Nigerian Journal of Technology (NIJOTECH)
Vol. 44, No. 3, September, 2025, pp.422 - 432
www.nijotech.com

Print ISSN: 0331-8443
Electronic ISSN: 2467-8821
https://doi.org/10.4314/njt.v44i3.6

DEVELOPMENT AND CHARACTERIZATION OF LEMONGRASS ESSENTIAL OIL-INCORPORATED CARBOXYMETHYL STARCH/POLY (VINYL ALCOHOL)/KAOLIN COMPOSITE FILMS: ANTIMICROBIAL EFFICACY AND BIODEGRADABILITY ASSESSMENT

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ARTICLE HISTORY:
Received: August 04, 2024
Revised: August 26, 2025
Accepted: September 10, 2025
Published: October 15, 2025.

ARTICLE INCLUDES:
Peer review

DATA AVAILABILITY:
On request from author(s)

EDITORS:
Chidozie Charles Nnaji

FUNDING:
None

KEYWORDS:
Lemongrass Essential Oil, Antimicrobial, Packaging, Biodegradable, Composites

Abstract
Food preservation has been a serious global concern due to increased microbial activity. Therefore, the development of antimicrobial bioplastic packaging is an active area of research aimed at enhancing food quality and shelf-life while also addressing the environmental challenges posed by synthetic plastic. This study explored the antimicrobial and biodegradability properties of composite films prepared from carboxymethyl starch (CMS), polyvinyl alcohol (PVA), kaolin and integrated with lemongrass essential oil (LGO). The CMS-based composite films were produced using solution casting techniques and characterized by scanning electron microscopy (SEM), X-ray diffraction (XRD), and thermogravimetric analysis (TGA). The films' biodegradability properties were determined by soil burial method. The antimicrobial activity of the LGO-integrated films against Escherichia coli, Salmonella typhimurium, and Staphylococcus aureus was done using a disk assay antimicrobial test. The results of SEM, XRD, and thermal stability analyses showed that the films had good homogeneity, satisfactory surface morphology, and relatively high thermal stability. Although LGO affected the films' morphology and thermal stability, these properties remained suitable for food packaging applications. The LGO-incorporated films demonstrated good antimicrobial activity against the tested pathogens with highest inhibition zones of 31.00mm and 19.00mm observed at 5.0 parts per hundred (pph) LGO concentration. All the films exhibited substantial biodegradability but decreased slightly by 16. 67% upon incorporation of 5.0pph LGO. Therefore, LGO-incorporated CMS/PVA/kaolin composite film holds significant promise as a sustainable and renewable alternative to conventional plastics in food packaging.

HOW TO CITE:
Omoike B. A, Okeimen F. E, and Imoisi C., "Development and Characterization of Lemongrass Essential Oil-Incorporated Carboxymethyl Starch/Poly (Vinyl Alcohol)/Kaolin Composite Films: Antimicrobial Efficacy and Biodegradability Assessment", *Nigerian Journal of Technology*, 2025; 44(3), pp. 422 – 432; https://doi.org/10.4314/njt.v44i3.6

1.0 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]. Over the years, conventional plastics have mainly been used for packaging food materials.

While these plastics have been effective in protecting food from external environmental factors, they fail to address the threat of microbial growth within the packaged product. This limitation underscores the need for an effective packaging solution that can both protect food materials from the external environment and inhibit internal microbial growth [2].

To overcome this challenge, antimicrobial packaging derived from bioplastic with functional properties comparable to conventional plastics, while also inhibiting the growth of microorganisms, offers a promising solution [3]. By incorporating antimicrobial agents into packaging materials, we 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, 7]. 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 [7, 8]. However, starch films exhibit inferior mechanical and barrier properties when compared to conventional plastics like polyethylene [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 [9, 10, 11, 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, reduced tendency for retro-degradation, 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 [10, 14].

Incorporating reinforcing inorganic fillers has been recognized as an effective method to alter polymer structures, enhancing their mechanical, barrier, and thermal properties significantly [15]. 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 [16]. Plant essential oils and leaf extracts are actively researched as natural and safe sources of antimicrobial compounds. Lemongrass essential 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 essential oil is derived from the steam distillation of lemongrass (*Cymbopogon spp*.), yielding between 0.28% to 1.4% of a pale yellowish liquid [17].

