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Production of Rice Bran Wax based Biodegradable Film

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***Abstract*:** Biodegradable films produced from plant source with binding agents to reduce the negative effects of non-degradable waste materials on the environment and human health. An attempt was made to prepare films by using corn starch, rice starch and rice bran wax solutions. Glycerol was added as plasticizer. The study was conducted to compare the cornstarch and rice starch films prepared with and without addition of rice bran wax. Film thickness of the treatments increased with the increase in binding agent. Highest value of thickness was observed for treatment, CS-10 g + glycerol (5 ml) as 1.21 mm. Similar trends was observed for thickness for the films prepared with rice starch. Moisture content of the films without addition of rice bran wax was higher than that of treatments added with rice bran wax. The transparency values for both corn starch and rice starch samples with and without addition of rice bran wax were reasonably less. The folding endurance test indicated that maximum number of double folds were 0.60. Biodegradability test reported that the films prepared using rice starch with addition of rice bran wax degraded faster than corn starch films with addition of rice bran wax.

***Keywords*:** Rice bran wax**,** Corn starch, Rice starch, Biodegradable film

Most degradable plastics are made from polyethylene and contain an additive to speed up the decomposition. Depending on the composition and thickness of the material, some biodegradable plastics disintegrate quickly, whilst others take longer (Le 2020). Though many types of plastic films are available for packaging, very few with certain level of gas permeability are suitable for MAP and CAP for storage of fruits and vegetables (Gopalaswamy et al 2016). Starch is an agricultural and biodegradable feed stock biopolymer in a variety of plants such as wheat, corn, rice and potato. The starch granules consist of amylose and branching points of amylopectin molecules. A semi-crystalline granule of starch is converted into a homogeneous material with hydrogen bond broken between the starch molecules. This process is called gelatinization and it leads to loss of both crystallinity and double helices (Talja et al 2007). Starch-starch interactions are replaced by starch plasticizer interactions to strengthen the linkages in bonds.

Lipid biopolymer based coatings include waxes or long chain fatty acids and they are the most efficient compounds to reduce moisture permeability due to their high Hydrophobicity. This characteristic is justified by their high content in esters of long-chain fatty alcohols and acids along with long-chain alkanes (Khwaldia et al 2010). Glycerol is a molecule that has ability to attract water. Addition of glycerol to the starch mixture increases holding capacity of water in the starch chain making it less crystalline and thus less brittle (Le 2020). Waxes are the esters of long chain carboxylic acid and long chain alcohol (Kolattukudy 1976). Wax content in rice bran oil may vary with oil extraction conditions such as the source of the rice bran, solvent used for extraction, temperature at which extraction occurs. These waxes can be used for preparation of films. The formation of wax-in-water emulsion is enhanced by the addition of arabic gum, which serves as an emulsifier (Kim and Ustunol 2001). The added rice bran wax acts as a function modifying agent for improved water vapour barrier properties of the composite films. In view of these, the project was undertaken to produce a biodegradable film from plant source with binding agents to reduce the negative effects of non-degradable waste materials on the environment and human health with the following objectives, to prepare film solutions from plant based materials in combination with rice bran wax, to produce a biodegradable film with plant based material with binding agents, and to analyze the physico-chemical properties of the films.

MATERIAL AND METHODS

Corn starch, rice starch glycerol was collected from the local shop in Bodhan, Nizamabad dist, Telangana state. Rice bran wax was collected from an oil refinery located at Vijayawada, Andhra Pradesh state. Glassware was used for casting of films.

Preparation of film solutions from the rice starch (RS) and corn starch (CS): The rice starch and cornstarch solutions were prepared for casting of films. The rice starch and corn starch solutions with and without addition of rice bran wax were studied to analyse barrier properties.

Different combinations of films were shown in Table 1. The procedure for preparation of film solutions was explained in Figure 1 and 2.

Preparation of film solutions using rice starch with and without addition of rice bran wax: Rice starch solutions were prepared by dissolving 2, 5 and 10g of rice starch in 100 ml, 250 ml, and 500 ml of distilled water. Glycerol was added as plasticizer. The solutions were made to gelatinize at 85˚C for 30 min and mixed thoroughly. The solutions were poured through sieve to avoid colloidal formations in the solution and subsequently cooled to ambient temperature. After preparation of rice starch solutions, 2g rice bran wax was added to some of the samples to analyse barrier properties.

Preparation of film solutions using corn starch with and without addition of rice bran wax: Similarly, corn starch solutions were prepared by addition of 2, 5 and 10 g of corn starch 100, 250, 500ml of distilled water (Fig. 1 and 2). Glycerol (1, 2.5, 5 ml) was also added. The solutions were made to gelatinize at 85˚C for 30 min and mixed thoroughly. 2g of rice bran wax added to samples to analyse the barrier properties.

