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Production of biopolymer films using groundnut oilcake

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Abstract

Protein-based biopolymer films were produced using Groundnut (*Arachis hypogea*) oilcake. Thin, semi-transparent, brownish films were obtained, the films were smooth and uniform. The film was plasticized using PVA (polyvinyl alcohol) and glycerol was added to improve the elasticity. This resulted in the film being more flexible and more like conventional plastics. The thickness of the film was found. The tensile strength and the elongation at break were calculated. The water absorption capacity of the films was also estimated. Further, FTIR and SEM analysis were done to find out the chemical structures and morphological microstructures of the film. X-ray diffraction studies were also done. Also, antimicrobial and antioxidant assays were performed to find out the potential of the film as active food packaging. All these tests prove that the GOC films are capable of being used as food packaging alternative for conventional plastics. Biopolymer films were prepared using the protein extracted from groundnut oilcake. Its various characteristics were evaluated. These films can be used as substitutes to conventional food packaging plastics. The development of new bio-materials from agricultural wastes/ by-products of oil industry, may be good and cheap sources of both energy and protein. The present study focused on obtaining useful protein-based biodegradable films for an eco-friendly option. Potential applications of the obtained bio-polymer films include wrapping of different fabricated foods for shelf-life extension. These types of protein-based films are very useful as they are readily biodegradable in nature and also, they are sourced from natural and renewable raw materials rather than petroleum-based plastics.

Keywords: Bioplastics; Biodegradable films; Protein films; Groundnut oilcake; PVA

1 Introduction

Plastic pollution is now a global concern. As per the Central Pollution Control Board (CPCB), plastics contribute for 8% of total solid waste in India. Petroleum derived plastics are used in all types of industries, especially in food packaging. These plastics are used as one time use containers, cartons, or packages and are thrown away after one time use. Only 10% of plastic produced is recycled every year, making plastics end up in oceans and landfills. Land dumping creates severe pollution concerns for the environment by negatively influencing soil physicochemical characteristics.

Plastic food packaging usually contains residues from the food it contains and other biological substances. So, complete recycling is impractical and economically inconvenient. As a result, tons of plastic packaging are thrown into nature, thus increasing the waste problem year after year. One of the major drawbacks to the widespread use of packaging produced from petrol is its environmental impact. An alternative to plastic, therefore, should be sought, and the development of biodegradable films and coatings made from natural bio- sourced polymers should be encouraged.

In the recent years, there has been an increased concern to reduce the use of petroleum derived plastics. This has led to the invention of novel bioplastics. These bioplastics ae produced using different biomaterials like starch, proteins, lipids, etc. derived from agricultural waste or byproducts of agriculture industry. Plastics produced from plants and plant-based byproducts are both biodegradable and are of renewable sources. In the 1980s, starch was discovered to be a

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filmogenic material, and while some basic research on the potential of this was conducted, scientists chose to focus more instead on other biopolymers such as proteins. Since protein films are biodegradable in nature, they breakdown significantly faster than films produced from crude oil. Not requiring special composting conditions, a protein film could potentially breakdown in less than a few weeks. Biopolymer films is a continuous matrix that acts as a layer protecting food quality and extending their shelf life. Hence, they act as a barrier for migration of moisture, oxygen, carbon dioxide, aromas and flavors from adjacent foods or between food and the environment. They can reduce mechanical damage while functioning as a vehicle for active compounds such as antioxidants and antimicrobials. Use of biopolymers in packaging allows use of natural resources that are often food system waste byproducts, consequently reducing solid waste pollution, among other benefits. Proteins (soy, gelatin, zein, sunflower) and polysaccharides (cellulose, starch, chitosan, pectin) are commonly used biopolymers in producing films.

Groundnut oilcake can be used as a raw material to produce protein-based films. Groundnut (*Arachis hypogea*) is a leguminous crop cultivated for edible purposes. It is exclusively found in tropical and subtropical regions of the world. It is considered as a grain legume because the seed can produce oil and the crop is categorized as an oil crop. After the extraction of oil from groundnut seeds, the waste is known as oilcake and it is a byproduct of the agricultural industry. It is rich in proteins and because of this, the oilcake is commonly used as animal feed and as a natural manure for plants in some regions of the world. Apart from that, groundnut oilcake is not extensively used in any other industries. So, this can be used as a good and renewable source of raw material for protein-based film production.

2 Material and methods

2.1 Materials

Groundnut oilcake (GOC) was purchased from an oil mill, Glycerol (Molecular weight 92.09) and PVA (Polyvinyl alcohol) were purchased. All other chemicals and solvents used for extraction of protein, preparation and analysis of films in this study were analytical grade commercial products.