Numerous studies have explored the antimicrobial efficacy of edible films containing lemongrass essential oil against pathogenic bacteria [12]. Perdana *et al*. [18] reported that incorporating lemongrass essential oil into cassava starch/chitosan film significantly enhanced its antimicrobial activity. Ahmad *et al*. [19] also reported that the gelatin film incorporated with lemongrass essential oil exhibited strong antimicrobial activity against selected microorganisms, with the highest inhibitory effect observed at 25% LGO concentration. However, while the addition of lemongrass essential 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 was detailed [20]. 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 essential oil was integrated into carboxymethyl starch/PVA/kaolin

https://doi.org/10.4314/njt.v44i3.6

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, to our knowledge, there are no reports on the biodegradability and antimicrobial properties of CMS/PVA/kaolin/LGO composite packaging.

2.0 MATERIALS AND METHODS

2.1 Materials

Carboxymethyl starch (degree of substitution = 0.71), polyvinyl alcohol (PVA), and glycerol of analytical grades were procured from a chemical supplier in Benin City. Kaolin clay was sourced from a factory in Auchi. 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, Benin City. All other chemicals and reagents used were of analytical grade.

2.2 Extraction of Lemongrass Essential Oil

The lemongrass essential oil was extracted using a standard steam distillation method as described by Dangkulwanich and Charaslertrangsi, [21]. The process began by gathering lemongrass leaves from a garden in University of Benin and identified by a botanist. It was then followed by washing and finely chopping to fit into a 250 mL round-bottom flask. The chopped lemongrass was then placed into the flask and covered with distilled water, before fitted with Clevenger apparatus. Heat was applied for two hours. At the end of the process the essential oil was collected into a centrifuge tube, and stored for subsequent use in film preparation.

2.3 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.* [22] with slight modifications. Initially, a known mass of kaolin clay was dispersed in 50 mL of distilled water and placed in a mechanical shaker for four hours to ensure uniform dispersion of the clay particles. Simultaneously, a 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 minutes 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 minutes 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 cm x 10 cm. The mold containing the solution was dried in an oven at 60°C for 16 hours. After drying, the films were carefully removed from the glass plate and stored in a desiccator prior to further analysis. Film thickness was controlled by pouring 100 mL of the film solution on the glass plate.

2.4 Preparation of Lemongrass Essential Oil-incorporated Carboxymethyl Starch/PVA/Kaolin Composite Films

To prepare carboxymethyl starch/PVA/kaolin composite films incorporated with lemongrass essential 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 essential oil in varying quantities was gradually introduced into the mixture while continuously stirring with a magnetic stirrer for an additional 20 minutes.

The resulting mixture was then cast onto a glass plate and allowed to stand at room temperature for 2 hours to facilitate evaporation and curing. Further drying of the films was carried out in an oven at 60°C for a duration of 16 hours. 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 100 mL of the film solution into the mold (glass plate). The specific compositions of the films used in this preparation are detailed in Table 1.

2.5 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 10kV. 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

https://doi.org/10.4314/njt.v44i3.6

surface of the films at magnifications ranging from 1500 to 2000 times.

2.6 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. 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.

Table 1: Composition of composite films

Films (Coded)	CMS (wt%)	PVA (wt%)	Kaolin (pph)	LGO (pph)
CMS/PVA/K (Control)	40	60	4.5	0.0
CMS/PVA/K/LGO1	40	60	4.5	1.0
CMS/PVA/K/LGO2	40	60	4.5	2.0
CMS/PVA/K/LGO3	40	60	4.5	3.0
CMS/PVA/K/LGO4	40	60	4.5	4.0
CMS/PVA/K/LGO5	40	60	4.5	5.0

*K = kaolin, LGO = lemongrass essential oil, PVA = poly (vinyl alcohol), CMS = carboxymethyl starch, pph = parts per hundred.

