POLYSACCHARIDE BASED FILM AS A FOOD PACKAGING MATERIAL

A PROJECT REPORT

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CANDIDATE'S DECLARATION

I, Tanvi Singh [2K21/MSCCHE/45], student of M.Sc. (Applied Chemistry), hereby declare that the project Dissertation titled "Polysaccharide based film as a food packaging material" which is submitted by me to the Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Masters, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or Other similar title or recognition.

Place: Delhi **(Tanvi Singh)**

Date: 23rd May 2023

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CERTIFICATE

I hereby certify that the project titled "Polysaccharide based film as a food packaging material" which is submitted by Tanvi Singh [2K21/MSCCHE/45] of Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Masters in Science, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Place: Delhi **(PROF. RAJINDER K.GUPTA)**

Date: 23rd May 2023 **SUPERVISOR**

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I am in debt to our parents and our family for their valuable support in every situation for of life. Last but not the least; I would like to thank God for his blessings in the form of support from all these wonderful people in my life to achieve a specified goal.

TANVI SINGH

ABSTRACT

Using the solvent casting approach, a new composite hydrogel film based on ascorbic acid loaded carboxymethyl guar gum (CMG) and citric acid (CA) was produced for food packaging applications. The addition of glycerol to the formulation increased the film's flexibility and mechanical strength. The films were tested for anti-oxidant and anti-microbial properties against Escherichia coli and Staphylococcus aureus using the DPPH+ assay. MTT assay was used to assess the film's cytotoxicity. FTIR, XRD, SEM, and TGA were used to perform structural, morphological, and thermal investigation on the film. The film's viscosity and mechanical strength were investigated. The film's fruit shelf life-enhancer qualities were also evaluated utilizing orange as a fruit sample.

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CHAPTER-1
INTRODUCTION

Plastic packaging has recently posed major disposal and environmental concerns, endangering human health and development. Food packaging contains a substantial amount of these plastic components. Only 9% of plastic garbage is recycled globally, while 22% is improperly disposed of. Given the need for improved food product safety, it is critical to examine new food packaging with a variety of functional aspects. When it comes to packaged food, consumers appreciate the naturalness of the product and the extended shelf life. According to Mintel study, two out of every five Indians (44% of the population) would choose one product over another if it had a longer shelf life. Synthetic plastics are not biodegradable, have limited reuse and recycling alternatives, and have additional negative environmental implications. The use of bioactive packing materials as a replacement for plastic packaging may be a solution to such issues. Natural polymer-based hydrogel films (bio-based films) can be an excellent choice for food packaging materials. Natural materials are emphasized and encouraged to be used instead of other possibilities since they are better for the environment. [2] Bio-based films could be made from lipids, proteins, polysaccharides, or a combination of these substances. Carbohydrate polymers stand out as attractive food packaging materials due to their exceptional mechanical properties (such as tensile strength, toughness, and elongation). [3]

Carboxymethyl guar gums cross-linked with citric acid hydrogel films and loaded with ascorbic acid (CMG-CA-AA) have been proposed as a potential replacement in this area. All of the raw materials used to make CMG-CA-AA films are non-toxic and low-cost, and the manufacturing method is easy and economical.

Guar gum (GG) is a seed gum derived from Leguminosae embryos of Cyamopsis tetragonolobus. [4-6] CMG is a GG derivative that is preferred over pure GG because to its low thermal stability, uneven hydration rate, lower viscosity upon storage, and higher risk of microbiological contamination. [7] Carboxymethylation is a chemical process that bonds pendant carboxymethyl groups to pure GG. [8]

Cross-linking is a common way for improving polymer performance. Citric acid, a polycarboxylic acid found naturally in citrus fruits such as lemons, is a low-cost and non-toxic example that has been used to improve the characteristics of polymers. [9, 10]

CMG cross-linked CA hydrogel sheets are devoid of intrinsic antioxidant and antibacterial capabilities. [1]Food packaging films with antimicrobial and antioxidant qualities can improve food quality and inhibit microbial development. [11] Ascorbic acid (AA) is a naturally occurring chemical with high polarity and antioxidant activity that can be found in a variety of foods such as mangoes, oranges, lemons, blackberries, and vegetables. [12, 13]

When designing packing material, mechanical strength is an important component to consider. A plasticizing chemical, glycerol, was added to the produced biofilm to improve this feature. Glycerol is an odorless, colorless, and slightly sweet-tasting naturally occurring alcohol that was discovered to improve the mechanical strength and flexibility of the films. [16]

The bioactive films were made from CMG that had been cross-linked by CA, with the addition of AA and glycerol to improve the film's characteristics. The film is biodegradable, non-toxic, colorless, and odorless, as well as having strong oxygen transmission barriers. [17] High oxygen transmission barrier materials are ideal for protecting packages. [18]

CHAPTER 2

LITERATURE REVIEW

Research work in the field of food packaging using guar gum based films has been explored in the recent times. Modified PVP-CMC hydrogel film based on bacterial cellulose and guar gum has been used as a packaging film. Food sample testing on fruit samples such as berries were done to evaluate its effectiveness. [19]

Applications and characterization of films made from GG and CMG have been studied. Various combination of the gum with other polysaccharide like chitosan, cellulose etc. [20]

GG AND CMG films cross linked with citric acid also showed studies as a food packaging material. CA as a cross linker being incorporated in packaging films increases the networking of the film thus enhancing the strength of films. It is also a natural derivative therefore is safe for usage in food packaging. [21, 22]

2.1 RESEARCH GAP

Reviewing the available research work on food packaging, it was concluded that use of CMG cross linked with CA, loaded with AA and glycerol as a plasticizer have not been explored. The research gap here leads to the studies of the work in this thesis.

CMG was used over GG due to its enhanced mechanical and other properties. CA was a natural cross linker for enhancing the network of polymer film. AA was incorporated to incorporate antimicrobial property in the film. Glycerol worked as a plasticizer to enhance the film’s mechanical strength.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 MATERIALS

CMG powder was purchased from local market and CA was purchased from CDH, New Delhi. AA was purchased from CDH. Glycerol used in the preparation was from Fisher chemical, UK.

3.2 METHODOLOGY

The polysaccharide films were created using a solvent casting method. CMG powder was first weighed and dissolved in deionized water. The gum mixture was stirred on a magnetic stirrer at 450 rpm for 1 hour to obtain a homogenous solution. After stirring for 1 hour, 0.1% (w/v) CA was added to the homogenized gum solution and again left for an hour of stirring. To the cross-linked CMG 0.1% (w/v) of AA was added. After 1 hour of stirring, 1ml of glycerol was added and the solution was thoroughly homogenized. The solution was sonicated for 30 min to remove any air bubbles in the solution. The final solution was oil cured for 5-7 minutes at a temperature of 140°C. Except for the addition of AA, controlled CMG-CA films were prepared with the same procedure. The f solution was transferred to petri dishes and dried for 24 hours at 45°C.

