Isolation and study of Starch and Starch composite Film from source Eleusine coracana

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ABSTRACT

Starch was extracted from the seeds of Ragi (*Eleusine coracana)*. Starch was characterized by Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimeter (DSC), Scanning Electron Microscopy (SEM), and X-ray Diffraction (XRD). Starches, Starch -glycerol, Starch- glycerol-PVA composite film were prepared. The surface morphology was checked by SEM. Biodegradation of starch and starch composite film was studied by enzymatic hydrolysis also by soil degradation, which shows within 8 days the starch film was degraded completely.

Keywords: Biodegradation, Starch based biopolymer, Enzymatic degradation, Composting

I. INTRODUCTION

Starch is the second most abundant natural polymer on earth. It is a renewable, inexpensive and widely available resource [1]. Starch has received considerable attention because of its totally biodegradable nature. Starch has a wide range of industrial applications .These include food, textile, and pharmaceutical, paper as well as in synthetic polymer industries [2]. Starch also plays a prominent role in technology development. The modification of starch can be done by physical and chemical means as well as enzymatically. Amongst naturally occurring biopolymers, starches have the greatest potential to produce biodegradable films by different processing techniques such as casting, injection or blow molding and so on [3]. Several studies have reported the use of starches from different sources as a raw material for forming films and coating with different properties showing the potential of this carbohydrate in different industrial applications [4]. In food industry the use of biodegradable starch based films is continuously increasing [5].

Biodegradation is the process by which organic substances are broken down by microorganism aerobically or anaerobically. Microorganisms such as bacteria and fungi are involved in the degradation of natural as well as synthetic polymers. Generally, an increase in the molecular weight of polymer results in a decline in its rate of degradation. Plastic material, being non-biodegradable is a significant source of environmental pollution [6].

II. METHODS AND MALERIALS

2.1 Materials

Eleusine coracana and seeds were procured from the local market, Pune, India, for isolation of starch respectively. Glycerol (99.5%purity) methanol were obtained from Merck chemicals Pvt.Ltd. Mumbai. Polyvinyl alcohol (with Mol. wt. 1.0×10^5 and degree of hydrolysis 98%) and toluene were obtained from Himedia Chemicals Pvt. Ltd. Mumbai.

2.2 Isolation of starch

Starch was isolated from ragi seeds (Eleusine coracana) essentially as described by Adkins and Green wood (1966) with slight modifications. 100g E. coracana seeds were soaked in water for 10 h followed by washing of the steeped material with water to remove the seed coat and then mixed with 500ml water. The mixture was homogenized in a blender to form slurry

which was sieved through a sieve of 85 micron opening. The residue was again dispersed in water, and the above process repeated. The slurry containing starch was pooled and centrifuged, at 10, 000 g for 10 minutes and then washed with mild alkali (0.1 N) saline (0.145M) and finally toluene to remove the contaminating proteins and pigments. Finally the isolated starch was air dried and preserved for further use at Room temperature (R.T) [7].

2.3 Characterization of E. coracana starch
2.3.1 Total reducing sugar content
Total reducing sugar content after mild hydrolysis was determined by Dintro salicylic acid (DNSA) method using maltose as standard [8].

2.3.2 Differential Scanning calorimeter
The gelatinization temperature and enthalpy of starch was determined by using Differential Scanning Calorimeter (Perkin Elmer). Native starch sample (1 mg) was hermetically sealed in a large volume high pressure aluminum DSC pan, equilibrated at room temperature for about 1 h prior to experiment and scanned in the differential scanning calorimeter to record the calorigram. The instrument was calibrated using Indium as standard material and was programmed to rise 10 °C/min. The thermal transition of starch in terms of temperature of onset (T_o), peak temperature (T_p) and endset temperature (T_e) were recorded from calorigrams automatically. Based on the area of triangle of the calorigram, the ΔH (enthalpy) associated with gelatinization of starch was calculated [9].

2.3.3 Fourier Transform Infrared (FTIR)
FTIR spectrum of starch was recorded using Tensor 37 spectrometer between 4000 to 400 cm^{-1} (16 times per sample) with a resolution of 4.The spectrum was Fourier transformed and recorded in absorption mode, single beam spectra of the sample were obtained and corrected against the background spectrum of sample holder. IR solution software was employed for getting the spectrum.

2.3.4 Scanning Electron Microscopy (SEM)
For scanning electron microscopy, the samples were fixed on aluminum stub using double adhesive carbon tape and to make the sample conductive a thin layer of platinum (4-5 nm) was vacuum deposited on it in sputter coater (Quarum technologies).Samples were visualized for surface and cross section morphology with field emission scanning electron microscope (FESEM –Nova nanosem 450, FEI Netherland). All samples were examined using accelerating voltage of 15kv.