2.2 GOC Protein Extraction

PC (protein concentrate) was obtained from GOC by using acid base extraction method. The process of extraction was done according to the method described by Prospero Di Pierro et al., 2017 [11] with little modifications. GOC was ground using mortar and pestle and mixer until fine powder was obtained and stored in airtight container under ambient temperature until use. 15 g of this powder was dispersed in 150 ml double distilled water (1:10 w/v) and brought to pH 12 using NaOH pellets. This solution was stirred at medium speed in a magnetic stirrer for 1 hour at room temperature. It was then centrifuged at 4000 rpm for 20 minutes and the supernatant was collected. The pH of the supernatant was adjusted to 4 using HCl to form a white precipitate which was then separated by centrifugation at 4000 rpm for 20 minutes. The pellet was then collected and dried at room temperature for one hour to produce the PC.

2.3 FFS Preparation and Casting

The process of film preparation and casting was done according to the methods described by Atefeh Solouk et al., 2019[1] with little modifications. PVA crystals were mixed with double distilled water (1:10 w/v) and stirred in a magnetic stirrer at 80 °C until the PVA was completely dissolved. This solution was mixed with the PC previously obtained (50:50 v/w) and to this, Glycerol (2% of the final volume) was added and the pH of this solution was adjusted to 8 by adding NaOH pellets and mixed in a magnetic stirrer at medium speed for 1 hour. The ratio of PC to PVA and Glycerol is based upon previous experiments. This FFS (film forming solution) (10 ml) was then casted onto a glass petri plate and dried in the hot air oven at 60 °C for 2 hours. The obtained films were further analyzed for various properties.

2.4 Quantitative protein assay (Lowry's method)

The amount of protein present both in the sample and the product were measured using Lowry's method at 640 nm in a colorimeter to prove that the protein extracted is high in concentration.

2.5 Film Thickness

The thickness of the film was measured using a digital micrometer at four different points on the film to the nearest 0.001 mm and the mean value was calculated [7].

2.6 Tensile Strength and Elongation at Break

The tensile strength and elongation at break were measured at 23 °C and 50% relative humidity. Four different weight loads were used and four values were obtained [12].

2.7 FTIR (Fourier Transform Infrared Spectrometry)

FTIR spectra were obtained for the film. FTIR measurements were recorded in the range of 4000–400 cm−1 at a resolution of 4 cm−1 using 40 scans. The results were used to find out the crosslinking and functional groups present in the films [1].

2.8 SEM (Scanning Electron Microscopy)

The surface structure of the films was studied by High Resolution scanning electron microscopy (HRSEM) at an acceleration voltage of 20 kV to observe the morphology and microstructure of the films [1].

2.9 Water Absorption Test

The film was cut into 1" x 1" piece and weighed (W1) and was immersed in water for 10 minutes.

It was taken from water and wiped with tissue to remove excess water. The film was then weighed (W2) [16]. The amount of water absorption was calculated using the formula,

$$\frac{w_1 - w_2}{w_1} \times 100$$

2.10 XRD (X- ray Diffraction Analysis)

X- ray diffraction analysis was performed using an X- ray diffractometer operated at 30kV and 30mA [6].

2.11 Antimicrobial Activity

Antimicrobial activity of the sample and product was tested against a gram-negative organism *E. coli* and a gram-positive organism *Staphylococcus aureus* using well- plate method and Muller Hinton agar was used as the media.

2.12 Antioxidant Activity

DPPH free radical scavenging activity was used for screening the antioxidant activity of the sample and product measured spectrometrically at 517 nm [14] and calculated using the formula,

$$\% \text{ Of Inhibition} = \frac{\text{A of control } - \text{A of treated cells}}{\text{A of control } - \text{A of treated cells}} \times 100$$

A= Absorbance

2.13 Soil Degradability

The films were buried under the soil to check landfill biodegradability.

3 Results and discussion

3.1 Method of Preparation of Protein- Based Film

GOC powder dissolved in double distilled water and pH adjusted to 12 using NaOH pellets.

Figure 1GOC powder

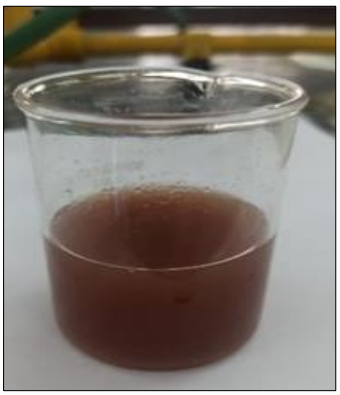

Figure 2 PC was extracted from the GOC powder

Figure 3 PC

This PC was mixed with PVA and casted on petri plates

Figure 4 PVA

The petri plates were dried in the hot air oven at 60ºC for 2 hours to obtain the films.

