Facile Synthesis of Bio-Composite Films Obtained from Sugarcane Bagasse and Cardboard Waste

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Abstract – In this study, we focus on the recycling of cardboard waste and sugarcane bagasse (SCB) for the preparation of carboxymethyl cellulose (CMC) and its conversion into a biodegradable film. Sodium alginate (SA) was added to form a biodegradable composite film. SA was used to increase film permeability. Glycerol, which is a plasticizer, was used to increase the tensile strength (TS) and film expansion. To characterize the CMC, X-ray diffraction, Fourier transform infrared spectroscopy, and scanning electron microscopy were used. The addition of olive oil to the CMC-SA matrix highlighted its antimicrobial property against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). A slight decrease in tensile strength was observed with the addition of olive oil (OO), which improved the functional properties of the control films as well as lowered moisture content and water solubility. But considering all other factors, the composite films obtained from sugarcane bagasse and cardboard waste incorporated with olive oil are suitable for applications in the field of food packaging.

Key words: Carboxymethyl cellulose, Sodium alginate, Olive oil, Biopolymer film, Cardboard waste, Sugarcane bagasse

1. Introduction

The development of new green products to reduce plastics has become popular with steadily increasing environmental pollution. Plastics are usually manufactured from organic materials that are synthetic or semi-synthetic. Plastic is manufactured from cellulose, biomass, natural gas, salt, and crude oil as raw materials, with petrochemicals accounting for the majority of synthetic plastics [1]. Plastics are currently used in 32 different ways, with worldwide production reaching 359 million tons in 2018. As a result of the covid-2019 pandemic, the consumption of plastic is expected to increase by 40% in packaging and 17% in other applications, including medical applications [2]. In India, 6.5-8.5 million tons of plastic as waste is collected by itinerant waste merchants and garbage collectors [3]. Even though cellulose is a widespread natural polymer, its application is restricted due to its poor solubility. To solve this problem and broaden the reach of applications, cellulose derivatives have been investigated. CMC is a derivative which is soluble in water and has been increasingly employed in a variety of industrial products, including dairy, petrochemicals, and construction materials [4]. Due to low-cost availability and good mechanical properties,

petrochemical-based plastics such as polyethylene, polypropylene, and polystyrene have been used extensively as packaging films. Therefore, plastic use is limited because they are highly resistant to biodegradation, which contributes to many problems in the environment. Using biopolymers like carboxymethyl cellulose, chitosan, starch, protein, and lipid, which can degrade within months or even years, we can replace traditional petrochemical packaging with green packaging materials that are recyclable, non-toxic, and environmentally friendly [3-8]. Cellulosic fiber's insolubility in different organic and inorganic solvents due to heavy hydrogen bonding is a major obstacle in direct cellulose conversion. However, derivatives of cellulose are one of the possible solutions. Mercerization and etherification processes produce carboxymethyl cellulose (CMC), a water-soluble derivative [9,10]. The carboxymethyl groups were replaced by polyelectrolyte hydrogen atoms, making them water-soluble and reducing inter-particle interactions. Because of its polyelectrolyte form, this linear chain polymer has seen many uses in detergents, pharmaceuticals, food, the paper industry, and as a thickener in the textile industry [11]. SA, a biopolymer obtained from brown algae, has been commonly used in biomedical applications and for the production of new materials because of its wound-healing properties, strong moisture absorption and permeability, high viscosity for aqueous solutions, and other properties. Since glycerol is hydrophilic, it reduces intermolecular forces between plasticizer molecules and increases polymer chain movement, which is primarily used to improve the durability and biodegradability of films [3,8].

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In previous studies, olive oil reduced the water vapor permeability of chitosan-based biofilms [11]. Olive oil could be used as a natural active additive on SA [12]. As an unsaponifiable and soluble fraction, olive oil (OO) is a natural source of many bioactive compounds, including phenolic compounds. It has excellent organoleptic properties, making it a good choice for use in the formulation of healthy foods. However, the source of oil impacts not only the sensory and nutritional properties of emulsions but also their rheology and stability. Each form of oil has its own set of difficulties, determined by physicochemical properties such as solubility, fatty acid chain length, and chemical stability. In the film emulsion, olive oil and fatty acids reinforce the water barrier [13].

