ISSN: 2249 - 7862 (Print)
Aureole, Vol. XIII, 2021 PP 114-127
www.aureoleonline.in

BIODEGRADABLE AND ANTIMICROBIAL NANOFILM FROM RICE STARCH (KANJIVELLAM) FOR FOOD PACKAGING AND PRESERVATION

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**** Dr. Megha Shejoy**

Abstract

Due to the negative environmental impacts of synthetic plastics, the development of biodegradable plastics for both industrial and commercial applications is essential today. The present work investigates the rice starch-based biofilms for packaging applications. Various samples of biofilms are produced, with different compositions of rice starch, glycerol, sorbitol and gelatin. The tensile properties were improved after adding rice starch. However, water absorption and water solubility were reduced. On the basis of these results, the best sample was analyzed for thickness testing, biodegradability properties and sealing properties of biofilms. The results show the suitability of rice -based thermoplastic starch for packaging applications. A greater attention has been paid to antimicrobial activity screening and evaluating methods.

Keywords **:** *biodegradable plastics, antimicrobial nano film, rice starch*

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Introduction

Plastics play a vital role today in both industries and household appliances. Plastics are widely used for various applications, such as hand baggage, cool drink bottles, toys, food packages, components and containers of electronic equipment, modules of vehicles, office block segments, furniture, dress materials, etc. During the manufacturing of plastic bags, the emission of carbon and many other dangerous gases causes environmental concerns. Generally, polyethylene plastic films, such as low-density polyethylene (LDPE) and high-density polyethylene (HDPE), are being used to produce a variety of polyethylene plastic films, and the drawback of this plastic is its non-degradability. Over 1000 million tons of plastic were predisposed of as unwanted elements, and they might take several hundreds of years to decay. When plastic wastes are dumped in landfills, they interact with water and form hazardous chemicals, and the quality of drinking water may also be affected. Hence, efforts are taken to reduce the use of synthetic plastics and to promote biofilms.

Biodegradable plastics are made from starch, cellulose, chitosan, and protein extracted from renewable biomass. The development of most biofilm is assumed to reduce fossil fuel usage, and plastic waste, as well as carbon dioxide emissions. Approximately 50% of the biofilms used commercially are prepared from starch. The production of starch-based biofilms is simple, and they are widely used for packaging applications. The tensile properties of starch are suitable for the production of packing materials, and glycerol is added into the starch as a plasticizer. Starch is a carbohydrate that contains a great amount of glucose units, combined through glycosidic links. Pure starch is white in color. The starch powder does not possess any specific taste or odor. Furthermore, pure starch cannot be dissolved in cold water or alcohol. It is non-toxic, biologically absorbable, and semi-permeable to carbon dioxide. The linear and helical amylose and the branched amylopectin are the two types of molecules present in starch. The amylose content may vary from 20 to 25%, while the amylopectin content varies from 75 to 80% by weight, depending on the type of plant.While heating, the starch becomes a paste and the viscosity is also increased. High amylose starch is a smart reserve for use as an obstruction in packing materials. Due to the low price, renewability, and having decent mechanical properties, it was used to produce decomposable films to partly or else completely substitute the plastic polymers.

In this scenario, this study aims to produce starch based biofilms in place of plastic packaging material . Since, the raw material rice water is a common house hold refuse in Kerala, we tried here to completely utilize the potentiality of starch . The main objectives included selection of ideal source of starch, cross linkage with effective plasticizers, isolation of food borne pathogens from spoiled food, analysis of antimicrobial activity of plant extracts against isolated food pathogens, Identification and incorporation of antioxidant property conferring bioextracts and Incorporation of antimicrobial and antioxidant components; casting of the biofilm.

