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Extraction and Characterization of Starch from Tubers and its Application as Bioplastic

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ABSTRACT

Introduction: *Biodegradable plastics manufacture is needed to replace conventional plastics.* ***Aims:*** *Potato starch and its alternative sources such as sweet potato and tapioca have been studied for their potential use in making bioplastics.* ***Methods and Material:*** *Starch was extracted and characterized by microscopic, physicochemical, and spectroscopic methods. Starch, along with other ingredients, was used to prepare bioplastics which were assessed for mechanical properties also biodegradability in soil and water.* ***Statistical analysis used:*** *One Way ANOVA test was used.* ***Results:*** *Tapioca and sweet potato starch had smaller granule size than potato starch. Sweet potato starch showed highest blue value indicating higher amylose content, followed by potato. Higher amylose content and smaller granule size are desirable in preparation of bioplastics. Sweet potato and potato starch, which were completely soluble in water within 2 hours and 91% to 96% biodegraded in soil within one month, were used to prepare bioplastics. Bioplastic with higher carboxymethyl cellulose content had the highest tensile strength of 8.38 MPa and maximum peak load of 2.4 kg. These bioplastics had 210 microns thickness.* ***Conclusions:*** *Sweet potato starch had good yield, smaller granule size and high amylose content. Addition of carboxymethyl cellulose in preparation of bioplastic improved its tensile strength and maximum peak load. These bioplastics can be replaced by the current plastic bags as they were completely biodegradable in soil and water. They can be safely used as bin bags or carry bags wherein they can carry load up to 2.4 kg.*

Keywords: *Biodegradability, Bioplastic, Starch, Sweet Potato, Potato, Tensile Strength, Carboxy Methyl Cellulose*

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1. INTRODUCTION

Biodegradable plastics can replace conventional plastics and have the ability to get decomposed after disposal to the environment (Tharanathan 2003). They can be made from agricultural wastes (Emadian et al. 2017). Wahyuningtiyas et al. (2017) made bioplastics using cassava starch. Marichelvam et al. (2019) investigated application of corn and rice starch-based bioplastics for packaging. Ghanbarzadeh et al. (2011) prepared corn starch films with citric acid (CA) and carboxymethyl cellulose (CMC). Higher tensile strength was obtained with higher percentage of CMC. Galindez et al. (2019) found that high amylose content was helpful in biofilm formation. Lopez et al. (2019) worked with wheat and corn starch films and concluded that starch with high amylose content has bigger crystalline domains, giving greater mechanical resistance for the films. To enhance the mechanical properties and water barrier properties, starch can be mixed with other polymers such as PLA, PBAT, PBS, PCL, and PHAs (Gadhave et al. 2018). These preparations showed great tensile strength but had high cost (Siakeng et al. 2019). Haider et al. (2018) concluded that polylactic acid is non-degradable in seawater and that biodegradation tests carried out in artificial environments can seldom be transferred to real conditions and, therefore they highlighted the necessity of testing conditions to be environmentally authentic and relevant. Indoor environments have essential roles in human health (Al-Rejaboo et al. 2019; AL-Kattan et al. 2019; Choudhary et al. 2019; Munir et al. 2018).

Thus, there is yet to be developed a bioplastic to be able to replace conventional plastic, which is truly biodegradable and not blended with other polymers. As corn and rice starch have been already used in earlier reports, it was imperative that newer sources of starch for making bioplastics be researched.

The aim of this study was to extract starch from potato, sweet potato and tapioca, to characterize extracted starch for granule shape and size by microscopic studies and to detect amylose content by finding Blue Value. Based on these observations it was our further aim to prepare bioplastics of starch along with few other ingredients. Finally, to test the prepared bioplastic films for their maximum peak load carrying capacity and tensile strength, as also for their thickness, moisture content, solubility and biodegradability in soil and water. The main objective of the study was to be able to arrive at a suitable preparation for a bioplastic which can replace the currently used plastic bags and be 100% biodegradable.