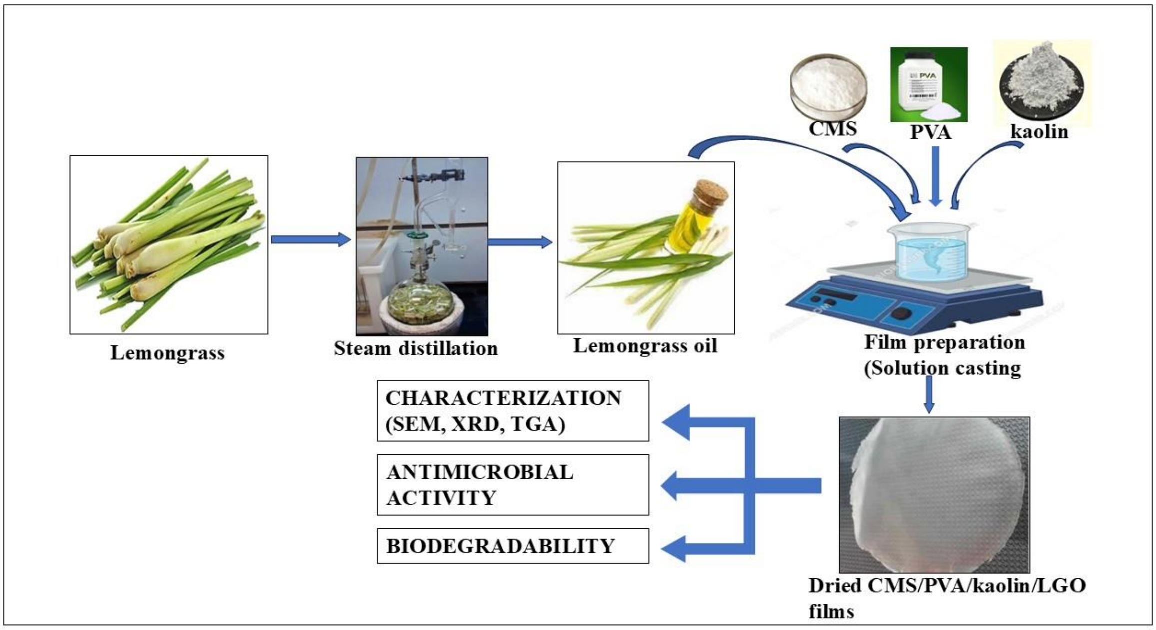

Figure 1: Schematic flow chart for the preparation, characterization and property evaluation of films

2.7 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°C 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.

2.8 Determination of Antimicrobial Activity

The antimicrobial activity of the composite films was determined using agar diffusion assay as per the method outlined by Omoike and Okieimen [23] 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 hours. Following incubation, the diameter of the clear zones devoid of bacterial growth surrounding each film was measured to evaluate their antibacterial efficacy.

2.9 Determination of Biodegradation

Soil burial degradation was performed using the soil burial method as described by Chaisuwan et *al.* [24] with slight modifications. Garden pots with an approximate capacity of 10 liters were filled with compost soil sourced from a local nursery farm.

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Rectangular pieces of the films, sized 3 cm x 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 an 8-week period. 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 (Eqn. 1).

$$\text{DSBD} = \frac{W_1 - W_2}{W_1} \text{ X } 100\% \dots\dots\dots\dots\dots\dots\dots (1)$$

3.0 RESULTS AND DISCUSSION

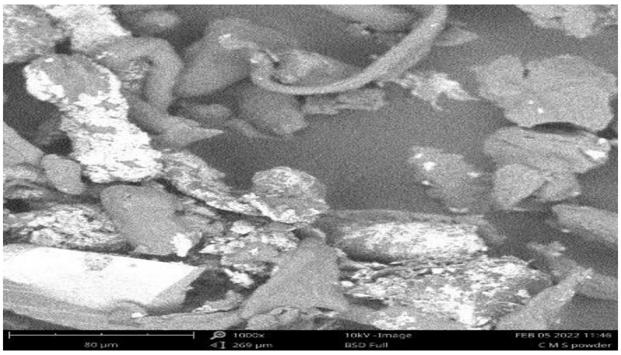
3.1 Scanning Electron Microscopy (SEM)

Figure 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 (Figure 2a) revealed a granular surface structure. Conversely, the SEM image of the CMS/PVA/kaolin composite (Figure 2c) displayed a smoother, more uniform surface with visible particle aggregates, likely due to the presence of kaolin. The lack of a granular surface structure (as 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 composite (Figure 2c) further alters the surface morphology. The dispersion level was reduced, resulting in a not so smooth and non-uniform surface. Visible LGO droplets or agglomeration zones were 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].