Methodology

1. Isolation and characterization of food borne pathogens from spoiled food

Pathogens such as Staphylococcus auerus and E.coli were isolated from different putrefied food sources using Mannitol Salt agar and MacConkey agar. Gram staining was done for further characterisation

2. Bioextract preparation

Crude extracts of Fresh leaves and flowers of Hibiscus (Hibiscus rosa-sinesis), Aloe vera(Aloe barbadensis miller),Tulsi (Ocimum tenuiflorum) and Drum stick(Moringa oleifera) were prepared with 70% ethanolic solution

3. Antimicrobial activity of the bioextracts:

In vitro antimicrobial activity of all different plant extracts was determined by standard agar well diffusion assay in order to check the potential of plant extracts against both E.coli&Staphylococcusaureus (Perez et al., 1990; Nair and Chanda, 2006). Minimum Inhibitory Concentration(MIC) was determined to find out the minimum concentration of selected plant extract to inhibit the growth of food borne pathogens by Tube dilution method and Broth micro dilution assay (Eloff method ;1989).

Turbidity was observed on the tube dilution method(fig 7).In this method all the tubes containing nutrient broth was inoculated with E.coli and one was kept as sterile control . Then plant extract was added in the concentration ranging from 100ml,200ml 300ml and 400ml and it was incubated at 37 0 c .The result was observed by visualizing the turbidity.

Minimum bactericidal concentration (MBC) was determined by chosing wells with no growth as well as with the lowest growth.

Micro dilution in 96 well plate is the quantitative method that can be used to determine the MIC and MBC values. This is a standardized ,accurate & inexpensive method to perform Resazurin dye acts as a Redox indicator and active bacterial cell reduces non Fluorescent Resazurin (blue) to the Fluorescent Resorufin (pink)which can be further reduced to hydroresorufin.

4. Optimized Biofilm Preparation :-

The film was prepared by mixing in proper quantity of rice water along with different sets of plasticizers, thus generating two sets of samples:-

Sample 1 with Rice water -100mL; Glycerin-5mL;Vinegar - 5mL & Appropriate antimicrobial bioextract - 2 mL

Sample2 with Rice water - 100mL ; Sorbitol - 5mL; agarose-2 g ; 1%glycerol- 70 mL & Appropriate antimicrobial bioextract - 2mL

A defined volume of film-forming suspension was spread over a clean smooth metallic surface. The mixture is left to dry for 48 hours under normal room temperature. The concentration of plasticizer was chosen based on strength of the formed film under varying quantity.After completion of drying, the starchy fim is carefully taken and subjected to scrutiny to check for various parameters.

5. Characterisation of Starch Wrap

Thickness

The thickness of the bioplastics was measured by using the thickness gauge. The thickness was measured by holding the work piece between stylus and anvil, reading the value directly.

Tensile strength and elongation

A thin strip of biofilm of dimension 15 X 2.5 cm was suspended by being tied over a straight plank and suspended over 2 wooden poles of about 100 cm. At the middle of film weight was added gradually incremented ranging from 10 gm, 20gm, 50 gm & so on. The weight at which the film get's cleaved from the middle was noted. Change in length caused to the film was also noted. By ploting load along the X- axis & extension(cm) along the Y-axis, the elastic limit & yield point was determined. By computing the stress & strain imposed upon the film. The tensile strength of the film was determined. An increase in length causes a simultaneous increase in tensile strength as well.

1.8.3 Test for Moisture Content

By measuring the weight loss of films, the moisture content was estimated. The TPS samples were cut into square pieces of 2.0 cm2. The samples were weighed accurately. The dry film mass was recorded upon drying in an oven at 110 °C until a fixed dry weight was acquired . Each film treatment was used with five replications, and the moisture content was measured:

Moisture Content in (%) = [(Wi ? Wf)/Wi] × 100,

where Wi is the weight at the beginning and Wf is the final weight

1.8.4 Water Solubility Test

The film samples were cut into square sections of 2.0 cm2, and the dry film mass was weighed accurately and recorded. The samples remained immersed in 100 mL distilled water and fixed agitation at 180 rpm was carried out for 6 h at 25 °C .The lasting portions of the film were filtered after 6 h. They were then dried in a hot air oven at 110 °C until an ultimate fixed weight was found,

The percentage of total soluble matter (% solubility) was calculated as:-

$WS (\%) = [(W_0 - W_f)/W_0] \times 100$,
where WS is solubility in water; W0 is the weight at the beginning of the bioplastics; and Wf is the final weight of the bioplastic.