In this study, the better moisture absorption, durability and extensibility, film-forming capability of SA, and good tensile strength of CMC were united to prepare composite film via a casting scheme. The structures of the CMCs were analyzed using FTIR, XRD, and SEM. In this perspective, commercial CMC (0.51), cardboard CMC (0.574), and sugarcane bagasse CMC (0.653) were used to manufacture the composite film. The antibacterial activity of CMC-SA-OO films against *E. coli* (Gram-negative) and *S. Aurious* (Gram-positive) bacteria was investigated by using the agar disc diffusion process.

2. Material and Methods

All the chemicals were acquired from different sources, and liquid chemicals were stored over molecular sieves (4 Å) in amber-coloured bottles for 72 hr before usage. The details of the chemicals used like CAS number, source, and purity are now reported in Table 1. Millipore water was used for preparing solutions and washing during this study.

2-1. Isolation of cellulose from cardboard waste and sugarcane bagasse

The waste cardboard and sugarcane bagasse were first cut into small pieces and then ground at 500 rpm, thoroughly washed with water, and dried in an oven for 24 h at 60 ℃. After that, dried waste was ground again up to 60 mesh size.

To extract hemicellulose, an alkali treatment was performed with NaOH (8% w/v) for sugarcane bagasse, and cardboard waste lower concentration of sodium hydroxide (3% w/v) was used. Alkali treatment reduces crystallinity and lignin structure destruction while improving internal surface swelling. The processed biomass fibers were washed and purified with water before being dried in an oven for 24 h at 70 ℃.

Then acid hydrolysis of dried waste cardboard/sugarcane bagasse was done with sulfuric acid (4% v/v) for enzymatic biomass hydrolysis and the elimination of hemicellulose sugars. This hydrolysis aids in the removal of the amorphous regions of the cellulose fibers. This has an impact on the morphology of biomass fibers, thermal stability, composition, and crystallinity [14]. The processed biomass fibers were washed and purified with water and dried for 24 h at 70 ℃ in an oven.

In the final step, $NaClO_2$ (4% w/v) was used to eliminate the color of fibers with acetic acid to maintain the pH between 3 and 4. The biomass fibers were then thoroughly washed with water until the pH of washing was 7. The processed biomass fibers were dried in the oven for 24 h at 70 ℃. The cellulose material was white.

2-2. Carboxymethyl cellulose (CMC) preparation from cellulose

There are two processes for making CMC from cellulose: mercerization and etherification. The mercerization of cellulose processed with NaOH (20% w/v) in an isopropanol solution aids in the breaking of -OH bonding between molecules. A 10 g of each cellulose sample, which was processed from cardboard waste, and sugarcane bagasse, was put into an Erlenmeyer flask. Each flask was filled with isopropanol (100 ml) and 20 ml of 20% w/v sodium hydroxide. The entire mixture was stirred at 55 ℃ for 1.5 h. After the prescribed time, the mercerized sample was treated with $C_2H_3ClO_2$ (20% w/v). During the etherification, the -OH groups were replaced with -CH$_2$COOH groups. Then 5 g of $C_2H_3ClO_2$ was added to the slurry and stirred for 1 h. in this process. The obtained material was rinsed with 70% v/v C_2H_5OH. The filtered sample was then dried in an oven to characterize or use in filmmaking [9,15].

