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Antimicrobial Activity and Physicochemical Analysis of Bio-degradable Films from *Cucurbita pepo* and *Musa paradisiaca*

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ABSTRACT

Cucurbita pepo and *Musa paradisiaca* can be considered as a large source of starch which makes it appropriate to be used for the preparation of Bio-plastic material. In this study, biodegradable films from *Cucurbita pepo* and *Musa paradisiaca* were developed and investigated for their properties like pH, ash, moisture content, amylose content, biodegradability, and antimicrobial activity. 1,2,3-Propanetriol, Gingelly oil, and Agarose gel was used to reduce the brittleness of the developed starch-based bioplastic films. The investigation of films was done by Spectroscopic and Surface analysis techniques. The developed Bio-films showed substantial properties like less engorgement and insolubility in water which makes it worth a commercial viable product for food packaging.

Keywords: *Cucurbita pepo*, *Musa paradisiaca*, Liquid food packaging material, Moisture and Amylose content, Spectral analysis, Anti-microbial studies.

INTRODUCTION

One of the foremost challenges in dry waste management is the plastic waste, which leaches out harmful chemicals inadvertently into the environment. The durability and increasing usage of plastics creates a major waste management problem which accounts for approximately 10 per cent of the waste generated[1] by man kind. Interaction of plastic waste with soil and water affects their quality and uniqueness[2], the toxic gases from plastics also pollute the environment and increase the global warming. When plastic waste is discarded in landfills, it is viable for the percolation of the hazardous chemical into the potable water[3] affecting its quality. Due to these environmental issues, some alternative methods are used for the preparation Bio-plastics. Bio-plastic is usually developed from a renewable source like starch, sawdust, cellulose, food waste, vegetable fats, and oils which is used for the packing of cutlery, crockery, food items etc.[4] The production of Bio-plastics can significantly reduce the emission of greenhouse gases and it can also decrease the consumption of non-renewable energy sources. The present study aims to prepare and analyze the Bio-films obtained from *Cucurbita pepo* and *Musa paradisiaca* using 1,2,3-Propanetriol, Gingelly oil, and Agarose gel as the plasticizing agents. In the last few years, Glycerol (1,2,3-Propanetriol) has been used in commercial applications because of its properties like high tensile strength, solubility in water, and biodegradability. It is also generally used as a plasticizing agent to increase the flexibility of the Bio-plastic film. Apart from 1,2,3-Propanetriol, Gingelly oil and Agarose gel has been used as plasticizers in this study. The bio-films obtained were further analyzed and characterized by different spectral and surface investigation techniques.

MATERIALS AND METHODS

Abstraction of starch

The dry pulp of *Cucurbita pepo* and peels of *Musa paradisiaca* was ground into a fine powder, soaked in water for about a day, filtered and used for the abstraction of its starch content. Finally, the dried starch from *Cucurbita pepo* and *Musa paradisiaca* was used for further studies.

$$\text{Yield (\%)} = \frac{\text{Mass of the starch extracted in g}}{\text{Mass of the peel/pulp in g}} \times 100 \quad (1)$$

The Moisture content of Starch

About 3 g of the starch samples were taken in the pre-heated, cooled Petri-dishes and kept in the oven at a temperature of 125^0C[5]. After an hour it was cooled to room temperature and the moisture content of the sample was found out using the following equation (2).

$$\text{Moisture content (\%)} = \frac{\text{Dried weight of the sample}}{\text{Wet weight of the sample}} \times 100 \quad (2)$$

Ash content

About 3 g of the starch samples were incinerated and completely carbonized in the pre-heated and cooled crucibles for about an hour at 100^0C. After cooling, the percentage of ash content[6] was calculated using equation (3).

$$\text{Ash Content (\%)} = \frac{\text{Dried sample weight}}{\text{Wet sample weight}} 100 \quad (3)$$

Starch pH

About 2 g of the starch sample obtained from *Musa paradisiaca* and *Cucurbita pepo* was shaken in distilled water for 25 min the starch was let on to clear and the pH was measured using a Systronics, Digital pH Meter 335 with 7% buffer solution.

