SI Science International

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Development of Lemongrass Oil-Based Starch/PVA/Kaolin Films: Antimicrobial Properties and Biodegradability

[1]Bright Ayemwenre Omoike, [2]Felix Ebhodaghe Okieimen and [1]Chinyere Imoisi

[1]Department of Industrial Chemistry, Mewar International University, KM 21, Abuja-Keffi Expressway, Masaka, Nasarawa State, Nigeria

[2]Department of Chemistry, University of Benin, P.M.B.1154, Benin, Nigeria

ABSTRACT

Background and Objective: The development of antimicrobial plastic packaging is an active research area focused on enhancing food quality and shelf-life while addressing the environmental challenges posed by plastic waste. This study explored the antimicrobial and biodegradability properties of the CMS-based composite films. **Materials and Methods:** The CMS-based composite films were developed from carboxymethyl starch (CMS), poly(vinyl alcohol) (PVA), kaolin and lemongrass oil (LGO) using solution-casting techniques. The films were characterized using SEM, XRD, TGA and DTA to assess their morphology, thermal stability and biodegradability. Antimicrobial properties were tested against *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus* using disk assays. Statistical analysis was performed using linear regression and ANOVA at $p<0.05$. **Results:** The results of SEM, XRD and thermal stability analysis showed that the films had good homogeneity, satisfactory surface morphology and relatively high thermal stability. Although LGO impacted the films' morphology and thermal stability, these properties remained suitable for food packaging applications. The LGO-integrated films demonstrated significant antimicrobial activity against the tested pathogens at a concentration of 5.0 parts per hundred (pph) of LGO. All CMS-based composite films exhibited substantial biodegradability. **Conclusion:** Therefore, the LGO-incorporated CMS/PVA/kaolin composite film holds significant promise as a sustainable and innovative alternative to conventional plastics in food packaging. It offers a unique combination of biodegradability, antimicrobial properties and thermal stability, which can help reduce environmental pollution, enhance food safety and meet the growing demand for eco-friendly packaging solutions.

KEYWORDS

Lemongrass oil, antimicrobial, packaging, biodegradable, composites

INTRODUCTION

Food spoilage due to microbial contamination is a significant challenge in Nigeria, leading to food scarcity, economic losses and health risks for consumers[1]. Traditional plastic packaging, while effective in protecting food from external environmental factors, fails to address the threat of microbial growth within

ISSN: 2305-1884 (Print)
https://doi.org/10.17311/sciintl.2025.01.12

Received: 03 Nov. 2024
Accepted: 09 Jan. 2025
Published: 11 Jan. 2025

the packaged product. This limitation underscores the need for innovative packaging solutions that not only prevent external contamination but also combat internal microbial growth[2]. Antimicrobial packaging materials, which in addition to their function as conventional packaging, can also inhibit the growth of microorganisms, offer a promising solution[3]. By incorporating antimicrobial properties into packaging materials, can enhance food safety, extend shelf life and reduce the risk of foodborne illnesses[3,4]. The development of effective antimicrobial packaging is, therefore, crucial to address the growing concerns of food spoilage and ensure the well-being of consumers.

Moreover, the biodegradability of packaging is crucial for environmental sustainability, as it enables the material to decompose naturally in soil environments after use, reducing plastic waste accumulation[5]. Currently, there is a significant focus on creating bio-based antimicrobial packaging materials from natural biopolymers like polysaccharides and proteins[6-8]. Starch is a promising biopolymer alternative to conventional plastic for food packaging due to its excellent biodegradability, availability, chemical stability and ability to form films[8,9]. However, starch films exhibit inferior mechanical and barrier properties when compared to conventional plastics like polyethylene[8,9]. To enhance the properties of starch films, various modification techniques have been developed[7]. These techniques include the use of chemically modified starch like carboxymethyl starch, blending with synthetic polymers, reinforcement with appropriate fillers and the incorporation of effective antimicrobial agents[8,10-12].

Carboxymethyl starch is a modified form of starch derived from the Williamson process, where carboxymethyl groups are introduced into the starch structure, primarily at positions C_1, C_2 and C_3[13]. Carboxymethyl starch differs from native starch in several advantageous ways: It exhibits a lower gelatinization temperature, a reduced tendency for retrodegradation and boasts good thermal stability and film-forming capabilities. In the realm of synthetic polymers, poly (vinyl alcohol) (PVA) is highly regarded for its food safety credentials, cost-effectiveness, high biodegradability and compatibility with starch[8,9].