Table 1. Detail of Chemicals used in this study

S. No	Chemical Name	CAS No	Source	Purity (%)
1	Carboxy Methyl Cellulose	9004-32-4	CDH	
2	Sodium Alginate	9005-38-3	SRL	
3	Sodium Chlorite	7758-19-2	CDH	80
4	Sulfuric Acid	7664-93-9	Rankem	98
5	Monochloroacetic Acid	79-11-8	CDH	99.5
6	Acetic Acid	64-19-7	CDH	99
7	Ethanol	64-17-5	Sigma-Aldrich	100
8	Sodium Hydroxide	1310-73-2	Rankem	98
9	Hydrochloric Acid	7647-01-0	Rankem	35
10	Methanol	7-56-1	Molychem	99
11	Nitric Acid	7697-37-2	CDH	70
12	Mono Chloro Acetic Acid	79-11-8	CDH	99

2-3. Preparation of CMC/SA composite films

1 g CMC was added in 100 ml of distilled water, and the mixture was stirred with a magnetic stirrer at 65 ℃ for 1 h to obtain a homogeneous viscous solution. Simultaneously, in 100 ml distilled water, we added 1 g sodium alginate (SA), glycerol (1% w/v), and 1% (v/v) CH_3COOH solution, and agitated this mixture by magnetic stirrer at 40 ℃ until SA completely dissolved. The solutions were allowed to stand for 12 h for de-aeration. The obtained solutions were mixed in the mass ratio of 17:3 (CMC: SA). The whole mixture was stirred at 45 ℃ for 2 h, and then it was poured in a petri dish and dried for 12 h in an oven at 60 ℃.

For CMC/SA/OO composite films, olive oil (1% w/v) was poured into the mixture of CMC: SA (in the mass ratio of 17:3) by using a micropipette. Then the whole mixture was stirred for 2 h at 45 ℃ and dried in petri dish for 12 h in an oven at 60 ℃.

2-4. Characterization of CMC

The degree of substitution (DS) is the number of -OH group that was substituted by the $-CH_2-COOH$ group in the cellulose configuration at C2, C3, and C6 positions. The degree of substitution and CMC content for commercial CMC and CMC made from cardboard waste and sugarcane bagasse was determined according to a previously reported method [8].

Fourier transform infrared spectroscopy (FTIR) was used to characterize the anionic polysaccharide CMC (FTIR). The samples were crushed with KBR to make pellets, and spectra in the 4000-400 cm^{-1} range were recorded on a Perkin Elmer BX 11-FTIR spectrophotometer. The surface morphology of various CMC was determined by using scanning electron microscopy (SEM). We coated dried samples with palladium gold alloy and mounted them on a double-sided carbon (C) tape (Zeiss 1555 VP). X-ray diffractometer models dried specimens were traced with Rigaku D/MAX-2400X using a Cu Ka radiation (λ = 1.5406 Å) in the 2θ range from 5º to 70º, with a scanning speed of 10º min^{-1} at 40.0 mA and 40 kV.

2-5. Characterization of the composite film

The film solubility, opacity, and mechanical properties (tensile strength and elongation) of the films were determined according to a previously reported method [8].

The antibacterial properties of the biopolymer films were studied using the agar well diffusion method for their antibacterial activity. The diffusion process was influenced by the shape, size, and polarity of the diffusion process [16]. We prepared agar (14 g in 500 ml distilled water) and nutrient broth (1.3 g in 100 ml distilled water) at the beginning of our experiment. Simultaneously in an autoclave at 121 ℃, petri plates were sterilized. The nutrient broth and agar were sterilized before being put in a laminar chamber. In due course, nutrient agar that had been sterilized was poured onto sterilized Petri dishes. The bacteria *E. coli* (Gram-negative) and *S. Aurious* (Gram-positive) were grown in nutrient broth before being dispersed onto solidified agar plates. Then, these bacteria cells were deposited on the agar surface. With the aid of a sterilized test tube, holes were designed in agar plates. Different biopolymer suspension solution in water was placed in the well. The zone of inhibition (ZOI) was calculated and analyzed after a 24 h incubation period at 37 ℃ (±1 ℃), [17].

