Original Research Article

PREPARATION AND ANTIMICROBIAL ACTIVITY OF CORN COB AND COIR REINFORCED BIODEGRADABLE STARCH BIOCOMPOSITE FILMS FOR FOOD PACKAGING APPLICATION

Abstract

Finding a bio-composite film to replace petroleum-based synthetic plastic has received considerable attention in recent years. In this research, biocomposite films were effectively prepared using corn cob powder and coconut coir as reinforcing materials in various concentrations using solution casting and annealing techniques. Corn starch was used as a co-biopolymer, glycerol (3% v/v) and acetic acid (5% v/v) as plasticizers were added as well. Biodegradability, mechanical, physical, FTIR, SEM, XRD, tensile and antibacterial tests were performed on the biocomposite samples. The biodegradability test showed that the samples were biodegradable and that the rate of biodegradation improved with time, reaching 87.37 % in just ten days. The SEM test was used to investigate the molecular structure of the biocomposite films, and the findings demonstrated that the raw ingredients had been well mixed, resulting in a smooth surface. The antibacterial test was conducted against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria. This study examines the influence of fiber reinforcement on the mechanical properties of biocomposites based on starch. Various natural fibers may boost the strength of biocomposites, as shown by the findings. According to the results, maize starch, corn cobs, and coconut coir are all suitable for food packaging purposes.

Keywords: *Biodegradable, biocomposite films, corn starch, corn cob, coconut coir, antimicrobial activity*

1. Introduction

Softness, lightness, and transparency are ideal characteristics of synthetic films and petro-based plastics, which have long been employed in the food packaging business to protect food products. However, the negative environmental effect of these materials, which is caused by their entire non-biodegradability, is a significant drawback for them[1]. Environmental damage is caused through typical plastic debasement processes like landfilling, burning, and substance treatment. Furthermore, plastics that are not biodegradable present a massive danger to human health and well-being[2, 3]. The petrochemical industry produces about 99 percent of plastic materials. The packing sector in India uses more synthetic polymers than anywhere else globally, accounting for over 43% of the country's total yearly production[4]. Approximately 50 % of commercial bioplastics are made from starch. To save petrochemical resources and decrease environmental impact, a great deal of research has gone into developing starch-based polymers[5-13]. However, starch-based materials have disadvantages such as weak mechanical characteristics and low long-term stability[10]. Compared to standard synthetic polymers, starch has an additional shortcoming, such as a robust hydrophilic character that makes it less suitable for some packaging applications[14]. A plasticizer, like glycerol, has been used to enhance the product's shelf life, flexibility, and limits[11]. The manufacture of starch-based bioplastics is uncomplicated, and their usage in packaging is widespread[15, 16]. The tensile characteristics of starch are excellent for the manufacture of

packaging materials, and glycerol is added to the starch as a plasticizer. By adjusting the amounts of additives, the desired features of the bioplastics may be attained. The starch-based polymers are often blended with eco-friendly polyesters for commercial purposes. Various bio-based plastics are produced from renewable resources such as wheat, potatoes, corn, and vegetable oil. These polymers are produced using chemical or biological processes such as acidification, microbial fermentation, hydrolysis [17].

Among the numerous resources found naturally, starch is one of the most frequently used by researchers. Pure starch has a white hue. The starch powder has neither a distinct flavor nor odor. In addition, pure starch is insoluble in cold water and alcohol. It is non-toxic, biologically absorbable and semi-permeable to CO_2[18]. The primary components of starch are amylase and amylopectin. Corn starch has 25% amylose and 75% amylopectin. The amylose molecules lose water, which increases biodegradation, while the amylopectin molecule is in charge of plasticizer characteristics. The size of their granules varies from 5 to 20 microns, i.e., good absorption capacity, rapid gel formation & good strength. The linear structure of amylase confers more robust and more flexible mechanical qualities—the branching structure of amylopectin results in reduced resistance to tensile strength and elongation characteristics[19]. Starch is a renewable substance with several benefits, including its endless supply, cheap cost, and ability to be recycled[20].

Bioplastics (green plastics) and green composites (Bio Composite = Bio Plastic + Bio Fibers) tend to degrade proportionally and are processed more sustainably. The mechanical qualities of bioplastics are restricted. However, composites produced from green polymers and natural fibers offer superior mechanical properties and a rapid decomposition rate[21]. Being inherently biobased and biodegradable, green biocomposites play a significant role in developing unique and creative materials for the burgeoning sustainable packaging sector[22].

The main objective of this study was to produce a cost-effective alternative to conventional petrochemical plastic by synthesizing eco-friendly and biodegradable biocomposite films from corn starch and different concentration of corn cob powder and coconut fibers that can have antimicrobial properties capable of restraining or inhibiting the development of spoilage and harmful bacteria. Using glycerol as a plasticizer, mixtures of corn cob powder, coconut fibers, corn starch and sodium benzoate may make biodegradable polymers with an antibacterial action against microbes. In addition, the material's physicochemical, mechanical and morphological properties were investigated to ascertain its suitability for use. The antimicrobial effect was tested on *Staphylococcus aureus* (gram-positive bacteria) and *Escherichia coli* (gram-negative bacteria) using the disc well-diffusion method on solid agar media.

The investigation aimed at determining the area of use for these materials and assessing their potential. This study resource may be used to develop and produce biodegradable materials.

2. Materials and methods

2.1 Materials

The biocomposite films were formed using corn starch, corn cob powder, and coconut coir. Fresh corn granules from a farm field in Bijnour village, Lucknow, Uttar Pradesh, India, were used to extract CS using the Ali *et al.* (2016) technique[23]. Corn cobs were torn to 5-7 mm in diameter, dehydrated, and ground into a fine powder, filtered through a 75 µm mesh screen. Coconut coir was collected in Lucknow, Uttar Pradesh, India, at a local market. It was rinsed with seawater, dried, and scissored to a 5-6 mm length before sieving to obtain a fiber length of mostly 5-6 mm. Glycerol, acetic acid as plasticizers, and sodium benzoate as a preservative were purchased online from Amazon.com, Inc.

