

## **Analysis of Biodegradable Films of Starch from Potato Waste**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Starch was extracted from potato wastes and its peels to prepare bio-degradable films using glycerol and sorbitol at various concentrations of 35%, 45% and 55 (w/w) of dry starch. The properties of films prepared with modification techniques (hydrothermal treatment (HTT) and acid-alcohol treatment (AAT)) were analyzed. Biodegradability test was done by incubating with amylolytic bacteria (*Bacillus lichneformis* and *Streptococcus bovis*) for 24 hours and fungi (*Aspergillus niger* and *Rhizopus stolonifer*) for 72 hours. All microbial species were isolated from soil except *S. bovis* which was from calf stool and identified by conventional methods. The properties of modified starches were significantly different from non-treated starches. Tensile strength (10 N/m<sup>2</sup>) and elongation (9.47%) were significantly ( $p < 0.05$ ) superior in HTT starch films whereas solubility (25.8%) was superior in AAT starch films. Elongation (8.91%) and solubility (29.98%) were significantly ( $p < 0.05$ ) superior in 35% and 55% glycerol used films respectively but tensile strength (13.02 N/m<sup>2</sup>) was superior in 35% sorbitol used films. WVTR (999 g/m<sup>2</sup>/d) was higher at 91% RH in 55% glycerol used films. Micro-organisms used showed a significant effect ( $p < 0.05$ ) on biodegradation of starch based films. Highest degradation was observed by *B.*

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*lichneformis* i.e, 57.85% while *A. niger* had minimum of 25.13%. Films prepared with 35% glycerol was significantly ( $P<0.05$ ) degraded by *B. lichneformis* i.e, 78.86%. Films prepared by using glycerol with acid alcohol treatment was significantly ( $P<0.05$ ) degraded by *S. bovis* (55.57%). Although the starch of same variety of potato waste was not studied, results of the study suggest possibility of preparation of starch based bio degradable films potato waste and its peels by using glycerol and sorbitol.

**Keywords:** *Potato starch; bio-degradable films; acid alcohol treatment; hydrothermal treatment; biodegradation.*

## 1. INTRODUCTION

The thriving use of non-biodegradable polymers has caused serious environmental problems and degradable polymers that are commonly prepared from renewable polymer sources such as starches, proteins, PLA (polylactic acid), PHA (poly-hydroxy-alkanoate), cellulose esters, and poly-anhydride have been paid more attention since the 1970s [1]. Owing to its biodegradability, low cost and renewability, starch is considered as a promising resource for developing starch-based polymers for conserving the petrochemical resources and reducing environmental impact. Potato peels and its wastes serve as good sources of starch, cellulose, hemicelluloses and fermentable sugars. In potato processing plants, a significant amount of the potato sludge, peels and damaged tubers are obtained as the by-product. Biodegradable polymer films are not meant to totally replace synthetic packaging films but to reduce their use and impacts. However, biodegradable and edible films can be satisfactorily used for versatile food products to reduce loss of moisture, to restrict absorption of oxygen, to lessen migration of lipids, to improve mechanical handling properties, to provide physical protection, or to offer an alternative to the commercial packaging materials [2]. Starch derived films are supposed to possess the favorable barrier properties and strength depending on the modification methods and plasticizers used [3]. Among microbes, bacteria are the preferred source for the production of amylase because of maximum production of enzyme within a short period of time and extracellular production of enzyme which are easy to isolate. Amylases from the microbial sources that are present in soil are significant in degrading the bio-polymers [4].

Though, the production of the starch biopolymer is not done in Nepal yet, there is an increasing interest in utilizing renewable resources as food packaging [5]. The use of biodegradable polymers for packaging offers an alternative and

partial solution to the problem of accumulation of solid waste composed of synthetic inert polymers and proper utilization of the waste starches. Thus, potato starches were used to prepare films and the rate of degradation of the films was studied by the use of different bacteria found in the soil.

## 2. MATERIALS AND METHODS

### 2.1 Extraction and Modifications of the Starch

Potato waste and its peels were collected from krishi bazaar of Dharan and canteen of Central Campus of Technology, Dharan. Starch was extracted and purified as described by Altemimi [6] with slight modifications. Two (2) kg of waste potatoes and peels were washed thoroughly, sliced and chopped into small chunks. After adding distilled water in the potato peel mixture, extraction process was carried out by using centrifuge at 1500, rpm for 15 min. Wet starch was obtained by filtering centrifuged samples through Whatman no. 1 and neglecting the supernatant. The obtained starch was dried at room temperature for 5-6 h, then crushed into a fine powder and stored in sealed containers for later use. The extracted starch from the waste potato and its peels (Non-Treated) was modified hydrothermal treatment (HTT) and acid-alcohol treatment (AA). HTT was conducted as described by [7]. The extracted starch (100 gram) was adjusted from 25% to 28% moisture, pH 6.7 and equilibrated at 4°C to 6°C overnight (refrigerated condition) and placed in a hot air oven for 3 hours at 110°C. The sample was shaken occasionally for even distribution of heat. The sample was cooled to room temperature (about 30°C) and dried at 50°C, equilibrated for 4 hours and sealed in polyethylene bags until use. AAT was conducted as described by Chang et al. [8]. 25 g starch was suspended in 100 ml of ethanol in a 500 ml conical flask. The hydrolysis reaction was initiated by adding 36% hydrochloric acid (20ml) and allowed to proceed for 1 hour at 45°C in a