Titratable acidity

0.1M of Sodium Hydroxide was used as the titrant for 3 g of the starch sample dissolved in 30 mL of deionized water using phenolphthalein as the indicator.

Test for Amylose and Amylopectin content

About 1 mL of ethanol and 9 mL of NaOH were added to 0.1 g of the starch sample in a test tube covered by an aluminum foil, after which it was completely mixed and heated for about 10minutes[7]. The cooled dispersion was diluted to10 times it's volume from which 0.5 mL of the extract was used for the analysis. To the extract, 0.1 mL of CH_3COOH and 0.2 mL of I_2 in CCl_4 were added and made up to 10 mL with distilled water. The Amylose content was determined using Aer Infra Digi, Digital Photo Colorimeter. The Amylose and Amylopectin content (%) was determined using equations 4 & 5.

$$\text{Amylose content (\%)} = \text{pH value} \times \text{Absorbance} \times 20 \quad (4)$$

$$\text{Amylopectin content (\%)} = 100 - \text{Amylose content} \quad (5)$$

Preparation of the Bio-film

The bio-films were made using the starch of *Cucurbita pepo* and *Musa paradisiaca*. The bio-plastic from *Cucurbita pepo* was developed by taking 3 g of its starch to which a mixture of 2 g of agarose gel and 2 mL of gingelly oil was added and heated in a low flame to get a colloidal gel. The gel was spread on a flat aluminum foil and dried[8] under the sunlight for two days to obtain the bio-film. About 3 g of *Musa paradisiaca* peel was soaked with sodium metabisulphite (Na_2SO_3) for about 45 minutes. Then, the peel was boiled for half an hour, filtered, dried, and ground into a paste. 2.5 g of *Musa paradisiaca* paste was taken to which, 3 mL of sodium hydroxide, 3 mL of hydrochloric acid, and 2 mL of 1,2,3-Propanetriolwere added and mixed well. The mixture was poured into a petri dish, and heated for about half an hour at 110^0C in an oven. After cooling a brown-colored bio-plastic film was formed.

Water Holding Capacity of the film

The bio-film was cut into 2 cm x 2 cm size and placed into a beaker containing 100 mL of water[9] for an hour. The initial and final weight of the films were noted from which the water holding capacity of the films was calculated using Equation (6),

$$\text{Water uptake (\%)} = \frac{\text{Final wt. of the bio-film in g} - \text{Initial wt. of the sample in g}}{\text{Final weight of the bio-film in g}} \times 100 \quad (6)$$

Biodegradability test

About 2 g of the bio-film was taken and buried under the soil in a beaker at the depth of 5 cm from the ground surface for about 15 days. The weight of the film was taken in a cycle of three days and results were recorded accordingly[10]. The weight of the sample before (W_0) and after burial (W_f) in the compost soil was noted and the weight loss of the samples was calculated using Equation (7),

$$\text{Weight loss (\%)} = \frac{W_0 - W_F}{W_0} \times 100 \quad (7)$$

Bio-films as Liquid food Packaging Material

Both the bio-films of *Cucurbita pepo* and *Musa paradisiaca* were tested as a packaging material for liquid food. The pre-weighed bioplastic (W_1) was cut into 2×2 cm and immersed in normal water and coconut oil at room temperature for three hours after which it was re-weighed (W_2). The weight change (%) of the bio-film was calculated using Equation.8. If the weight change of bio-film after being plunged into the liquid food products is less than 15% then the bio-film is to be compatible with the liquid food product and can be used as a packaging material for the same.

$$\Delta W\ (\%) = \frac{W_2 - W_1}{W_2} \times 100 \quad (8)$$

Characterization of the Bio-films

The investigation of the bio-films was carried out by different Spectral and Surface Analyzing techniques.

