

Antibacterial Activity of Lemongrass (*Cymbopogon citratus*) Against *Staphylococcus aureus* in Cellulose Nanofibers from Lignocellulosic Biomass and Glycerol

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Abstract. Lemongrass oil (LGO) has great antimicrobial effects, it increases shelf life as a food coating. Incorporating LGO into cellulose nanofibers (CNFs)-(Glycerol)-(Starch) was done by a mixing procedure and the antibacterial characteristics were evaluated. The interaction of LGO in the composite systems was studied by Scanning electron microscopy (SEM), microbial analysis by total plate count, antimicrobial by inhibitory zone, film thickness, and by the support of Fourier-transform infrared spectroscopy (FT-IR). The results indicated that the best composite systems could maintain LGO by 0.15 g at all predetermined cellulose concentrations. Its antibacterial substance can be integrated into a polymer matrix for active coating.

1 Introduction

Based on historical factors, most of the existing food coating is low-cost and has good mechanical and barrier properties originally made of petrochemical products or cellulose [1-2]. As time goes by, this faces pressure from environmental problems, so it is necessary to gradually eliminate the use of petrochemical materials, and replace them with innovative and biodegradable polymer coating such as chitosan [3], alginate [4], cellulose [5], starch [6], pullulan [7], polylactic acid [8], etc., where demand continues to increase. The food industry is forced to develop and adopt new antimicrobial materials for coating due to the need to reduce food waste, increase food safety, and extend the shelf life of food [9]. According to references, a biopolymer has been identified with antimicrobial properties, chitosan [10], so the polymer matrix needs to be mixed with various antimicrobial agents, to have antibacterial or antifungal properties.

2 Literature Review

Cellulose is a natural biopolymer that is easy to find and attracts the attention of many

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people. This is due to cellulose nanofibers (CNF), which have extraordinary properties such as biocompatibility and biodegradability. These properties make biopolymers that can be used in various industries, including pharmaceutical, biomedical, electronics, and textiles, as well as in the food packaging industry [11-12]. Due to its molecular strength, cellulose fibers can also function as reinforcement.

Spices and essential oils (EOs) don't represent a serious environmental or human health risk because they are natural products with potent antibacterial and antioxidant qualities [13-14]. Plant hydrodistillation produces essential oils rich in phenolic chemicals, including monoterpenes, flavonoids, and phenolic acids [15]. These oils have various medical and pharmacological uses [16-17]. Numerous essential oils have been thoroughly researched for producing polymer composite systems in packaging films, air fresheners, and wound healing because of their potent antibacterial qualities [18].

A variety of films, both biodegradable and non-biodegradable, including low-density polyethylene, ethylene vinyl alcohol, fish skin, whey protein isolate, chitosan, and polypropylene coated or mixed with essential oils, were evaluated for their antibacterial properties [19]. This work suggests that lemongrass (*Cymbopogon citratus*) essential oil is manufactured as filler to the matrix of pure CNF with starch and glycerol.

In this context, many antimicrobial compounds present in essential oils have demonstrated their importance for microbial population control, so they help in the production of food products of higher quality and safety [20-21]. For instance, the essential oil of lemongrass (LGO) has antimicrobial activity against a diverse range of microorganisms including molds, yeasts, and gram-positive and gram-negative bacteria [22,23,24]. The best of our knowledge, there are no studies on the application of essential oils of lemongrass, sawdust cellulose nanofiber, starch, and glycerol for edible coatings application. Thus, the goal of this study was to evaluate the effects of different edible coatings on the physical-chemical parameters (cellulose purity test, total plate number, inhibitory test, observing microstructure, soil burial test, and sensory evaluation).

3 Materials and Methods

The equipment needed to support this research includes a blender, Mesh 40, chemical beaker, hotplate, stirring rod, filter cloth, filter paper, oven, analytical balance, funnel, volumetric flask, measuring cup, erlenmeyer, spatula, thermometer, volumetric pipette, pipette measuring, micropipette, bulb, glass plate, petri dish, test tube, disc, tweezers, wire tube, spirit burner, laminar air flow, caliper, bunsen, porcelain dish, furnace, desiccator, pH Meter, Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FTIR).

The materials used in this research were sawdust obtained from wood craftsmen, approximately 20 km from campus, starch manufactured by Gujarat Ambuja Export Ltd. Supplied by P.T Tatarasa Primatama, distilled water, sodium hydroxide, sodium hypochlorite, sulfuric acid, nutrient agar, plate count agar, buffer peptide water, chloramphenicol, planting media, *Staphylococcus aureus* bacterium.

3.1 Material and Procedure

In the preparation of cellulose from sawdust, the powder was ground using a blender and sieved using a 40-mesh. Then, boil in distilled water at 100 °C for one hour and dry in the oven at 45 °C for 24 hours.