3. Results and Discussion

3-1. Effect of CMCs after isolation

The CMC content, DS, NaCl, and sodium glycolate for both CMCs were determined and illustrated in Table 2. The by-products obtained from cardboard waste CMC (NaCl 1.02% and sodium glycolate 0.83%) and SCB CMC (NaCl 0.72% and sodium glycolate 0.41%) were less than in amount as compared to commercial CMC (NaCl 1.74% and sodium glycolate ($C_2H_3NaO_3$) (1.4%). $C_2H_3NaO_3$ is toxic, and the ratio of $C_2H_3NaO_3$ to sodium chloride does not exceed 0.5 % in food additives. The undesirable side reaction was responsible for the formation of by-products. This is because, in the etherification process, NaOH reacts with the $C_2H_3ClO_2$ to form CMC and the unreacted
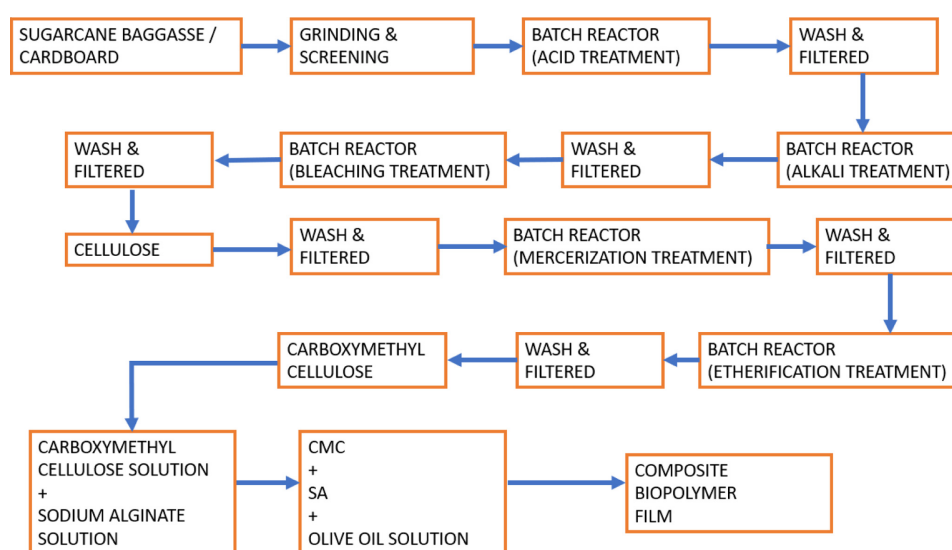

**Fig. 1. Flow sheet diagram for composite biopolymer film preparation from cardboard waste/sugarcane baggase.**

Table 2. Degree of substitution (DS) and content of CMCs and byproducts for film preparation

CMCs	DS	CMC content (%)	NaCl content (%)	Sodium glycolate content (%)
Commercial CMC	0.51	96.86	1.74	1.40
Cardboard CMC	0.58	98.15	1.02	0.83
Sugarcane bagasse CMC	0.65	98.87	0.72	0.41

NaOH may react with $C_2H_3ClO_2$ to form $C_2H_3NaO_3$ and NaCl. This reduces the availability of acetic acid, hence decreasing the DS [18,19,23]. The CMC content for cardboard waste CMC (98.15), sugarcane bagasse CMC (98.87), and commercial CMC was 98.15%, 98.87%, and 96.86%, respectively.