1.8.5 Migration test

Migration is the transfer of chemical substances from food contact materials into food. Usually they are checked for : - 1) Overall Migration Limit - sum of all the substances that can migrate from food contact material to the food. It is a measure of the inertness of the material.
2) Specific Migration Limit - applies to an individual substance and is based in toxological studies.

1.8.6 Biodegradability Test

Biodegradability is important for a polymer. It was carried out by keeping the film in open atmosphere and soil. It was kept for 10 days and then the biodegradability was visually monitored.

1.8.7 Temperature resistance

A 10 ×10 cm film was subjected to the heat of microwave oven for a duration of 15 minutes and checked for any deterioration in it's properties. Similarly a film of about the same dimension was exposed to 4? within a refrigerator for 30 minutes. This was done to check for any wear and tear under extreme temperature conditions.

1.8.8 Sealing property of biofilm

To produce a seal in most form/fill/seal machines, bar sealing is the best technique. The sealing pressure, sealing temperature, and dwell time are the parameters in the heat-sealing procedure. The heat sealing was done using the sealing machine at Sagar Polybags Limited, Palakkad, Kerala.

1.8.9 Enhancement of shelf life

To check for it's potential as as additive packaging material (i.e; enhancing the shelf life of food items, fruits and vegetables easily prone to rotting) they were packaged with the starch wrap and subjected for visual inspection.

Result and Discussion

Isolation of Food pathogens

Fig 1 .E.coli in Mac-conkey agar medium and Staphylococcus aureus in Mannitol salt agar medium.

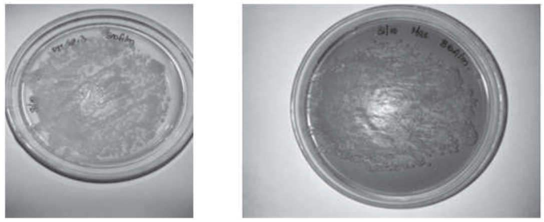


Fig.2 Gram staining

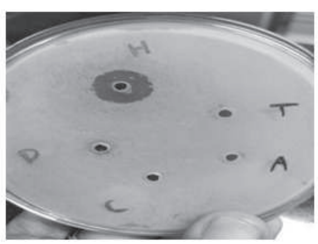
Escherichia.coli
Pink colored Gram negative rods

Staphylococcus auerus
Purple colored Gram positive cocci

2. Bioextract preparation: Ethanolic extract of leaves of Tulsi (Ocimum tenuflorum), Aloe vera (Aloe barbadensis miller) and drumstick (Moriga olifera) and that of flowers of Hibiscus (Hibiscus rosa-sinesis) were prepared after overnight incubation on rotary shaker at 250C

3. Antimicrobial activity of the bio-extracts:

Fig 3 Screening for antimicrobial activity (agar well diffusion assay) of the plant extracts was done and got the zone of inhibition as follows



H-Hibiscus extract -13.2 mm for E.coli 10mm for S. aureus
D-Drumstick leaves extract- 5 mm for E.coli 3 mm for S. aureus
T-Tulsi extract, A-Aloe vera extract and C-Control - no zone of inhibition was observed
Since Hibiscus extract showed greater inhibition, it was selected for further study

4. Determination of MIC and MBC

Tube dilution method

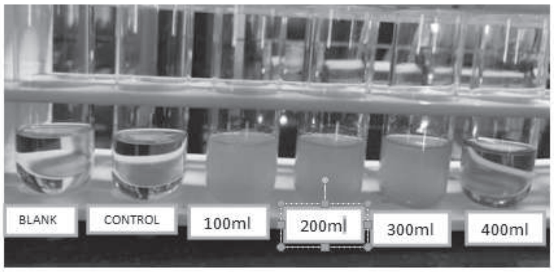


Plate.7 Estimation of Minimum Inhibition Concentration (MIC) of Hibiscus extract

The tube with concentration of 400ml Hibiscus extract showed the maximum rate of inhibition and it was confirmed by inoculating a suspension of it into a culture plate for measuring the MBC and the results were confirmed.