water bath. The reaction was stopped by neutralizing the solution with 1M NaOH. The sample was transferred into 50 ml centrifuge tubes and centrifuged at 3500 rpm for 5 minutes. The supernatant was collected and the precipitate was held with 50% ethanol until neutral to litmus. The starch sample was filtered using Whatman's No.1 filter paper and dried in an oven at 50°C and sealed in polyethylene bags until use.

## 2.2 Preparation of Starch Films

The starch films (Non-Treated starch film, HTTS film and AATS film) were prepared according to the method described by Müller et al. [9] with slight modifications. The films were prepared by a casting technique using a film-forming solution containing 5% starch. Glycerol and sorbitol at various concentrations (35% (w/w), 45% (w/w) and 55% (w/w) of dry starch) were used as plasticizer. The mixture was heated to boiling temperature with constant stirring for 10 minutes by magnetic stirrer on a hot plate. The mixture was then cooled until the bubbles vanished, and 45 ml of mixture was poured homogeneously into the non-sticky plastic trays of diameter 9 cm. The trays containing the film forming solution were then dried in a cabinet drier at 50°C for 5h. The dried films were peeled from the trays and kept in air tight polyethylene bags until further analysis.

## 2.3 Analysis of Starch Based Films

**Solubility of the film:** The solubility of the film was measured as a percentage of dry matter remaining in the film after being immersed in water for 24h [10].

**Tensile strength and elongation of the film:** The tensile force and elongation of sample films were measured according to the standard method D882-02 ASTM (2006) at room temperature using a texture analyser (TA.XT plus model, Stable Micro Systems). 100 x 35 mm (length x breadth) films were taken for analysis. Initial grip separation and crosshead speed were set at 50 mm and 50 mm/min, respectively. Tensile strength was calculated by dividing the maximum force by initial specimen cross-sectional area. Percent elongation at break was calculated as follows:

$$E = \frac{Da - Db}{Db} \times 100$$

Where, Da and Db were the distance between grips holding the specimen before elongation and after the break of the specimen respectively.

**Water Vapour Transmission Rate (WVTR) of the film:** WVTR (g water/m<sup>2</sup>/d) of the starch film was measured as described by (Robertson, 2005). A film of 2.5 cm diameter was used to seal a testing cup containing salt solutions (75% by volume of cup). The cup was then placed in a controlled chamber. The weight of the cup was measured intermittently at intervals of 24h up to 4 days. The weight of the arrangement was plotted against time, and WVTR was calculated from the slope of curve. KNO<sub>3</sub> at 35°C, NaI.2H<sub>2</sub>O at 30°C, NaNO<sub>2</sub> at 20°C were used for 91%, 40% and 65% RH respectively [11].

## 2.4 Bio-degradation of the Films

**Collection of sample for isolation of microorganisms:** Soil sample was collected from agricultural waste dump site of Dharan Sub-Municipality (Krishi bazar) using sterile spatula in a sterile petri-plate and was transported to the laboratory in controlled conditions for the isolation of *Bacillus licheniformis*, *Aspergillus niger* and *Rhizopus stolonifer*. The dung of new born calf was taken to isolate *Streptococcus bovis*. All microbial specimens are identified by the conventional methods.

**Isolation of amylase producing microorganisms:** One (1) gram of sample was weighed and added to 9 ml of sterile distilled water. Sterile dilutions were prepared up to 10<sup>-9</sup> dilution and 0.1 ml of each dilution was added using spread plate method to starch agar plates. The agar plates were incubated at 37°C for 24 h for bacteria (*Bacillus licheniformis* and *Streptococcus bovis*) and at 25°C for 5 days for fungi (*Aspergillus niger* and *Rhizopus stolonifer*) and then were flooded with lugol's iodine. The colonies producing halo zones were designated as amylase [12].

**Biodegradation of starch films:** Dried Starch polymers sterilized at 15psi. 25 ml of prepared broth were taken in a test tube. Weighted polymer was immersed in test tube and 0.1 ml of respective bacterial/ fungal culture was added. Incubation was carried out at 37°C for 2 days for bacterial culture and 6 days for fungal culture in a shaker incubator. After that they were dried to constant weight and final weight was taken. For bacterial degradation, nutrient broth was taken and for fungal degradation, potato dextrose broth

was taken. Degradation of starch films were determined by measuring the residual weight of the polymers. The percentage weight change was calculated by comparing the dry weight of residual films with the original weight of the films using the formula [13].

$$\text{Biodegradation (\%)} = ((\text{Initial weight} - \text{final weight after incubation}) / (\text{Initial weight})) \times 100$$

## 2.5 Statistical Analysis

Each experimental analysis was done in triplicate. The experimental data were analyzed by one- and two- way analysis of variance (ANOVA), which was carried out by using software Genstat twelfth edition (VSN International). In case of significant difference, means were separated using Tukey's HSD post hoc test at 5% level of significance.