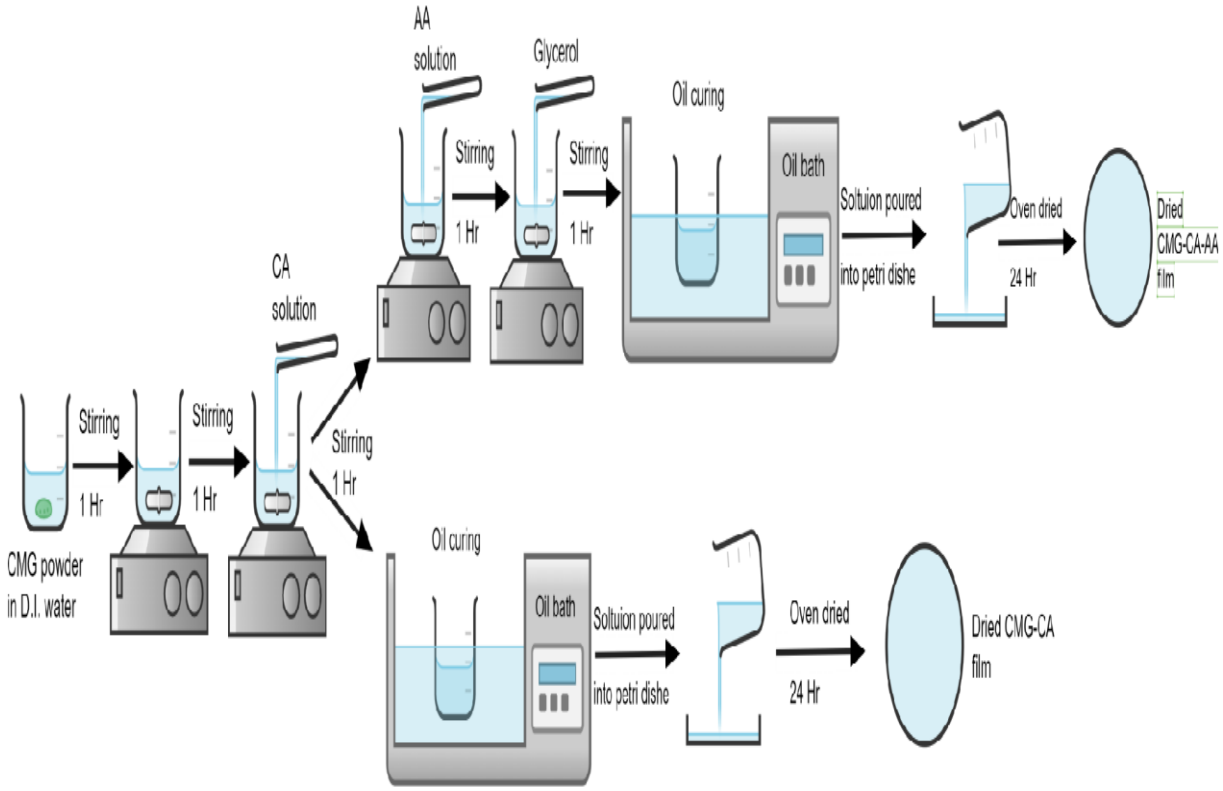


FIGURE 1 Preparation method of CMG-CA and CMG-CA-AA films

CHAPTER 3

CHARACHTERIZATION

3.1 MECHANICAL PROPERTIES

UTM (Instron RP01 5982) was used to study the mechanical properties of the CMG-CA and CMG-CA-AA films, such as thickness, elongation at break (EB %), and tensile strength (TS). The machine's load range was 1-20 N, its extension range was 1000 mm, and its gauge length was 25 mm. With no preload, the approach and test speeds were 500 mm/min. Each film was sliced into a 15 mm wide by 150 mm long strip for mechanical testing. Under room temperature, each sample received four readings, and the average result was utilized to analyze the mechanical feature.

FIGURE 2 UTM (Instron RP01 5982

3.2 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

The FTIR spectra of CMG-CA and CMG-CA-AA films were obtained using a Perkin-Elmer model 2000 Fourier transform infrared spectrophotometer. At room temperature, the spectral range observed was preserved between 4000-400 cm^{-1}.

FIGURE 3 Perkin-Elmer model 2000 Fourier transform infrared spectrophotometer

3.3 X-RAY DIFFRACTION (XRD)

To obtain crucial information regarding the solid-state structure of CMG-CA and CMG-CA-AA films, an Expert Pro MDR, Panalytical X-Ray diffractometer was used. The scan rate was 10 per minute in the range of 2θ = 50-800 with a Copper diode emitting Kα radiation of λ = 1.5406 Aº. [23]

FIGURE 4 Expert Pro MDR, Panalytical X-Ray diffractometer

3.4 THERMO GRAVIMETRICANALYSIS (TGA)

Thermal degradation of CMG-CA and CMG-CA-AA films was measured by thermo gravimetric analysis (Perkin Elmer, USA, TGA 4000). In a N_2 gas environment, roughly 5 mg of each sample was heated in a platinum crucible at a heating rate of 100 °C/min in a temperature range of 25-800°C. [24]

FIGURE 5 Perkin Elmer, USA, TGA 4000

3.5 SCANNING ELECTRON MICROSCOPY (SEM)

The morphological and physical properties of CMG-CA and CMG-CA-AA were examined using a scanning electron microscope (SEM) (ZESIS EVO MA15). During the investigation, the samples were gold-coated to prevent electrostatic charge under a strong electron beam and low resolution. At 10,000 magnifications, film samples were seen with an accelerating voltage of 5.0 kV. [24]

FIGURE 6 SEM ZESIS EVO MA15

3.6 IN VITRO ANTIMICROBIAL ACTIVITIES OF THE HYDROGELS

To assess the antibacterial activity of the films, a disc diffusion susceptibility test was performed. [25,26] The microorganisms tested positive on agar plates were gram-positive Staphylococcus aureus and gram-negative Escherichia coli. The experiment was performed using overnight broth cultures of both microorganisms. The agar solution was placed into Petri dishes under sterile conditions and allowed to harden. Bacterial inoculums were evenly distributed across the agar plates. A 1cm disc of CMG-CA and CMG-CA-AA film was cut and placed on agar plates exhibiting bacterial spread. These plates were sealed and placed in an incubator at 35°C for 24 hours. The inhibitory zone width (mm) around each sample was measured. The inhibition zones were measured in millimeters using a digital ruler.

3.7 ANTIOXIDANT ACTIVITY BY DPPH RADICAL SCAVENGING ASSAY

The free radical $DPPH^+$ (2,2-diphenyl-1-picrylhydrazyl) has an absorbance maxima of 515 nm. [25] Antioxidants diminish DPPH absorption. The $DPPH^+$ radical scavenging capacity of the samples was determined using the $DPPH^+$ radical scavenging assay. To be employed as a radical solution, 1 mg $DPPH^+$ was dissolved in 20 ml of methanol. DPPH produces a violet or purple color in methanol solution. When an antioxidant is present, the violet color fades to bright yellow. 1 ml of CMG-CA-AA homogeneous solution was mixed with 3 ml of methanol to make the antioxidant solution. For the photometric experiment, 1 mL of DPPH+ solution was mixed with 4 mL of antioxidant solution. [29] First, the absorbance of 1 mL DPPH+ was measured as a blank (using Ultraviolet-visible spectroscopy), and then the DPPH+ solution was mixed with the antioxidant solution. The readings were then taken promptly and at 30-minute intervals. The identical technique was followed for each of the five different CMG-CA-AA concentrations. The antioxidant activity was assessed by analyzing and estimating the

reduction in DPPH+ absorbance at various doses. [23] The % DPPH+ radical scavenging was calculated using the following equation (Blois and Desmarchelier)

$$[1 - \frac{a-b}{c}] \times 100 \quad [3.1]$$

where, a is the absorbance of sample solution, b is the absorbance of blank solution and c is the absorbance of DPPH+

A percentage higher than 85 indicates antioxidant activity. [29]

3.8 CYTOTOXICITY EVALUATION BY MTT ASSAY

An SRB assay was used to assess the cytotoxicity of the films on the HepG2 cell line. The cells were cultivated in 96-well plates for 24 hours in DMEM media supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO2. The following day, cells were treated with varied concentrations (0.075-5%). (Several concentrations were produced in an inadequate media.) After incubating for 24 hours, Tri Chloro Acetic Acid (TCA, 10%) was added to each well and incubated for an additional hour. After that, the plate was rinsed with DM water and left to dry at room temperature. Each well was given 0.04% SRB Solution, which was then added and left for an hour. After 1 hour of incubation, the plates were allowed to air-dry at room temperature. They were then rinsed with 1% (v/v) acetic acid to remove any leftover unbound color. Tris base solution (pH=5) was added to solubilize the protein-bound dye, which was then agitated for 10 minutes on an orbital shaker and read at 510 nm in an Elisa plate reader (iMark, Biorad, USA).

3.9 FOOD SAMPLE TESTING

The bioactive films' capacity to preserve fresh fruit oranges was tested in packaging studies. Oranges are commercially stored at a temperature of 3-9°C with an ideal humidity of 90-95% to avoid spoiling and extend shelf life. [30]Food spoils and wastes when such ideal circumstances are not available. The bioactive film that was created is

an attempt to produce sustainable packaging material with the fewest additional storage conditions. Three oranges were obtained and placed in separate petri dishes. The first orange was left unpackaged, that is, without any protective film. Then it was wrapped in CMG-CA film. Third, it's wrapped in a bioactive CMG-CA-AA film. The coating fully sealed the fruits. For 8 days, all of the samples were evaluated. The samples were stored at room temperature under natural light. The samples' deterioration and freshness levels were examined between the first and last days (8th). These modifications were examined in order to get a conclusion about the preservation capabilities of the produced bioactive film (CMG-CA-AA).