Preparation of casting films from rice starch and corn starch film solutions: The rice starch and corn starch film solutions with and without addition of rice bran wax were casted by pouring the solutions into Petri dishes. The films casted with different compositions were shown in plate 3.3. A sample of 50 ml was poured in each petri dish uniformly and dried at 55 °C for 10 hours in hot air oven. After drying, the films were peeled gently and stored in desiccators.

Physico-chemical Analysis

Film thickness (mm): Film thickness was measured by the procedure given by Brodnjak et al (2018) at 5 random locations on each film.

Moisture content (%): Moisture content of the films was determined by assessing the weight loss upon drying. Initial weight of the films was taken, and then the films were dried at 105°C for 24 h in a laboratory oven (Nordin et al 2020). Then, the dried films were weighed to obtain the final weight**.**

Solubility test: Solubility test determines the ability of dissolution of the sample. In this work, solubility of film was analysed using ethanol, chloroform, diethyl ether and distilled water till five days.

Folding endurance: The folding endurance was calculated as (Sharmila et al 2021).

$$F = \log_{10} d$$

Where F=Folding endurance, D=Number of double folding

Transparency (%mm^{-1}): The transparency of film was recorded at 600nm by cutting the films into a rectangular piece (15 × 50 mm) and placed inside a UV-Vis spectrophotometer.

Transparency (%mm^{-1})= $\frac{-\log T_{600}}{x}$, Where T600 is light transmission at 600 nm

Biodegradability in soil: Biodegradation was tested in soil

Take distilled water of known volume (eg: 100ml)
↓
Add starch powder (eg:2%w/v)
↓
Add the glycerol (eg:1ml)
↓
Add vinegar (eg:1ml)
↓
Gelatinize the mixture at 85˚C for 30min
↓
Pour 50ml of sample in petridish
↓
Dry the mixture in hot air oven at 55˚C for 10h
↓
Gently peel the film and store in desiccator

Fig. 1. Flow chart for preparation of films without addition of rice bran wax

Take distilled water of known volume
↓
Add starch powder (eg:2%w/v)
↓
Add vinegar (eg:1ml)
↓
Add 2g of rice bran wax and 0.35% of Arabic gum
↓
Gelatinize the mixture at 85˚C for 30min
↓
Pour 50ml of sample in Petri dishes
↓
Dry the mixture in hot air oven at 55˚C for 10h
↓
Gently peel the film and store in desiccator

Fig. 2. Flow chart for preparation of films with addition of rice bran wax

Table 1. Different composition of films

Treatment	Binding agent (g)	Plasticizer (ml)
Without addition of rice bran wax		
T1	CS–2	1
T2	CS-5	2.5
T3	CS-10	5
T4	RS-2	1
T5	RS-5	2.5
T6	RS-10	5
With addition of Rice bran wax		
T7	CS–2	1
T8	CS-5	2.5
T9	CS-10	5
T10	RS-2	1
T11	RS-5	2.5
T12	RS-10	5

*0.35%w/v Arabic gum was added to all samples added with rice bran wax under controlled laboratory conditions (25˚C, Relative humidity 25% and pH=7). The film samples were placed in plastic container filled with soil.

RESULTS AND DISCUSSION

Thickness (mm): The highest thickness was observed for T_3; CS-10g + glycerol (5ml) as 1.21 mm. The lowest f thickness in T_1; CS-2g + glycerol (1ml) as 0.50 mm. Similar trend was observed for thickness for the films prepared with rice starch. The thickness increased as the quantity of binding agent increased. Similar results was obtained by Brodnjak et al (2018) while preparation of films. The thickness of corn starch and rice starch films prepared with addition of rice bran wax was measured (Fig. 2). Among all the treatments, highest value of thickness was observed for T_{12}; RS-10g+glycerol (5ml)+rice bran wax (2g) as 1.78 mm. The lowest thickness observed for T_{10}; RS -2 g+ glycerol (1ml) + rice bran wax (2g) as 1.02 mm. In all the treatments, the thickness increased upon increase in binding agent. The amylose content in corn starch is high compared to rice

Table 3. Solubility test in distilled water for different composition of films with and without addition of rice bran wax

Solvent (Distilled water)	Cornstarch + glycerol (T_1)	Rice starch + glycerol(T_4)	Cornstarch + glycerol + rice bran wax (T_7)	Rice starch + glycerol + rice bran wa (T_{10})
Day 1	Insoluble	Insoluble	Insoluble	Insoluble
Day 2	Insoluble	Insoluble	Insoluble	Insoluble
Day 3	Insoluble	Insoluble	Insoluble	Insoluble
Day 4	Partially soluble	Partially soluble	Partially soluble	Partially soluble
Day 5	Partially soluble	Partially soluble	Partially soluble	Partially soluble