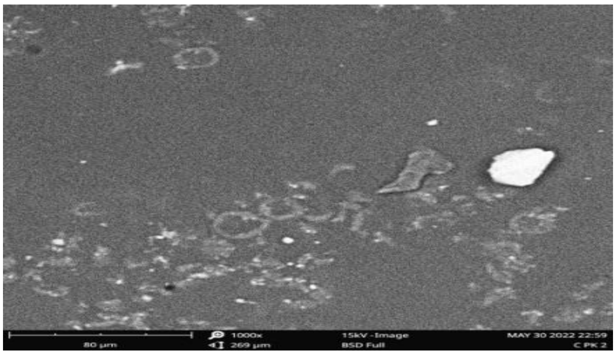
3.2 X-ray Diffraction

Figure 3 displays the XRD diffractograms of CMS powder and the composite films. The diffractogram of CMS powder (Figure 3a) shows a distinct peak at 2θ=28.6°, reflecting the presence of crystallites within its granular structure. Conversely, the diffractogram of the CMS/PVA/kaolin film (Figure 3b) indicates a notable decrease in crystallinity, as seen by the broadened peak and diminished intensity. This change suggests that the granular structure has swelled due to the gelatinization of CMS [25] and the addition of PVA and kaolin disrupts the starch's crystalline structure and decreasing 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].



(a)



(b)

(c)

Figure 2: SEM micrographs of CMS powder and composite films **(a)** CMS powder **(b)** CMS/PVA/K and **(c)** CMS/PVA/K/LGO5 films

The addition of 5.0 pph LGO to the CMS/PVA/kaolin composite (Figure 3c) 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

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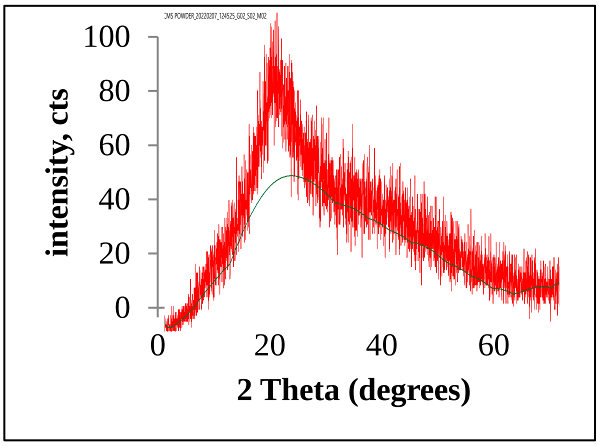
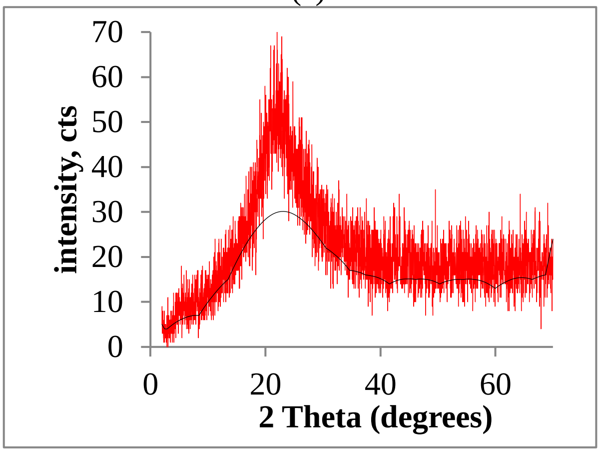
hydrophobic nature and plasticizing effect of LGO. These characteristics likely diminished the hydroxyl groups and weakened the intermolecular bonding within the composite, as observed in the SEM images.