Broth micro dilution assay

Upon serially diluting the content of the well where no resazurin colour change was observed (MIC value) a substantial reduction in the bacterial count was observed as in Fig.4.Plates after 24 h in modified resazurin assay [pink colour indicates growth and blue means inhibition of growth; the test organism was E. coli;
sterility control (test compound in serial dilution + broth + DMSO+ indicator), no bacteria; control without drug (bacteria + broth + indicator);
positive control (antibiotics in serial dilution + broth + indicator + bacteria)
A-D, extract (in serial dilution in wells 1-12 + broth + indicator + bacteria.

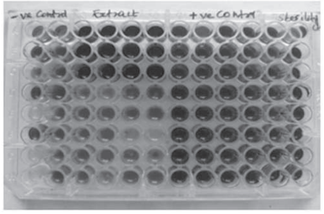


Fig 4. Broth microdilution assay

Other papers have reported the use of different concentrations of resazurin dye, some of which are significantly higher than what has been reported in this study. When these higher resazurin concentrations were tested (data not shown) with an untreated bacterial control, false negative results were observed, which can be attributed to the lack of ability of the bacteria to metabolise resazurin at such high concentrations (Singh et al. 2014).

Determination of MBC.

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a bacterium over a fixed, somewhat extended period, such as 18 hours or 24 hours, under a specific set of conditions.The MBC is identified by determining the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculum by a pre-determined reduction such as ?99.9%. The MBC is complementary to the MIC.Antibacterial agents are usually regarded as bactericidal if the MBC is no more than four times the MIC. MBC testing can be a good and relatively inexpensive tool to simultaneously evaluate multiple antimicrobial agents for potency.

5. Optimized Biofilm Preparation

Biofilm was casted using a varying range of two different plasticiers and was let to dry. Biofilm obtained from ingredients of sample 2was found to be satisfactory as in fig. 5 Then the film was optimized further by trying it at varying concentration as in table. 3

Sample 2 of varying concentration.	Rice water	Sorbitol	Agarose	1% Glycerol	2%Antimicrobial extract.
S2A	100ml	3	1	60ml	5ml
S2B	100ml	2	0.5	50ml	5ml
S2C	100ml	1.5	0.25	40ml	5m
S2D	100ml	1	0.05	30ml	5ml

Table.3 sample 2 with varying concentration.

The film casted with varying concentration of sample 2(fig 5) was visually inspected and in which sample C was found to be much similar to that of conventional plastic. Hence it was subjected to scrutiny by testing for various physical parameters.

Fig.6 Sample 1: Optimization of composition of biofilm formation

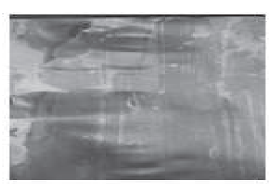
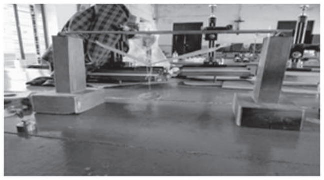


Fig.6 Sample 2: Optimization of composition of biofilm formation

6. Characterisation of Biofilm

Thickness

The thickness of the bioplastic is measured at 10 different places using a thickness gauge, and the average is calculated. The average thickness of the bioplastic is found to be 0.7 mm (700 microns). As per the regulations of the Government of India, the thickness of plastic bags should not be less than 50 microns. The results show that the prepared bioplastic can be used for preparing carry bags. However, several works have been reported on the thickness of starch films. Research by Fakhouri et al. studied the thickness of several starch films made up of potato, rice, wheat, gelatin, and sorghum and found 53 to 63 microns, which is much lower than the present study.



7. Measurement of Tensile strength and Elongation

Water solubility

The initial weight of the sample was recorded.The sample was then placed into a beaker containing 60 mL of water at room temperature for 24 hours. The sample was then taken out from the water and wiped off. .After that, the specimens were dried and the weight taken. And then, we observed the decrease in the weight of the bioplastic material and results were recorded accordingly, each experiment was done in triplicate in order to ensure results. A pre-weighed piece of samples were prepared were taken in the test tube to check the protuberance and other morphological changes. The sample showed the highest water solubility, with a higher ratio of diffusion compared to other bioplastics.