Table 4 . Solubility test in chloroform for different composition of films with and without addition of rice bran wax

Solvent (chloroform)	Corn starch + glycerol (T_1)	Rice starch + glycerol (T_4)	Corn starch + glycerol + rice bran wax (T_7)	Rice starch+glycerol +rice bran wax (T_{10})
Day 1	Insoluble	Insoluble	Insoluble	Insoluble
Day 2	Insoluble	Insoluble	Insoluble	Insoluble
Day 3	Insoluble	Insoluble	Insoluble	Insoluble
Day 4	Partially soluble	Partially soluble	Partially soluble	Partially soluble
Day 5	Partially soluble	Partially soluble	Partially soluble	Partially soluble

Table 5. Solubility test in ethanol for different composition of films with and without addition of rice bran wax

Solvent (ethanol)	Cornstarch + glycerol (T_1)	Rice starch+ glycerol (T_4)	Corn starch +glycerol+ rice bran wax (T_7)	Rice starch+ glycerol +rice bran wax (T_{10})
Day 1	Insoluble	Insoluble	Insoluble	Insoluble
Day 2	Insoluble	Insoluble	Insoluble	Insoluble
Day 3	Insoluble	Insoluble	Insoluble	Insoluble
Day 4	Partially soluble	Partially soluble	Partially soluble	Partially soluble
Day 5	Partially soluble	Partially soluble	Partially soluble	Partially soluble

Table 6. Solubility test in diethyl ether for different composition of films with and without addition of rice bran wax

Solvent (diethyl ether)	Cornstarch + glycerol (T_1)	Rice starch+ glycerol (T_4)	Corn starch +glycerol+ rice bran wax (T_7)	Rice starch+ glycerol +rice bran wax (T_{10})
Day 1	Insoluble	Insoluble	Insoluble	Insoluble
Day 2	Insoluble	Insoluble	Insoluble	Insoluble
Day 3	Insoluble	Insoluble	Insoluble	Insoluble
Day 4	Partially soluble	Partially soluble	Partially soluble	Partially soluble
Day 5	Partially soluble	Partially soluble	Partially soluble	Partially soluble

starch, which may have accounted for strong, close binding among the corn starch molecules that led to decrease in thickness of films (Ali et al 2014). The addition of plasticizers also led to the increase in thickness of films (Nordin et al 2020). Higher values of thickness were recorded for treatments with addition of rice bran wax than the treatments without addition of rice bran wax. Regardless of rice bran wax content, the corn starch and rice starch films with the addition of rice bran wax were translucent.

Moisture content (%): Moisture content of the corn starch and rice starch film prepared without addition of rice bran wax is evaluated (Fig. 3). The highest moisture content was observed for treatment T_6; RS-10 g + glycerol (5ml) as 11.4% and lowest was for $T_{1;}$ CS-2g + glycerol (1 ml) as 9.5%. Similar results was obtained by Brodnjak et al (2018) while preparation of chitosan and starch films. The lower moisture content which is desirable is because of the presence of rice starch and corn starch in the films. These starch molecules form highly cross-linked systems, preventing water molecules from penetrating into composite films (Thakur et al 2016). The moisture content values of the films with addition of rice bran wax were presented in Figure 4. The highest moisture content was observed for T_9; CS-10 g + glycerol (5ml)+ 2 g rice bran wax as 10 % (Fig. 4). The lowest moisture content was in $T_{7;}$ CS-2g+glycerol (5ml)+ 2g rice bran wax as 9%. Similar results was obtained while preparation of films by Brodnjak et al (2018). The moisture content of the films without addition of rice bran wax was higher than of added rice bran wax. Hence, the added rice bran wax is a function modifying agent for improved moisture absorption of the composite films (Abhirami 2019).

Transparency (% mm^{-1}): The transparency of the films was higher in T_3; rice starch (10 g)+ glycerol (5ml) as 1.3 % mm^{-1} and the lower value for T_4; corn starch (2 g) + glycerol (1ml) + rice bran wax (2g) as 1.2%mm^{-1} (Fig. 4). The transparency values for both corn starch and rice starch samples were reasonably less. Xu et al (2005) worked on starch films with grape pomace extract, emphasized that opacity is directly related to the degree of film homogeneity. In present study, the presence of glycerol in CS-glycerol and RS-Glycerol film may block or lower the intensity of the scattering light passing through the film resulting in higher opacity values. The corn starch and rice starch samples with addition of rice bran wax shown similar trend of decrease in transparency as compared to samples without addition of rice bran wax (2g). Although rice bran wax (2g) reduced the film transparency, this could be advantageous for food packaging, especially for foods that are light-sensitive since it provides barrier against UV.