2. MATERIALS AND METHODS:

Sources used for extraction of starch were tubers; potato *(Solanum tuberosum* (L)), sweet potato (*Ipomoea batatas* (L) Lam.), and tapioca (*Manihot esculenta)*.

Extraction of starch: 50g of the sample was ground finely in mortar and pestle, suspended in 100 ml of saline solution (0.145M NaCl), and stirred mechanically as well as with the help of a magnetic stirrer. The extract was filtered through muslin cloth. This filtrate was allowed to stand for sedimentation of starch to occur. After decantation, the sediment was dried at room temperature. The residue was re-extracted with same volume of saline as before. The extract was filtered, and this process was continued till all of the starch was extracted (till the residue tested with iodine was negative for starch). The filtrates were pooled and then allowed to stand. The filtrates were then centrifuged at 5000rpm for 10 minutes. The extracted starch was dried. The dry residue was weighed and % yield was calculated (Surendra Babu and Parimalavalli 2014, Moorthy and Nair 1989).

Characterization of starch: Shape and size of starch granule was studied by preparing slides stained with dilute Iodine solution and observing under microscope (45 X).
Loss on drying was found by keeping 1g starch sample in oven at 100º C for 3 hrs and then weighing the starch again.
Blue Value indicates the amylose content; higher the amylose content, higher will be the Blue Value. It was determined as follows, (Nwokocha et al. 2014):
0.1 g dry starch sample was weighed in a tube, 1 ml ethanol (95%) was added followed by 9 ml of 1 M NaOH solution and heated in a boiling water bath for 10 min to solubilize the starch. The starch solution was cooled and transferred into a 100 ml standard volumetric flask and the volume made up to 100 ml mark with distilled water. 2.5 ml of starch solution was taken into 50 ml standard flask; 0.5 ml of 1 M acetic acid was added followed by 1 ml of stock iodine (0.2 g I_2 / 2.0 g KI/ 100 ml) and the solution made up to the 50 ml mark with distilled water. Color was allowed to develop for 20 min and then absorbance reading was measured at 620 nm using UV/ visible spectrophotometer. Iodine solution of same concentration as above but without starch sample was used in the reference cell. The Blue Value was calculated according to the method of Gilbert and Spragg (1964) using the formula:

Blue Value = Absorbance at 620 nm X 4 / Concentration (mg / dl).

Preparation of Bioplastic: According to Lopez et al. (2019) starch with high amylose content, usually has bigger crystalline domains, giving greater mechanical resistance for the films. Sweet potato and potato showed higher amylose content, hence they were used for bioplastic preparation. All ingredients, as given in Table 1, were added and mixed in blender. After blending, the slurry was poured in a Petri plate and was heated in the microwave till the slurry clarified to become semi-transparent (~100ºC, for 7 to 10min). The plate was then kept for drying in incubator at 40 ºC for 24 hrs.

Table 1: Composition and concentration of ingredients for preparation of bioplastics:

Sr. No	Contents	Bioplastic A	Bioplastic B	Bioplastic C
1	Carrageenan	4.2 g	4.2 g	4.2 g
2	Potato starch	2.6 g	2.6 g	2.6 g
3	Sweet potato starch	2.6 g	2.6 g	2.6 g
4	Vinegar	25 ml	25 ml	25 ml
5	1% Glycerol	20 ml	20 ml	20 ml
6	CMC	1 g	-	2 g
7	Distilled water	60 ml	60 ml	60 ml

Testing of Bioplastics: (Marichelvam et al. 2019)
Thickness Measurement: The thickness of the bioplastics was measured by using the digital caliper (Mitutoyo Absolute, P65, least count 0.001mm).

Moisture Content: The samples were cut into square pieces of 2.0 cm^2 and weighed. The weight of the samples was recorded upon drying in an oven at 110ºC until a fixed dry weight was acquired.

Moisture Content in (%) = [(Wi – Wf)/Wi] × 100, where Wi is the initial weight and Wf is the final weight.

