *African Journal of Food Science*, 5(1), 6-11.
- Perdana, M. I., Ruamcharoen, J. S., Panphon, S., & Leelakriangsak, M. (2021). Antimicrobial activity and physical properties of starch/chitosan film incorporated with lemongrass essential oil and its application. *LWT*, 141(19), 110931. <https://doi.org/10.1016/j.lwt.2021.110934>
- Perdana, M. I., Panphon, S., Ruamcharoen, J. S., & Leelakriangsak, M. (2022). Antimicrobial property of cassava starch/chitosan film with

- lemongrass essential oil and its shelf life. *Journal of Pure and Applied Microbiology*, 16(4), 2891–2900. <https://doi.org/10.22207/JPAM.16.4.64>
- Petkoska, T., Daniloski, D., D'Cunha N. M., Naumovski, N., and Broach, A. T. (2021). Edible packaging: Sustainable solutions and novel trends in food packaging. *Food Research International*, 140, 109981. <https://doi.org/10.1016/j.foodres.2020.109981>
- Resianingrum, R. Atmaka, W., Khasana, L., Kawiji, Utami, R., & Praseptianga, D. (2016). Characterization of cassava starch-based edible film enriched with lemongrass oil (*Cymbopogon citratus*). *Nusantara Bioscience*, 8(2), 278-282. <https://doi.org/10.13057/nusbiosci/n080223>
- Schlievert, P. M., & Peterson, M. L. (2012). Glycerol monolaurate antibacterial activity in broth and biofilm cultures. *PloS one*, 7(7), e40350. <https://doi.org/10.1371/journal.pone.0040350>
- Shanbhag, C., Shenoy, R., Shetty, P., Srinivasulu, M. & Nayak, R. (2023). Formulation and characterization of starch-based novel biodegradable edible films for food packaging. *Journal of Food Science and Technology*, 60(11): 2858–2867. <https://doi.org/10.1007/s13197-023-05803-2>
- Utami, R., Khasanah, L. U., Manuhara, G. J. & Ayunigrum, Z. K. (2019). Effects of cinnamon bark essential oil (*Cinnamomum burmannii*) on characteristics of edible film and quality of fresh beef. *Pertanika Journal of Tropical Agricultural Science*, 42(4), 1173 – 1184.
- Wang, B., Sui, J., Yu, B., Yuan, C., Guo, L., Abd El-Aty, A. M., & Cui, B. (2020). Physicochemical properties and antibacterial activity of corn starch-based films incorporated with *Zanthoxylum bungeanum* essential oil. *Carbohydrates Polymers*, 254. <https://doi.org/10.1016/j.carbpol.2020.117314>
- Youssef, A. M. & El-Sayed, S. M. (2018). Bionanocomposites materials for food packaging applications: concepts and future outlook. *Carbohydrate Polymers*, 193, 19-27. <https://doi.org/10.1016/j.carbpol.2018.03.088>
- Zubair, M., Shahzad, S., Hussain, A., Pradhan, R. A., Arshad, M. & Ullah, A. (2022). Current trends in the utilization of essential oils for polysaccharide- and protein-derived food packaging materials. *Polymers* 14, 114. <https://doi.org/10.3390/polym14061146>