3.10 RHEOLOGICAL ANALYSIS

By integrating basic principles with other qualities, rheology is a useful tool for measuring the flow and deformation of matter. At 25°C, rheological experiments of liquid CMG-CA-AA were carried out using the Anton Paar Modular Compact Rheometer 302 (MCR). For the tests, 40 mm parallel plates were used. For the steady shear rheology tests, the constant shear rate ranged from 0.1 to 500 s^{-1}, whereas the variable shear rate ranged from 10 to 100 s^{-1}. [31]

FIGURE 7 Anton Paar Modular Compact Rheometer 302 (MCR

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 MECHANICAL PROPERTIES

The thicknesses of the CMG-CA and CMG-CA-AA films were found to be 0.08 mm and 0.03 mm, respectively. The mechanical testing results for both films are shown in Table 1. CMG-CA could withstand a maximum load of 17.65 N, whereas CMG-CA-AA could withstand a maximum force of 19.41216 N. CMG-CA and CMG-CA-AA had tensile strengths of 14.708 MPa and 43.138 MPa, respectively. The results show that the bioactive film CMG-CA-AA has a higher tensile strength than CMG-CA, indicating that it can withstand more force without breaking. The presence of glycerol in bioactive films accounts for their improved mechanical characteristics.

TABLE 1 Mechanical properties of the films.

Film	Width (mm)	Thick-ness	Max Force (N)	Tensile Strength (MPa)	Elongation at Break (%)
CMG-CA	15.00	0.0800	17.650	14.708	11.392
CMG-CA-AA	15.00	0.0300	19.412	43.138	13.240

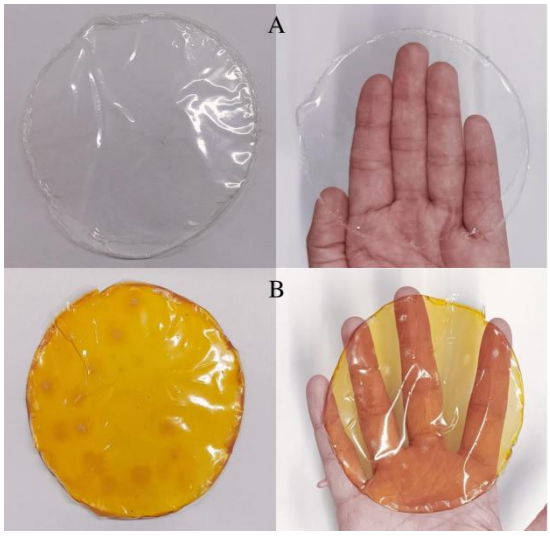


FIGURE 8 A) CMG-CA film, B) CMG-CA-AA film

4.2 FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY

The functional groups of the films were determined using FTIR spectroscopy. The spectra of CMG, CMG-CA, and CMG-CA-AA films are shown in Figure 9. Large, conspicuous peaks in the CMG spectrum between 3500 and 3000 cm^{-1} demonstrated the vibrational stretching of -OH. The asymmetric stretching of -CH was confirmed by the medium's peaks at 2852 cm-1 and 2925 cm^{-1}. [28] The maximum presence was attributed to the ester groups -CO at 1720 cm^{-1}. The C-O-C length of CMG's glycoside bond, resulting in its peak existence at 1023 cm^{-1}. The ester cross-links and carbonyl bond of free carboxylic acid were visible in the FTIR spectra of the cross-linked films CMG-CA and CMG-CA-AA at 1738cm-1 and 1726 cm-1, respectively. [33]

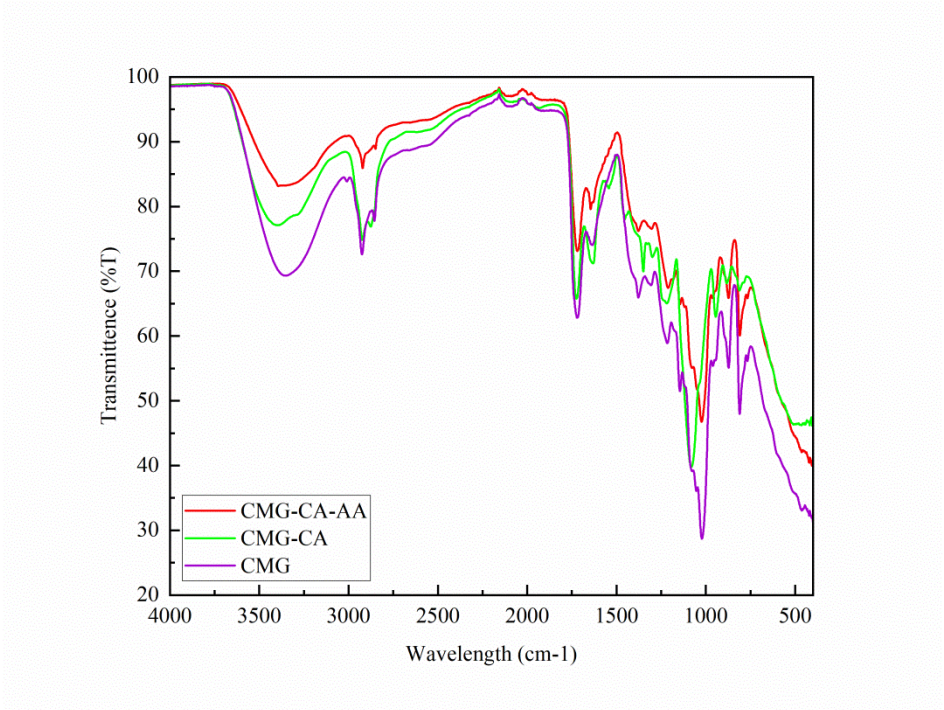


FIGURE 9 FTIR graphs of CMG, CMG-CA and CMG-CA-AA

4.3 X-RAY DIFFRACTION (XRD) ANALYSIS

The XRD results provide crucial information about how composition influences crystal structure and shape. The X-ray diffractograms of CA, CMG, CMG-CA, and CMG-CA-AA are shown in Figure 10. At 19.08°, CMG films display a large peak. The amorphous

nature of the CMG-CA and CMG-CA-AA films was indicated by large peaks at 2θ = 18.16° and 17.66°, respectively. Powder CMG is less amorphous, as evidenced by the large peak at 2 = 21.2°. Cross linking via CA led the hydroxyl groups in CMG-CA and CMG-CA-AA to be replaced by carboxymethyl groups, resulting in an increase in amorphous structure. The breaking of CMG crystal hydrogen bonds by cross linking promotes the amorphous character due to lower crystal stability. The moderately broader peak of CMG-CA-AA in comparison to CMG-CA at 18.36° suggests a smaller particle size which in turn suggests a higher amorphous nature of CMG-CA-AA. [29]

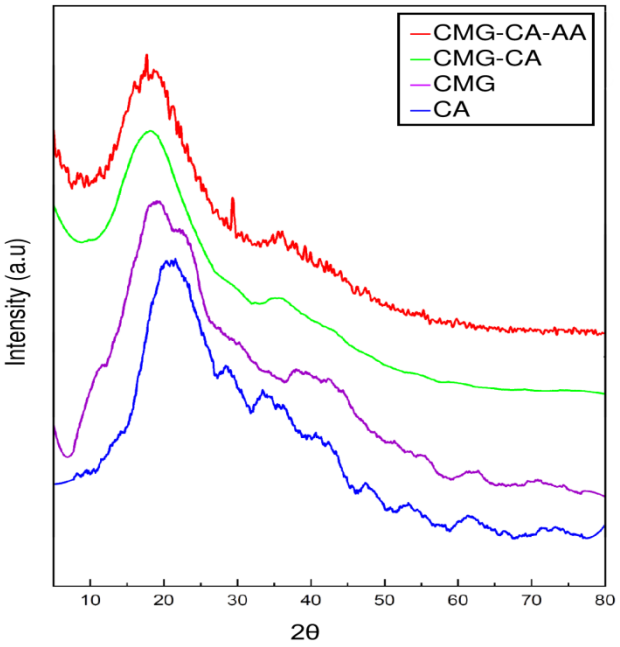


FIGURE 10 XRD graphs of CA, CMG, CMG-CA and CMG-CA-A

4.4 THERMAL ANALYSIS (TGA) ANALYSIS

The TGA curves, (Figure 11) of CA, CMG, CMG-CA, and CMG-CA-AA were studied to determine the thermal stability of the bioactive film. CMG-CA and CMG-CA-AA films showed 4 stages of thermal degradation due to water evaporation, decomposition of CA and AA, breaking of polymer linkage bonds, and degradation of polymer backbone. The 1st stage of mass loss at is due to the evaporation of water molecules in all the samples tested.