Water Solubility Test: The samples were cut in square pieces of 2.0 cm^2 and weighed. The samples were put in 100 mL distilled water and were agitated at 30 rpm for 6 h at 25ºC. The portions of the samples left were filtered after 6 hrs. They were then dried in a hot air oven at 110ºC until fixed weight was found. The percentage of solubility was calculated as;

S (%) = [(Wi – Wf)/Wi] × 100, where Wi is the initial weight of the bioplastic and Wf is the final weight of the bioplastic.

Biodegradability Test: The samples were cut in pieces of 4.0 cm^2. Garden soil was collected, moistened, and stored in a container. The samples were buried 2 cm inside the soil and kept at room temperature. They were monitored for 30 days. The weight of the samples was measured before (day zero) and after the testing (day 30). The biodegradability was measured by:

Weight Loss (%) = [(Wi – Wf)/Wi] × 100, where Wi and Wf are the weights of samples before and after the test.

Tensile Strength, Peak Load and Break Elongation tests: These tests were performed using Star Testing Systems at MSME, Mumbai, India, (as per IS 694, 1554 (PL-1) and 7098) (Ministry of Micro, Small and Medium Enterprises, Govt of India). The cross-head speed was fixed at 25 mm/min. Dumbbell shaped specimens were cut from the bioplastic samples (three different specimens and triplicates for each). During the stretching of the samples, tensile strength (MPa), break elongation (%), and peak load carrying capacity (N and kg) were recorded.

3. RESULTS:

Table 2: Microscopic studies of starch:

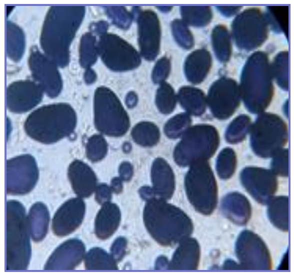
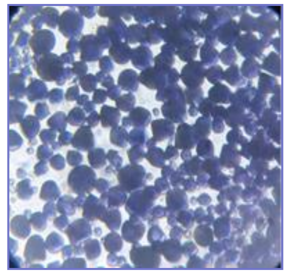
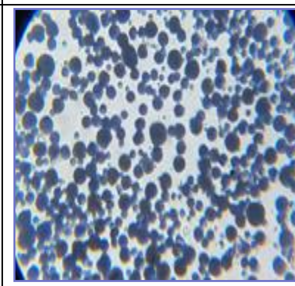
Parameters	Potato starch	Sweet Potato starch	Tapioca starch
45X Magnification			
Granule shape	Oval, triangular	Circular, polygonal	Circular
Granule size (μm)	38-104.4	23.2-69.9	11.6-58

Table 2 shows that granule size is smallest for tapioca starch and largest for potato starch.

Table 3: Yield, Loss on Drying, and Blue Value of starch:

Parameters	Potato starch	Sweet Potato starch	Tapioca starch
Yield	8.21%	16.05%	9.51%
Loss on Drying	22.4%	14.8%	14.1%
Blue Value (mg/dl)	0.456	0.512	0.376

Table 3 shows that sweet potato had highest yield of starch. Ratnayake and Jackson (2003) have reported 12% starch yield from potato. Thao and Noomhorm (2011) have reported 12.38 to 17.52% starch yield from sweet potato. Also, the Blue Value is highest for sweet potato, followed by potato, which indicates high amount of amylose content in sweet potato, followed by potato. Since small granule size and high amylose content are suitable for bioplastic preparation, potato and sweet potato starch were selected to prepare them. Granule size was smallest for tapioca but its Blue Value was lowest, hence it was not used in bioplastic preparation.

Table 4: Bioplastic Moisture content, Biodegradability in soil, and Solubility in water:

	Bioplastic A	Bioplastic B	Bioplastic C
Moisture Content	11.36%	7.95%	15.74%
Biodegradability in soil (in 10 days)	61.36%	78.48%	66.21%
Biodegradability in soil (in 30days)	94.02%	91.68%	96.83%
Complete solubility in water	Within 2 hrs	Within 1.5 hrs	Within 2 hrs