Incorporating reinforcing inorganic fillers has been recognized as an effective method to alter polymer structures, enhancing their mechanical, barrier and thermal properties significantly[14]. Kaolin is preferred for its widespread availability, abundant supply in Nigeria and its non-toxic nature in food applications. Its hydrophilic nature ensures excellent compatibility with starch-based films.

Furthermore, biopolymers like starch can be adapted to serve as carriers for antimicrobial agents. There is a growing demand for natural antimicrobial components driven by concerns over the health risks linked to synthetic antimicrobial agents[15]. Plant essential oils and leaf extracts are actively researched as natural and safe sources of antimicrobial compounds. Lemongrass oil was chosen for this study due to its potent antimicrobial activity and associated health benefits. It is widely available and economically feasible, being the most common and affordable essential oil on the market. Lemongrass oil is derived from the steam distillation of lemongrass (*Cymbopogon* spp.), yielding between 0.28 to 1.4% of a pale yellowish liquid[16].

Alkaabi *et al.*[12] have explored the antimicrobial efficacy of edible films containing lemongrass oil against pathogenic bacteria. Perdana *et al.*[17] reported that incorporating lemongrass oil into cassava starch/chitosan film significantly enhanced its antimicrobial activity. Ahmad *et al.*[18] also reported that the gelatin film incorporated with lemongrass oil exhibited strong antimicrobial activity against selected microorganisms, with the highest inhibitory effect observed at 25% LGO concentration. However, while the addition of lemongrass oil impacted the organoleptic properties of the film, its effective antimicrobial efficacy must remain within levels acceptable to consumers.

In earlier research on carboxymethyl starch/PVA/kaolin composite packaging, the compositions aimed at enhancing mechanical and barrier properties were detailed. The findings from these formulations were crucial for integrating antimicrobial agents, such as LGO, as an extension of the work.

In this current research, lemongrass oil was integrated into carboxymethyl starch/PVA/kaolin composites to evaluate its efficacy as an antimicrobial agent in CMS-based packaging suitable for food applications. Additionally, the biodegradability of these films to promote waste reduction and environmental sustainability was investigated. While there is scarce research on the compatibility and film-forming characteristics of CMS-based composites, there are no reports on the biodegradability and antimicrobial properties of CMS/PVA/kaolin/LGO composite packaging.

MATERIALS AND METHODS

Study area and sites: This research took place in Benin City, Edo State, Nigeria, which is positioned at a Latitude of 6.34°N and a Longitude of 5.63°E. The city's elevation is 88 m above sea level. With a population of over 2,125,058, Benin City is the largest urban area in Edo State.

Sample collection and analysis: The 500 g of carboxymethyl starch (with a degree of substitution of 0.71), poly (vinyl alcohol) (PVA) and glycerol of analytical grade were purchased from a chemical supply store in Benin City. Kaolin clay was sourced from a factory in Auchi, Edo State. Lemongrass was harvested from the Ugbowo campus of the University of Benin. Microorganisms (*Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*) used in this study were obtained from the Department of Microbiology at the University of Benin. All other chemicals and reagents of analytical grade were obtained from the Chemistry Laboratory, University of Benin, Benin City. This study spanned from September, 2021 to July, 2023.

Extraction of lemongrass oil: The lemongrass oil was extracted using a standard steam distillation method as described by Dangkulwanich and Charaslertrangsi[19]. The process began by gathering lemongrass leaves from a garden, followed by washing and finely chopping them to fit into a 250 mL round-bottom flask. The chopped lemongrass was then placed into the flask and covered with distilled water. A steam distillation setup was assembled and the flask was heated to boiling for approximately 2 hrs. The oil extracted from the lemongrass was separated from the water using a Clevenger apparatus, collected in a centrifuge tube and stored for subsequent use in film preparation.

Preparation of carboxymethyl starch/PVA/kaolin composite films: Carboxymethyl starch/PVA film reinforced with kaolin clay was prepared by solution casting as described by Garavand *et al.*[20] with slight modifications. Initially, various masses of kaolin clay were dispersed in 50 mL of distilled water and placed in a mechanical shaker for 4 hrs to ensure uniform dispersion of the clay particles. Simultaneously, CMS slurry was prepared by suspending 2.0 g of CMS in 20 mL of distilled water. In a separate container, a PVA solution was created by dissolving 3.0 g of PVA in 100 mL of distilled water at 90°C for 30 min until complete dissolution occurred. Once the PVA was dissolved, the mixture of clay/CMS was added to the PVA solution, followed by the addition of glycerol (30% of the total dry weight of CMS and PVA). The entire mixture was stirred using a magnetic stirrer for 30 min at 80-90°C until complete gelatinization and homogenization were achieved.