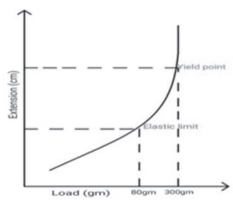
Starchfilm kept in water.	Initial weight Wo (in grams)	Final weight WF(in grams)	Solubility in (%).	Appearance
2 hours	0.301	0.265	11.9%	Visible
6 hours	0.301	0.154	48.8%	Partially
18 hours	0.301	0.032	89.36%	Invisible

Table 4. Water solubility of starchfilm in %.

Tensile strength and elongation

Tensile strength is the maximum stress that can be retained by the film before breaking.Good film should has a tensile strength value that meets the standards. Moderate film, such as biodegradable film, should has a tensile strength 1-10 MPa . The greater tensile strength indicates the stronger the film. Lower value of tensile strength indicate the more fragile the film.

Elongation process is a change in maximum length during stretching until the film broke. Elongation is associated with the average flexibility of the mechanical properties of the film.The tensile strength of the TPS, Young's Modulus, and the elongation of the film at the breakingpoint were found for the sample. The data obtained from this sample was presented in Figure 2.It was found that the biofilm was able to hold a maximum of 300gm for a length of 10x3 cm and above that the film breaks. Therefore the maximum stress attained will be at this point.



Temperature resistance

The temperature resistant of the biofilm was tested at varying temperature. It could withstand extreme temperature ranging from boiling point to freezing point(1'-100'c) from the table. It is observed that the biofilm show no particular change in their mass after keeping at different temperatures. Hence it can be used under microwave condition increasing its applicability in food packaging industry.

	Temperature conditions	Initial weight of biofilm.	Final weight of biofilm	Appearance
Sample 2	4°c (30min)	0.43g	0.40g	Good
	37°c	0.43g	0.43g	Good
	100°c(15min)	0.43g	0.39g	Good

Table.5.Temperature resistance of starch film at various temperatures

Enhancement of shelf life

In fresh food packaging, bioplastics can enhance the product's lifespan compared to using petrochemical plastics.The starchfilm was tested for packaging the food material. The food was fresh when compared to uncovered one and it was proved that additive film was suitable for food packaging and also it shows shelf life of the packed food was able maintain inthe same rate as plastic and hence emhances the shelf life of the food material.

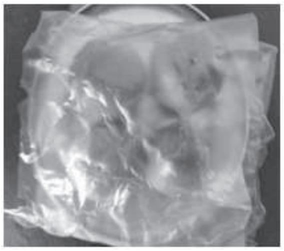


Fig. 7 Estimation of shelf life of vegetables and fruit

Food Packed with:	Appearance	
	3 days	7 days
Biofilm	Good	Good
Plastic	Good	Initial stage of spoilage
Without packing	Good	Rotten

Table 6:Shelf life

Migration rate

The use of food stimulants is an approximation for actual migration in to food .for plastics food contact materials the rules for migration testing are clearly stipulated in the commission regulation EU10/2011.The overall migration limit of a food graded plastics according to EU are (10mg/dm2). The overall migration rate of the bioplastic is 0.92 mg/dm2 , which is considerably less compared to normal plastic . Hence the biofilm is highly safe for packaging the food material .(Fig.8)

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TEST REPORT

ULR - TC648920000007166F
Page 1 of 1
Date : 10/01/2020

Ref H-13201/20
Name & Address of Customer : Dr. Megha P M, Mercy College, Palakkad-678006

Sample Drawn by : The Party
Particulars of the Sample : Bio degradable material
Sample code : H-13201/20
Date of receipt of sample : 03/01/2020
Date of analysis : 03/01/2020-09/01/2020

Sample Quantity Received : 1No.
Type of packing : Plastic Pouch
Condition of sample : Ambient, Sample fit for analysis

CHEMICAL TESTING

PLASTIC & RESINS

Parameters	Unit	Test Method	Result
Overall Migration with n-heptane at 38°C for 30mins	mg/dm^2	IS:9845:1998 :R 2015	0.92

Fig. 8 Test report of Migration rate

Biodegradability Test

A biodegradable test was conducted on the sample. The weight loss indicated the process of biodegradation on the specimenby the soil microbes.