(a)



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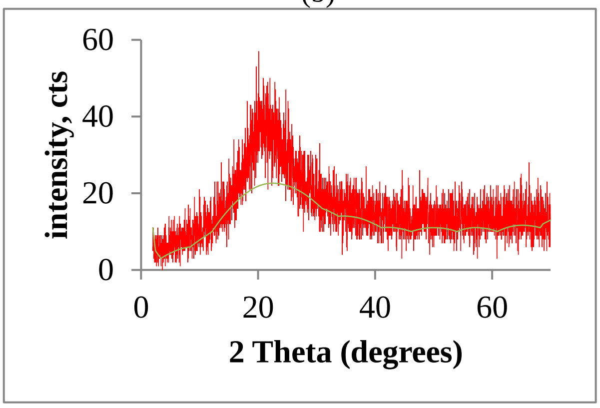

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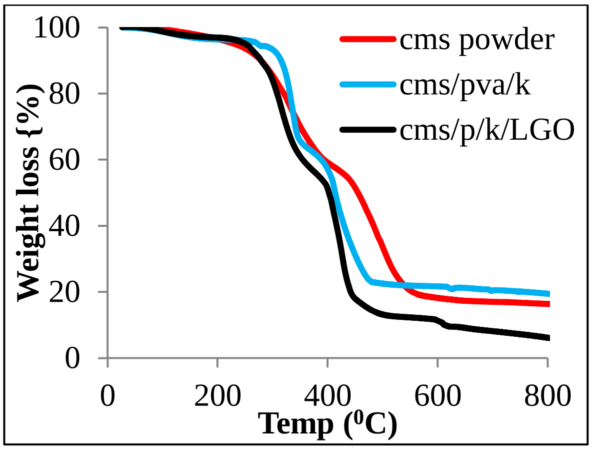
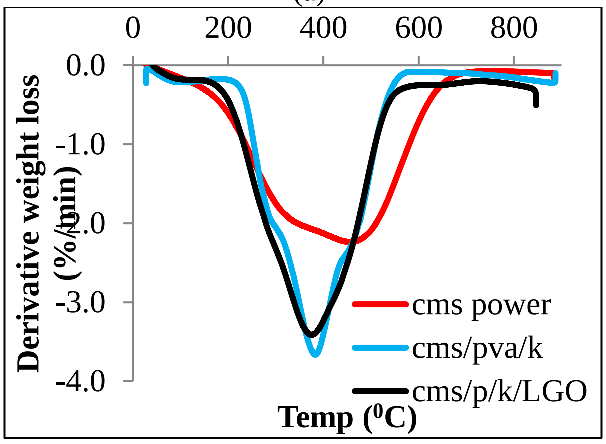
Figure 3: X-ray diffraction patterns of **(a)** CMS powder **(b)** CMS/PVA/kaolin composite and **(c)** CMS/PVA/K/LGO5 film.

3.3 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 Figure 5.



(a)



(b)

Figure 4: (a) TGA and (b) DTA curves of CMS powder, CMS/PVA/kaolin and CMS/PVA/K/LGO5 composite film

The 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. The subsequent steps corresponded to the decomposition of the composite constituents: CMS, PVA, and kaolin. The final degradation temperatures of the films were determined from the DTA curves, and the specific values are detailed in Table 2.

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Table 2: Ultimate degradation temperature of CMS composite films

Films	**T_{dmax} (°C)**
CMS powder	508
CMS/PVA/K	395
CMS/PVA/K/LGO5	390

Tdmax is the Ultimate degradation temperature, (°C)

According to Table 2, 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 5.0 pph LGO.

3.4 Antimicrobial Properties of Carboxymethyl Starch-based Composite Films

Initially, the minimum inhibitory concentration (MIC) of lemongrass essential oil against the tested pathogens was determined and is detailed in Table 3. This information was crucial for determining the appropriate concentration of lemongrass essential 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 essential oil respectively against the same pathogens.