Next, the film-forming solution was cast onto a waxed glass plate measuring 10×10 cm. The mold containing the solution was dried in an oven at 60°C for 16 hrs. After drying, the films were carefully removed from the glass plate and stored in a desiccator before further analysis. Film thickness was controlled by measuring a known volume of the film solution on the glass plate.

Preparation of lemongrass oil-incorporated carboxymethyl starch/PVA/kaolin composite films: To prepare carboxymethyl starch/PVA/kaolin composite films incorporated with lemongrass oil, the following procedure was employed. Initially, the filmogenic mixture comprising carboxymethyl starch, PVA and kaolin was prepared following the method outlined in the previous section. The temperature of the filmogenic mixture was subsequently lowered to 40°C and lemongrass oil in varying quantities was

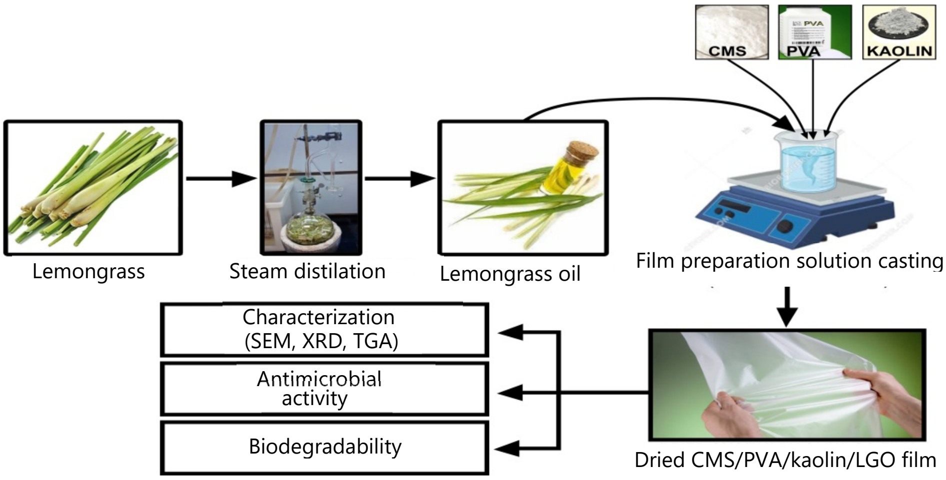

Fig. 1: Schematic flow chart for the preparation, characterization and property evaluation of films, CMS: Carboxymethyl starch, PVA: Polyvinyl alcohol (PVA) and LGO: Lemongrass oil

Table 1: Composition of composite films

Films	CMS (%)	PVA (%)	Kaolin (pph)	LGO (pph)
CMS-PVA-	40	60	4.5	0.0
kaolin	40	60	4.5	1.0
incorporated	40	60	4.5	2.0
with LGO	40	60	4.5	3.0
	40	60	4.5	4.0
	40	60	4.5	5.0

gradually introduced into the mixture while continuously stirring with a magnetic stirrer for an additional 20 min. The resulting mixture was then cast onto a glass plate and allowed to stand at room temperature for 2 hrs to facilitate evaporation and curing. Further drying of the films was carried out in an oven at 60°C for 16 hrs. Once dried, the films were carefully removed from the glass plates and stored in a desiccator for subsequent analysis. The film thickness was controlled by pouring a measured volume of the film solution into the mold (glass plate). The specific compositions used in the preparation of the films and the schematic flowchart for the preparation, characterization and properties evaluation of the composite films are displayed in Table 1 and Fig. 1.

Scanning electron microscopy (SEM): The morphological characteristics of the films were examined using a scanning electron microscope (Phenom ProX by phenomWorld Eindhoven, Netherlands) operated at an accelerating voltage of 10 kV. Before imaging, the film specimens were affixed to a brass stub and coated with a thin layer of gold to enhance electrical conductivity. Scanning electron micrographs were captured of the upper surface of the films at magnifications ranging from 1500 to 2000 times.

X-ray diffraction (XRD): The crystalline structure of the film samples was analyzed using an X-ray diffractometer (Rigaku Miniflex 600, Japan) equipped with copper Kα radiation (λ = 1.5444 Å). The X-ray tube operated at 40 kV and 15 mA. The A 1° divergence slit was employed for the incident beam path. Diffraction patterns were recorded over a range of 2θ = 2°-70° at room temperature to determine the crystallinity and structural characteristics of the films.