Before Degradation After Degradation

Fig 9. Qualitative estimation of biodegradation of biofilm in soil.

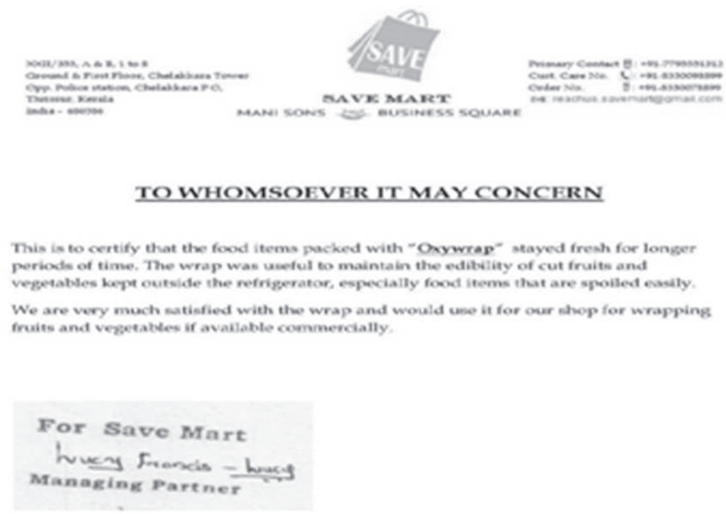
Moisture content

The moisture content of starch is the amount of moisture present in it. The higher the moisture content the lower the amount of dry solids in the flour. Apart from being within acceptable limit, the moisture content recorded in this work was lower than kaffir potato starch of 17.16%, rice starch with moisture content of 6% and maize starch with moisture content of 4% . This indicates a higher solid content and that this starch could stay longer under storage as compared with those other starches (kaffir potato, rice and maize). The low moisture content of starch makes it easy to store at room temperature and less prone to fungal and microorganism infections.

2.8.9 Sealing property of bioplastic

It is observed that no single temperature is accepted for the heat-sealing process. This may occur due to the reason that the plastic could be sealed at moderately molten or melting conditions. The results indicated that the prepared bioplastic samples have good sealing capabilities. The sealed sample seems have excellent sealing properties.

Fig10. Durable food packages made out of biofilm.



SAVE MART

MANI SONS BUSINESS SQUARE

TO WHOMSOEVER IT MAY CONCERN

This is to certify that the food items packed with "Oxywrap" stayed fresh for longer periods of time. The wrap was useful to maintain the edibility of cut fruits and vegetables kept outside the refrigerator, especially food items that are spoiled easily.

We are very much satisfied with the wrap and would use it for our shop for wrapping fruits and vegetables if available commercially.

For Save Mart

Managing Partner

Fig 11. Review report

Summary and Conclusion

With the creation of starch based from bioplastics from rice starch, the potential ofstarch has been completely utilized in the creation of an ecofriendly alternative tothe conventional plastics. The availability of raw material is of little concern asrice is the staple diet of Keralites. The overall strength of the formed bioplastic wasenhanced with the addition of plasticizers which in turn was selected as a result ofintense research over food grade organic substances. As food materials are easilyprone to the attack of food born pathogens, measures were taken to combat themwith the addition of antimicrobial substances derived from plant sources. The plantmaterial chosen was also subjected to scrutiny to check for it's potential. Bilofilmwas casted using appropriate concentration of the plasticizers. The film formedwas found to be satisfactory after checking for various parameters such as 7 micronthickness, tensile strength of 10MPa, no migration of components to the food withwhich is being wrapped as well as quick degradability on soil within 4 weeks,ability to get thermally sealed proves it to be effective against conventional plastic bags. Therefore with theintense exploitation of the potentiality of starch, this research wasfulfilled that resulted in the creation of an effective alternative to conventionalplastic bags.

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10. Minimum Bacterial concentration assay (Akinyemi et al., 2005, Pavithra et al., 2009).
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