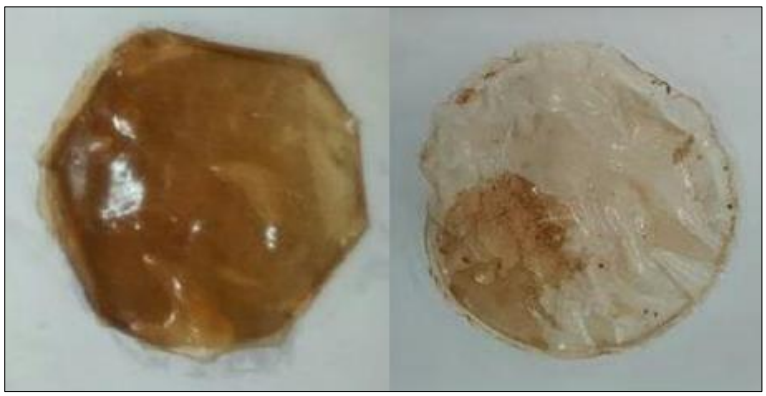

Figure 5a and 5b Protein Films

3.2 Quantitative protein assay(Lowry's method)

C= negative control; S= sample; P = product

Figure 6 Lowry's test

The concentration of protein in the sample (GOC) and film were found to be 70 mg/100ml and 47 mg/100ml respectively. So, it is concluded that GOC contains high protein content.

3.3 Film Morphology

Uniform, brownish, semitransparent films were obtained. The surface of the films was smooth and without cracks. Fragile films were formed when PVA was not added. Increased concentration of PVA resulted in more rigid films. The films were sticky if the concentration of glycerol exceeded 5% of the total volume. Adding 2% glycerol gave non-sticky films. The films could be easily peeled from the petri plates. The appearance of two sides of the film was not the same. The side of the film facing the petri plate was shiny, while the other side was dull.

Figure 7 Film morphology

3.4 Film Thickness

Based on the measurement of film thickness using a digital micrometer, the mean thickness of the films was found to be 0.40mm

Table 1 Film thickness

Thickness in mm
0.15
0.17
0.72
0.55
MEAN = 0.40

3.5 Tensile Strength and Elongation at break

Table 2 Tensile strength

	Maximum Load [N]	Tensile Strength [MPa]
1	5.74	3.83
2	7.25	4.26
3	8.67	1.20
4	8.63	1.57
Mean	7.57	2.72

Table 3 Elongation at break

Elongation At Break [%]	Extension at Maximum Load [mm]
303.84	91.15
239.84	71.95
323.84	97.15
237.34	71.20
276.21	82.86

The average tensile strength of the film was found to be 2.72 MPa and the elongation at break was found as 276.21%, which indicated that the film had good mechanical properties.

3.6 FTIR (Fourier Transform Infrared Spectrometry)

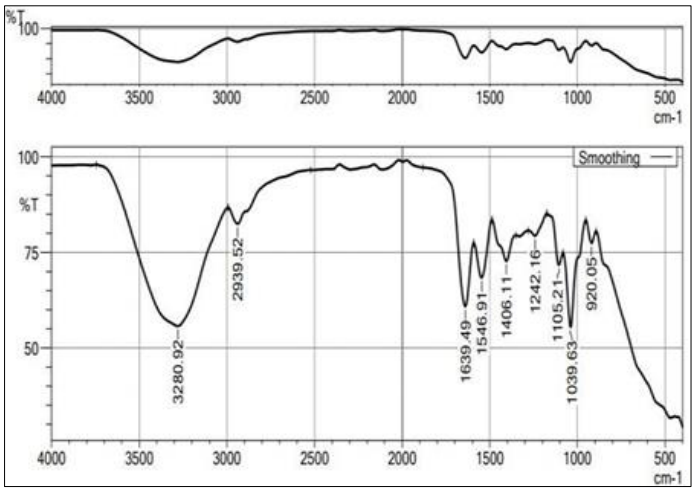

Figure 8 FTIR spectra for GOC film

FTIR spectra obtained for the film showed peaks at 3280.92 which indicate OH groups, 2939.52 which indicates CH and CH_2 stretchingaliphatic groups, 1639.49 which indicates C=O Amine I band, 1546.91 which indicates C-N amide III band, 1406.11 which indicates stretching- C=O inorganic carbonate, 1242.16 which indicates C-N amide III band, 1105.21 which indicates C-O carbohydrate, 1039.63 which indicates-O carbohydrate and 920.05 which indicates bending -C=O inorganic carbonate.