FT-IR analysis

The functional groups present in the films were determined through an Attenuated Total Reflectance (ATR) accessory with 8 scans at the range of 4000-400 cm^{-1}(Perkin Elmer FT-IR Spectrometer Frontier). The Bio-film was mixed KBr in a ratio of 1:5[11]. The peaks were obtained in the range of 4000 to 400 cm^{-1}.

SEM analysis

The surface morphology of a biopolymeric film can be visualized using scanning electronic microscope[12] under normal atmospheric conditions. The SEM analysis utilizes a focused beam of high-energy electrons to produce an assortment of signals at the surface of the specimens.

Antimicrobial Activity Test

The antimicrobial activity of the bio-film for micro-organisms was done by the Disc-diffusion method[13]. The Petri dish was prepared with Muller Hinton Agar and immunized with test organisms. Sterile disc of 6-millimeter width was infused with the bioplastic film at different concentrations of 20-100 microgram per milliliter respectively. The impregnated disc was placed on the top layer of agar plates for 30 min at room temperature for compound diffusion. The dishes were incubated for 24 h at 37°C and the zone of inhibition was recorded in millimeters and the experiment was repeated twice.

RESULTS AND DISCUSSION

Yield of Starch

The percentage yield of starch from *Cucurbita pepo* and *Musa paradisiaca* is given in Table 1 From the values it can found that bio-films prepared from the starches can be used as a packaging material for food products.

Table 1: Results of Physical, Chemical parameters and Proximate analysis of Cucurbita pepo and Musa paradisiaca

No	Parameters	Curcubita pepo	Musa paradisiaca
1	Appearance	Yellow	Brown
2	Odor	Odorless	Odorless
3	% yield	76.25	82.9
4	pH	5.30	6.64
5	Titrable acidity	3.8	3.5
6	Moisture content	11.99	11.9
7	Ash content	5.86	3.9
8	Amylose Content	20.27	34
9	Water Holding Capacity	63.68	89

Moisture content

The moisture content for *Cucurbita pepo* starch and *Musa paradisiaca* starch was found to be in the range of 11-12% (Table 1). The result shows that the moisture content value for both starches is low when compared to other starches[14] which may be due to reasons like handling problems and level of ripping. High moisture content in food can lead to microbial spoilage and short shelf life to the food items, leading to its deterioration. Less percentage of moisture content in food items is acceptable and good.

Ash content

The mineral content of the bio-film[15] is identified by the ash test. The ash content of both *Cucurbita pepo* and *Musa paradisiaca* starches was found in the range of 3-5%. The mineral and organic salt in the tubers is expressed as the ash content which is the food residue after the combustion process. The low value of ash indicates a low mineral content in the fruit or the vegetable under investigation.

pH of the starch

The pH value of the *Cucurbita pepo* and *Musa paradisiaca* starches were found to be around 5-7. If the pH of the starch is in the range of 4-7[16] it can be used as a packaging material in food industry.

Amylose content

The Amylose content test will help to detect the Amylose and Amylopectin percentage in the starch. The Amylose content present in the *Cucurbita pepo* (20.27%)and *Musa paradisiacal* (34%) is given in Table 1. The Amylose content leads to chain formation in the bioplastic preparation.

Water Holding Capacity of the Bio-film

The water holding capacity is an important study to determine the suitable source for bio-polymers.[17] The water uptake value of the bio-plastic sheet from *Musa* had 89% percentage followed by *Cucurbita pepo* bio-plastic sheet with 63.68% of absorption capacity (Table 2). Due to the hydrophilic nature of the films the water upholding capacity was found to be more than 50% and this may be due to the plasticizing agent 1,2,3,-propanetriol. It is a low molecular carbohydrate that tends to absorb water according to the molecular weight of its structure[18] and the number of hydroxyl groups present in it.

