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Evaluation of Tapioca Starch Films for Biodegradability and Food Storing

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The present study was undertaken to know the effectiveness of tapioca starch films for storing of five different food materials (four different spices and tea granules). The net change in weight for non-hygroscopic items was recorded from gain of 0.38 % to loss of 3.74 %, after 168 days of storage at room condition. The starch films were stored at room temperature and refrigerated condition for six months and the total microbial count was calculated. The biodegradability of the films was determined by CO_2 emission test. Starch film having starch 3 %, chitosan 1 % and glycerol 20 % was found to be the most susceptible to microbial contamination, which produced net 0.019 g carbon dioxide evolution per g film during 105 days of soil burial.

Keywords: Tapioca, Starch films, Chitosan, Biodegradability, CO_2 emission.

INTRODUCTION

Biodegradable plastics were first started with hydroxy butyrate valerate co-polyster, which is accumulated in special bacterium [1], polythene containing starch [2], *etc*. Polysaccharide such as starch and cellulose has been studied [3]. Starch is a polymer synthesized in plants. The principal crops used for its production include potatoes, corn, rice and tapioca. In all of these plants, starch is produced in the form of granules, which vary in size and in composition from plant to plant. Starch has been widely used as a raw material in film production because of increasing prices and decreasing availability of conventional film-forming resins. Starch having a thermoplastic property upon the disruption of its molecular structure, enhanced biodegradability by microbial and oxidative degradation [4]. Tapioca is an economical source of starch [5]. Plasticizers such as glycerol, sorbitol, ethylene glycol are used which improves the film flexibility [6]. Glycerol is a popular plasticizer used in film making technique, due to its stability and compatibility [7]. The type of plasticizer influences the properties of films. As a plasticizer, glycerol with suitable size and three hydroxyl groups, easily enters in between the film forming polymeric chains and weakens the intermolecular forces between the polymers and decreases the crystallinity. Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). Chitosan provides good mechanical strength, higher moisture barrier and water resistance. It is biodegradable, non-toxic, relatively more hydrophobic and antimicrobial [8,9].

Several reports [8,10-12] are available on properties of starch-chitosan films. However, a few reports are available on effectiveness of starch film for food packaging [12,13]. Though, starch itself is biodegradable, research work on determination of the rate of biodegradability of starch films are very few [2,14]. Considering paucity of research work on the above mentioned area, the present study involving the evaluation of tapioca starch films for storage of food materials and its susceptibility to the microbial contamination and biodegradability was conducted.

EXPERIMENTAL

Starch was extracted mechanically from tapioca at the laboratory of All India Coordinated Research Project on Post Harvest Technology, Department of Agricultural Engineering, Assam Agricultural University, Jorhat, India. The tubers for this purpose were collected from Horticultural Experimental Farm, Department of Horticulture, Assam Agricultural University, Jorhat, India. Starch used during the present study had moisture content of 6 % (wet basis) and amylose content of 28 % (dry basis). Glycerol (Analytical Reagent) was procured from Ranbaxy Fine Chemicals Limited, New Delhi. Chitosan was collected from Sisco Research Laboratories Private limited, Andheri, Mumbai, Maharastra. Acetic acid was collected from Merck limited, Mumbai, Maharastra. Nutrient Agar Medium was collected from HiMedia Laboratories Private Limited, Mumbai, Maharastra. Polypropylene plastic (0.03 mm) was collected from local market of Jorhat city.

Preparation of starch films: The films were prepared according to the reported methods [15]. The films (T1) were used for packaging purpose within 1 week of preparation.

Following three types of films were used for the present study:

Treatment 1 (T1): Starch (3 % w/v of casting solution) and glycerol (20 % v/w of starch), casting depth 0.50 cm. Final thickness, moisture content (on wet basis) and tensile strength after drying were 0.15 mm, 11.90 % and 9.23 MPa, respectively.

Treatment 2 (T2): Starch (3 % w/v of casting solution) and glycerol (30 % v/w of starch), casting depth 0. 50 cm. Final thickness, moisture content (on wet basis) and tensile strength after drying were 0.17 mm, 15.42 % and 4.69 MPa, respectively.