Table 3: Minimum inhibitory concentration (MIC) of LGO against tested pathogens

Microorganisms	**MIC (%)**
Escherichia coli	0.50
Salmonella typhymurium	0.60
Staphylococcus aureus	0.45

5 shows pictorially the inhibition zones produced by the CMS composite films at varying concentrations of LGO against each tested microorganism using *Staphylococcus aureus* as a typical example

Figure 6: Inhibition zones of CMS-based composite films (a) control (b) CMS/PVA/K/LGO1 (c) CMS/PVA/K/LGO2 (d) CMS/PVA/K/LGO3 (e) CMS/PVA/K/LGO4 & (f) CMS/PVA/K/LGO5[L-R]

Table 4 presents the results of the antimicrobial properties of the composite films against *Staphylococcus aureus*, *Salmonella typhymurium* and *Escherichia coli* as depicted by their inhibition zones The results indicated that the film lacking LGO (CMS/PVA/K) exhibited no inhibition zones against the tested pathogens, except for a small zone of 5.0 mm against *Salmonella typhimurium*. It was also observed from the result that increasing the concentration of LGO in the films resulted in increase in inhibition zones, an indication of increased antimicrobial activity against all the microorganisms.

Most notably, CMS/PVA/K/LGO5 film containing 5.0 pph LGO exhibited the highest antimicrobial activity as revealed by the inhibition zones of 31.00 mm, 19.00 mm and 19.00 mm against *Staphylococcus aureus*, *Salmonella typhymurium* and *Escherichia coli* respectively. The result also shows that the antimicrobial activity of the films against *Staphylococcus aureus* (a Gram-positive bacterium) was highest, as 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.

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Table 4: Antimicrobial properties of composite films against tested microorganisms

Films	Inhibition zones (mm)		
	Staphylococcus aureus	***Salmonella typhymurium***	***Escherichia coli***
CMS/PVA/K (Control)	0.00	5.00	0.00
CMS/PVA/K/LGO1	14.00	10.00	10.00
CMS/PVA/K/LGO2	20.00	14.00	12.00
CMS/PVA/K/LGO3	23.50	16.00	16.00
CMS/PVA/K/LGO4	26.00	17.00	17.00
CMS/PVA/K/LGO5	31.00	19.00	19.00

This reduced antimicrobial action against the Gram-negative bacteria is likely 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.* [19] who studied the physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils.

3.5 Bio-degradation of CMS Composite Films

Biodegradation involves the decomposition of materials by microorganisms such as bacteria, yeasts, and fungi found in the soil [10]. The biodegradability of CMS/PVA/kaolin and LGO-incorporated composite films was evaluated using soil burial method. This approach offers a valuable method for assessing the biodegradability of biopolymer films as it replicates the conditions these films would face during disposal [30].

The films were buried in the soil, and their weight loss was monitored at 7-day intervals over a period of 56 days (Table 5). The extent of biodegradation as assessed through their percentage of weight lost was used to track the degradation progress of the composite films. The findings showed that there was a rapid initial biodegradation rate across all the films as revealed by their substantial weight loss within the first 7 days. However, this degradation rate decreased 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. [31]. 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 incorporated with 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 essential oil, which potentially inhibited microbial degradation of the films [18]. However, the exact mechanism and implications of this observation require further investigations.

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Table 5: Percentage weight loss of films (CMS-based) for 56days

Films	Percentage weight loss of films(g) after								
	0 days	**7 days**	**14 days**	**21 days**	**28 days**	**35 days**	**42 days**	**49 days**	**56 days**
CMS/PVA/K	0	16	27	35	38	42	46	52	60
CMS/PVA/K/LGO1	0	15	25	35	41	47	51	54	56
CMS/PVA/K/LGO2	0	14	23	30	36	42	47	52	55
CMS/PVA/K/LGO3	0	14	22	30	34	38	43	48	53
CMS/PVA/K/LGO4	0	12	20	27	33	36	40	45	50
CMS/PVA/K/LGO5	0	12	21	26	32	37	40	43	50

4.0 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 antimicrobial activity against these pathogens. The highest inhibitory effects were observed at a concentration of 5.0 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 the films exhibited significant biodegradation properties over the testing period. Despite the introduction of lemongrass essential 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.

5.0 ACKNOWLEDGEMENT

The authors gratefully acknowledge the head of 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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