In CA 1st stage of degradation was observed at 29–220°C with 13.66% weight loss.2nd stage was at 220–585 °C (13.21%).

In CMG gum 1st stage of degradation was observed at 60–24°C with 15% weight loss.2nd stage occurred at 245–384 °C (38.56%).

In CMG-CA 1st stage of degradation was observed at 58.27–180°C with 6.88% weight loss. 2nd stage was observed at 1800–2670 °C (15.23%). The 3rd stage degradation ranged between 267-485ºC (45.27 %). The 4th and final stage of decomposition ranged from 485-7920C (26.99%).

In CMG-CA-AA 1st stage of degradation was observed at 5–220°C with 11.906% weight loss. 2nd stage was observed at 2200–3430 °C (39.39%). The 3rd stage degradation ranged between 343-493ºC (10.539 %). The 4th and final stage of decomposition ranged from 493-714 (15.76%).

In CMG-CA-AA, a slower thermal degradation occurred initially. Cross linking decreased the mass loss in both films in comparison to CMG. The temperature ranges responsible for major weight loss were increased in cross linked films, indicating improved thermal stability. CMG-CA-AA showed higher thermal stability than CMG-CA. [30]

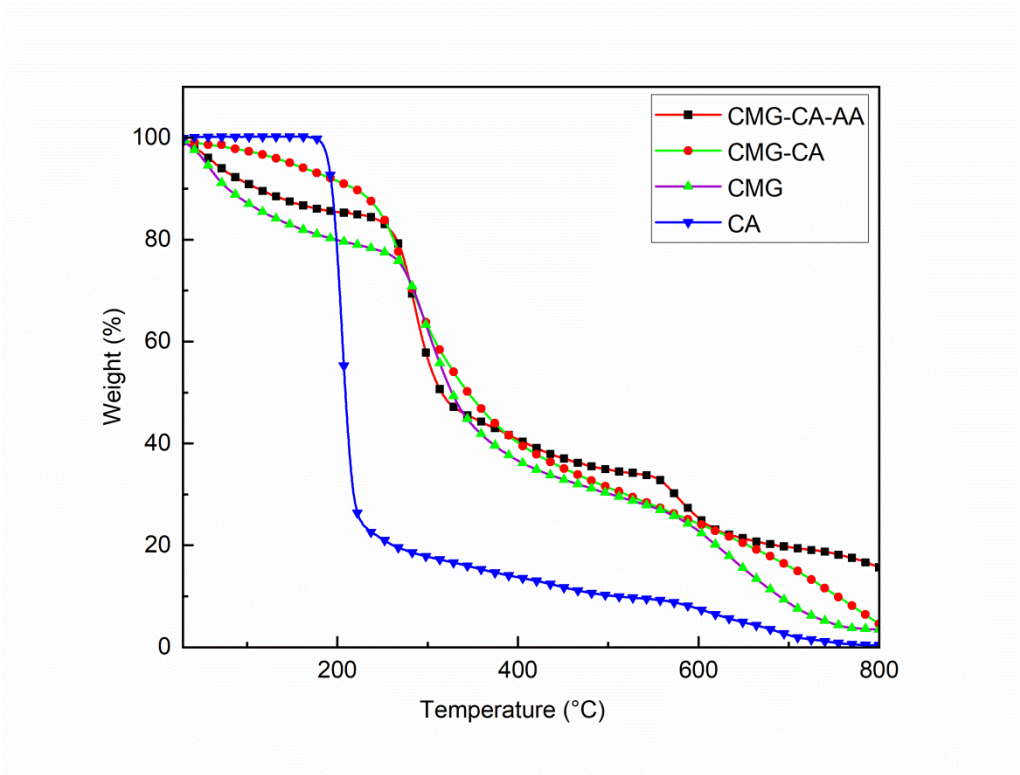


FIGURE 11 TGA curves of CA, CMG, CMG-CA and CMG-CA-AA

4.5 SCANNING ELECTRON MICROSCOPY (SEM)

Figure 12 shows scanning electron micrographs of the surface morphology of CMG-CA and CMG-CA-AA films. Because of their higher electrical conductivity than CMG-CA, SEM pictures of CMG-CA-AA were considerably easier to generate. Both films have particles of small sizes and unusual shapes. According to Z. Wu et al. (2019), SEM image analysis shows that the control CMG film has a non-porous and compact surface with a few clusters of particles visible on the surface. [34] CMG-CA-AA active films, on the other hand, have a smoother, more compact and homogenous surface with a patterned distribution of particle clusters.

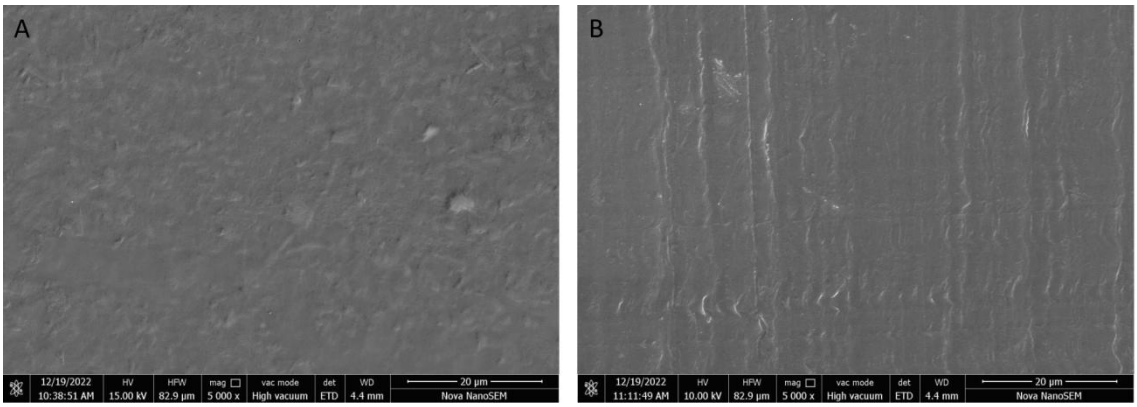


FIGURE 12 A) Scanning electron microscope (SEM) image of CMG-CA film and B) CMG- CA-AA film at 5000 magnification

4.6 ANTIBACTERIAL ACTIVITY EVALUATION

The antibacterial activity of CMG-CA and CMG-CA-AA films is depicted in Figure 13. The images show that controlled CMG films had no antibacterial action, but CMG-CA-AA had good antibacterial activity. CMG-CA-AA inhibited both gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli) bacteria in a different zone. The presence of AA contributed to CMG-CA-AA's ability to limit bacterial growth. Incorporating 0.5 mg of AA into CMG-CA films resulted in a 15 mm zone of inhibition against Staphylococcus aureus and an 18 mm zone of inhibition against Escherichia coli. As a result of the findings, it is possible to conclude that CMG-CA-AA films can inhibit the growth of bacterial strains. [35] CMG-CA-AA films' antibacterial qualities may be important for their use as a food packaging material. [36]