The cellulose extraction was done, by weighing wood powder into a beaker in the amount of 1 g. Add 30 mL of 0.5 M NaOH. Then, delignify using ultrasonics at 30°C for 90 minutes. Separate the filtrate from the residue using Whatman filter paper 40. Bleached the residue using 5% NaOCl solution at 50 °C until the residue turns white. Washed the residue using distilled water until it reached the neutral pH of 7. After obtaining a neutral pH, dry the residue in the oven at a temperature of ±45 °C for 24 hours.

Table 1. The Formulation of Native Cellulose with Glycerol and Lemongrass Oil (LGO)

Sample Cell-LGO (g)	Cellulose (g)	LGO (g)	Glycerol (g)	Starch (g)	Sample Cell-LGO (g)	Cellulose (g)	LGO (g)	Glycerol (g)	Starch (g)
Cell 1_0.1	1	0.1	7	10	Cell 1_0.25	1	0.25	7	10
Cell 2_0.1	2	0.1	7	10	Cell 2_0.25	2	0.25	7	10
Cell 3_0.1	3	0.1	7	10	Cell 3_0.25	3	0.25	7	10
Cell 4_0.1	4	0.1	7	10	Cell 4_0.25	4	0.25	7	10
Cell 5_0.1	5	0.1	7	10	Cell 5_0.25	5	0.25	7	10
Cell 1_0.15	1	0.15	7	10	Cell 1_0.30	1	0.3	7	10
Cell 2_0.15	2	0.15	7	10	Cell 2_0.30	2	0.3	7	10
Cell 3_0.15	3	0.15	7	10	Cell 3_0.30	3	0.3	7	10
Cell 4_0.15	4	0.15	7	10	Cell 4_0.30	4	0.3	7	10
Cell 5_0.15	5	0.15	7	10	Cell 5_0.30	5	0.3	7	10
Cell 1_0.20	1	0.2	7	10					
Cell 2_0.20	2	0.2	7	10					
Cell 3_0.20	3	0.2	7	10					
Cell 4_0.20	4	0.2	7	10					
Cell 5_0.20	5	0.2	7	10					

The lemongrass extraction was done after being washed clean and then dried at 105 °C using ethanol solvent in a ratio of 3:7 (v/v) for three days using a maceration process and then filtered using filter paper. Separate the oil and solvent using the distillation process. The fabrication of biodegradable plastic was done by weighing 10 g of starch and adding 70 mL of water. The starch solution was heated with a magnetic stirrer hotplate for 15 minutes at 70 °C until gelatin formed. Then, add 7 mL of glycerin. Add cellulose with variations of 1 g; 2 g; 3 g; 4 g, and 5 g, then homogenize. Reheat for about 10 minutes, and add lemongrass oil with variations of 0.10 g; 0.15 g; 0.20 g; 0.25 g, and 0.30 g with respective concentrations. Once homogeneous and gelatinized, pour into glass molds, and dry at room temperature for 1x24 hours.

3.2 Analysis Procedure

1. Fourir Transformed-Infra Red (FTIR) for FTIR for Identification of Native Cellulose to Standard

The sample is weighed at 0.5 g. Then measured using an FTIR tool.

2. Cellulose Ash Content Test

Weigh 1 g of sample into a porcelain cup that has been adjusted. Add H₂SO₄ to the porcelain cup containing the sample. Heat until completely cooked using bunsen. Add 1-3 drops of H₂SO₄ again, then heat until white smoke does not form. Put the sample into the furnace at 600 °C for 1 hour until ash forms. Remove the cup, then put it in the desiccator for ±10 minutes. Weigh the cup containing the sample. Repeat from stages 5 to 7 until the weight is constant.

3. Organoleptic Test

The sensory evaluation of organoleptic qualities such as scent, color, and texture was conducted by thirty trained panelists, and the data was run by ANOVA (Analysis of Variance) and Duncan's Multiple Range Test (DMRT).

4. Thickness Test

Cut the biodegradable film to a size of 2 cm x 5 cm. Measure the thickness using a digital caliper with an accuracy of 0.01 mm.

5. SEM (Scanning Electron Microscope)

The biodegradable film samples are cut to size 1 cm x 1 cm. Place it on the carbon tape. Then the sample is coated using platinum. Then analyzed using a Scanning Electron Microscope.

6. Microbiological Analysis by Total Plate Count

Weigh 1 g of sample, put it in 9 mL of buffered peptone water (BPW) with dilution 10-1, and homogenize with a vortex. A total of 1 mL of the 10-1 dilution from the homogenization results in sample preparation was taken and put into the first tube which had been filled with 9 mL of BPW (10-2 dilution) homogenized with a vortex. A total of 1 mL of the 10-2 dilution from the homogenization results in sample preparation was taken and put into the first tube which had been filled with 9 mL of BPW (10-3 dilution) homogenized with a vortex. Pipette 1 mL of each sample dilution into a sterile petri dish. Then 15 mL of thawed plate count agar media was poured in and cooled to a temperature of 45°C. The petri dish was immediately shaken and rotated until the media was evenly distributed and homogeneous. The experiment included a petri dish containing media and BPW diluent solution as a blank. After the medium has solidified, the petri dish is incubated at 37 °C for 24 - 48 hours in an inverted position. Count the colonies that grow in each petri dish.