2.2 Extraction of Starch

As reported in research by Ali *et al.*[2, 23] corn starch was extracted. Briefly, 1 kg of corn was steeped in 4 L of distilled water at 4 °C for 12 hours. The goal of steeping was to enhance the moisture content of the grain to aid the grinding process. After the water was removed, the grains were pulverized in a laboratory electric blender (Maharaja Electric Blender) (wet milling) until the minuscule possible fraction was obtained. After passing the crushed fractions through a filter with a 75 µm screen, they were left to settle for 8 hours. The supernatant was extracted, and the deposited particles were

washed in distilled water to eliminate any excess starch protein. Next, 20 minutes of centrifugation at 3000 rpm segregated the starch and liquid slurry. The resultant corn starch was dried in an air circulation oven (Type Venticell 22, Planegg, Germany) at 50 °C for 12 hours; the dried corn starch was blended and sieved to achieve a uniform particle size distribution. It was then placed in an airtight plastic container and kept in the dark area for future use. About 400 grams of starch was extracted from 1,000 grams of corn.

2.3 Fabrication of biocomposite films

Biocomposites were prepared using the solution casting technique by varying the amount of corn cob and coconut fiber while maintaining a constant starch concentration. A film-forming solution containing 10 gm and 50 ml of corn starch and distilled water respectively was used to prepare the composite starch films for the matrix and different amounts of corn cob and coconut fiber as reinforcement. All samples had same amounts of glycerol (3 ml), acetic acid (5 ml), and sodium benzoate (0.2 gm).

2.3.1 Mechanism: The experiment's mechanism was broken down into two parts: Gel formation & Drying

2.3.2 Gel formation: Corn starch had 75 % amylopectin molecules, which contributed to its ability to gel quickly when heated. When raw, uncooked starch granules were subjected to heat, they soaked up the water and swelled, causing some amylose molecules to escape and collapse. The gelation and casting processes were finished, and the collapsed granules began to compress and approach the fibers, forming a narrow junction. The fibers became entrapped in the tight junctions and were mainly accountable for drying corn starch bioplastics and composites.

In a thermal bath, the mixture containing all the constituents shown in Figure 1 was heated to 85 °C and cooled down for 20 minutes while being constantly stirred. The mixture was then stored in a vacuum desiccator to prevent the formation of air bubbles. After that, the solution (30 gm) was distributed evenly onto 160 mm diameter mold plates. To ensure the smooth surface finish of the biocomposite film, thin plastic sheets were used to line the top and bottom of the mold plate. In transparent matrices, corn cob and coconut fibers were distributed randomly but uniformly.

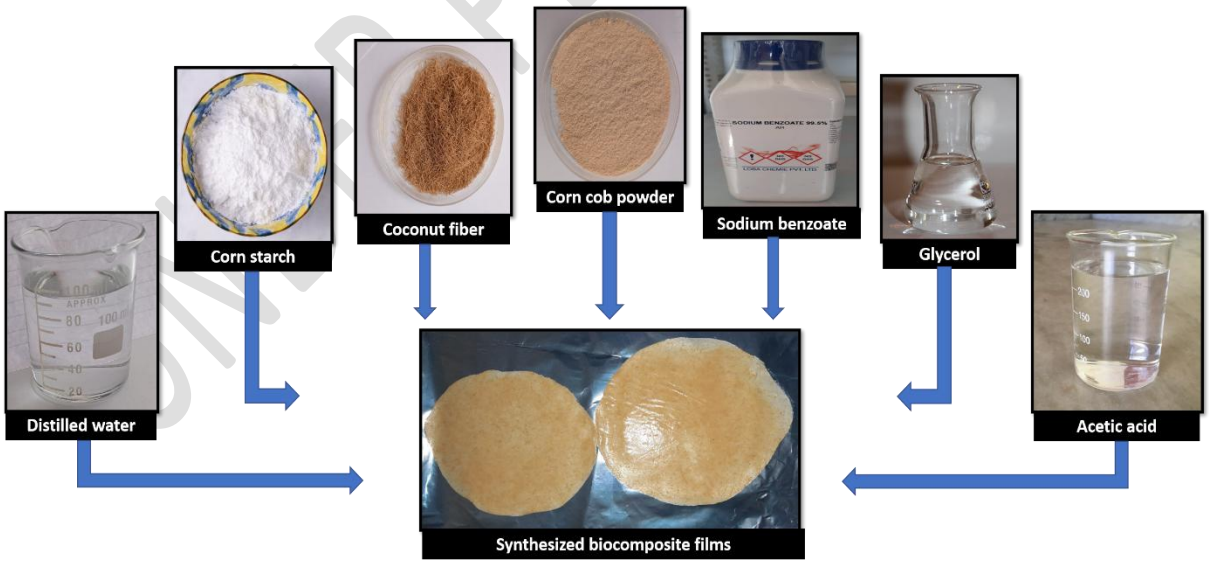

Fig. 1. Synthesis of biocomposite films from natural ingredients[3]

After conditioning, laminates of corn starch matrix and composites with varying amounts of corn cob powder and coir fibers were peeled off the plastic sheets and heat-treated (annealed)[4] for 12 hours in a hot air oven set to 60 °C. Finally, one week prior to characterization, the food wrappers were

stored in different plastic bags at room temperature. As indicated in Table 1, the resulting films were labeled according to their corn cob powder and coconut fiber compositions.