Treatment 3 (T3): Starch (3 % w/v of casting solution), chitosan (1 % w/v of casting solution) and glycerol (20 % v/w of starch), casting depth 0.50 cm. Final thickness, moisture content (on wet basis) and tensile strength after drying were 0.18 mm, 10.10 % and 3.11 MPa, respectively.

Treatment 4 (T4): Polypropylene plastic of thickness 0.03 mm, tensile strength 11.95 MPa.

Different food items (tea granules, mustard, black pepper, mixed spice 1 composed of cardamom, cinnamon and clove and mixed spice 2 composed of cumin, mustard, fenugreek, fennel black cumin) were collected from market, cleaned manually, dried in oven at 50 °C, to constant weight (to prevent insect infestation during storage). These were taken out of oven, cooled to room temperature and kept inside the starch bags (T1) the same day (Fig. 1). Each food item was replicated four times. The weight of empty starch bags and the bags with stored items after sealing of the bags were recorded separately. All the packed food items were stored in a dark place at room temperature. During storage period, (Average temperature 26.98 °C and Relative humidity 85.58 %), the weights of the packed food items were recorded at different intervals. This was continued till any breakage was observed in starch film bag. Food item stored in polypropylene bags (T4) were used as control. Infestation on food items by pest during storage was also noticed carefully. The physiological change in weight was expressed as cumulative percent change (loss or gain).

Tea granules Mixed spices 1 and 2, Mustard and Black Pepper seed

Fig. 1. Storage of food items in bags of tapioca starch film (T1)

Detection of microbial contamination on starch films: The starch film samples stored inside polypropylene bags, both at room temperature and refrigerated condition, were analyzed for total microbial count using standard method. 0.1 g of the film sample was washed with 10 mL of autoclaved distilled water in a test tube, from which 1 mL of the uniform solution was taken and serial dilution was done. Microbial enumeration was done in triplicate with Nutrient Agar media by colony counting after an incubation period of 72 h at 25 °C and expressed as colony forming units (c.f.u.) per gram of sample. Temperature and relative humidity during storage of film for detection of microbial contamination were 29.3 °C and 80.5 % and 0 °C and 40.0 %, for room storage and refrigerated storage, respectively.

Determination of biodegradability of starch films by carbon dioxide evolution from the starch films buried inside soil: Biodegradability of starch films were determined by measuring the carbon dioxide evolution from the starch films buried inside soil. The carbon dioxide evolution in soil was determined according to Crossono *et al.* [16]. Each plastic container of 2 L capacity with air tight plastic lid was filled with 500 g of soil. A film of size 2.5 cm × 2.5 cm was buried at 5 cm depth of soil. The soil was collected from the surroundings of the dairy farm of Assam Agricultural University, Jorhat, India. Prior to use, the soil was sieved through 250 µ sieve and total microbes present in the soil was estimated, which was found to be 3150×10^6 cfu/g. The moisture content of the soil (wet basis) was adjusted to 25 %. A test tube containing 5 mL of 0.1 N sodium hydroxide was placed inside the container. Then the lid of the container was closed air tight. The carbon dioxide gas evolved, both from the soil and from degradation of the film was absorbed in sodium hydroxide solution and converted into an equivalent amount of sodium carbonate. The resulting mixture consisting of excess sodium hydroxide and sodium carbonate was titrated against standard HCl (0.1N). Titration to the first colourless phenolphthalein end point was done which neutralized the excess sodium hydroxide and converted all the sodium carbonate into sodium bicarbonate. Continuation of the titration to the second methyl orange endpoint converted the sodium bicarbonate to water and carbon dioxide.

RESULTS AND DISCUSSION

The data presented at Tables 1 and 2, after 168 days of storage, showed net change in weight from gain of 0.38 % (mixed spice 2) to loss of 3.74 % (mixed spice 1). However, the same for the food items stored inside polypropylene bags recorded a loss in weight from 0.007 % (mixed spice 2) to 0.56 % (black pepper seed). It was observed that the moisture content of the oven dried food items stored inside starch films increased to a range of 0.96 % (mixed spice 2) to 7.16 % (mixed spice 1) after 168 days of storage. However, all the food items except mixed spice 2 showed net decrease in weight during storage. This may represent loss of moisture by the starch film used for packaging during storage. However, it has been reported that there was an increase in cumulative percent physiological weight loss from 3.5 to 7.5 % in mango during storage for 18 days at room temperature using low density polyethylene film and biodegradable chitosan films, respectively [17].