3-2. FTIR analysis

Figs. 2-3 show the FTIR spectrum of sugarcane bagasse and cardboard waste CMCs. The band at 980 to 1140 cm^{-1} is attributable to the $>CH-O-CH_2$ group. The extremely strong specific band at (1450 to 1540 cm^{-1}) is due to asymmetric stretching anion reconsolidated throughout peaks at (1410 to 1460 cm^{-1}) associated with the symmetrical carboxylate anion. The C-H (aliphatic) is assigned to the band at (2900 to 2950 cm^{-1}) respectively. The pure CMC IR spectra specify an extensive absorption band at (3300 to 3400 cm^{-1}), attributable to the -OH group stretching rate [20,21]. The peak at around 1600 cm^{-1} (-COO) in the FTIR spectrum of CMC from different CMC confirmed the carboxymethylation [24].
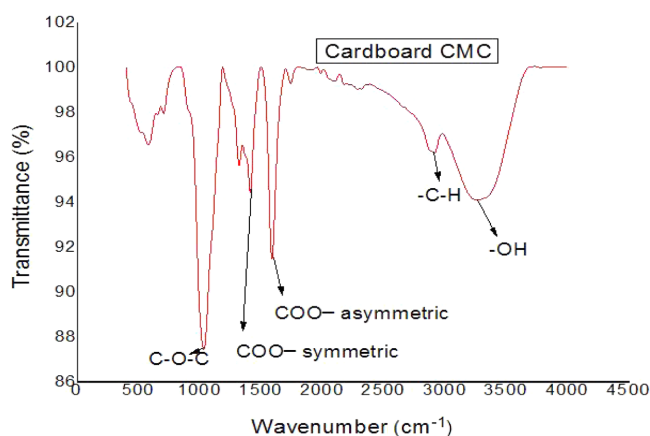
Fig. 2. FTIR spectra of cardboard CMC.
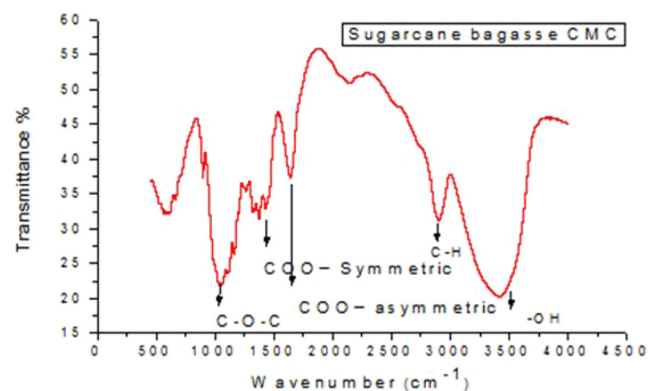
Fig. 3. FTIR spectra of sugarcane bagasse CMC.
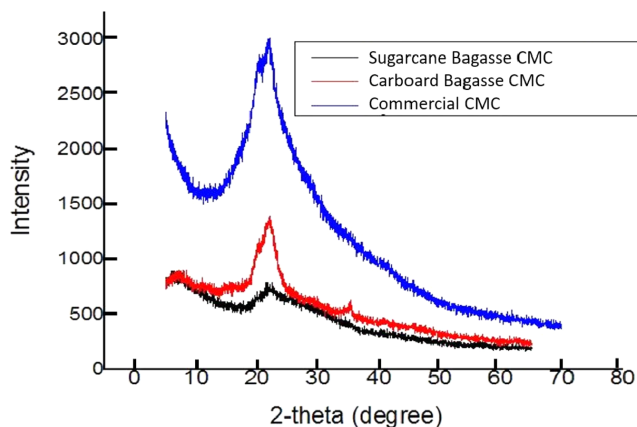
Fig. 4. X-ray diffractogram of CMC.

3-3. XRD analysis

The crystallinity of sugarcane bagasse CMC and cardboard waste CMC in the ratio of 20% NaOH and 20% MCA was analyzed by XRD [9]. Fig. 4 demonstrates the crystalline peaks of both CMCs. XRD spectra reveal that in SCB CMC, peaks show less crystallinity than the cardboard CMC and commercial CMC. The XRD type peak's height and width were significantly altered by a -OH bond that preserved the crystalline arrangement by replacement of -OH with $-CH_2-COOH$ groups. Applications such as hydrogel production and the increased ability of CMC to absorb water due to increasing amorphous regions show a significant decrease in crystallinity. CMC peaks in SCB CMC are lesser than the cardboard and commercial CMCs peaks due to the rupture of -OH bonding during mercerization treatment with sodium hydroxide. Due to the breakdown of β-1, 4 glycosidic bonding liable for increasing the gap between the cellulose molecules, the XRD spectrum crystalline peaks in CMCs change. Higher DS is responsible for the lesser crystallinity results in broader crystalline peaks [22,26].