Table 1 - Composition of prepared biocomposite films

Sample	Corn Starch (gm)	Corn Cob (gm)	Coconut Coir (gm)	Distilled water (ml)	Glycerol (ml)	Acetic acid (ml)	Sodium benzoate (gm)
Control	10	0	0	50	3	5	0
F1	10	0	0.25	50	3	5	0.2
F2	10	1	0	50	3	5	0.2
F3	10	1	1	50	3	5	0.2
F5	10	2	0.5	50	3	5	0.2
F7	10	3	1	50	3	5	0.2
F8	10	4	1.5	50	3	5	0.2[5]

2.4 Characterization

2.4.1 Thickness of films

An electronic caliper was used to measure the film thickness with 0.01mm accuracy (Mitutoyo Co., Japan). Three random measurements were taken for each film sample, and an average value was calculated.

2.4.2 Density (ρ)

The film's density was calculated using its volume (V) and weight (m). The thickness obtained from the film thickness study was used to compute the size of each film based on the specified dimensions (10mm×30mm). The film density was calculated with the help of Eq. (1)

$$\rho = \frac{m}{V} \quad (1)$$

Where, m = mass (g), V = volume (cm^3)

2.4.3 Moisture content

The moisture content of each film sample was determined using Eq. (2). The moisture content of the film specimen was evaluated by weighing each sample separately (W_i) on a digital weighing scale. The film samples were dried in an oven for 24 hours at 105 °C (W_f). The experiment was repeated three times, with the mean value for each film being the final moisture content.

$$MC\ \% = \frac{W_i - W_f}{W_i} \times 100 \quad (2)$$

2.4.4 Folding endurance

The folding endurance and strength properties of a film are related to its flexibility and, as a result, represent the film's physical stability during production, packing, and use[24]. The folding endurance of the prepared film was measured by repeatedly folding a 2 cm × 2 cm sample strip at the same point until it broke. The number of times (n) the strip was folded without breaking defined the folding endurance value[25]. It was manually determined by repeatedly folding a film tightly across its centre.

2.4.5 Biodegradability of Films (Soil burial test)

All the samples were subjected to biodegradation tests according to the scientific method steps by Ibrahim *et al.*[6, 26]. Each film specimen was placed in a control condition and buried 5 cm deep in wet soil with an equal volume and known weight (W_o). The weight loss (W_L) examination was done in triplicate by collecting soil samples at various time points and gently rubbing them with a brush. After

6 hours of dehydration at 105 °C, the samples were reweighed (W_t). Degradation analysis was conducted on the collected samples and determined using equation (3) every couple of days.

$$Weight\ loss\ (\%) = \frac{W_o - Wt}{W_o} \times 100 \quad (3)$$

2.4.6 Sealing property

Bar sealing is the optimal technique for producing a seal in most form/fill/seal machines. The parameters used in the heat-sealing procedure are the sealing pressure, the sealing temperature, and the dwell time. When creating a decent seal, these three aspects should be balanced appropriately. In the heat-sealing process, heat is applied to melt the sealing layer to a molten or partially molten state to affect the sealing. The heat sealing was performed at the Food Science Analysis laboratory, School for Home Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, using an 8-inch Hot Bar Bag sealing machine.

2.4.7 Scanning electron microscopy [27]

The morphological surface characteristics of the samples were analyzed using a scanning electron microscope model (JSM 6490 LV, JEOL, Japan)[28]. Multiple magnifications were used to examine the sample, including ×200, ×1000, and ×1800. The film samples were coated with a thin layer of platinum to provide electricity before scanning. 7.4 mm was chosen as the working distance, and 15 kV was chosen as the acceleration voltage. The SEM apparatus was operated at a current of 58 µA.

2.4.8 Fourier transform infrared spectroscopy (FTIR)

The Fourier Transform Infrared Spectrometer (FTIR), Model: Nicole 6700, Make: Thermo-Scientific, USA, was used to monitor the samples' FTIR spectrum to determine the functional groups present in the film samples. Each sample was analyzed using 16 scans between 4000-400 cm^{-1} with a spectral resolution of 4 cm^{-1}.

2.4.9 X-ray diffraction (XRD)

Corn starch, corn cob powder, coconut coir powder, and biocomposite film were analyzed using a D8 Advance Eco X-ray diffractometer (Bruker, Germany). The angle of scattering (2θ) was varied at a rate of 0.02° per angle between 5- 60°C. The operational current and voltage were set to 35 mA and 40 kV during the test, respectively. Equation (4) was used to calculate the crystallinity index (C_i) of samples using the amorphous area [29] and crystalline area (A_c) calculus.

$$C_i = \frac{A_c}{A_c + A_a} \times 100 \quad (4)$$

2.4.10 Tensile properties

The tensile characteristics of the produced films were evaluated at room temperature using a 5 kN INSTRON Universal testing machine (UTM) following ASTM standard D882 (ASTM, 2002)[30, 31]. Tensile clamps were employed to hold the 10 × 70 mm film strip. Initially, the gauge length was set at 30mm, and the crosshead speed was maintained at 2mm/min. The tensile strength, elastic modulus, and elongation at break were determined by measuring five duplicates of each specimen. Each specimen was tested five times to establish its tensile strength, elastic modulus, and break elongation.

2.4.11 Antimicrobial activity

Antibacterial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria was determined using the agar well disc diffusion method. First, nutrient broth (14 g in 500 mL distilled water) and agar (1.3 g in 100 mL distilled water) were prepared. Next, Petri dishes

were sterilized in an autoclave for 15 minutes at 121 °C[7] temperature and 15 psi pressure. Following that, sterilized nutritional agar was added to sterilized Petri dishes. Then, on solidified agar plates, *Staphylococcus aureus*[8] (Gram-positive bacteria) and *Escherichia coli*[9] (Gram-negative bacteria) were cultivated and dispersed in nutrient broth. Bacterial cells were then deposited on the agar surface[10]. To create wells in the agar plates, sterilized test tubes were used. Biocomposite films with 8 mm diameter were positioned in the well. To determine the inhibition zone, Petri dishes were incubated at 37 ± 1 °C for 24 hours [32, 33].