Table 2: The weight change of the bio-plastics after being immersed in test samples for one hour

No	Test samples	*Curcubita pepo*	*Musa paradisiaca*
1	Distilled water	63.68	89
2	Vegetable oil	2.9	4.87

Application of bio-films as a Packaging Material for Liquids

From Table 2 it can be seen that the penetration of vegetable oil is low compared to the penetration of water in the synthesized bio-films. The weight of the biofilm increased to a maximum than its original weight. This indicates that the bio-plastic of *Cucurbita pepo* and *Musa paradisiaca* can be used as a packaging material for vegetable oils.

Biodegradability test

The degradation tests of bio-films was conducted using the soil burial technique. The biodegradability of bioplastics was determined by allowing them to degrade in the soil for a month. The degradation of *Cucurbita pepo* and *Musa paradisiaca* bio-film was monitored regularly. Fig. 1 & 2 show the percentage weight loss of the samples within one month for *Cucurbita pepo* (87%) and *Musa paradisiaca* (93%) respectively. Generally, the results show the increase in the percentage of degradation of the bio-film gradually after five days. The weight loss was monitored periodically for every five days and after 30 days it was found to be fully degraded. Visually the texture of the bio-plastics showed literal damage on the surface of the bio-plastics[19].

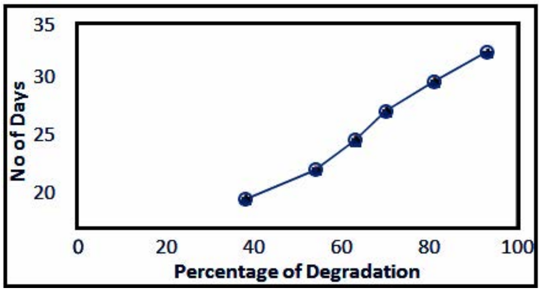

Fig. 1. Degradation percentage of bio-film from *Curcubita pepo*

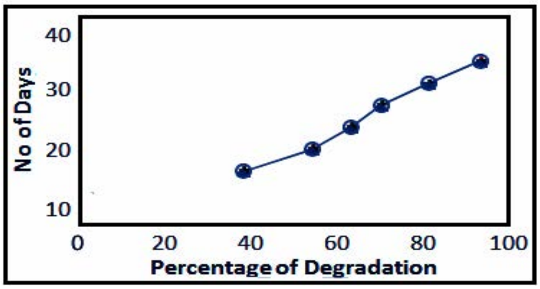

Fig. 2. Degradation percentage of bio-film from *Musa paradisiaca*

Spectral Analysis of the Bio-films

Figure 3 and 4 show the IR spectra of *Cucurbita pepo* and *Musa paradisiaca*. In the IR spectrum of *Cucurbita pepo* bio-film the characteristic peak at 3605.99 cm^{-1} shows the presence of O-H stretching bond[20], the peak at 2326.18 cm^{-1} shows O=C=O stretching bond[21], the absorption bands at 2155.99 cm^{-1} and 1743.92 cm^{-1} shows the presence of N=N=N and C=O stretching and the band at 1472.50 cm^{-1} shows C=C ring stretching aromatic bond. The characteristics peaks at 3205.90 cm^{-1}, 2957.66 cm^{-1}, 2132 cm^{-1}, 1421 cm^{-1} and at 857 cm^{-1} of the *Musa paradisiacal* biofilm were attributed to N-H stretching (secondary amine) hydrogen-bonded nitriles, strong C-H stretching (alkanes)[22], C≡Calkynes, C-H stretching (alkene) and aromatic C-H stretching respectively in the FT-IR Spectrum.