Solubility test: Test was conducted for five days by

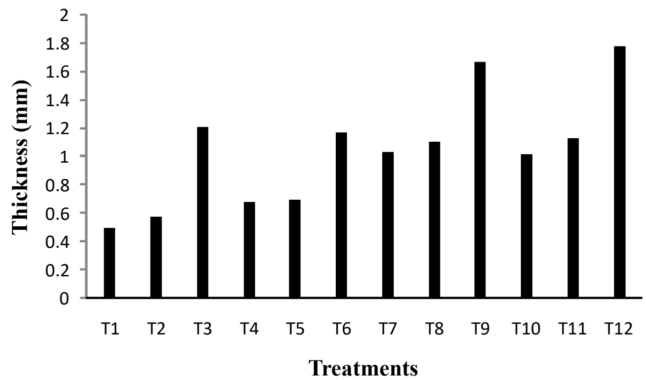

Fig. 1. Changes in thickness of corn starch and rice starch films prepared without addition of rice bran wax

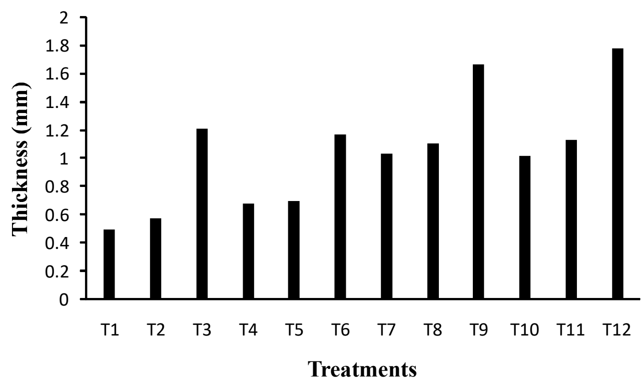

Fig. 3. Changes in moisture content of corn starch and rice starch films with and without addition of rice bran wax

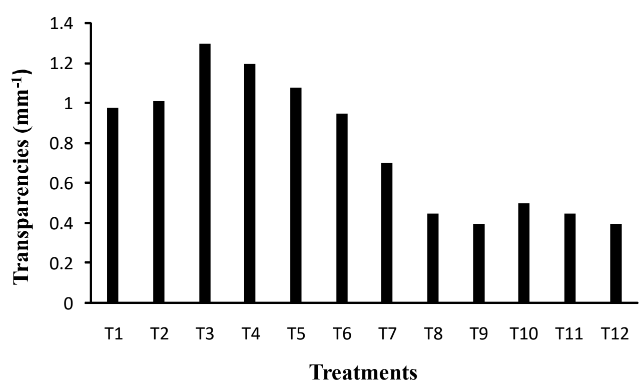

Fig. 4. Changes in transparency (%mm^{-1}) of corn starch and rice starch films without addition of rice bran wax

Table 7. Folding endurance for different composition of films with and without addition of rice bran wax

Treatments	No. of double folds	Endurance
Cornstarch + glycerol (T_3)	4	0.60
Rice starch + glycerol (T_6)	4	0.60
Corn starch + glycerol +rice bran wax (T_9)	3	0.47
Rice Starch + glycerol +rice bran wax (T_{12})	4	0.60

dissolving films in distilled water, diethyl ether, ethanol, and chloroform (Table 3). $T_{1;}$ Corn starch (2g) + glycerol (1ml), T_4; Rice starch (2g)+glycerol (1ml), T_7; Corn starch (2g)+glycerol (1ml)+rice bran wax (2g), T_{10}**;** Rice starch (2g)+glycerol (1ml)+rice bran wax (2g) was dissolved in chloroform for five days. Similarly, all the treatments are insoluble for three days and partially soluble on day-4and day-5 in chloroform. These exhibited no change for 5 days in ethanol and di-ethyl ether. All the films were insoluble for first three days in all solvents. Due to hydrophilic nature of the plasticizer and binding agent, the films were partially soluble in water. The result confirms that the films were easily and naturally degradable by water.

Folding endurance: Maximum number of double folds is for T_3;corn starch(10g) + glycerol (5ml), $T_{6;}$ rice starch (10g) + glycerol (5ml), $T_{12;}$ rice starch (10g) + glycerol (5ml)+rice bran wax (2g) were recorded as 0.60. The minimum folding endurance was reported for $T_{9;}$ Corn starch(10g) +glycerol(5ml)+rice bran wax (2g) as0.47. Slight increase in relative humidity causes decrease in folding endurance. It may be due to an increase in ductility (Sharmila et al 2021).

Biodegradability: The film prepared using rice starch with addition of rice bran wax degraded faster than corn starch film with addition of rice bran wax. The surface of samples included cracks, holes and colour changes. Both films with rice bran wax were completely degraded after 15days in soil.

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