3. Result & Discussion

3.1 Fabrication of biocomposite films

The glycerol-plasticized film was easily peeled off after drying the corn starch-based biocomposite film-forming solutions [11]. At increased corn cob powder and coconut fiber concentration, the translucence of film was lowered[12].

3.2 Thickness and density

The various starch-based biocomposite films seemed to have similar densities and thicknesses. The thickness increased, but the density decreased with small changes when corn cob powder and coconut fiber were incorporated, as indicated in Table 2. On the other hand, biomaterials have a lower density than synthetic composite materials like fiberglass (2.500 g/m^3), making them more suitable for biomass production[34]. A consistent quantity of dry mass per unit area was maintained throughout the casting process, which resulted in CS control and composite films with different thickness values. The biocomposite film has a slightly higher density than the control film. It might be attributed to the film matrix's increased filler content[35].
The quantity of reinforcing material used influenced the thickness and density. Corn cob and coconut fiber levels were high, resulting in thicker, rougher films. Chemical properties of the fiber might provide insight into the situation: an excess of hydroxyl groups across a broad surface area, leading to partial destruction of strong fibre connections and the formation of a new strong inter-facial adhesion between the fibres and the starch matrix during film processing[36]. The free space inside the starch biopolymer was reduced by forming a strong bond between the matrix and the reinforcement material, which made it more compact than plain starch. Density, or density of volumetric mass, is the amount of a material that fits into a certain amount of space. The higher the density of the biocomposite films, the smaller their volume. In addition, this study's results supported those of Ibrahim et al. [37] and Sayang et al. [18].

Table 2 - Physical attributes of the films[13]

Sample	Thickness (cm)	Density (g/cm^3)	Moisture content (%)
Control film	0.026 ± 25.4	1.45 ± 0.08	12.18 ± 0.30
F1	0.029 ± 23.2	1.33 ± 0.06	11.82 ± 0.50
F2	0.028 ± 25.4	1.30 ± 0.07	10.56 ± 0.11
F3	0.032 ± 22.6	1.24 ± 0.04	7.56 ± 0.20
F5	0.042 ± 22.2	1.18 ± 0.06	6.72 ± 0.19
F7	0.059 ± 25.6	1.10 ± 0.08	6.36 ± 0.11
F8	0.078± 25.4	1.04 ±0.07	5.29 ± 0.20

3.3 Moisture content (MC)[14]

Moisture content is one of the most important things to consider when choosing natural fibers to use as reinforcement in making new biocomposite materials. The low water content is important because a high water content could damage the biocomposite material's shape stability, especially in terms of mechanical performance, porosity development, and its ability to hold water[38]. Nevertheless, as shown in Table 2, when reinforcing materials like corn cob and coconut fiber were added to the biocomposite samples, the amount of water in the samples dropped down. Ilyas et al.

(2018)[36] explained this phenomenon regarding how cellulose works in the hybrid composite. Adding more fiber increases the amount of cellulose in the composite, making it less likely to absorb water. Due to a hydroxyl group in cellulose's structure, it is insoluble in water. This hypothesis is congruent with the findings reported by Soykeabkaew et al. [39].

3.4 Folding endurance

Figure 2 shows the average folding endurance values for various film compositions. It was discovered that folding endurance ranges from 10.67 to 19.67. Folding resilience is a measurement of the product's packaging quality. These allow the products to be carried safely without breaking and demonstrate the pliability of the film. The incorporation of corn cob and coconut fiber and a gradual increase in their concentration led to a significant decrease in folding endurance[15].

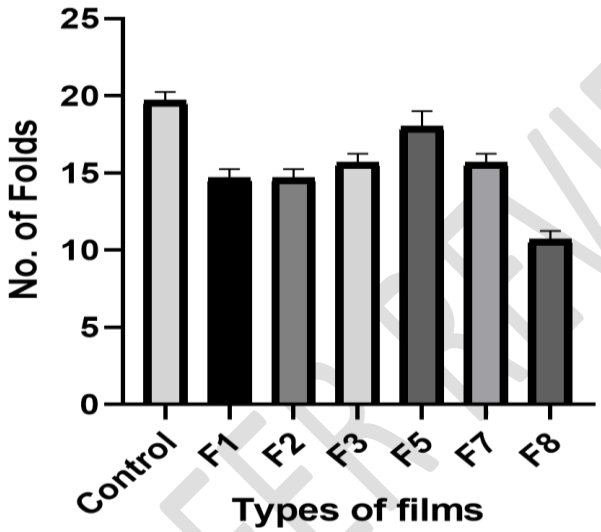

Fig. 2. Evaluation of biocomposite film formulation's pliability

3.5 Biodegradation of the biocomposite starch films

Biodegradation characteristics study is critical for using biocomposite films in the environment. This research conducted soil burial experiments on both the unreinforced CS film and the biocomposite film specimen. Biodegradation is the breakdown of a substance by microorganisms, fungus, or other biological decomposers[40]. The interaction of microorganisms with biodegradable polymers often initiates polymer degradation via an enzymatic or metabolic process; these microorganisms decompose the polymer into smaller molecules with a lower average molecular weight. This accelerated the breakdown of environmental trash. After biodegradation is complete, the process is termed mineralization[41]. Mass loss as a function of soil burial time was used to evaluate the biodegradability of the samples (Figures 4 & 5). The obtained mass loss values for all samples on day 1 were zero. The weight loss of control film, F1, F2, F3, F5, F7 and F8 biocomposite films as a function of biodegradation time showed the degradation of 78.19, 62.70, 92.94, 44.12, 87.37, 50.50 and 58.11% respectively at the end of 10 days, as presented in Fig. 3. The highest rate of weight loss was observed in the F2 film. After 12 days, the control film was found to have entirely degraded. In the meanwhile, biocomposite film degradation required 15 days. The matrix of the biocomposite film had less weight loss than the control films. Water absorption and the degree of crystallinity of maize starch in biocomposite films were two potential contributors to this issue. The biocomposite films based on corn starch lost less weight for each subsequent degradation test than the starch control film. Due to their physical qualities, biocomposites based on maize starch absorbed more water than control films, but the inclusion of sodium benzoate made them less susceptible to microbial attack. Films with less fiber concentration disintegrated more rapidly, mainly when the fiber size was small. The results indicated that biocomposite films with a smaller size and lower fiber content had a greater