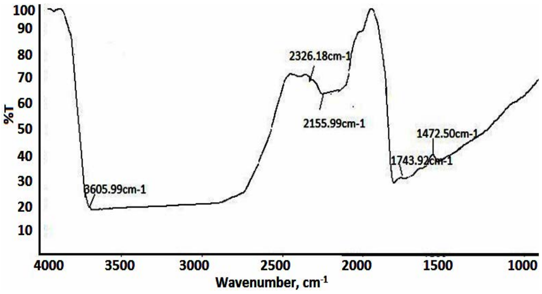

Fig. 3. FT-IR Spectra of the bio-film from *Cucurbita pepo*

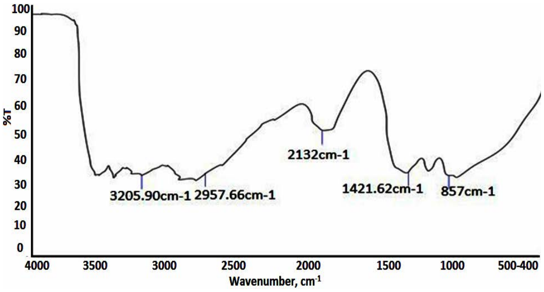

Fig. 4. FT-IR spectra of the bio-film from *Musa paradisiaca*

Surface analysis technique

The scanning electron microscopy studies showed that *Musa paradisiaca* starch granules[23] are rectangular (Fig. 5) with a smooth surface. The starch granules of *Cucurbita pepo* were found to be spherical with a smooth surface (Figure 6).

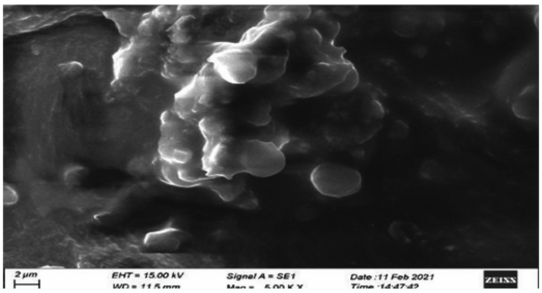

Fig. 5. Sem image of the developed bio-film from *Musa paradisiaca*

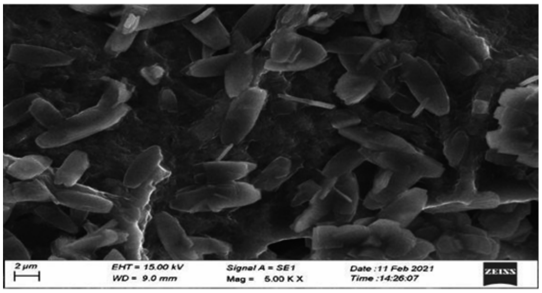

Fig. 6. Semimage of the developed bio-film from *Cucurbita pepo*

Anti-microbial Activity

The anti-fungal and anti-bacterial results of *Cucurbita pepo* and *Musa paradisiaca* bio-films reveal that both the starches are effective against *Aspergillus niger*[24] and *Staphylococcus aureus*[25] compared to *Escherichia coli* and *Candida albicans*. The results are given in Tables 3 and 4.

Table 3: Antifungal activity of the plant starches

Starch	Concentration (μl/disc)	Organisms/Zone of Inhibition *Candida albicans*	*Aspergillus niger*
Curcurbito pepo	100	5	6
Musa paradisiaca	100	3	4

Table 4: Anti-bacterial activity of the plant starches

Extract	Concentration (μl/disc)	Organisms/Zone of Inhibition *Escherichia coli*	*Staphylococcus aureus*
Curcurbita pepo	100	7	6
Musa paradisiaca	100	5	8

CONCLUSION

The development of packaging material for food products involves sustainability and environmental responsibility. In this study, *Cucurbita pepo* and *Musa paradisiaca* starch have been used for the preparation of bio-films. The bio-film also investigated by spectral and surface morphological analysis. Anti-microbial studies showed that the bio-films were effective against *Aspergillus niger* and *Staphylococcus aureus*. The different physical and chemical properties like percentage yield of starch, pH, Titrable acidity, proximate analysis like amylose content and moisture content of the biofilm showed that it can be used as an alternative food packaging material for vegetable oils.

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Conflict of interest

The author declare that we have no conflict of interest.

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