TABLE-1
CHANGE IN WEIGHT (g) OF DIFFERENT FOOD SAMPLES STORED IN BAGS OF TAPIOCA STARCH FILMS AT DIFFERENT PERIOD OF STORAGE

Duration of storage	Mixed spice 1	Tea leaf granules	Mustard seed	Black pepper seed	Mixed spice 2
Initial	26.519 (20.309)	60.879 (63.770)	42.750 (39.513)	34.903 (31.452)	42.643 (40.645)
16 days	26.379 (20.308)	60.575 (63.772)	42.872 (39.489)	34.991 (31.460)	43.048 (40.833)
33 days	25.979 (20.290)	60.153 (63.781)	42.820 (39.460)	34.591 (31.458)	42.998 (40.770)
40 days	25.938 (20.300)	59.802 (63.796)	42.553 (39.463)	34.552 (31.433)	42.923 (40.747)
50 days	25.773 (20.28)	59.626 (63.816)	42.305 (39.470)	34.416 (31.463)	43.009 (40.727)
59 days	26.146 (20.325)	59.757 (63.842)	42.534 (39.478)	34.516 (31.331)	43.128 (40.720)
67 days	25.974 (20.323)	59.866 (63.85)	42.666 (39.484)	34.650 (31.327)	43.528 (40.713)
70 days	25.942 (20.324)	59.837 (63.844)	42.614 (39.487)	34.552 (31.329)	43.368 (40.714)
80 days	26.084 (20.326)	59.954 (63.839)	42.708 (39.486)	34.71731.328)	43.695 (40.709)
98 days	25.802 (20.316)	59.588 (63.816)	42.382 (39.473)	34.694 (31.317)	43.312 (40.697)
113 days	25.906 (20.316)	59.938 (63.812)	42.540 (39.473)	34.870 (31.320)	43.341 (40.697)
121 days	25.792 (20.309)	59.483 (63.798)	42.460 (39.463)	34.622 (31.310)	43.164 (40.688)
134 days	25.772 (20.295	59.462 (63.800)	42.438 (39.454)	34.671 (31.394)	43.019 (40.679)
142 days	25.737 (20.283)	59.218 (63.804)	42.382 (39.433)	34.711 (31.283)	42.961 (40.653)
151 days	25.713 (20.280)	59.577 (63.749)	42.412 (39.436)	34.704 (31.285)	43.079 (40.656)
158 days	25.734 (20.275)	59.708 (63.751)	42.517 (39.441)	34.749 (31.289)	43.254 (40.661)
168 days	25.525 (20.273)	59.220 (63.727)	42.232 (39.426)	34.358 (31.275)	42.806 (40.642)
Net change in weight	-3.74 % (-0.17 %)	-2.72 % (-0.06 %)	-1.21 % (-0.22 %)	-1.56 % (-0.56 %)	+0.38 % (-0.007 %)

Data in parentheses represent respective weight of food item in control (T4) bags; Weight of starch bag used for tea: 6.8 g, Weight of starch bag used for black pepper, two types of mixed spice and mustard seed = 5.0 g, Weight of polypropylene bag (control): 1.257 g

TABLE-2
MOISTURE CONTENT (WET BASIS) OF THE FOOD SAMPLES AFTER STORAGE

Food samples	After storage (%)
Tea granules	2.900
Black pepper	1.105
Mustard	5.440
Mixed spice 1	7.160
Mixed spice 2	0.964

TABLE-3
GROWTH OF MICROBES ON TAPIOCA STARCH FILMS

Treatment	1 Month after storage (cfu/g)		6 Months after storage (cfu/g)	
	Room storage (29.3 °C and 80.5 % RH)	Refrigeration storage (0 °C and 40 % RH)	Room storage (29.3 °C and 80.5 % RH)	Refrigeration storage (0 °C and 40 % RH)
T1	2×10^6	Nil	28×10^6	22×10^6
T2	Nil	Nil	15×10^6	Nil
T3	2×10^6	2×10^6	40×10^6	15×10^6
T4	Nil	Nil	Nil	Nil

The observed difference in the cumulative percent physiological weight change might be due to hygroscopic nature of food items and the fluctuations of relative humidity in the surrounding atmosphere during the period of storage.