Thermal stability: The thermal stability of the films was determined by thermogravimetric analysis using a PerkinElmer thermal analyzer (TGA 4000, Netherlands) under a nitrogen atmosphere. The heating rate was set at 10°C/min, covering a temperature range from 30 to 950°C. The thermogravimetric analysis (TGA) and derivative thermogravimetric analysis (DTG) curves were generated and used to assess the thermal stability of the films.

Determination of antimicrobial activity: The antimicrobial activity of the composite films was determined using agar diffusion assay as per the method outlined by Sadiq *et al.*[21] with some modifications. In this experiment, circular films measuring 10 mm in diameter were cut and placed onto Petri dishes coated with Mueller-Hinton agar. These agar plates had previously been inoculated with bacterial cultures adjusted to a standard optical density of 0.5 McFarland. The plates were then incubated at 37°C for 24 hrs. Following incubation, the diameter of the clear zones devoid of bacterial growth surrounding each film was measured to evaluate their antibacterial efficacy.

Determination of biodegradation: Soil burial degradation was performed using the soil burial method as described by Omoike *et al.*[8]. Garden pots with an approximate capacity of 10 L were filled with compost soil sourced from a local nursery farm. Rectangular pieces of the films, sized 3×5 cm, were buried in the soil at a depth of 10 cm. These pots were maintained at room temperature in a laboratory environment. The soil moisture was regulated by watering twice daily. To monitor degradation, the films were periodically removed from the soil at intervals of 7 days over 8 weeks. Each film was gently washed with water to remove any adhering soil and then weighed to determine the dry residual film weight. The percentage weight loss of each film sample over time provided a measure of its degradation rate in the soil burial test. The degree of soil burial degradation (DSBD) was calculated using a specific equation (Eq. 1)[8]:

$$\text{DSBD (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

where, W_1 is the original weight of the film and W_2 is the final weight of the film after soil burial degradation.

Tools and equipment manufacturers: The instruments used in this study, such as the digital caliper, oven, analytical balance, desiccator and other equipment, were obtained from well-known manufacturers, including Hanna Instruments (Woonsocket, Rhode Island, USA), Thermo Fisher Scientific (Waltham, Massachusetts, USA) and Mettler Toledo (Columbus, Ohio, USA), among others[22,23].

Statistical analysis: The statistical analysis was carried out using the BMDP 2R software for stepwise multiple regression. Data were presented as the mean of three replicate measurements. The findings of the study were considered statistically significant at $p<0.05$[24].

RESULTS AND DISCUSSION

Scanning electron microscopy (SEM): A Fig. 2 presents SEM images illustrating different stages of the composite film's development. The SEM images of the three samples showed notable differences in their surface morphology. The SEM micrograph of CMS powder (Fig. 2a) revealed a porous and granular surface structure[8]. Conversely, the SEM image of the CMS/PVA/kaolin composite (Fig. 2b) displayed a smoother, more uniform surface with visible particle aggregates, likely due to the presence of kaolin. The lack of the porous, granular structure observed in CMS powder suggests successful gelatinization of CMS and enhanced cohesion between CMS, PVA and kaolin in the composite film[25,26]. The inclusion of LGO in the CMS/PVA/kaolin/LGO composite (Fig. 2c) further alters the surface morphology. The porosity is significantly reduced, resulting in a smoother and more uniform surface. Visible LGO droplets or agglomeration zones are evident, which could indicate an increase in hydrophobicity. This distribution of LGO on the surface suggests it may affect the compatibility of the components and the overall properties of the material[27].

X-ray diffraction: The Fig. 3 displays the XRD diffractograms of CMS powder and the composite films. Figure 3a depicts that the diffractogram of CMS powder shows a distinct peak at $2\theta=28.6°$, reflecting the presence of crystallites within its granular structure[8]. Conversely, the diffractogram of the CMS/PVA/kaolin film in Fig. 3b illustrates a notable decrease in crystallinity, as seen by the broadened peak and diminished