potential for biodegradation, making them more susceptible to attack by microorganisms. In essence, the results demonstrated that the maize starch-based biocomposite films would have no negative environmental impact, indicating that the prepared films were utterly biodegradable.

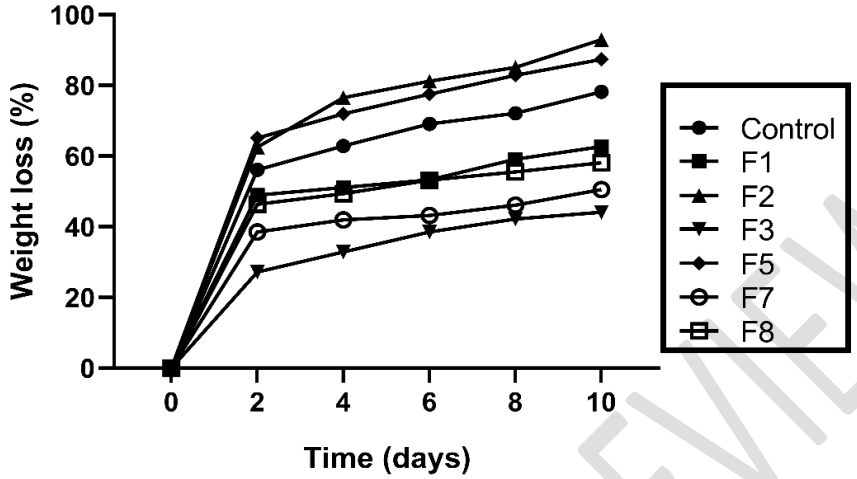

Fig. 3 Weight loss of corn starch-based biocomposite films

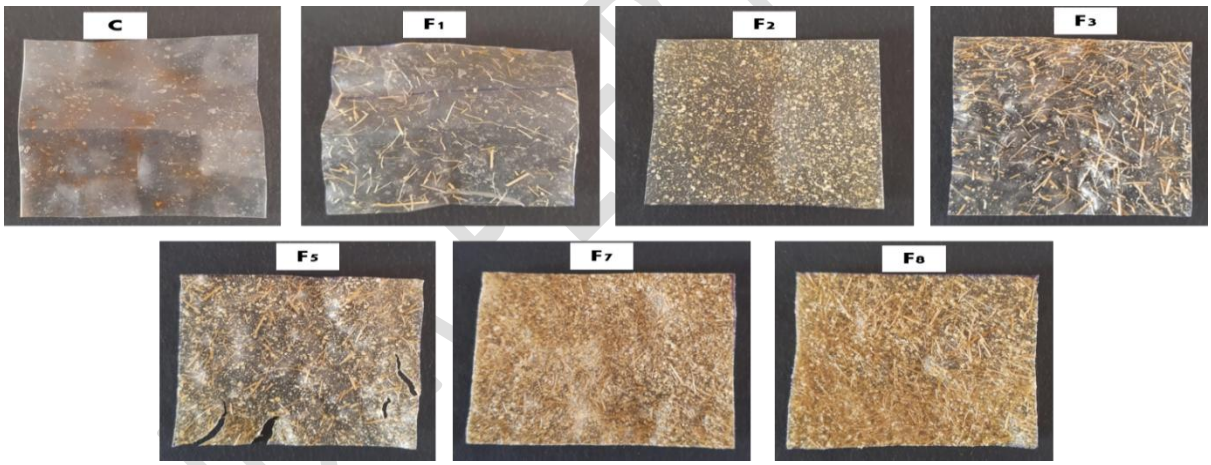

Fig.4 Soil burial biodegradation test sample before burial[16, 17]