## 3. RESULTS AND DISCUSSION

The yield and moisture content of the starch was  $20.5 \pm 2.6\%$  and  $9.2 \pm 1.1\%$  (wet basis) from the potato waste from the different places of Dharan. Similar result of yield for the kaffir potato starch was found [14]. Four to five films (moisture content  $12.5 \pm 1.3\%$  (wet basis)) of  $1.5 \pm 0.45$  g were produced from 5 g starch of thickness  $125 \pm 8.5$  microns and diameter of 9 cm. The yield of the starch might have varied due to the variation of potatoes types and various extents of deteriorations. The yield of starch films might have varied due to the variations in thickness. The thickness varied due to inconsistent pouring in the petri-plate while forming the films.

### 3.1 Physicochemical Properties of Starch Based Films

**Solubility of the films:** Solubility is an important property of biodegradable films. Mean solubility of starch films for different treatments (Non treated, HTTS and AATS) and varying plasticizers (glycerol and sorbitol) concentration (35%, 45% and 55%) varied from 12.11% for HTTS film (45% sorbitol) to 32.03% for AATS film (55% glycerol). Glycerol concentration and modifications using sorbitol had significant effect ( $P < 0.05$ ) in solubility of films. As the glycerol concentration increased, solubility of the films also increased. The momentous increase in solubility with increase in glycerol concentration ( $P < 0.05$ ) might be due to the hydrophilic nature of

glycerol. The result is in accordance with the findings of Bertuzzi et al. [1]. HTTS film had minimum solubility. This may be due to case hardening during hydrothermal treatment which is resistant to water adsorption. Several researchers have reported that after HTT structural changes within the starch granule cause reduction in swelling capacity and solubility [15]. HTT decreases granular stability, due to unraveling of double helices that are present in crystalline array in the native granule. The interactions between amylose–amylose and amylopectin–amylopectin chains during HTT might be the reason for decrease in solubility, [16]. The degradation of amylopectin upon acid alcohol treatment might have resulted in increased number of exposed hydroxyl groups which are responsible for greater water absorption, ultimately caused the starch granules swell and disrupt resulting in increased solubility in AATS films.

**Tensile strength of the films:** Mean tensile strength of starch films for different treatments (Non treated, HTTS and AATS) and varying plasticizers (glycerol and sorbitol) concentration (35%, 45% and 55%) varied from  $2.833 \text{ N/m}^2$  for ATTS film (55% glycerol) to  $23.133 \text{ N/m}^2$  for HTTS film (35% sorbitol). Plasticizers concentration showed significant differences ( $P < 0.05$ ) among tensile strength of films. Modifications by use of glycerol didn't show significant difference ( $P > 0.05$ ) in tensile strength. Decrease in the tensile strength of film was observed by increasing the concentration of plasticizers both glycerol and sorbitol. This might be due to degradation of mechanical integrity. Similar result was revealed by Shrestha et al. [17] in sorghum based starch films. Higher values of tensile strength were found in HTT films in both glycerol and sorbitol containing films. HTT may make the granules resistant to deformation by strengthening the intra-granular binding force. Rodriguez et al. [18] had also found that increase in glycerol content decreased the tensile strength of the starch-based films. Low tensile strength of AAT films might be due to increased exposure of hydroxyl groups that formed weak bond which have been re-formed during film formation.

**Elongation of films:** Elongation (E %) helps to determine the flexibility and stretch-ability of films. The greater the elongation, the greater will be the flexibility. Mean elongation of starch films for different treatments (Non treated, HTTS and AATS) and varying plasticizers (glycerol and

sorbitol) concentration (35%, 45% and 55%) varied from 2.5% for ATTS film (55% sorbitol) to 11.5% for HTTS film (35% glycerol). Plasticizers concentration and modifications showed significant effect ( $P < 0.05$ ) in elongation of films.

HTTS film with low concentration of the glycerol addition increased the elongation of the films. Glycerol having less molecular weight produced more plasticization [19] might have caused more elongation. Similarly, AATS films treated with the higher concentration of the sorbitol decreased elongation significantly. This occurrence can be explained by the anti-plasticization behavior or phase separation of highly plasticized starch films. During the processing of potato starch film, the granular and crystal structures of starch are mostly destroyed by high temperatures and shear forces, and the resulting structures are considered to exist mainly in an amorphous phase [20]. HTT adds to the breakdown of the structures resulting in increase of elongation at breakdown.

**Water Vapor Transmission Rate (WVTR) of the film:** The water vapor transmission rate (WVTR), is a mass of water vapor transmitted through a unit area in a unit time under specified condition of temperature and humidity. Water Vapor Transmission Rate (WVTR) of the plasticized potato starch film was measured as described by Robertson [21]. Increase in weight of the arrangement was plotted against time, the slope of which gives WVTR.