7. Antimicrobial Test by Inhibition Zone

Accurately weigh 23 g of Nutrient Agar powder. Measure 1000 mL of distilled water in a suitable container. Gradually add to the distilled water while stirring continuously to ensure even dispersion and prevent clumping. Continue to stir the mixture until the Nutrient Agar powder is completely dissolved. Bring the mixture to a boil while stirring constantly, this ensures the agar is fully dissolved and the medium is sterilized. The Nutrient Agar ready for microbiological work, typically sterilizes the medium by autoclaving at 121°C for 15-20 minutes. Allow the medium to cool to about 45-50°C. Microbial culture is carried out by regrowing microbes that have previously grown on old media into new media. Microbes are taken 1 dose and inoculated on new media, incubated for 24 hours at 32 °C.

The sample is weighed 50 mg into 10 mL of sterile distilled water, and homogenized. Pour 20 mL of sterile media into a sterile petri dish, then smear it. Make a microbial suspension by dissolving the bacteria taken using a loop needle and homogenizing it in sterile distilled water. The suspension is adjusted to McFarland standards. Pour 100 µL of the suspension onto the surface of the agar and smear it using a sterile cotton swab three times.

Prepare a paper disc that has been soaked in the sample solution. The paper disc is placed on the media etched using test bacteria. Incubate for 24 hours at 32 °C. The final results of this test can be calculated in mm, namely by calculating the size of the inhibition zone formed.

The sterile solution of chloramphenicol 50 µg/mL was served as a control positive and added to the cooled liquid Nutrient Agar. Mix thoroughly to ensure even distribution of the antibiotic throughout the medium. Pour the chloramphenicol-supplemented Nutrient Agar into sterile petri dishes, covering the bottom of each dish with a thin layer. Allow the agar to solidify at room temperature. The role of chloramphenicol was to detect antimicrobial activity and the active ingredient in the film can inhibit the growth of microorganisms.

8. Degradation Test

Prepare a container filled with planting medium to a height of 3 cm. Place the film with a size of 2 cm × 4 cm. Then add another 3 cm of planting medium until it covers the entire surface. Visually observe the changes that occur over 14 days.

4 Result and Discussion

1. FTIR of Native Cellulose Nanofibril in Comparison to Cellulose Standard

FTIR testing was carried out to determine the cellulose bonds of the sawdust sample and to confirm the material used was suitable. These chemical bonds are indicated by different peaks and functional groups. The morphology of cellulose-derived sawdust has a slightly rounded, irregular shape, slightly sharp corners, and an uneven surface. Meanwhile, microcrystalline cellulose has a slightly irregular round morphology, sharp corners, and a flat surface. Overview of the Microcrystalline Cellulose (MCC) spectra: The large O-H stretching band (~3330-3400 cm⁻¹) corresponds to the hydroxyl (O-H) groups present in cellulose due to

inter- and intramolecular hydrogen bonding. C-H Stretching ($\sim 2890\text{ cm}^{-1}$): This peak is caused by the C-H stretching vibrations of aliphatic chains in the cellulose structure. O-H Bending ($\sim 1640\text{ cm}^{-1}$) is often connected with the absorbed water in cellulose. C-H Bending ($\sim 1430\text{ cm}^{-1}$ and $\sim 1370\text{ cm}^{-1}$): These peaks represent the bending vibrations of C-H groups. C-O-C stretching ($\sim 1160\text{ cm}^{-1}$ and $\sim 1110\text{ cm}^{-1}$): These bands show glycosidic linkage in cellulose. C-O Stretching ($\sim 1050\text{ cm}^{-1}$ and $\sim 1030\text{ cm}^{-1}$): These peaks represent the C-O stretching vibrations seen in cellulose. An overview of the FTIR spectrum of cellulose from sawdust: O-H Stretching ($\sim 3330\text{--}3400\text{ cm}^{-1}$): Similar to MCC, except this band may be broader or slightly displaced due to changes in hydrogen bonding. This could indicate various degrees of crystallinity or impurities. The C-H stretching peak ($\sim 2890\text{ cm}^{-1}$) should be present and similar to that of MCC. However, the strength may vary depending on the purity and crystallinity of the cellulose recovered from sawdust. O-H Bending ($\sim 1640\text{ cm}^{-1}$): This peak may be more apparent if the sawdust-derived cellulose has a higher moisture content or contains lignin or hemicellulose impurities, which are also absorbed in this region. C-H Bending ($\sim 1430\text{ cm}^{-1}$ and $\sim 1370\text{ cm}^{-1}$). These peaks may exist but vary in intensity, reflecting changes in cellulose purity. C-O-C and C-O stretching (about $1160\text{--}1030\text{ cm}^{-1}$). The occurrence of these peaks in the sawdust sample would confirm the glycosidic connections seen in cellulose. However, minor changes or variations in intensity may suggest variances in structure or the presence of different polysaccharides. Key differences to consider: The MCC has a high crystalline content, as evidenced by strong and well-defined FTIR peaks. Sawdust cellulose may have a lesser degree of crystallinity, resulting in larger peaks or modest shifts in wavenumbers; it may also contain residual lignin, hemicellulose, or other organic impurities, which could introduce new peaks or cause existing peaks to shift or alter in strength. Lignin peaks at $1500\text{--}1600\text{ cm}^{-1}$, indicating the presence of aromatic rings, while hemicellulose peaks at $1200\text{--}900\text{ cm}^{-1}$. The FTIR result of cellulose can be seen in Fig1.