Analysis for detection of microbial growth on starch film stored inside polypropylene plastic at room and refrigerated condition: The higher microbial growth on starch films (Table-3) stored in room condition compared to starch films stored at refrigerated condition might be due to higher room temperature favouring the growth of microbes. Growth of microbes on starch films stored for 6 months in room condition was higher in comparison to starch films stored for 1 month, which might be due to extension of storage time. However, in T4 (control, polypropylene plastic) there was no microbial growth at both refrigerated and room condition after one month and six months of storage. It was also observed that in tapioca starch-chitosan films, there was higher growth of microbes than the starch films without chitosan at both 1 month and 6 months of storage. Though, certain reports are available [8,10-12] on antimicrobial property of chitosan, the present study revealed that certain microorganisms living in the experimental location might be resistant to chitosan at the concentration used in the film. It was reported that mould growth was observed by visual inspection at edges and surfaces during the end of storage period between 20 to 24 days in plasticized rice starch-chitosan biodegradable films [18].

There was a gradual increase in carbon dioxide evolution from 7 days to 105 days (Fig. 2). Net carbon dioxide evolution during first one week of soil burial was not detected. After 1 month of soil burial, the highest (0.0041 g) net carbon dioxide evolution was recorded in T3 and the lowest (0.0029 g) was recorded in T2. The highest (0.0078 g) net carbon dioxide evolution after 75 days of soil burial was recorded for T3 and the lowest (0.0057 g) was recorded for T1. After 105 days of soil burial, the highest (0.019 g) net carbon dioxide evolution was recorded in T3 and the lowest (0.0093 g) was recorded in T2. There was no net carbon dioxide evolution in control T4 (polypropylene plastic) up to 105 days of soil burial. It was observed that the starch film containing chitosan is more rapidly biodegradable than the other two kinds of starch films. This might be due to higher growth of microbes on the film T3, which is also supported by detection of higher microbial growth on this film during storage at both room and refrigerated condition. It was reported that the biodegradability is 0.08 % to 4.83 % for degradable polyethylene films containing 3.43 and 0.44 % stearate [13]. In the present study, on 105 days after storage maximum 0.019 g net carbon dioxide was evolved/g of dry film (T3), which was found to be considerably lower than the cumulative net carbon dioxide (2000 μM) evolved from 8 % starch-PE film observed by some other workers [2]

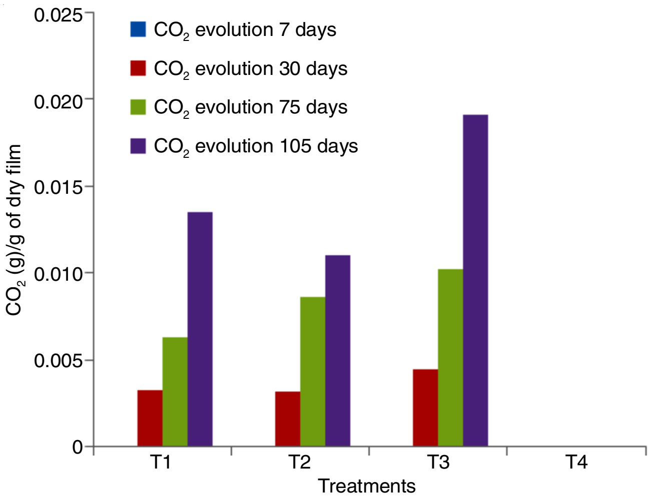

Fig. 2. Net carbon dioxide evolution (g) per gram of dry film at different periods of soil burial

which might be for amendment of soil used for degradation studies for better microbial activities by them.

Conclusion

The starch film T1 was found to be the best with intermediate susceptibility to microbial contamination and biodegradability. The starch film (T1) can be successfully used for storage of preferably, up to 50 g non-hygroscopic, low initial moisture content food items for 5 months at room temperature.

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