WVTR of all the treated samples ranged from 65.62 g/m<sup>2</sup>/day to 1255 g/m<sup>2</sup>/day. The lower the WVTR, the higher will be the quality of films. There is significant effect on the WVTR of starch films with respect to modification-types of plasticizers at different concentration at different RH.

The use of 35% sorbitol and 55% glycerol in Non-treated starch film can increase the WVTR at 40% RH. Use of 55% sorbitol in AATS and of 55% glycerol in AATS can increase the WVTR at 65% RH. Use of 55 % sorbitol in AATS and 45 % glycerol in HTTS can increase the WVTR at 91% RH.

Hydrophilic and hydrophobic nature of the materials, presence of the cracks and pores, tortuosity of the pathway through the film and the integrity of the film are the determining for the barrier properties of the films [22].

For films prepared from the HTT starch with sorbitol at 55% at RH 40% had least WVTR and Non treated starch with sorbitol at 35% at RH 40% had the highest WVTR among all the treatments. It reveals that the modification of the starch has great influence in the WVTR of the films. Among all treated starches HTT starches had superior wettability and water binding capacity. This might be the reason for the increase in the WVTR which helped to bind water for longer period of time. It can be seen that the concentration of sorbitol has also played the pivotal role for the decrease in WVTR. The more is the concentration of plasticizer, less will be the intermolecular interaction [23].

Higher values of WVTR at high RH have been observed which may be due to the absorption of more available moisture. Lower value of WVTR for AAT treated starch films might be due to low water binding capacity of AAT starch. AAT basically reduces the amorphous region in the starch granules. This reduces the number of available binding sites for water in the starch granule [5]. Considering the main effect, the concentration of the plasticizers seem to be insignificant ( $P > 0.05$ ) whereas the RH significantly affected ( $P < 0.05$ ) the WVTR. WVTR values were found to be increased with increase in RH. This might be due to the formation of the hydrogen bonds between the hydroxyl groups of biofilm chain segment and water. Water in the films act as the plasticizer and swells the film. The water vapor diffuses more readily across the swollen membrane than the drier films [23]. WVTR was found to be increased with increase in the glycerol concentration. This might be due to high affinity of the water to glycerol. The addition of the plasticizer increases the molecular mobility of the film simultaneously increasing the diffusivity of the permeating molecules and thus increasing the overall permeability [24]. At RH 40%, AAT and HTT of the potato starch decreased the WVTR of the biofilms. Addition of the acid in the starch film provided the additional barrier to water vapor. Similar result was discussed by [25].

### 3.2 Biodegradability of Films

Among all microbes, *Bacillus lichneformis* was found to be the most degradative of biopolymers and *Aspergillus niger* showed the least the degradation ability. The starches with different modifications were converted into biofilms and biodegradability test was done by incubating with isolated bacteria (*Bacillus lichneformis* and

*Streptococcus bovis*) and fungi (*Aspergillus niger* and *Rhizopus stolonifer*) at controlled conditions.

Fungi were successfully screened from the soil samples by using a modification of the screening techniques described by Malloch [26]. In each of the samples, screening showed the dominance of a single-type, morphologically distinct fungal colony. Bacteria isolated from dump site soil of (Krishi bazar, Dharan) exhibited lighter color and less spores in starch agar medium. Under conditions described in the methodology, isolated bacteria and fungi were found to be starch hydrolyzing. The bacterial isolates were identified on the basis of morphological, cultural and biochemical characteristics. The isolates were characterized according to IMViC test [12].

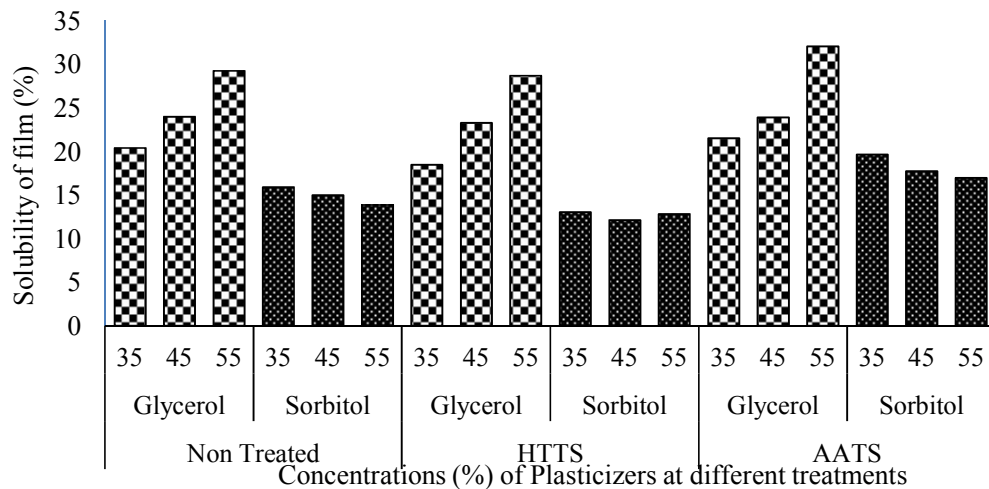
Micro-organisms used had significant effect ( $p < 0.05$ ) in biodegradation of starch based films. Highest degradation rate was observed by *Bacillus licheniformis* 57.85% while *Aspergillus*

*niger* had minimum of 25.13% degradation of films.