3-4. Morphological analysis

The morphology of CMCs isolated from bagasse and cardboard as well as commercial CMC was investigated using their SEM images. It shows a distinct, elongated, and regular fibrous structure, as displayed in Fig. 5. The higher the DS values, the more cracks will appear [27]. This incident explains that the fiber surface had been cracked during the carboxyl methylation reaction, and interruption of the outer layer of the cellulose fibers appears [17]. Also, during mercerization, a higher concentration of NaOH increased the surface area of the remaining hemicellulose, allowing for better contact during the etherification process (MCA). MCA also changed from a
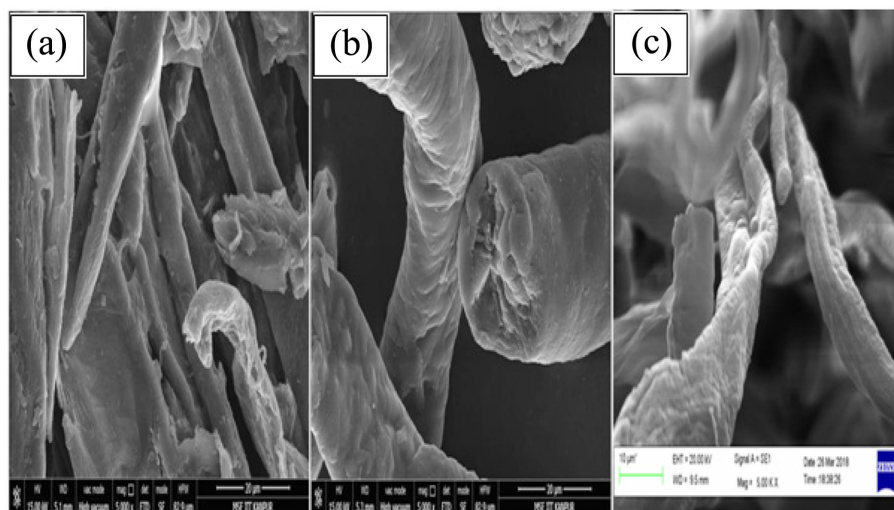
Fig. 5. SEM images of (a) cardboard CMC, (b) commercial CMC and (c) sugarcane bagasse CMC.

smooth to a rough surface because of the deposition of sodium acetate ions on the surface of CMCs [9,28,29].

3-5. Effect of moisture absorption, opacity, and solubility on composite films

The moisture absorption, solubility, and opacity of the composite films are reported in Table 3. The moisture absorption for commercial CMC and cardboard CMC was less than the bagasse CMC, although an increase in DS also enhanced moisture absorption ability. Glycerol supports the durability and smooth surface of the films. Plasticizer increases mobility, increasing the free volume space in the film network because of the more amorphous nature of bagasse CMC compared to cardboard CMC and commercial CMC. With the increase in CMC content or DS, film opacity significantly increased. But in general, when olive oil was used, the film's clarity decreased, resulting in active films being slightly yellow compared to the control film.

SA and CMC both are readily soluble in water. A small quantity of CMC has a marginal effect on water absorption and coefficient of moisture absorption. Since SA has poor water resistance, to further enhance the property of solubility, the inclusion of hydrophobic content may be advantageous. The inclusion of oil improves the stability of droplets stabilized by colloidal particles dispersed inside the CMC/SA matrix and provides hydrophobic properties to decrease the adsorption of water molecules. From Table 3, it is visible that the solubility of the edible film has been reduced due to the concentration of olive oil.