2.3.5 X- ray diffraction (XRD) study of E.coracana starch:
Isolated E. coracana starch was examined by X-ray diffraction to check its crystallinity, preferred orientation and average crystalline size. XRD was performed using an X-ray diffractometer (Philips PW1710, Holland) with CuKα radiation λ= 1.5405Å over wide range of Bragg angle 10-90°C, for identification of different phases. XRD analysis gives inter- planer spacing "d" (which is calculated using the Bragg law nλ =2d sinθ, where, λ is the wavelength of CuKα radiation, n is the order of diffraction and θ is the angle between the incident beam and the diffraction plane) [10,11].

2.4 Preparation of starch composite films
2.4.1 Starch film
E.coracana starch was processed into a biodegradable film as follows. An aqueous solution of starch (5%, 40 ml) was heated at 90° C under stirring for 30 minutes to get a viscous solution. The hot solution was then poured in a polystyrene petri dish and air dried at room temperature (25 ± 3°C) for 24 h to form film. The film thus obtained was carefully removed from the petri plate and used for further studies.

2.4.2 Starch Glycerol film
Starch glycerol films were prepared as follows: To 39.2 ml starch solution (5% w/v), 0.8 ml glycerol (2% v/v) was added. The hot homogeneous mixture was then spread over the polystyrene petri dish and air dried at

room temperature (25 ± 3°C) for 24 h to form film. Glycerol was used as a plasticizer.

2.4.3 Starch Glycerol Polyvinyl alcohol film

Starch glycerol polyvinyl alcohol (PVA) film was prepared as follows: 2 g PVA was dissolved in 10ml of water at 80-90 °C. To this E. coracana starch solution (2g in 20 ml distilled water) was added and the mixture heated at 80°C- 90°C. Then to the above mixture 0.8ml glycerol (2%) and 9.2 ml of distilled water were added to have a final volume 40 ml. The above slurry thus obtained containing 5% starch and 5 % PVA was stirred for 1 h at R.T .The hot homogeneous mixture was then spread over polystyrene petri dish and air dried at R.T (25 ± 3°C) for 24 h to form film. The film thus obtained was carefully removed from the petri plate and used for further studies.

2.5 Enzymatic hydrolysis of films

The biodegradation of the above film was evaluated by performing enzymatic hydrolysis using the enzymes α amylase (pancreatic) , glucoamylase (Rhizopus) β amylase (Barley) and fungal amylase (Aspergillus niger) at 37°C as follows:1x1 cm film (70 mg)was incubated with enzyme solution (0.2 mg/ml ,1 ml) and 1ml phosphate buffer pH (7.0, 10 mM)for different time intervals (0,2,4,6,8,12,24,48,72,96 h)After incubation, the reaction was terminated and the reducing sugar content determined by DNSA method [8]. Corresponding controls without enzymes were run simultaneously.

2.6 Soil degradation by soil burial

The biodegradation of the above film was also evaluated by soil burial for different time intervals upto 60 days at R.T .For this, film (2.6g) was cut into pieces and the pieces were buried under 400 gm of a 1:1mixture of   soil and compost containing organic matter for different time intervals. The pH of compost was fixed within range of 7.0-8.0. From time to time 20ml tap water was added to maintain humidity to

enable aerobic conditions. After incubation, the films were washed, air dried and weighed to check the biodegradation of films [12] .The weight loss was calculated as follows:

$$\text{Weight Loss (\%)} = \frac{Wi - Wd}{Wi} \times 100$$

(Equation 1)

Wd= Dry weight of film after washing with distilled water

Wi= Initial weight of film.

III RESULTS AND DISCUSSION

In the present work starch was isolated from the seeds of Eleusine coracana followed by its characterization and evaluation of its efficacy as an inert biodegradable support for immobilization of peroxidase.

3.1 Characterization of Starch

Starch was isolated from the seeds of E. coracana. The yield of starch was 49g / 100g seeds. The starch was characterized by DSC, FTIR spectra , FESEM and XRD.

3.1.1 DSC studies: DSC provides a quantitative measurement of enthalpy (Δ H) and facilitates measuring the energy required for gelatinization of starch. [13]. Table 1 shows the thermal properties of E. coracana starch.

Table 1. Thermal properties of E. coracana starch.