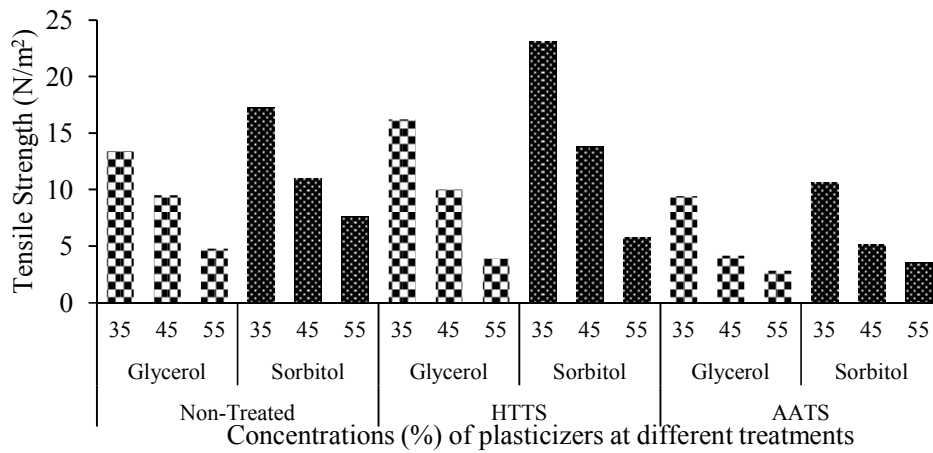
Glucoamylase, a hydrolyzing enzyme was reported to be produced by many fungi like *Aspergillus* spp., *Rhizopus* spp., *Mucor* spp., *Penicillium* spp. and Yeast were reported to produce Glucoamylase, a hydrolyzing enzyme [27,28] and among these, *Rhizopus* spp. are considered good producers of amylolytic enzyme [29,30]. The relative rates of hydrolysis of amylase by *B. licheniformis* was higher than *S. bovis* [31]. *Rhizopus stolonifer*, *Aspergillus niger* and *Streptococcus bovis* didn't show any significant biodegradation by the use of glycerol at 35% (w/w), 45% (w/w) and 55% (w/w). But films prepared with 35% (w/w) glycerol was significantly ( $P < 0.05$ ) degraded by *B. licheniformis*, i.e., 78.86%. This might be due to more amylase activity of the *B. licheniformis* and hydrophilic nature of glycerol. Except *S. bovis*, all microorganisms showed significant ( $p < 0.05$ )

**Table 1. Identification of microbial specimens**

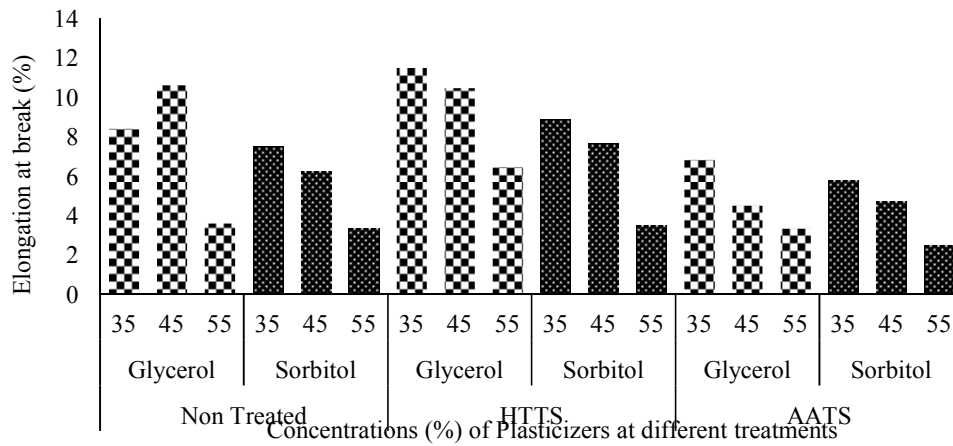
Microbe	Features for identification
<i>Bacillus licheniformis</i>	Gram positive, rod shaped, catalase positive, starch hydrolysing, gelatinase producing, Ellipsoidal, central endospore and motile
<i>Streptococcus bovis</i>	Gram positive cocci in pairs and short chain, catalase negative, no growth at 6.5% NaCl, hydrolysed starch, hydrolyse esculin in Bile-Esculin agar (turn bile esculin into black), acid from lactose
<i>Rhizopus stolonifer</i>	White cottony colonies in potato dextrose agar of 3-4 days of incubation and color changed to black (due to sporangium) after 5-6 days, no septa on hyphae was observed, single sporangia at the top of sporangiospore in tape staining with lacto phenol cotton blue staining, no biochemical tests performed
<i>Aspergillus niger</i>	Septate hyphae, cottony, white colon in 2-3 days and turned to black, biseriate sporangia