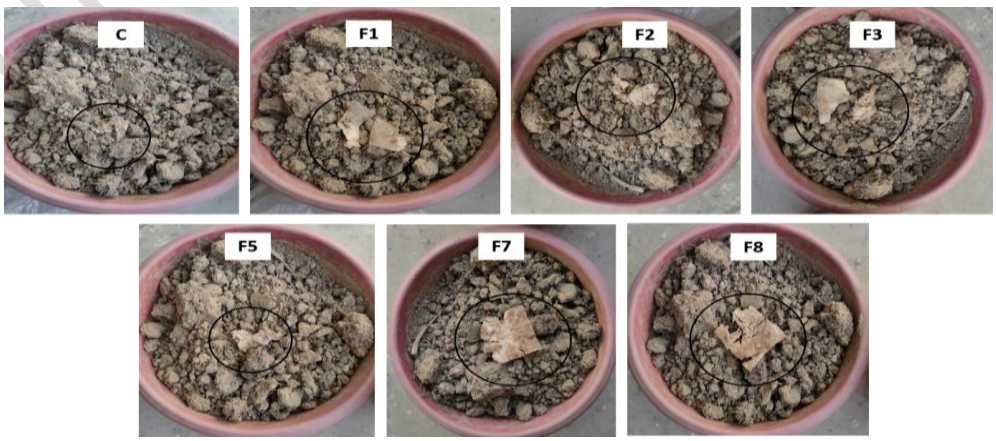

Fig. 5 Degraded soil buried biocomposite sample on day 10

3.6 Sealing property

It has been noted that no temperature for the heat-sealing procedure is acceptable. This might be because the biocomposite sheets can be sealed at moderately hot or melting temperatures. Therefore, an acceptable sealing temperature range is established, within which a satisfactory seal may be made if the seal is prepared within this temperature range. The dwelling time is when the coated film is in close contact with the heated film. The findings showed that the biocomposite samples generated had excellent sealing properties. The main goal of the sealing crimp is to "squeeze" the two layers of film together to establish as much molecular contact as possible across as much of the sealing area as feasible while staying within the bag/pouch design limits. Visual examination was used to assess the heat-sealing characteristic.
The sample was manually examined. The sealed sample seems to be quite well sealed. Because sealing qualities are vital for pouch preparation, it is inferred that the biocomposite films developed in this work may be utilized to make biocomposite carry bags. Figure 6 illustrates a sample bag made from biocomposite film.

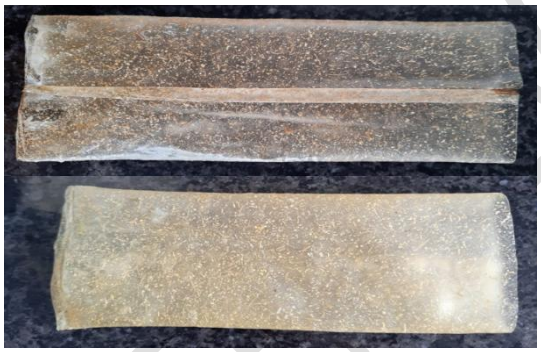

Fig. 6 Sealed biocomposite pouch

3.7 Scanning electron microscopy

Figure 7 depicts the scanning electron microscope [27] morphology of the biocomposite film's surface. The structure of the control film was compact, uniform, and largely porous-free; undissolved starch particles were also observed. In addition, film cross-sections revealed that the surface of the control film was smooth, but the surface of the biocomposite film was quite rough. This phenomenon was caused by the increasing fiber concentration and apparent fiber aggregation inside the biocomposite matrix, which led to the formation of irregularities on the biocomposite film's surface. The roughness of the structures evidenced low interfacial adhesion between coconut fiber and maize starch.

3.8 Fourier transform infrared spectroscopy (FTIR)

Figure 8 (a) illustrates the FTIR spectra of pure corn cob, corn starch, coconut coir, and biocomposite film. There is a strong peak at 3375.2 cm^{-1}, which corresponds to the stretching vibration peak of hydroxyl in the molecule. This is because corncob cellulose includes a high concentration of hydroxyl groups. The peak at 2936.2 cm^{-1} of the antisymmetric stretching vibration of alkyl C–H is the distinctive absorption peak of glycerol in the starch film, suggesting that glycerol has been effectively incorporated into the starch molecule. At 1660.1 cm^{-1}, the C-O bending associated with the OH group emerged as a strong peak. The absorption peak at 1079 cm^{-1} corresponds to the stretching vibration peak of cellulose's C–O in C–O–H. The peaks at 1153.8 and 1037.4 cm^{-1} correspond to the C-O-C and C-O stretching of alcohol, phenol, and ester groups. Carbohydrates exhibit C-O-C ring vibrations at 855.7 and 572.3 cm^{-1}.

3.9 X-ray diffraction (XRD)

Corn fibre, corn starch, coconut coir, and biocomposite film diffraction patterns were obtained and shown in Figure 8 (b). Raw chemicals and biocomposite film have identical pattern behaviour. The biocomposite film's crystalline structure was developed by gelatinization and retrogradation of starch molecules caused by heating, resulting in sharp 2θ peaks diffracted at angles 17.62°, 22.41°, and 24.19°, exhibiting the typical A-type pattern of natural plant starches. The addition of fibers boosted the relative crystallinity of the biocomposites by increasing the intensity of the major peaks.