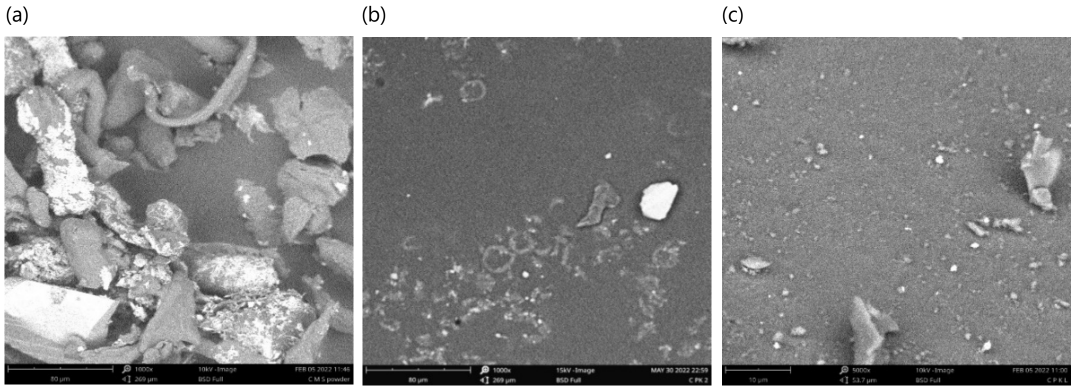

Fig. 2(a-c): SEM micrographs of CMS powder and composite films, (a) CMS powder, (b) CMS/PVA/kaolin composite and (c) LGO incorporated CMS/PVA/kaolin film

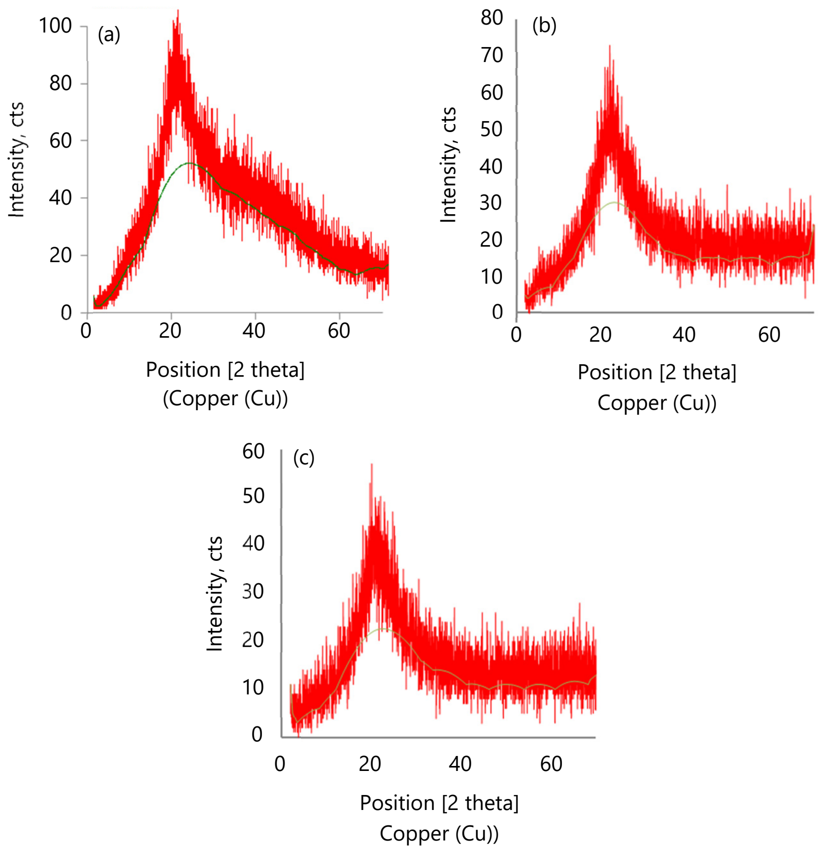

Fig. 3(a-c): X-ray diffraction patterns, (a) CMS powder, (b) CMS/PVA/kaolin composite and (c) LGO incorporated CMS/PVA/kaolin composite

intensity. This change suggests that the granular structure has swelled due to the gelatinization of CMS[8,25] and the addition of PVA and kaolin disrupts the starch's crystalline structure and decreases the overall crystallinity. The alteration in peak positions suggests interactions between starch and PVA/kaolin, likely resulting from hydrogen bonding or other intermolecular forces[26,27].

Figure 3c illustrates the XRD diffractogram of LGO-incorporated CMS/PVA/kaolin composite film. The result reveals that the addition of LGO to the CMS/PVA/kaolin/LGO composite further disrupted the crystalline structure, resulting in a more amorphous material as evidenced by the decreased peak intensity. This increase in amorphousness can be attributed to the hydrophobic nature and plasticizing effect of LGO. These characteristics likely diminished the hydroxyl groups and weakened the intermolecular bonding within the composite, as observed in the SEM images.