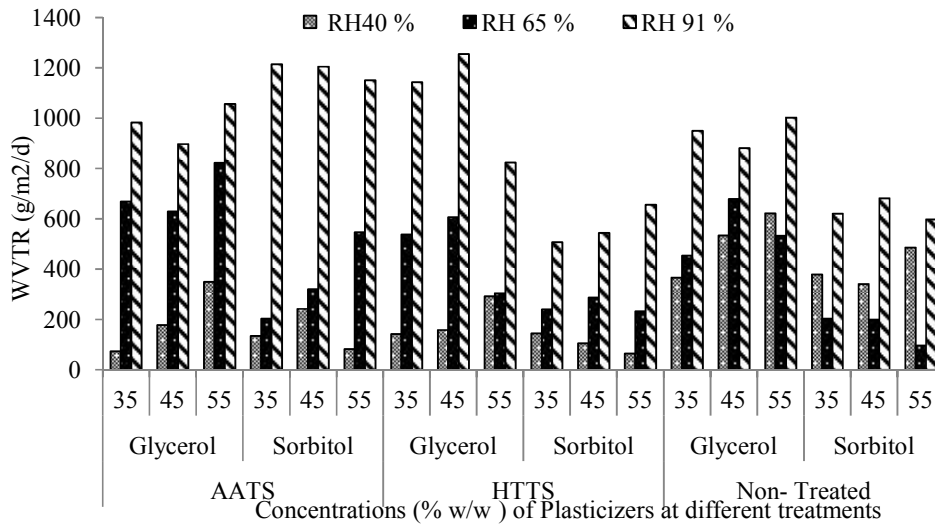
**(a)**



(b)

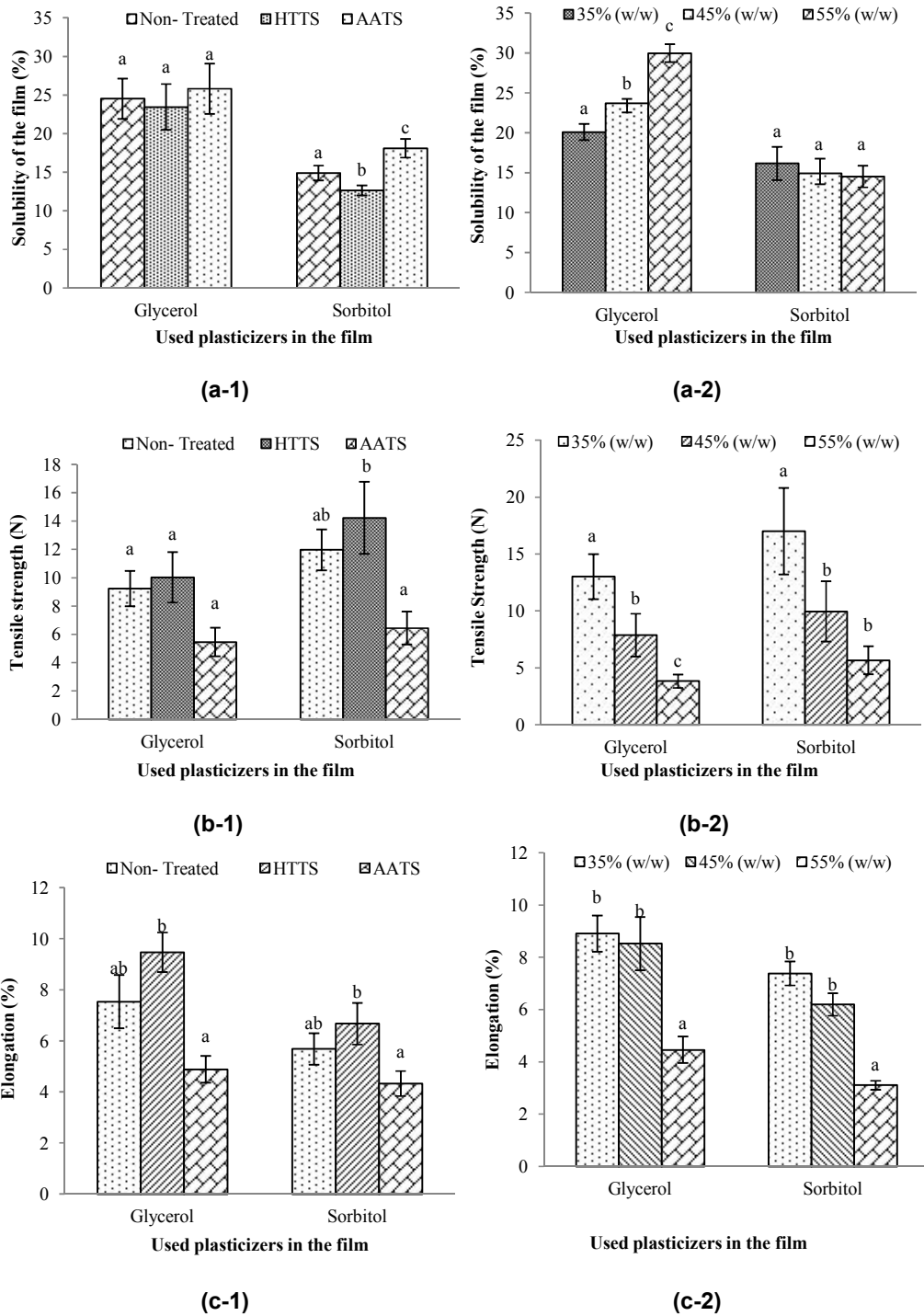


(c)