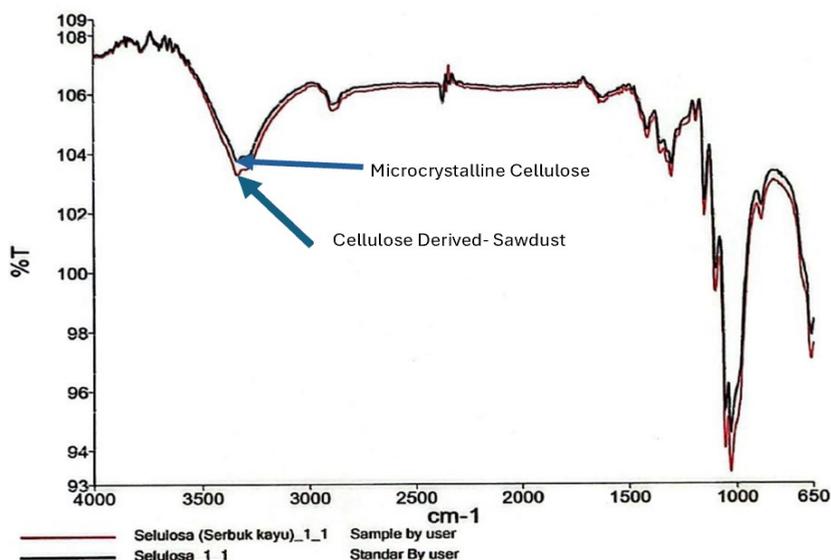


Fig 1. FTIR to Compare the Microcrystalline Cellulose (MCC) Spectra as a Standard to Cellulose Derived- Sawdust

2. Ash Content Test

Ash concentration indicates the amount of inorganic compounds remaining after burning. Cellulose, with a low ash concentration, implies purity, whereas a high ash content may indicate contamination or undesirable additions. Ash content testing can be used to monitor the manufacturing process and guarantee that no additives or impurities are introduced during

the extraction and processing of cellulose. Cellulose that has been thoroughly processed should contain relatively little ash, indicating that the majority of the inorganic impurities have been eliminated. The ash content can affect the final material's qualities such as strength, stability, and formulation success. The principle of testing ash content by dry digestion is burning using crucibles in a furnace at a high temperature of around 500°C-600°C for several hours, then weighing to obtain a constant weight. The purpose of measuring the ash content itself is to see the amount of mineral components in the organic sample that are left behind during the ashing process. In the ash content analysis, the porcelain cup was initially washed thoroughly and dried in a kiln at 600°C for 30 minutes, then cooled in a desiccator for 15 minutes and weighed until a constant weight was obtained. Then weigh 1 g of cellulose from sawdust and put it in a porcelain cup of known weight before ashing. The cup containing the sample is added with 1 ml of H₂SO₄, the function of adding H₂SO₄ is to convert carbon into carbon dioxide and reduce the evaporation of inorganic substances during the annealing process, then heated using a bunsen until completely cooked, then 1 drop of H₂SO₄ has added again, heated again with a bunsen until the white smoke disappears after the white smoke disappears, place it in the furnace at 600 °C for 1 hour. The next stage was cooling in a desiccator and weighing, the repetition until the weight was constant. The ash content in cellulose isolated from wood dust was an inorganic substance left over from combustion. In this study, the cellulose ash content obtained was 0.12%.

3.Organoleptic Test

Since the used concentration of glycerol and starch is constant which are 7 g and 10 g respectively, so on analyzing data we used the varied data of cellulose and LGO. Based on the 30 trained panelist test results on the color of the data, then processed using the ANOVA test, it was found that the F count of 52.98 was greater than the F table in the significance test of P 0.05. In further tests using Duncan, it was found that the color of each sample had significant differences. The color of the thin film is influenced by the type of starch and other components. The bleaching and rinsing of the wood powder cellulose results in a change in color from slightly yellow to white. Meanwhile, the water content of cellulose is lowered as a result of a 24-hour oven drying procedure. The inclusion of ingredients other than starch affects the color of the film. Cellulose consists of long chains of polymers formed from glucose monomers.