Fig. 1: Bioplastic

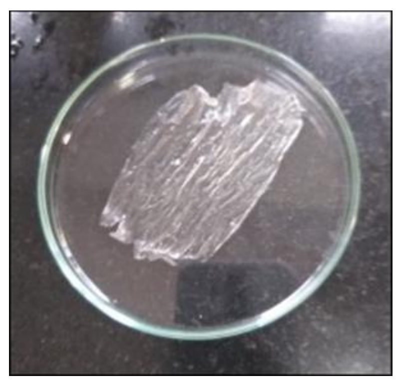


Fig. 2: Biodegradability: day1

Fig. 3: Biodegradability: day 30

Figure 1 shows the transparent bioplastic prepared in this study. Figure 2 shows the piece of bioplastic which was buried in soil on day 1 of its biodegradability test. The same piece is shown in Figure 3 after 30 days of biodegradation in soil. Table 4 shows that the bioplastics are more than 90% biodegraded in soil within 30 days. In addition, they are completely soluble in water within 2 hrs. The biodegradability results are consistent with those obtained by Prakash et al. (2014). Thus, these bioplastics are suitable for being disposed in either water bodies or soil.

Table 5: Mechanical properties of Bioplastics:

Mechanical Properties	Bioplastic A	Bioplastic B	Bioplastic C
Average Thickness (mm)	0.26	0.44	0.21
Tensile Strength (MPa) ***Significant at p< 0.0001	5.147	0.874	8.384***
Break Elongation (%) *Significant at p< 0.05	85.3	114.3*	60.3
Maximum (Peak) Load (kg) **Significant at p< 0.001	1.882	0.51	2.39**

One-way ANOVA test was used, variance ratio values were obtained and compared with table values (Mahajan 2008).
Table 5 shows that the average thickness of the bioplastics was found to be more than 0.21 mm (210 microns). As per the regulations of Government of India, the thickness of plastic bags should not be less than 50 microns (Marichelvam et al. 2019). Bioplastic C which had the highest CMC showed highest tensile strength of 8.384 ($p < 0.0001$) and highest peak weight carrying capacity of 2.39 kgs ($p<0.001$).

4. DISCUSSION:

Yield of sweet potato was the highest, hence it can be extensively used to prepare bioplastics. Sweet potato starch also showed high amylose content and small granule size which is best suited for bioplastic preparation. Bioplastics made in this study were completely soluble in water as well as degradable in soil environment. Average thickness of the bioplastics was found to be more than 0.21 mm (210 microns). These bioplastics could carry load up to 2.4 kgs.

5. CONCLUSION:

Currently, small quantities such as some polymers are added to the present bioplastics in the markets. They can make the bioplastics more tensile, but they do not guarantee complete biodegradation. Thus, bioplastic needed to be developed to be able to replace conventional plastic yet, which is truly biodegradable and is not blended with other polymers. In this paper potato starch and alternative sources of starch such as sweet potato and tapioca were studied for their potential use in making bioplastics. Sweet potato starch had good yield, smaller granule size and high amylose content. Hence, they are very well suited to make bioplastics. Addition of carboxymethyl cellulose in preparation of bioplastics improved their tensile strength and weight carrying capacity. With even higher amount of carboxymethyl cellulose, they can still be further strengthened. These bioplastics can be replaced by the current plastic bags as they are completely biodegradable in soil and water.

6. ACKNOWLEDGMENT:

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Key Messages:

Sweet potato starch has high yield, smaller granule size, and high amylose content, making it suitable to make bioplastic. Addition of carboxymethyl cellulose increases tensile strength and weight carrying capacity of the bioplastic. These bioplastics can replace conventional plastics as they are completely soluble in water and degradable in soil.

Conflict of Interest: No conflict of interest

Contribution of authors: Ms. Madhura Patkar, Ms. Kshitija Rahate, Ms. Rani Prasad, Mr. Pratik Shetye, Mr. Rohan Sinha, and Dr. Kanchan Chitnis were all involved in consequential inputs to the concept and design, actual experimental work, data analysis, and interpretation. Dr. Kanchan Chitnis was also involved in preparing the article, but along with her, all other authors also revised the article and gave their confirmation for the final article ready to be published in the journal.

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