(d)

**Fig. 1. Properties of films prepared at glycerol and sorbitol concentration of 35% (w/w), 45% (w/w) and 55% (w/w) respectively (a) Solubility of the film (%), (b) Tensile strength (N/m<sup>2</sup>), (c) Elongation (%) and (d) WVTR (g/m<sup>2</sup>/d) of the film at different RH**



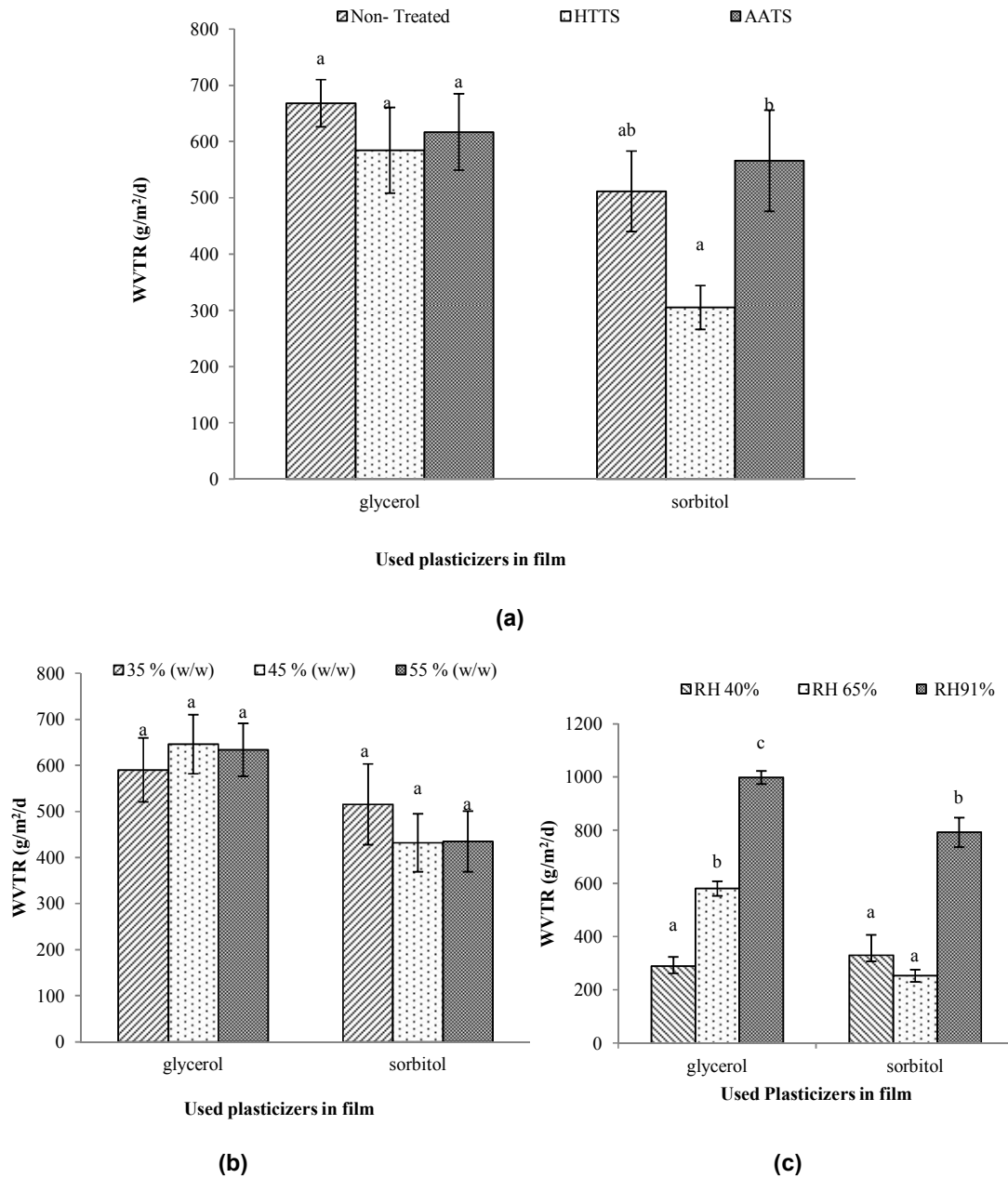
**Fig. 2.** Effect of modifications and concentration of plasticizers respectively in the properties of the films (a-1) and (a-2) Solubility of the film (%) (b-1) and (b-2) Tensile strength (N) (c-1) and (c-2) Elongation (%)

degradation of the films prepared by different modifications using glycerol. Degradation of mechanical integrity of films during modifications might have caused this variation, although this variation is not clearly understood. *B. lichneformis*, *R. stolonifer* and *A. niger* didn't