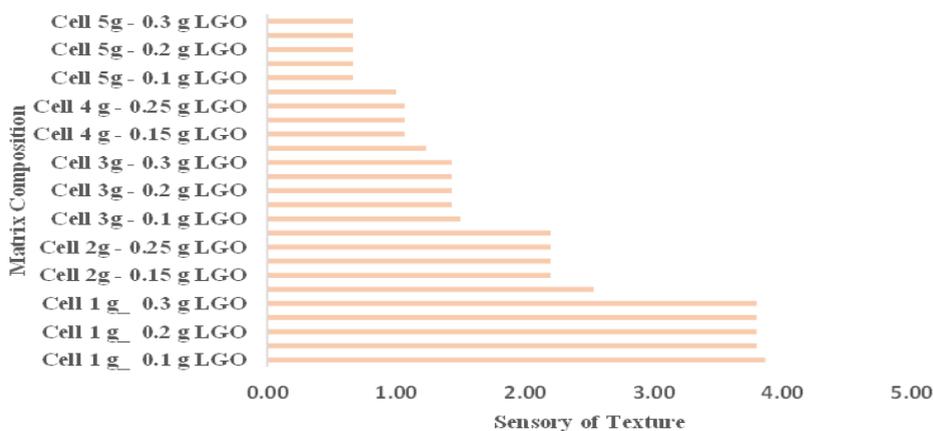


Fig.2 Plot Sensory of Texture

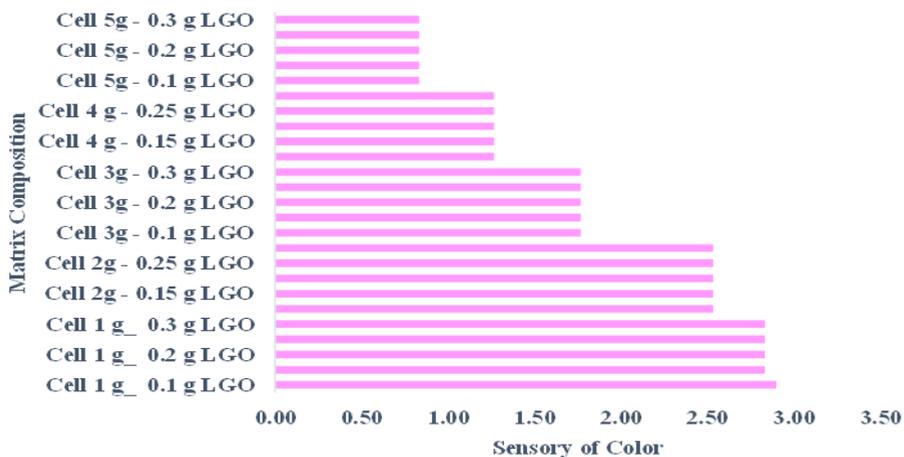


Fig.3 Plot Sensory of Color

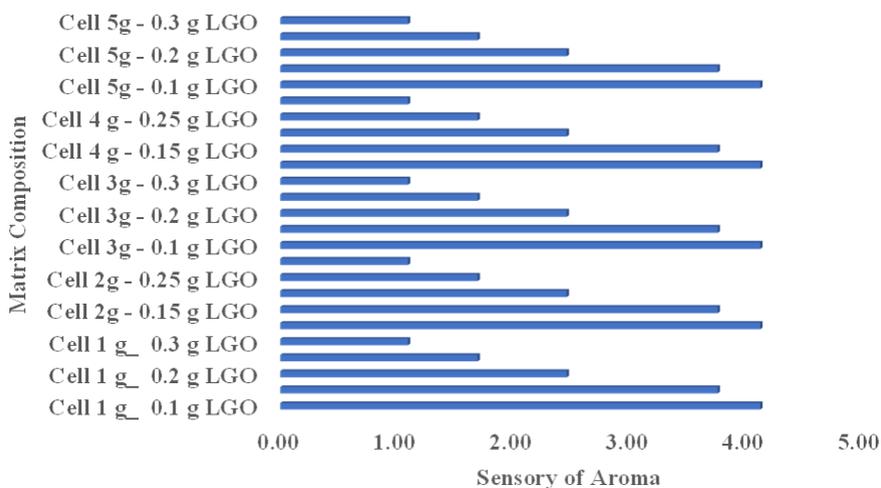


Fig.4 Plot Sensory of Aroma

Based on the results of the panelists' observations for texture, it was found that the F count was 106.3, which was greater than the F table at a significance figure of P 0.05. The surface texture of films can be influenced by the presence of glycerol and cellulose. In addition, the surface texture is also influenced by the printing technique. The fairly even distribution of glycerol causes the texture of the bioplastic to become smoother. The thin film shows white dots which depict the size distribution of cellulose particles. The aroma in bioplastic comes from the addition of lemongrass extract. Apart from its role as an aromatic agent, lemongrass extract also acts as an antimicrobial agent which can inhibit the growth of microbes in films which will affect the shelf life of the food product. The panelists' preference level for the aroma criteria has an insignificant difference, which can be seen from the undefined P-value to the significance value of P 0.05, so it can be concluded that there was no significant difference in the aroma of each sample. The plot sensory texture, color, and aroma can be seen in Fig 2., Fig 3, and Fig 4.