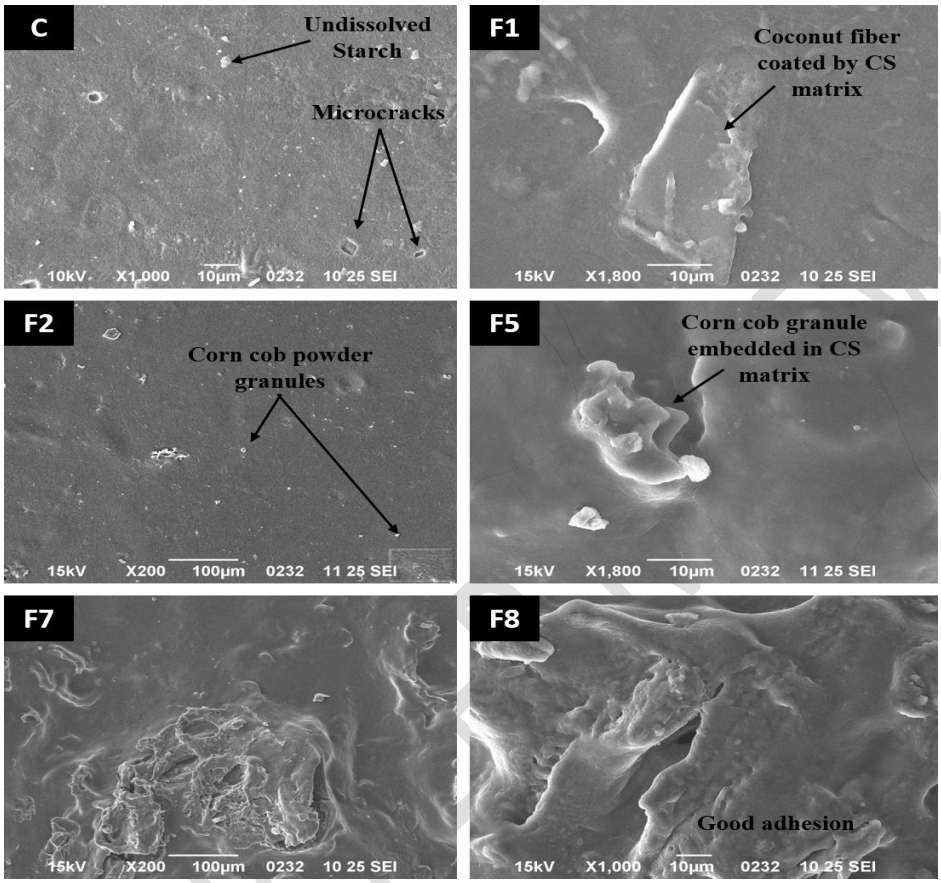

Fig. 7 – Scanning electron micrograph of biocomposite films. (C: control, F1, F2, F5, F7, F8: formulations)[18]

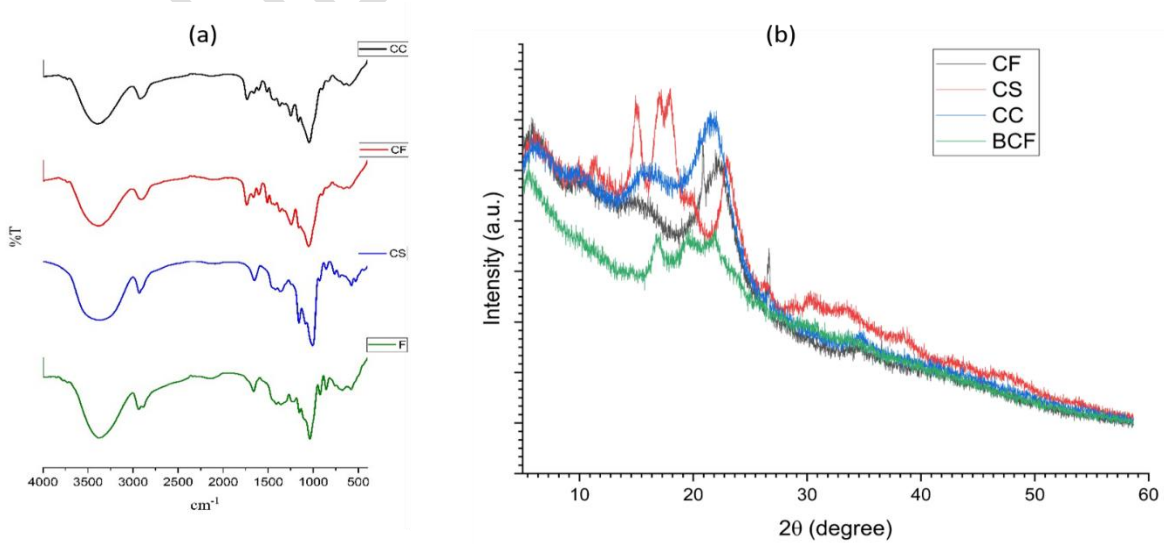

Fig. 8 – (a) FTIR spectra and (b) XRD spectra of corn starch, corn cob, coconut fiber and biocomposite film[19]

3.10 Tensile properties

Tensile strength is the amount of maximal strength necessary to rupture the biocomposite film. The tensile strength of produced biocomposite films is shown in Table 3. As a plasticizer, glycerol made the film more flexible by changing the mechanical characteristics and weakening the intermolecular interactions between polymer chains. According to the results, the incorporation of corn cob and coconut fiber and a steady increase in its concentration led to a significant increase in tensile strength due to the substitution of more hydroxyl groups with CH3COOH groups, indicating that the biocomposite film has become more rigid and stiff with less flexibility, resulting in more durable materials. Furthermore, cross-linking starch with corn cob and coir fiber reinforces intermolecular connections by generating a covalent bond, enhancing the biocomposites film's tensile strength. Additionally, the addition of glycerol and acetic acid substantially increases elongation percentage.

Table 3 - Tensile properties of Biocomposite films

Films	**Tensile Strength [25]**	**Elongation at Break (%)**
Control	14.67 ± 2.07	24.624 ± 1.56
F1	15.26 ± 1.93	26.98 ± 1.89
F2	16.82 ± 2.19	32.876 ± 2.31
F3	15.13 ± 1.81	34.302 ± 2.36
F5	20.37 ± 3.01	54.568 ± 2.19
F7	16.31 ± 2.17	32.437 ± 2.34
F8	21.23 ± 2.49	58.666 ± 2.31

3.11 Antibacterial activity

CS control film and fibers reinforced biocomposite blend film were screened for antibacterial activity against *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria) by employing the agar disc diffusion technique as shown in Fig. 9 (a) & (b). As a standard (+control), the antibiotics Vancomycin and Ampicillin (10 µl/disc) were used. The inhibitory impact was tested using the clear zone of the circular film disc. Measurement of exact zone diameter included the diameter of the film disc; hence, if a clear zone was present, the figures were consistently greater than the diameter of the film disc. If there is no surrounding clear zone, it implies that there is no inhibitory zone, and the diameter was assigned a value of zero. The existence of a distinct inhibitory zone around the disc of each film shows activity against *E. coli* and *S. aureus*. The interaction of positively charged and negatively charged bacterial cell membranes causes membrane permeability loss and intracellular component leakage, ultimately killing the bacterium[42]. The antibacterial activity of films against *Staphylococcus aureus* and *Escherichia coli* bacteria was moderate.