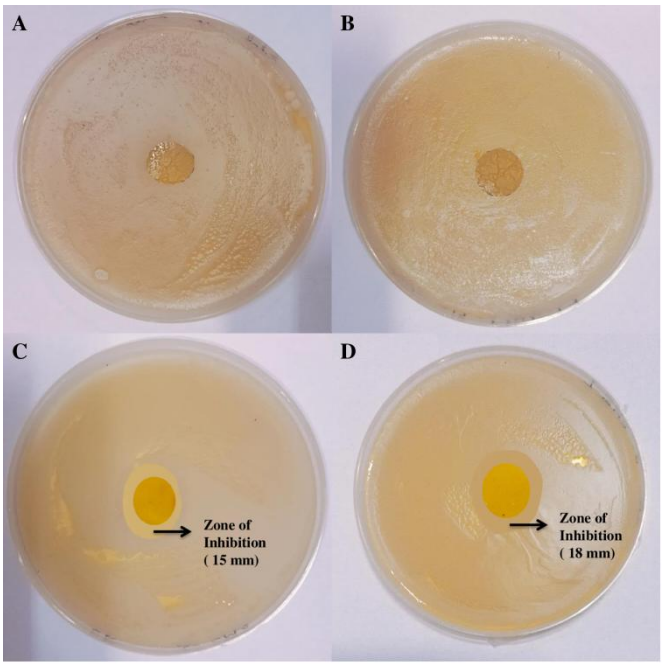


FIGURE 13 Antibacterial Assay, A) CMG-CA film in *Staphylococcus aureus*, B) CMG- CA film in *Escherichia coli* C) CMG-CA-AA film in *Staphylococcus aureus*, D)CMG-CA- AA film in *Escherichia coli*

4.7 ANTIOXIDANT ASSAY

CMG-CA-AA at five different doses were examined for antioxidant activity by analyzing the decrease of $DPPH^+$ radicals. Figure 8 shows plots of % DPPH scavenging against concentration. At max, the absorbance of DPPH+ was measured and found to be 0.863. The percentage of scavenging was highest (95%) at 100 L concentration and lowest (85.2%) at 500 L. As a result, increased scavenging was seen as concentration decreased. The scavenging activity of the samples was measured in the following order: 100 L, 200 L, 300 L, 400 L, and 500 L. Antioxidants can diminish DPPH free radicals by donating hydrogen, which is critical for preventing free radicals' damaging function in a variety of illnesses, including cancer. Because of their hydrogen donating or electron transfer capabilities, the results indicate that all of the loaded CMG-CA-AA samples have antioxidant qualities or radical scavenging activity.

TABLE 2 Percentage radical scavenging activity

Concentration (μL)	Absorbance (after 30 min)	Percentage scavenging activity
100	0.044	95%
200	0.077	91.1%
300	0.103	88.1%
400	0.113	86.8%
500	0.128	85.2%

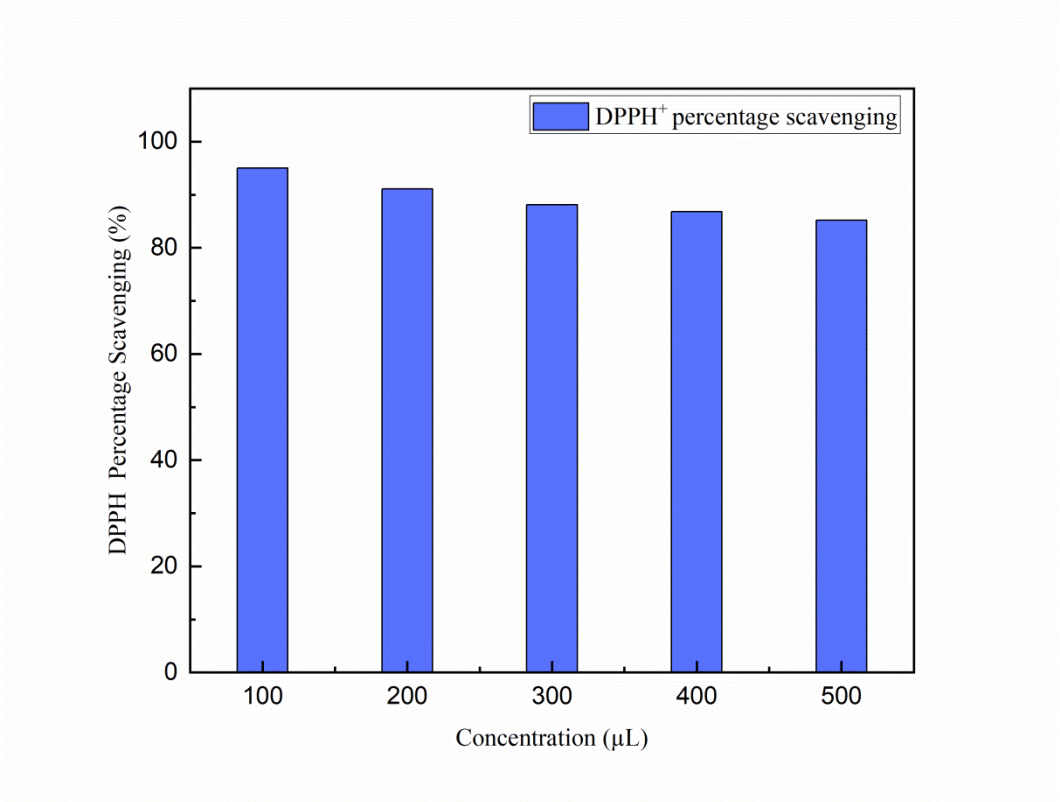


FIGURE 14 $DPPH^+$ scavenging percentage (antioxidant activity) graph of CMG-CA-AA

4.8 CYTOTOXICITY EVALUATION BY MTT ASSAY

The cytocompatibility of CMG-CA-AA films was evaluated using human hepatic cells (HepG2) as a model cell line. Figure 15 depicts the cytotoxicity data for various concentrations. The cells could stick to the film surfaces, distribute, and grow there, according to the results of the HepG2 cell culture experiment. After incubation, the cells

on the CMG-CA-AA films had a normal morphology compared to the control. [37] Figure 9 shows that all film samples of varying doses had cell viability values greater than 80%. This demonstrates the films' good biocompatibility with HepG2 cells. [37,38] As a result, the decreased cytotoxicity of CMG-CA-AA films verifies the bioactive film's cytocompatibility.

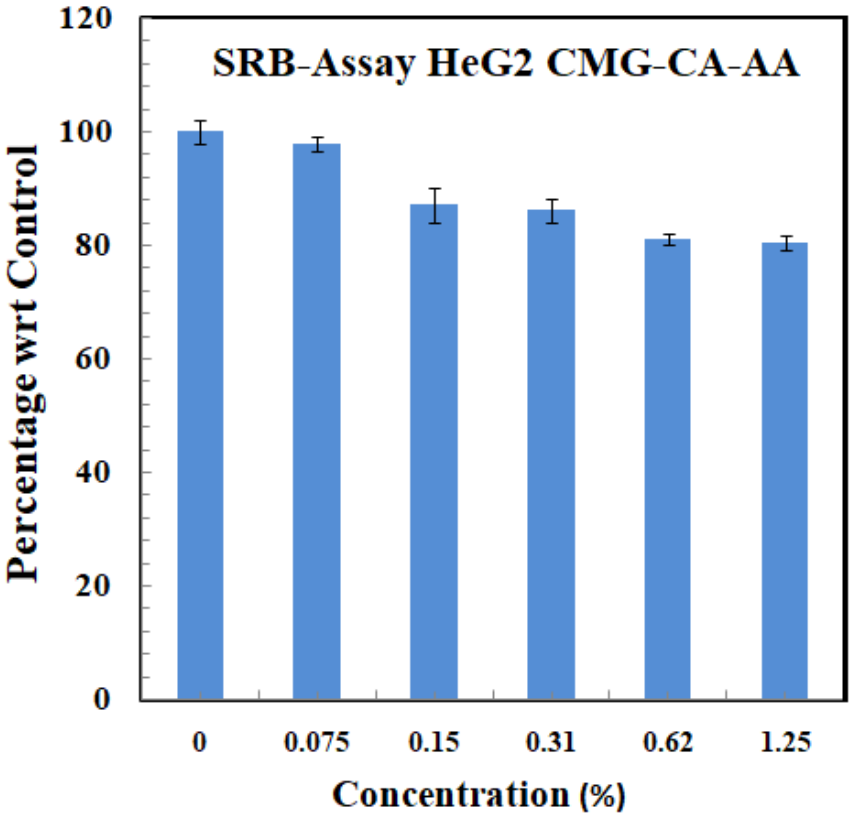


FIGURE 15 SRB assay-HepG2 of CMG-CA-AA at different concentrations

4.9 FOOD SAMPLING TEST EVALUATION

The dietary trials lasted 8 days in order to acquire precise findings, as shown in Figure 16. On the first day, all fruit samples seemed fresh and orange, with no microbial development. The oranges in the first and second Petri dishes began to ripen on day 2, while the third stayed unaltered. On day 3, the oranges in the first two Petri dishes began to darken, while the third orange maintained the same color. On days 4 and 5,

brown spots appeared on the oranges in the first and second Petri dishes, but there were no visible changes in the third. The brown stains on the first and second oranges grew larger on days 6 and 7, and white microbiological growth was noticed on the first orange. The third orange began to ripen. On day 8, the first two oranges had deteriorated dramatically and were no longer fit for consumption; however the third orange, which had been covered with the bioactive film, appeared exactly the same as on day 1 and was perfectly safe to eat. These findings suggest that the bioactive film protected the fruit successfully and might be employed as a packaging material.