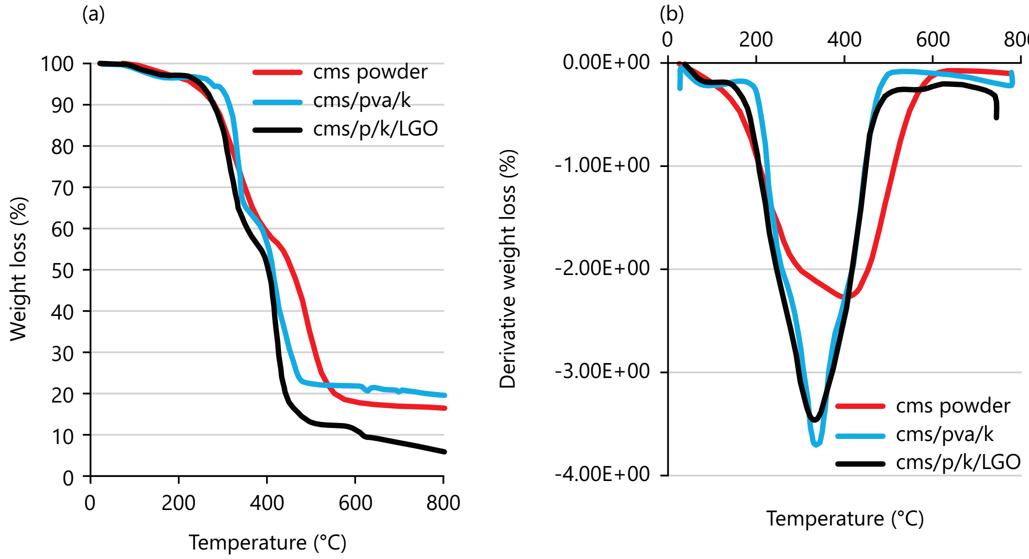

Fig. 4(a-b): (a) TGA and (b) DTA curves of CMS powder, CMS/PVA/kaolin and CMS/PVA/kaolin/LGO composite films

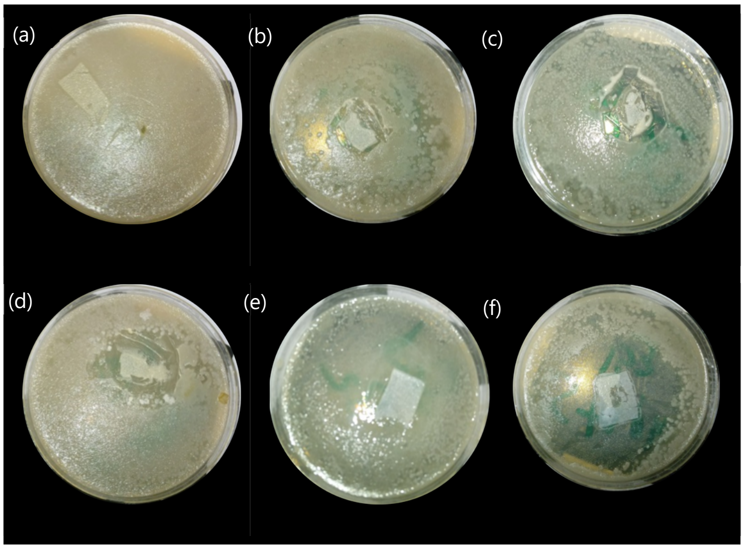

Fig. 5(a-f): Inhibition zones of CMS-based composite films, (a) Control and (b-f) Lemongrass incorporated films at concentrations of 1, 2, 3, 4 and 5pph respectively

Table 2: Minimum inhibitory concentration (MIC) of LGO against tested pathogens

Microorganisms	MIC concentration (%)
Escherichia coli	0.50
Salmonella typhimurium	0.60
Staphylococcus aureus	0.45

Table 3: Antimicrobial properties of composite films against tested microorganisms

		Inhibition zones (mm)		
Films	LGO (pph)	*Staphylococcus aureus*	*Salmonella typhimurium*	*Escherichia coli*
CMS-PVA-kaolin	0	0.00	5.00	0.00
incorporated with	1.0	14.00	10.00	10.00
LGO	2.0	20.00	14.00	12.00
	3.0	23.50	16.00	16.00
	4.0	26.00	17.00	17.00
	5.0	31.00	19.00	19.00

Thermal stability of CMS-based films: Thermogravimetric analysis was employed to study the thermal stability of the CMS-based composite films and the resulting TGA and DTA curves are depicted in Fig. 4.