3.7 SEM (Scanning Electron Microscopy)

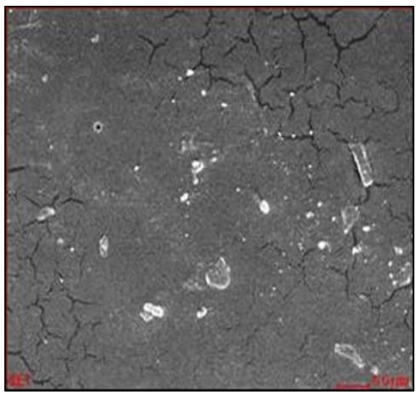

Figure 9 SEM image revealing the surface morphology of the films

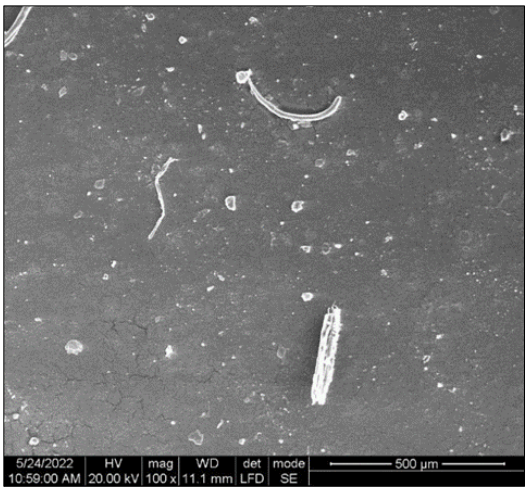

Figure 10 Surface morphology at 500 µm resolution

SEM images show that the film is of almost uniform structure and has very minute pores in the structure.

3.8 Water Absorption Test

The percentage of water absorption of the films was found to be 20%, which shows that the films can be effectively used for food packaging.

3.9 XRD (X- ray Diffraction Analysis)

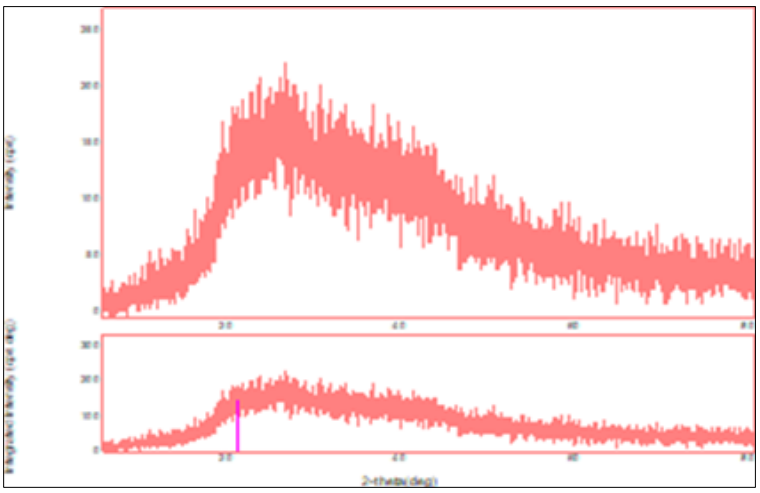

Figure 11 XRD patterns obtained for the films

The XRD analysis shows that the addition of glycerol to PC and PVA film slightly reduced the crystallinity. The overall crystallinity of the film is slightly reduced by the addition of PC with PVA.

3.10 Antimicrobial Activity

The antimicrobial activity of the films was tested against *E. coli* and *Staphylococcus aureus* and it was found out that the films did not have any antimicrobial activity against *E.coli* and *Staphylococcus aureus.*

3.11 Antioxidant Activity

The antioxidant activity assay was done only for the aqueous solution of GOC powder and the end product film, which were significantly good values.

Table 4 Antioxidant activity

Sample	Absorbance	% of inhibition
GOC powder	0.432	2.8
FILM	0.384	7.2

3.12 Soil Biodegradability

Based on the observations of burying the films under soil for two weeks, these films were able to partially degrade in two weeks.

4 Conclusion

Biopolymer films were prepared using the protein extracted from groundnut oilcake. Its various characteristics were evaluated. These films can be used as substitutes to conventional food packaging plastics. The development of new bio-materials from agricultural wastes/ by-products of oil industry, may be good and cheap sources of both energy and protein. The present study focused on obtaining useful protein-based biodegradable films for an eco-friendly option for uses others than animal feed or fertilizers. Potential applications of the obtained bio-polymer films include wrapping of different fabricated foods for shelf-life extension. These types of protein-based films are very useful as they are readily biodegradable in nature and also, they are sourced from natural and renewable raw materials rather than petroleum-based plastics.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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