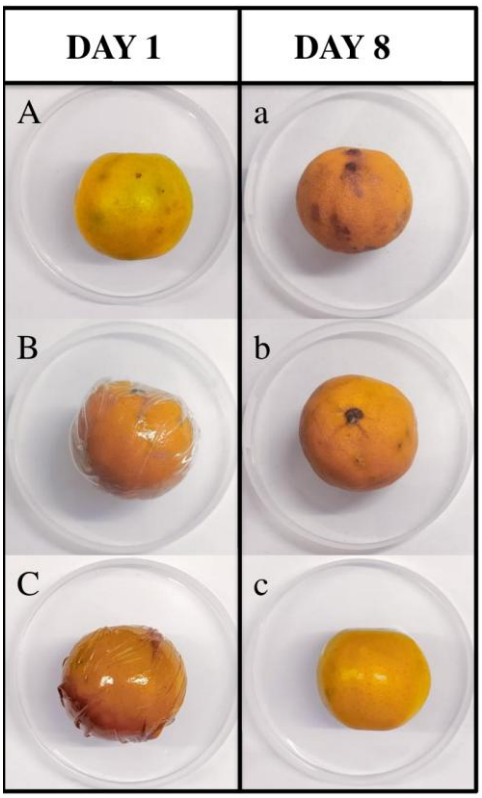


FIGURE 16 Food sampling assay A) Uncovered orange at day 1, B) Orange covered with CMG-CA film at day 1, C) Orange covered with CMG-CA-AA film at day 1, a)Appearance of uncovered orange after 8 days, b)CMG-CA covered orange after 8 days and c) CMG-CA-AA covered orange after 8 days.

4.10 RHEOLOGICAL PROPERTIES

4.10.1 Viscosity

Rheology, or the study of matter's movement and deformation, reveals the relationship between force, deformation, and time. The rheological experimental method is used to determine the rheological properties of materials. The CMG-CA-AA solution was shown to exhibit stronger shear thinning behavior than the CMG-CA solution. The breakdown of junctions initially stabilized by non-covalent associations (e.g., hydrogen bonding, van der Waals, etc.), which can be disrupted as shear rate increases, could be the reason of the junctions' considerable shear-thinning propensity. In either scenario, shear-induced structural alignment along the flow direction reduces viscosity significantly. [39]

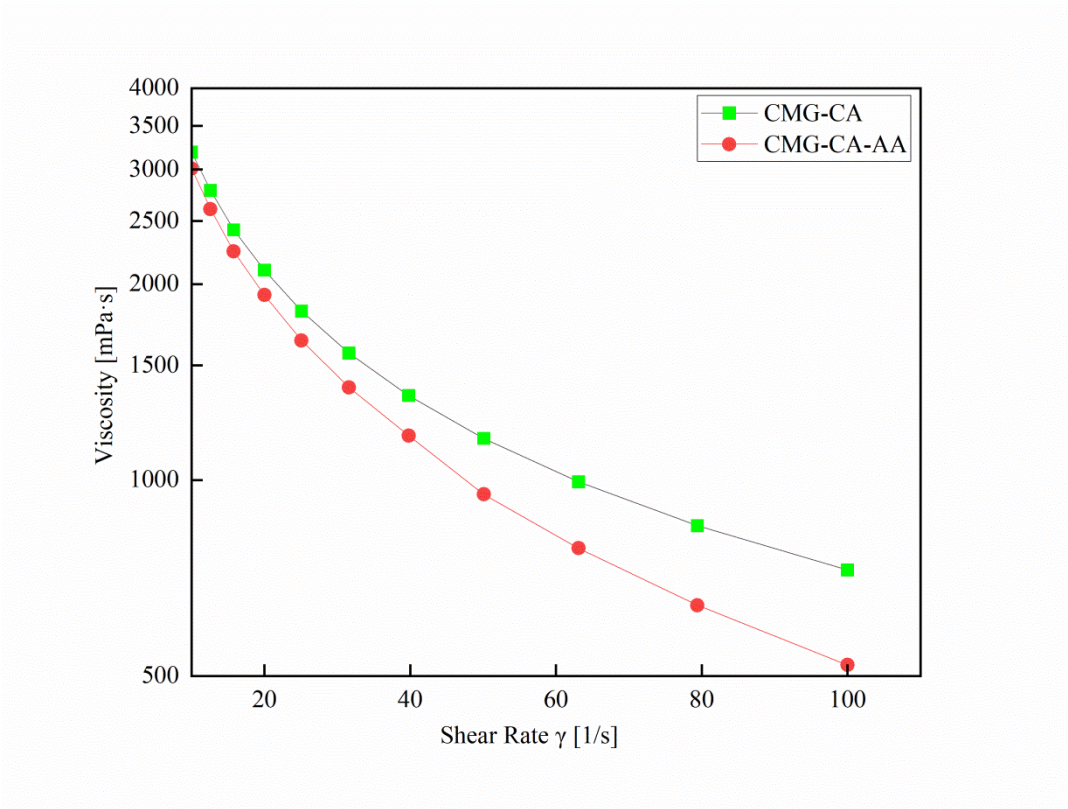


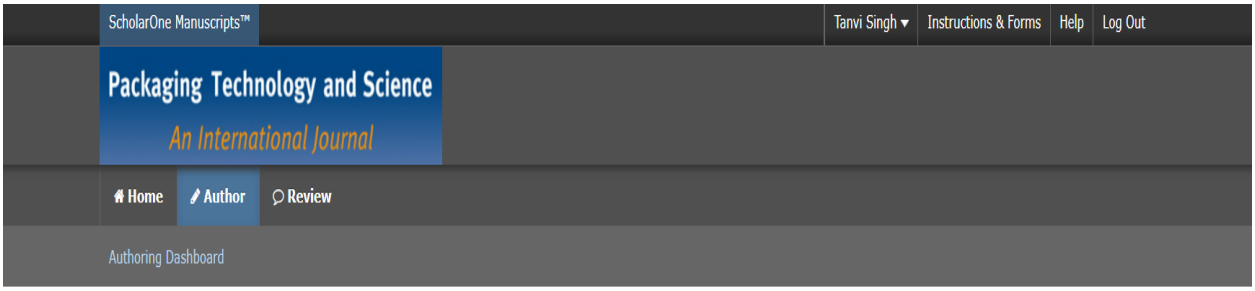
FIGURE 17 Viscosity sweeps of CMG-CA and CMG-CA-AA solutions