Figure 4a illustrates the TGA curves of the CMS powder, CMS/PVA/kaolin film and LGO-incorporated CMS/PVA/kaolin film. From the curves, the thermal degradation process, of the composite films was characterized by three distinct steps. The initial step referred to as incipient degradation, involved the evaporation of adsorbed water and volatile components such as glycerol[8]. The subsequent steps corresponded to the decomposition of the composite constituents: CMS, PVA and kaolin. The final and ultimate degradation temperatures of the films were determined from the DTA curves as illustrated in Fig. 4b. It is evident that CMS powder exhibited the highest thermal stability among the samples. The CMS/PVA/kaolin composite film also demonstrated good thermal stability, albeit slightly reduced upon the incorporation of LGO.

Antimicrobial properties of carboxymethyl starch-based composite films: Initially, the minimum inhibitory concentration (MIC) of lemongrass oil against the tested pathogens was determined (Table 2). This information was crucial for determining the appropriate concentration of lemongrass oil to be integrated into the CMS composite films to impart antimicrobial properties. Specifically, the MIC values against *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus* were found to be 0.5, 0.6 and 0.45%, respectively. Iamareerat *et al.*[28] had earlier reported similar results with cinnamon oil and lemongrass oil respectively against the same pathogens.

Figure 5 and Table 3 show the inhibition zones produced by CMS composite films at varying concentrations of LGO against each tested microorganism namely *Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli*.

The control sample shown in Fig. 5a is a petri dish containing CMS/PVA/kaolin without LGO. The remaining Fig. (5b-f) demonstrate the inhibition zones produced by the CMS/PVA/kaolin composites films at different LGO concentrations. Specifically, Fig. 5b, shows the Petri dish containing CMS/PVA/kaolin with 1pph LGO, while Fig. 5c-e shows Petri dishes containing CMS/PVA/kaolin with 2, 3 and 4 pph LGO, respectively. Finally, Fig. 5f depicts the petri dish containing CMS/PVA/kaolin with 5 pph LGO.

The inhibition zone observed in Fig. 5b-f indicates the antimicrobial efficacy of CMS/PVA/kaolin composite films against *Staphylococcus aureus* (as a typical case) with increasing LGO concentrations corresponding to larger inhibition zones.

Table 3 shows the inhibition zones produced by CMS composite films at varying concentrations of LGO against each tested microorganism namely *Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli*. The results indicated that films lacking LGO exhibited no inhibition zones against the tested pathogens, except for a small zone against *Salmonella typhimurium* measuring 5.0 mm. Increasing concentrations of LGO in the films demonstrated significant antimicrobial activity against all microorganisms. Particularly notable was the highest antimicrobial efficacy against *Staphylococcus aureus* (a Gram-positive bacterium), evidenced by larger inhibition zones. However, the films displayed comparatively lower antimicrobial effectiveness against the other two bacteria, *Salmonella typhimurium* and *Escherichia coli*, both Gram-negative species. This reduced efficacy against Gram-negative bacteria may be attributed to their additional outer membrane, composed of lipopolysaccharides, which hinders the diffusion of hydrophobic compounds like LGO[29]. Similar findings have also been reported by Ahmad *et al.*[18]who studied the physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils.

Table 4: Percentage of weight loss of films (CMS-based) for 56 days

		Percentage weight loss of films(g) after								
Films	LGO	0 days	7 days	14 days	21 days	28 days	35 days	42 days	49 days	56 days
CMS-PVA-	0.0	0	16	27	35	38	42	46	52	60
kaolin	1.0	0	15	25	35	41	47	51	54	56
with LGO	2.0	0	14	23	30	36	42	47	52	55
	3.0	0	14	22	30	34	38	43	48	53
	4.0	0	12	20	27	33	36	40	45	50
	5.0	0	12	21	26	32	37	40	43	50