3-6. Impact of the tensile strength (TS) and elongation on composite films

Table 3 displays that DS also influences the TS, and the film made up of higher CMC (DS) has stronger TS. DS (0.6534) and TS (17.02 MPa.) for bagasse CMC were higher than the cardboard and commercial CMCs, due to lower NaCl and sodium glycolate content. CMC has a well-structured internal network. Still, without plasticizers, it cannot make the film by itself. Glycerine is the most effective plasticizer between the polymers to minimize intermolecular forces. It reduces the tensile strength and decreases the elongation at breakage. The highly viscous nature of SA and film-forming capabilities allow molecules to closely connect and decrease intermolecular spaces and increase the composite film TS. Indirectly, the SA material influenced the TS of composite films. The addition of olive oil decreased the TS and increased the elongation. Olive oil decreases the polymer network's cohesion forces and improves SA/CMC mobility. The presence of oil droplets causes an increase in films porosity after drying, which decreases the mechanical resistance of films. This study confirms that bagasse CMC shows better tensile strength than cardboard CMC and commercial CMC because cross-linker effects adversely on the CMC and sodium alginate structure and hence increase the TS and decreases the elongation [6,8,28,29].

3-7. Antibacterial test

The antibacterial properties of different films were studied with the help of *E.coli* and *S. aureus*. SA has antibacterial properties, so it

Table 3. Characterization of composite films different films made from CMC with sodium alginate (SA) and olive oil (OO).

CMCs	Opacity (%)	Moisture content (%)	Solubility (%)	Tensile strength (MPa)	Elongation (%)
Commercial CMC + SA	2.94	10.82	39.14	13.66	10.9
Cardboard CMC + SA	4.55	13.27	51.50	14.20	21.08
Sugarcane CMC + SA	6.74	17.49	57.32	17.02	16.44
Commercial CMC/SA/OO	3.50	9.25	33.92	12.23	15.5
Cardboard CMC/SA/OO	5.35	12.33	40.25	12.57	28.35
Sugarcane CMC/SA/OO	7.12	15.67	43.37	15.90	25.07

Table 4. Zone of inhibition (mm) of different biopolymer suspension solution

Test Cultures	Commercial CMC/SA	Cardboard CMC/SA	Sugarcane CMC/SA	Commercial CMC/SA/OO	Cardboard CMC/SA/OO	Sugarcane CMC/SA/OO
E. *coli*	8.5	10.5	9.5	10.0	12.0	11.5
S. *aureous*	10.0	10.5	11.0	12.5	13.0	13.5

shows antibacterial properties without olive oil. As observed, in CMC/SA, there was an inhibition zone in every film. But with the incorporation of olive oil, the inhibition zone increased. This showed that olive oil enhanced the antibacterial property. Therefore, to enhance the antibacterial property, it is better to use olive oil. The biopolymer film inhibition zone (mm) against the bacteria *E.coli* and *S. aureus* is demonstrated in Table 4. From Table 4, it is observed that composite films are more efficient while using *S. aureus* bacteria than *E.coli*. The inhibition zone (13.5 mm) is highest in the case of SCB CMC/SA/OO biopolymer film.

4. Conclusions

A composite film was produced from commercial, cardboard, and bagasse CMCs. The film prepared from bagasse CMC showed better mechanical properties. The composite film composed from bagasse CMC showed more TS (17.02MPa) as compared to a film made from cardboard CMC TS (14.20 MPa) and commercial CMC TS (13.66 MPa) without olive oil. With the addition of olive oil, TS slightly decreased but other water resistance property increased. Bagasse CMC had more DS as compared to cardboard CMC and commercial CMC because of lower NaCl and sodium glycolate content. Moisture content, solubility and opacity also increased through increases in DS. FTIR, SEM, and XRD data further authenticate that CMCs were successfully synthesized. Other properties like moisture content, solubility, and antibacterial are important properties for food packaging material. The results of this study show that adding olive oil to SA-CMC films did not significantly affect the moisture content and solubility, but olive oil improved their antibacterial properties also. Due to its flexibility, and better antibacterial and mechanical properties, olive oil-incorporated synthesis films made from sugarcane bagasse and cardboard waste could serve as food packaging materials.

Acknowledgments

Conflict of interest
The authors declare that they have no conflict of interest with anyone.

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