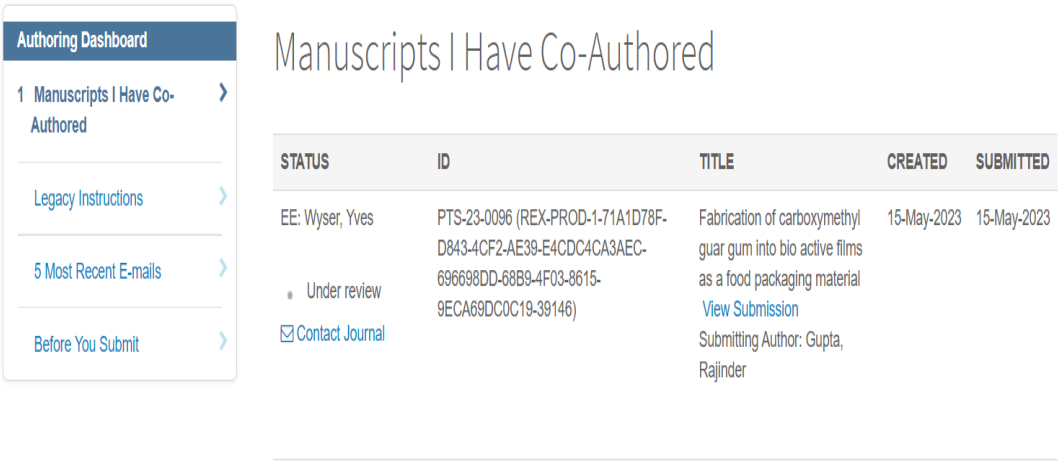
CHAPTER-5
CONCLUSION

The findings of the study reveal that CMG manufacturing was effective in producing a bioactive film. The generated biofilm outperformed the controlled film in terms of thermal stability and tensile strength. SEM images revealed small particle size, while FTIR measurements confirmed cross linking in the films. The XRD results showed that the films were amorphous, and the shift in peaks showed that crystallinity had been lost due to cross-linking. CMG-CA-AA films effectively suppressed both gram positive (Staphylococcus aureus) and gram negative (Escherichia coli) bacterial growth. The films are both antioxidant and biocompatible. The research on food packaging revealed its utility as a packaging material by enhancing the freshness and shelf life of fruits.

APPENDICES

Journal publication





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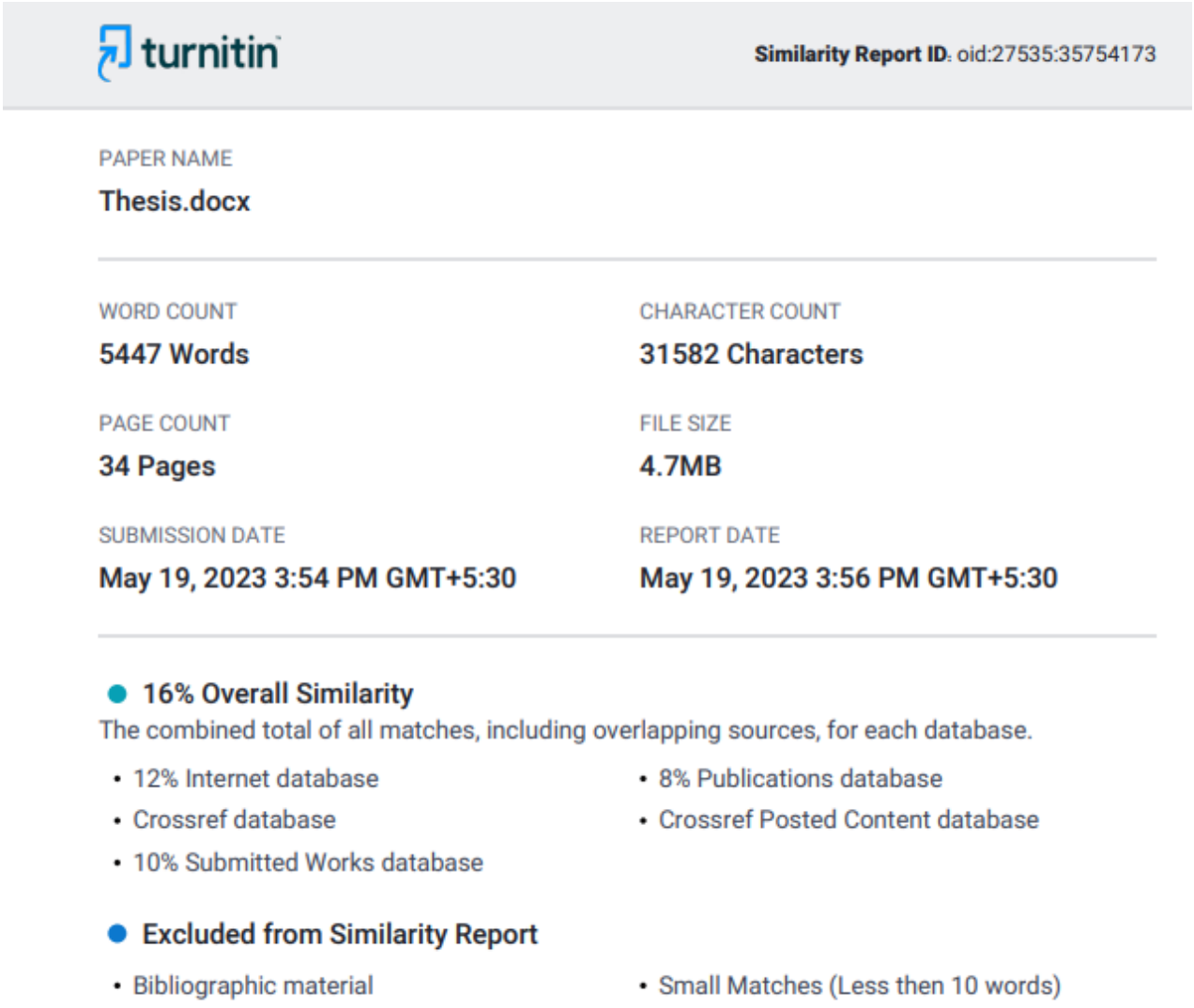

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REFERENCES

[1] Huang K, Wang Y. Recent applications of regenerated cellulose films and hydrogels in food packaging. *Curr Opin Food Sci*. 2022;43:7-17. doi:https://doi.org/10.1016/j.cofs.2021.09.003

[2] Sharma R, Kaith BS, Kalia S, et al. Biodegradable and conducting hydrogels based on Guar gum polysaccharide for antibacterial and dye removal applications. *J Environ Manage*. 2015;162:37-45. doi:https://doi.org/10.1016/j.jenvman.2015.07.044

[3] Janani N, Zare EN, Salimi F, Makvandi P. Antibacterial tragacanth gum-based nanocomposite films carrying ascorbic acid antioxidant for bioactive food packaging. *Carbohydr Polym*. 2020;247:116678. doi:https://doi.org/10.1016/j.carbpol.2020.116678

[4] Whistler RL, Hymowitz T. Guar Agronomy, Production, Industrial Use, and Nutrition. *Brittonia* . 1980;32:1-18.

[5] Prem D, Singh S, Prakash Gupta P, Singh J, Pal S, Kadyan S. *Callus Induction and de Novo Regeneration from Callus in Guar (Cyamopsis Tetragonoloba). Plant Cell Tiss Organ Cult 80, 209–214 (2005).*; 2005. doi:https://doi.org/10.1007/s11240-004-0738-9

[6] Cheng Y, M. Brown K, K. Prud'homme R. Characterization and Intermolecular Interactions of Hydroxypropyl Guar Solutions. *Biomacromolecules*. 2002;3(3):456-461. doi:https://doi.org/10.1021/bm0156227

[7] Chakka VP, Zhou T. Carboxymethylation of polysaccharides: Synthesis and bioactivities. *Int J Biol Macromol*. 2020;165:2425-2431. doi:https://doi.org/10.1016/j.ijbiomac.2020.10.178

[8] Gao J, P. Grady B. Reaction Kinetics and Subsequent Rheology of Carboxymethyl Guar Gum Produced from Guar Splits. *Industrial & Engineering Chemistry Research*. 2018;57(22):7345-7354. doi:https://doi.org/10.1021/acs.iecr.8b00782

[9] Yang CQ, Wang X, Kang in S. Ester Crosslinking of Cotton Fabric by Polymeric Carboxylic Acids and Citric Acid. *Textile Research Journal*. 1997;67(5):334-342. doi:https://doi.org/10.1177/004051759706700505

[10] Yanc CQ, Andrews' BAK. *Infrared Spectroscopic Studies of the Nonformaldehyde Durable Press Finishing of Cotton Fabrics by Use of Polycarboxylic Acids*. Vol 43. John Wiley & Sons, Inc; 1991. doi:https://doi.org/10.1002/app.1991.070430904

[11] Sharma R, Jafari SM, Sharma S. Antimicrobial bio-nanocomposites and their potential applications in food packaging. *Food Control*. 2020;112:107086. doi:https://doi.org/10.1016/j.foodcont.2020.107086