Biodegradation of CMS composite films: Biodegradation involves the decomposition of materials by microorganisms such as bacteria, yeasts and fungi found in the soil[30]. The biodegradability of CMS/PVA/kaolin/LGO composite films was evaluated using the soil burial method. This approach offers a valuable method for assessing the biodegradability of biobased films as it replicates the conditions these films would face during disposal[31]. The films were buried in soil and their weight loss was monitored at 7-day intervals over 56 days (Table 4). The extent of biodegradation, assessed through the percentage of weight lost, tracked the degradation progress of the composite films. The findings showed a rapid initial biodegradation rate across all films, characterized by substantial weight loss within the first 7 days. However, this degradation rate decelerated in subsequent periods until the final 56 days. The rapid initial biodegradation rate can be attributed to favorable conditions such as high microbial activity, rapid moisture absorption, readily available carbon sources and the presence of hydrophilic groups[32]. After 56 days, the findings indicated that CMS/PVA/kaolin (without LGO) experienced a weight loss of 60%, signifying substantial degradation of the composite films. In contrast, composite films incorporating LGO (CMS/PVA/Kaolin/LGO) exhibited weight losses ranging from 56 to 50%, depending on the specific composition. This suggests that the addition of LGO to the composite films marginally decreased weight loss by approximately 16.7% (at 5.0 pph LGO concentration) compared to films without LGO. However, the LGO-containing films still underwent significant degradation, albeit at a slightly slower rate than those without LGO. The reduced weight loss in LGO-containing films may be attributed to the antimicrobial properties of lemongrass oil, which potentially inhibited microbial degradation of the films[17]. However, the exact mechanism and implications of this observation require further investigation.

The development of lemongrass oil-incorporated carboxymethyl starch/poly(vinyl alcohol)/kaolin composite films has significant implications for the food packaging industry. These films offer a sustainable and innovative alternative to conventional plastics, which are widely used in food packaging but have negative environmental impacts. The antimicrobial properties of the films can enhance food safety and extend shelf-life, reducing foodborne illnesses and food waste. Additionally, the biodegradability of the films can reduce environmental pollution and promote eco-friendly packaging solutions.

The composite films have various potential applications in different industries. In the food packaging industry, they can be used as a sustainable packaging material for various food products, including meat, dairy and bakery products. The antimicrobial properties of the films make them suitable for packaging pharmaceutical products, such as pills and capsules, in the pharmaceutical industry. Furthermore, the films can be used as a sustainable packaging material for cosmetic products, such as creams and lotions, in the cosmetic industry.

To further advance the development and application of these composite films, several recommendations can be made. Firstly, further research should be conducted to optimize the formulation and processing conditions of the composite films. This can involve experimenting with different ratios of lemongrass oil, carboxymethyl starch, poly(vinyl alcohol) and kaolin to achieve the best balance of properties. Secondly, the scalability of the production process should be investigated to ensure commercial viability. Finally, the films should be tested for their compatibility with various food products and pharmaceuticals to ensure their safety and efficacy.

While the study demonstrates the potential of lemongrass oil-incorporated carboxymethyl starch/poly(vinyl alcohol)/kaolin composite films, there are some limitations to consider. Firstly, the study only investigated the antimicrobial properties of the films against three specific pathogens and further research is needed to determine their effectiveness against a broader range of microorganisms. Additionally, the biodegradability of the films was only tested under controlled laboratory conditions and their degradation in real-world environments needs to be studied. Finally, the films may have limited mechanical strength and barrier properties compared to conventional plastics, which could affect their performance in certain applications.

CONCLUSION

Based on the findings of this study, it has been demonstrated that LGO effectively functions as an antimicrobial agent against *Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli*, which are known for posing significant health risks in food products. The antimicrobial tests conducted showed that incorporating LGO into CMS/PVA/kaolin composite films resulted in substantial activity against these pathogens. The highest inhibitory effects were observed at a concentration of 5 parts per hundred (pph) LGO, which is below the minimum standard set by regulatory bodies such as the Food Standards Agency. Additionally, the biodegradability study indicated that all films exhibited significant biodegradation properties over the testing period. Despite the introduction of lemongrass oil compromising some material properties such as morphological and thermal stability, the films maintained sufficient integrity for various food packaging applications. In conclusion, the combination of effective antimicrobial efficacy and biodegradability in LGO-incorporated CMS-based composite films positions them as promising candidates for food packaging applications. These films have the potential to preserve food quality, extend shelf life and contribute to environmental sustainability by naturally degrading after use.

SIGNIFICANCE STATEMENT

The development and characterization of lemongrass oil-incorporated carboxymethyl starch/poly (vinyl alcohol)/kaolin composite films highlight their potential as a sustainable and innovative solution for food packaging. The significance of this research lies in its contribution to addressing two major global challenges: food safety and plastic waste management. By creating biodegradable and antimicrobial packaging materials, this research offers a promising alternative to conventional plastics, reducing environmental pollution and enhancing food quality and shelf-life. The findings of this study have important implications for the food packaging industry, providing a novel solution that meets the growing demand for eco-friendly packaging options.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the head of the Department of Chemistry, University of Benin for granting access to the laboratory facilities and to Mr. Isa Yakubu of Ahmadu Bello University for the technical support.

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