The larger inhibition zones around *Staphylococcus aureus* indicate that the samples are more effective against *Staphylococcus aureus* than against *Escherichia coli*. Due to *Escherichia coli*'s impermeable, lipid-based outer membrane, *S. aureus* is more resistant to antibiotics than *E. coli*.[20] Similar findings were shown in prior publications[43, 44]. The F5 film showed the best results in Gram-positive and Gram-negative bacteria. Figure 10 (a) & (b) is a bar graph displaying the zones created by each formulation.

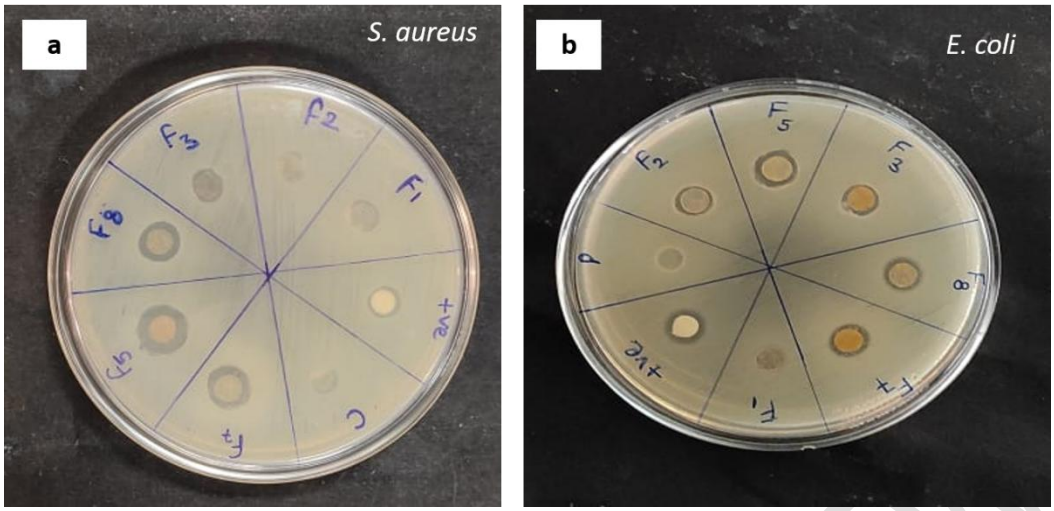

Fig. 9 – Inhibition zones of the biocomposite film discs (a)S. aureus (b) E. coli[21]

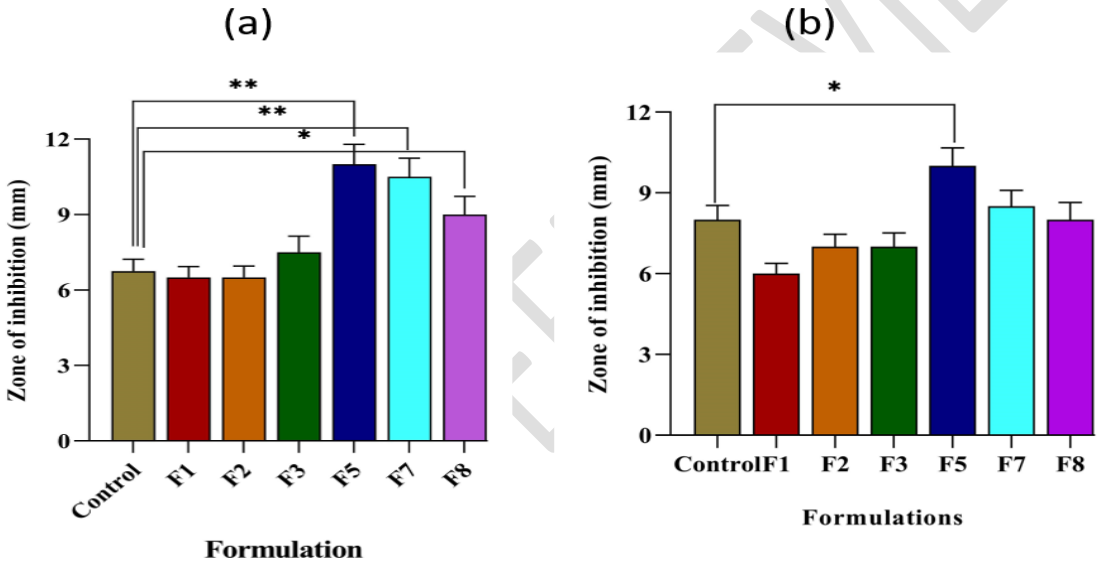

Fig. 10 - Bar diagram of agar disc diffusion assay results (C: control, F1, F2, F5, F7, F8: formulations) (a) S. aureus (b) E. coli. The error bars indicate the mean ± S.D. (n = 3) and * indicates significant p = 0.05; ** indicates significant p = 0.01.

Conclusion

Solution casting and annealing techniques were used to develop innovative maize starch-based biocomposite films reinforced by corn cob powder and coconut fibres. The biocomposite films were 0.042 cm thick on average. The average moisture content of the films was 8.64 %. The produced films were characterized using FTIR, XRD, and SEM techniques. After the hybridization technique, the biocomposite film's efficiency increased significantly, according to the characterization results. The findings demonstrated that the biocomposite films developed were fully biodegradable[23] and degraded in a short period. It may be inferred that biocomposite films derived from corn starch, reinforced by corn cob powder and coir fibers, can be utilized as a lower-cost option to produce sealable packaging pouches. Tensile strength and elongation at break (%) of the biocomposite films were higher than the maize starch-based control film. Gram-positive bacteria were more susceptible to the antibacterial action of the prepared biocomposite samples. Investigating the hybridization of suggested starch composites with other biomaterials and other plasticizers would be a fascinating research objective.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used in our research area and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather, it was funded by the personal efforts of the authors.

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