S. No	Parameter	Result
1	Onset temperature(T_o)°C	48.41
2	Peak temperature(T_{op})°C	84.47
3	End set temperature(T_e)°C	131.23
4	Gelatinization temperature(T_e-T_o)°C	82.82
5	Heat Enthalpy(ΔH)(kJ/g)	79.43

3.1.2 FTIR

In the IR spectra of the ragi starch (Fig.1) the peaks obtained at low wave numbers correspond to the skeletal mode vibrations of the glucose pyranose ring, whereas the band at 929cm^{-1} was due to the glycosidic linkages in starch. The vibrational band related to the C and H atom was observed between1500–1300cm^{-1}. Water adsorbed in the amorphous region of starch was identified as a broad band at 1640 cm^{-1} while the C-H and O-H stretching mode showed bands between 2800-3000 cm^{-1} and 3000-3600 cm^{-1} respectively [14].

Figure 1. FTIR spectra of starch

3.1.3 Scanning Electron Microscopic studies

Figure 2 shows the scanning electron micrograph of native starch As seen in the micrograph, most of the native starch granules were of polygonal and irregular shape with sizes between 3 -7 μm [13].
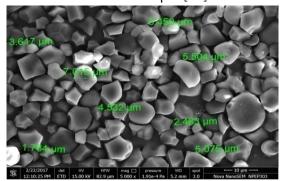

Figure 2. SEM of starch.

3.1.4 XRD studies:

Scattering angle 2θ at which diffraction intensity was observed and the spacing was used to discriminate the plane of different sites on ragi starch granules. X ray diffractogram of native starch showed a typical A type of diffraction pattern with strong reflection at15& 23°C & degree of crystallinity of native starch was 30.09% [13].
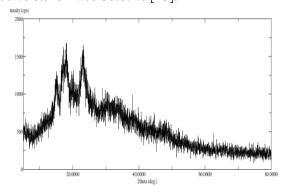

Figure 3. XRD of starch.

3.2 Biodegradation of Starch and starch composite films

All the films were degraded by different enzyme amylases within 24 h. After 24 h there was no change in degradation pattern.As seen in figure 4, the degradation of starch and starch glycerol films was more as compared to the starch glycerol PVA films (15).
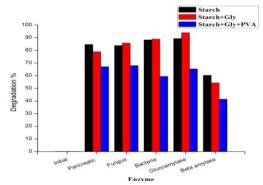

Figure 4. Degradation of starch and composite film by DNSA for 24 h.

3.3 Soil degradation

The weight loss rate during the soil burial compost tests of all starch based films was higher than the weight loss rate of the cellulose film used as a control. Most of the degradation was observed within the first 8 days due to composting (Table 2). It has been suggested that composting leads to an increase in the temperature which results in rapid microbial activity. As compost is rich in organic matter aerobic degradation is observed. Overall the degradation of starch, starch glycerol film was faster compared to starch -glycerol-PVOH composite film. The highest

percentage of weight loss was observed for ragi starch film and starch glycerol composite film within 8 days, whereas the lowest percentage of weight loss was observed for starch glycerol polyvinyl alcohol within 60 days (15).
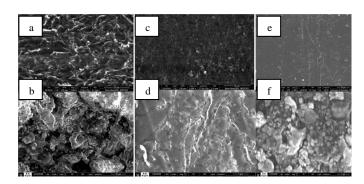

Figure 5. SEM images of a.Starch film(Before degradation) b.Starch film(After degradation) c. Starch glycerol film(Before degradation) d. Starch –glycerol film (After degradation) e. Starch glycerol PVA film(before degradation) f. Starch glycerol PVA film(after degradation).

Figure 6. images of a.Starch film(Before degradation) b.Starch film(After degradation) c. Starch glycerol film(Before degradation) d. Starch –glycerol film (After degradation) e. Starch glycerol PVA film(before degradation) f. Starch glycerol PVA film(after degradation).
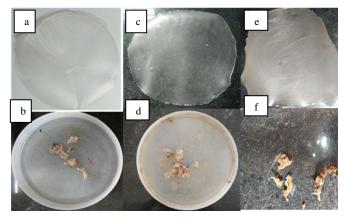
Tabel 2. Degradation of different starch and starch composite film in soil.

Film	Degradation %(w/w)	No of days
Starch (5 %)	74.7%	8
Starch glycerol (5%,2%)	85.4%	8
Starch gly PVA composite (5%,2%, 5%)	93.23%	60

IV. CONCLUSION

New processing techniques and the current demands of biodegradable and renewable resources have highlighted the versatility of starch and introduced it to new markets. Today starch has moved from its traditional role as food to being an indespersible safe, effective tool in industry, food and medicine, due to its adhesive thickening, gelling, swelling and film forming properties.

In the present work, starch from *E. coracana* seeds has been isolated and some of its properties have been studied. Biodegradable Starch, Starch glycerol, starch glycerol films were prepared and their biodegration were checked by Enzymatic degradation and soil buried, Compost environment is the ideal environment for degradation due to microbial environment and their diverse enzyme. Enzymatic method of is rapid method to check biodegradation.

V. REFERENCES

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