4. Thickness Test

As the concentration of cellulose in the mixture grows, so does the overall mass of the film-forming material. The film thickness is proportional to the overall amount of material utilized, more cellulose in the mix equals more film material, resulting in a thicker film. Cellulose helps to increase the viscosity of the solution. As the concentration of cellulose in the solution increases, it becomes more viscous and difficult to spread evenly across the surface resulting in a thicker film when dried. Cellulose fibers can form dense networks in films. As the concentration of cellulose increases, so does the number of cellulose fibers in the film, densifying the network and increasing film thickness.

Glycerin and starch interact with cellulose, influencing the film forms and dries. Gelatinized starch acts as an essential matrix, while cellulose strengthens the structure. This matrix becomes stronger as the cellulose concentration increases, resulting in a thicker film. Increasing the cellulose concentration can affect the drying rate because cellulose absorbs and retains more water in its filament structure.

Higher cellulose concentrations may make homogenization more challenging, jeopardizing cellulose distribution uniformity in the film. This can result in varied film thickness, however thicker films are often generated in areas with higher cellulose concentrations.

Overall, a combination of these factors contributes to the increase in film thickness as cellulose content increases. Cellulose contributes to the film's mass, viscosity, strength, and structure, all of which help to build thicker films. Based on our observations, it was found that the concentration of cellulose in the matrix formula of 1;2;3;4; and 5 g, shows that 5 g of cellulose addition will exhibit the highest thickness as can be seen in Table 2.

Table 2. The Film Thickness

Sample Cell-LGO	Thickness (mm)	Sample Cell-LGO	Thickness (mm)
Cell 1-0.1	0.37	Cell 1-0.25	0.44
Cell 2-0.1	0.37	Cell 2-0.25	0.45
Cell 3-0.1	0.37	Cell 3-0.25	0.44
Cell 4-0.1	0.36	Cell 4-0.25	0.46
Cell 5-0.1	0.38	Cell 5-0.25	0.43
Cell 1-0.15	0.38	Cell 1-0.30	0.44
Cell 2-0.15	0.38	Cell 2-0.30	0.44
Cell 3-0.15	0.38	Cell 3-0.30	0.46
Cell 4-0.15	0.39	Cell 4-0.30	0.45
Cell 5-0.15	0.4	Cell 5-0.30	0.47
Cell 1-0.20	0.41		
Cell 2-0.20	0.41		
Cell 3-0.20	0.41		
Cell 4-0.20	0.42		
Cell 5-0.20	0.4		

5. Scanning Electron Microscopy

Compositions containing 1 g of cellulose may have higher mechanical strength than those with 5 g of cellulose due to greater homogeneity and cohesiveness. Organized threads or particles can be observed at a magnification of 500x. Glycerol does not have a distinct

structure in SEM since it is more uniform and liquid, whereas LGO shows as minute particles or aggregates with varying structures depending on their shape and distribution. Glycerol has a composition of 7 g, which is higher than cellulose, and the reference image shows that it is cellulose. The content and appearance of glycerol and cellulose can differ, with cellulose typically displaying a fibrous structure in pictures. This is because cellulose has a rather dense and organized structure. The existence of relatively big white lumps shows that the cellulose particles are agglomerating in groups, causing the distribution of cellulose in the film layer to be unequal; otherwise, if white lumps are not visible it indicates that the cellulose is equally distributed due to the uniform stirring. At magnification, cellulose frequently appears as bigger, structured particles with a form resembling fibers or larger particles. At normal temperatures, glycerol is a liquid without a solid component. However, if dried or mixed with other components the microstructure of glycerol images appears as minute particles or residues with no discernible structure, because of the low viscosity and does not form a solid structure. However, microscopic particles that appear uneven or dispersed could be glycerol. The glycerol's increased composition compared to cellulose may not be immediately seen on SEM since it lacks a solid structure.