[12] Yang YC, Mei XW, Hu YJ, et al. Fabrication of antimicrobial composite films based on xylan from pulping process for food packaging. *Int J Biol Macromol*. 2019;134:122-130. doi:https://doi.org/10.1016/j.ijbiomac.2019.05.021

[13] Huang T, Qian Y, Wei J, Zhou C. Polymeric Antimicrobial food packaging and its applications. *Polymers (Basel)*. 2019;11(3). doi:https://doi.org/10.3390/polym11030560

[14] Baniasadi H, Madani Z, Ajdary R, Rojas OJ, Seppälä J. Ascorbic acid-loaded polyvinyl alcohol/cellulose nanofibril hydrogels as precursors for 3D printed materials. *Materials Science and Engineering: C*. 2021;130:112424. doi:https://doi.org/10.1016/j.msec.2021.112424

[15] Gemili S, Yemenicioğlu A, Altinkaya SA. Development of antioxidant food packaging materials with controlled release properties. *J Food Eng*. 2010;96(3):325-332. doi:https://doi.org/10.1016/j.jfoodeng.2009.08.020

[16] Li L, Guan S, Yang L, Qin X, Feng W. Mechanical and Adhesive Properties of Ploy(ethylene glycerol) Diacrylate Based Hydrogels Plasticized with PEG and Glycerol. *Chem Res Chin Univ*. 2018;34(2):311-317. doi:https://doi.org/10.1007/s40242-018-7337-5

[17] Nazarzadeh Zare E, Makvandi P, Tay FR. Recent progress in the industrial and biomedical applications of tragacanth gum: A review. *Carbohydr Polym*. 2019;212:450-467. doi:https://doi.org/10.1016/j.carbpol.2019.02.076

[18] Bhat VG, Narasagoudr SS, Masti SP, Chougale RB, Shanbhag Y. Hydroxy citric acid cross-linked chitosan/guar gum/poly(vinyl alcohol) active films for food packaging applications. *Int J Biol Macromol*. 2021;177:166-175. doi:https://doi.org/10.1016/j.ijbiomac.2021.02.109

[19] Orsu P, Matta S. Fabrication and characterization of carboxymethyl guar gum nanocomposite for application of wound healing. *Int J Biol Macromol*. 2020;164:2267-2276. doi:https://doi.org/10.1016/j.ijbiomac.2020.07.322

[20] V. G. Bhat, S. S. Narasagoudr, S. P. Masti, R. B. Chougale, and Y. Shanbhag, "Hydroxy citric acid cross-linked chitosan/guar gum/poly(vinyl alcohol) active films for food packaging applications," *Int J Biol Macromol*, vol. 177, pp. 166–175, Apr. 2021, doi: 10.1016/j.ijbiomac.2021.02.109.

[21] S. Bandyopadhyay, N. Saha, U. V. Brodnjak, and P. Sáha, "Bacterial cellulose and guar gum based modified PVP-CMC hydrogel films: Characterized for packaging fresh berries," *Food Packag Shelf Life*, vol. 22, Dec. 2019, doi: 10.1016/j.fpsl.2019.100402.

[22] S. S. Lal and S. T. Mhaske, "Old corrugated box (OCB)-based cellulose nanofiber-reinforced and citric acid-cross-linked TSP–guar gum composite film," *Polymer Bulletin*, vol. 78, no. 2, pp. 885–915, Feb. 2021, doi: 10.1007/s00289-020-03138-y.

[23] Orsu P, Matta S. Fabrication and characterization of carboxymethyl guar gum nanocomposite for application of wound healing. *Int J Biol Macromol*. 2020;164:2267-2276. doi:https://doi.org/10.1016/j.ijbiomac.2020.07.322

[24] Kaur M, Santhiya D. UV-shielding antimicrobial zein films blended with essential oils for active food packaging. *J Appl Polym Sci*. 2021;138(7). doi:https://doi.org/10.1002/app.49832

[25] Schlesier K, Harwat M, Böhm V, Bitsch R. Assessment of antioxidant activity by using different in vitro methods. *Free Radic Res*. 2002;36(2):177-187. doi:https://doi.org/10.1080/10715760290006411

[26] Bursali EA, Coskun S, Kizil M, Yurdakoc M. Synthesis, characterization and in vitro antimicrobial activities of boron/starch/polyvinyl alcohol hydrogels. *Carbohydr Polym*. 2011;83(3):1377-1383. doi:https://doi.org/10.1016/j.carbpol.2010.09.056

[27] C. Desmarchelier, M.J. Novoa Bermudez, J. Coussio, G. Ciccia, A. Boveris. Antioxidant and Prooxidant Activities in Aqueous Extracts of Argentine Plants,International. *International Journal of Pharmacognosy* . 2008;35(2):116-120. doi:https://doi.org/10.1076/phbi.35.2.116.13282

[28] Blois, Marsden S. M. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature 181*. Published online 1958:1199-1200. doi:https://doi.org/10.1038/1811199a0

[29] Le TA, Guo Y, Zhou JN, Yan J, Zhang H, Huynh TP. Synthesis, characterization and biocompatibility of guar gum-benzoic acid. *Int J Biol Macromol*. 2022;194:110-116. doi:https://doi.org/10.1016/j.ijbiomac.2021.11.180

[30] Snart, Jennifer E. *Oranges: Safe Methods to Store, Preserve, and Enjoy,UCANR Publications*.; 2006.

[31] Ur R, Mohammed R. *Rheological Behavior of Dspc-, Dbpc-, and Dppc-Oxygen Rheological Behavior of Dspc-, Dbpc-, and Dppc-Oxygen Microbubbles and Their Effectiveness in Improving Microbubbles and Their Effectiveness in Improving Survival in a Rat Model of Lipopolysaccharide-Induced Survival in a Rat Model of Lipopolysaccharide-Induced Acute Respiratory Distress Syndrome Acute Respiratory Distress Syndrome.Mechanicals (and Materials)Engineering-Dissertation,Theses and Student Research*.; 2022. doi:https://digitalcommons.unl.edu/mechengdiss/183

[32] Mali KK, Dhawale SC, Dias RJ. Synthesis and characterization of hydrogel films of carboxymethyl tamarind gum using citric acid. *Int J Biol Macromol*. 2017;105:463-470. doi:https://doi.org/10.1016/j.ijbiomac.2017.07.058

[33] Mudgil D, Barak S, Khatkar BS. X-ray diffraction, IR spectroscopy and thermal characterization of partially hydrolyzed guar gum. *Int J Biol Macromol*. 2012;50(4):1035-1039. doi:https://doi.org/10.1016/j.ijbiomac.2012.02.031

[34] Wu Z, Wu J, Peng T, et al. Preparation and application of starch/polyvinyl alcohol/citric acid ternary blend antimicrobial functional food packaging films. *Polymers (Basel)*. 2017;9(3). doi:https://doi.org/10.3390/polym9030102

[35] Bakht J, Ali H, Khan MA, et al. Antimicrobial activities of different solvents extracted samples of Linum usitatissimum by disc diffusion method. *Afr J Biotechnol*. 2011;10(85):19825-19835. doi:https://doi.org/10.5897/AJB11.229

[36] Verghese R, Mathew S, David A. Antimicrobial activity of Vitamin C demonstrated on uropathogenic Escherichia coli and Klebsiella pneumoniae. *Journal of Current Research in Scientific Medicine*. 2017;3(2):88. doi:https://doi.org/10.4103/jcrsm.jcrsm_35_17

[37] Peng N, Wang Y, Ye Q, et al. Biocompatible cellulose-based superabsorbent hydrogels with antimicrobial activity. *Carbohydr Polym*. 2016;137:59-64. doi:https://doi.org/10.1016/j.carbpol.2015.10.057

[38] Song Y, Sun Y, Zhang X, Zhou J, Zhang L. Homogeneous Quaternization of Cellulose in NaOH/Urea Aqueous Solutions as Gene Carriers. *Biomacromolecules*. 2008;9(8):2259-2264. doi:https://doi.org/10.1021/bm800429a

[39] Yadav R, Purwar R. Influence of metal oxide nanoparticles on morphological, structural, rheological and conductive properties of mulberry silk fibroin nanocomposite solutions. *Polym Test*. 2021;93:106916. doi:https://doi.org/10.1016/j.polymertesting.2020.106916

[40] Characterization instrument images- google images