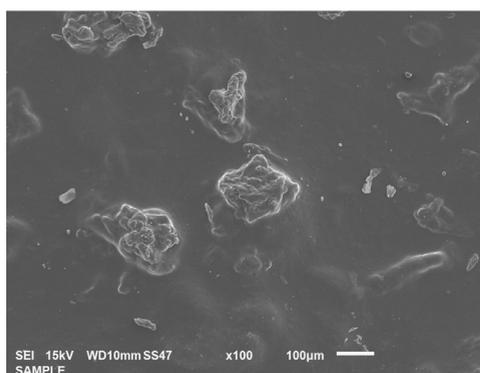


Fig 5. Cellulose 1 g with 0.1 g LGO

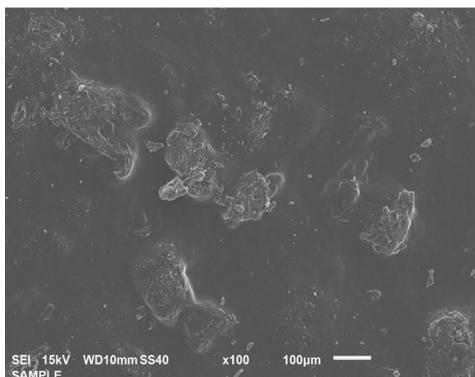


Fig 6. Cellulose 3 g with 0.1 g LGO

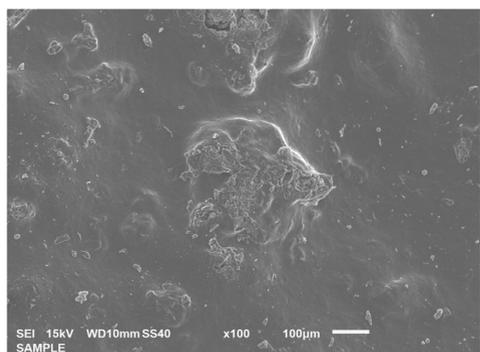


Fig 7. Cellulose 5 g with 0.1 g LGO

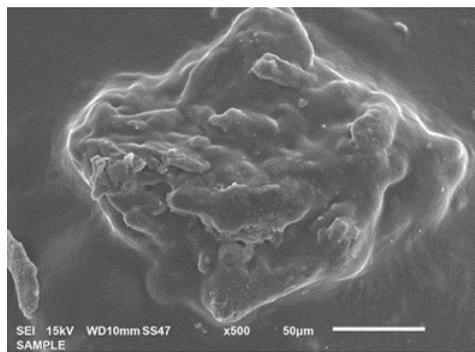


Fig 8. Cellulose 5 g and 0.1 g LGO. Magnification 500 x

6. Microbiological analysis

This study conducted the microbiological analysis by counting the total plate number method. At the 10^{-1} dilution, mostly all the formulas of cellulose 1 g, 2 g, and 3 g with the LGO 0.1 g, 0.15 g, and 0.2 g are too numerous. Furthermore, at dilutions 10^{-2} and 10^{-3} it was found that the total viable count (TVC) ($P > 0.05$) is not significant.

Logical the the bacterial count at 10^{-3} declined compared to 10^{-2} of the dilution. However, the magnitude of this decline is important to the analysis. The decline is 10-fold in pronounced to the consistency of the dilution effect [25].

From the observation results, it was found that the number of bacteria significantly decreased by more than 10-fold at LGO concentrations 0.15 for all cellulose concentration 1, 2, 3, 4, and 5. This indicates that the antimicrobial agent in the matrix may be more effective at lower bacterial concentrations, which leads to a disproportionately larger kill rate. In some cases, higher dilutions may reduce the protective effects of the matrix on the bacteria, making them more vulnerable to the antimicrobial. If the decline is less than expected like in cellulose 1 gr (LGO 0.1 g); and cellulose 2 g (LGO 0.1 g and 0.15 g), this can happen of bacteria clumping or aggregation, because of the uneven distribution in the diluent by matrix effect (cellulose, glycerol) protecting the bacteria.

Increasing the cellulose concentration to 5 g, bacteria become more embedded within the thicker cellulose matrix, potentially limiting mobility and access to nutrients which might reduce their ability to grow and multiply. The existence of cellulose also can happen similar to TVC count at both 10^{-2} and 10^{-3} dilution, indicating that the bacteria are surviving equally well despite the dilution at the lowest research formula, LGO 0.1 g addition as can be seen in Fig 9 and Fig 10.

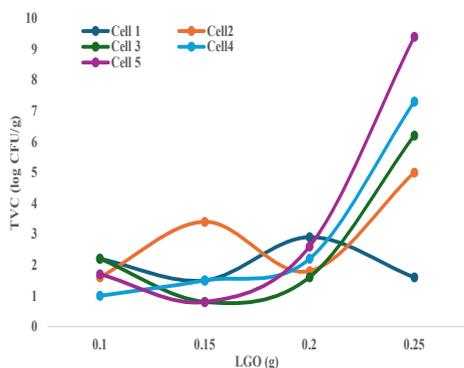


Fig 9. Total viable counts (TVC) of cellulose to LGO in dilution of 10^{-2}

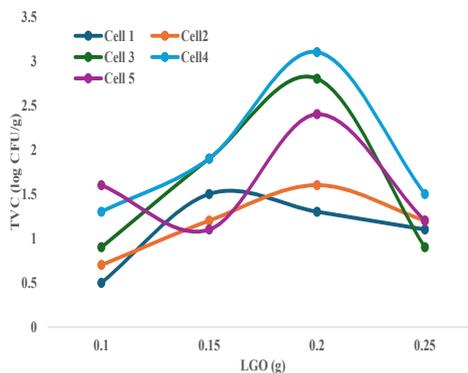


Fig 10. Total viable counts (TVC) of cellulose to LGO in dilution of 10^{-3}

7. Antimicrobial Test

Research on lemongrass essential oil based on derived cellulose coating was expanded following the advent of antibiotic resistance issues [26], primarily because bacteria, such as *Staphylococcus aureus*, a gram-positive bacterium, are responsible for a variety of foodborne disorders. LGO metabolites play a key role in preventing microbial development. LGO's mode of action is a synergistic collaboration between active compounds in LGO (citral, citronellal, geraniol, etc.) and numerous additional metabolites (fatty acids, phenol-soluble modulins (PSMs), staphyloxanthin), which cause bactericidal action against pathogenic bacteria. Each of these chemicals exhibits a distinct antibacterial object level [27]. The active compound in LGO functions largely by breaking the bacterial cell membrane, resulting in cell death. Their combined presence in LGO increases its efficacy, making it a promising natural alternative for treating *S. aureus* infections, even those produced by antibiotic-resistant strains. Citral is particularly efficient against *S. aureus*, slowing growth and potentially eradicating the organism [26]. Like citral, citronellal functions as a membrane disruptor. It penetrates the bacterial cell membrane, increasing permeability and causing the loss of essential ions and chemicals, killing the bacteria. Geraniol is effective against *S. aureus*, inhibiting its reproduction capacity and inducing cell death. The bacteria cultures were evaluated at 48 hours, with the following inhibitory zone results: 20.89 in pathogenic bacteria *S. aureus* 9.61; 9.706; 9.798; 10.06; 10.254 mm respectively in LGO 0.1; 0.15; 0.2; 0.25; 0.3 g.

Table 3. Antibacterial Activity of LGO against *S. Aureus* in Film Matrix Cellulose Derived-Sawdust/Glycerol

Concentration of LGO	Inhibition Zone (mm)					Average
	Cell 1	Cell 2	Cell 3	Cell 4	Cell 5	
0.1	9.62	9.6	9.62	9.58	9.63	9.61
0.15	9.78	9.74	9.76	9.73	9.52	9.706
0.2	9.94	9.88	9.9	9.86	9.41	9.798
0.25	10.08	10.04	10.05	10.01	10.12	10.06
0.3	10.17	10.1	10.08	10.15	10.77	10.254
Average	9.918	9.872	9.882	9.866	9.89	9.8856

The sensitive category comprised antibiotic inhibition zones that were established within 48 hours. This is consistent with accepted standards for the diameter of a bacterial inhibitory zone, which state that chloramphenicol is resistant if the diameter of the bacterial growth inhibition produced is less than 20.89 mm and sensitive if the resulting inhibitory diameter exceeds 21 mm [28]. The antibacterial activity test for LGO bacteria against *S. aureus* utilizing filtrate form and the agar diffusion method yielded an average inhibition zone of 9.8856 mm after 48 hours. The inhibitory zone linked with LGO utilizing filtrate was graded as strong for 0.25 and 0.3 (g) and moderate for concentrations less than 0.25 and 0.3 (g) as can be seen in Table 3.

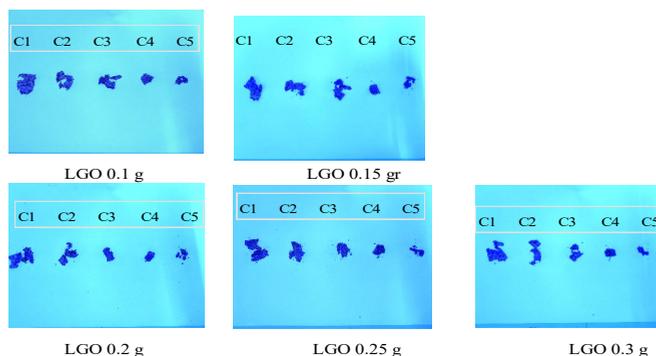


Fig 11. Biodegradation of Films After Fourteen Days of Observation

8. Degradation Test by Soil Burial Method

Biodegradation tests are performed to assess how rapidly films are decomposed by microorganisms under environmental exposure. The media is utilized as a planting medium because of the significant number of microorganisms (fungi, bacteria, and algae) in the soil, which will aid in the degradation process. The cellulose content in the composite formula affects film breakdown. The more cellulose, the faster the bioplastic degrades. So, cellulose contributes to biodegradability because of is a natural material decomposed organically by bacteria in the soil. Aside from that, the combination of starch and cellulose is limited since cellulose is difficult to disintegrate, resulting in a film with hydrophilic characteristics. Natural polymers with hydroxyl groups (-OH) are the most susceptible to natural degradation. Water and cellulose conditions both have an impact on biodegradability. We have set 14 days to determine the decomposing rate of the film. It was found that the rate of degradation depends on the concentration of cellulose in the matrix. The higher the concentration of the cellulose the faster the matrix to degrade. It was found the rate of degradation of composite in C5 (Cellulose 5 g) is faster than C4 > C3 > C2 > C1 as can be seen in Fig 11.

5 Conclusion

The research findings on film characterization have demonstrated that the active component in LGO was effective in suppressing *S. aureus* bacteria, one of the food spoilage strains. In this study, it was applied to a specified matrix, which included natural cellulose, starch, and glycerol. The findings of the organoleptic test on the aroma of LGO on the film, even at low concentrations, indicate that additional experiments on the influence of aroma migration on the quality of the wrapped product are required. Overall, this film can be used for packaging to extend the shelf life. Furthermore, in the food sector, it can be incorporated into active packaging purposes. The combination of LGO and natural cellulose can reinforce the idea that natural materials can be a robust and environmentally responsible source for the coating/packaging sector.

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