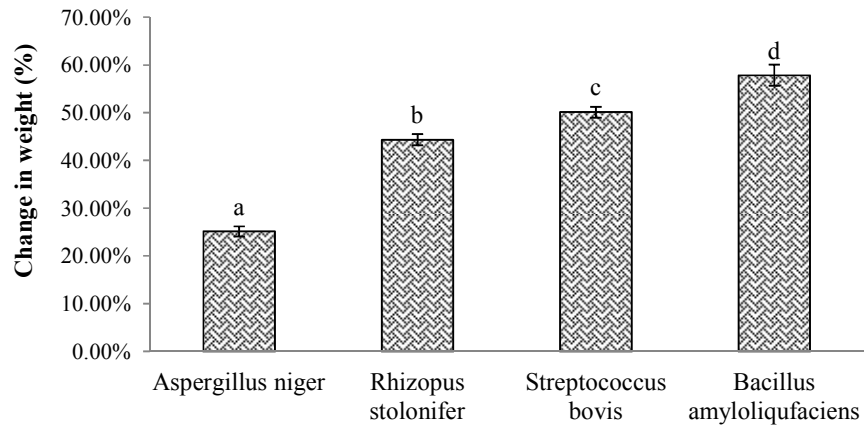


show any significant biodegradation of the films prepared by different modifications using sorbitol. But films prepared by using glycerol with AA treatment was significantly ( $P < 0.05$ ) degraded by *S. bovis*.e, 55.57%. Films prepared with glycerol at 35% (w/w), 45% (w/w) and 55% (w/w) was significantly ( $P < 0.05$ ) degraded by all

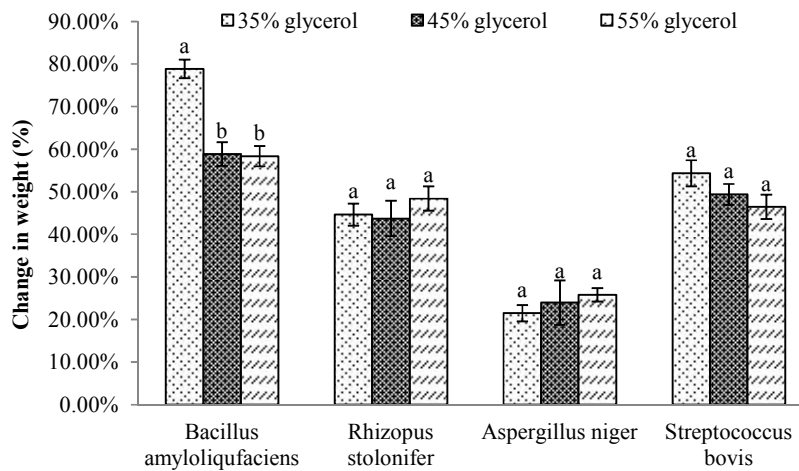
microorganisms. Microbial genetic diversity present in the environment, high enzymatic activity in wide range of conditions (extreme pH, temperature, pressure etc.), amylases from the microbial sources present in soil are significant in degrading the bio-films [4].



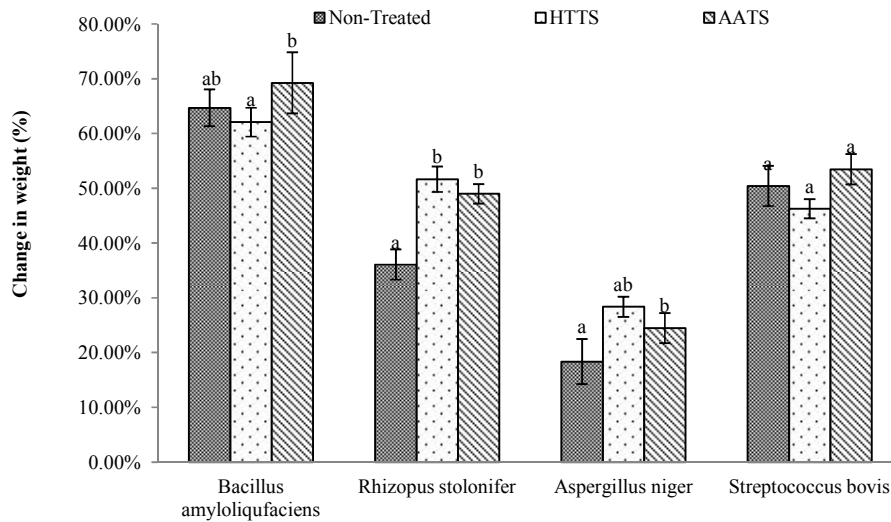
**Fig. 3. Effect of modifications, concentration of plasticizers and RH respectively in the WVTR of the film (a), (b) and (c)**



(a)

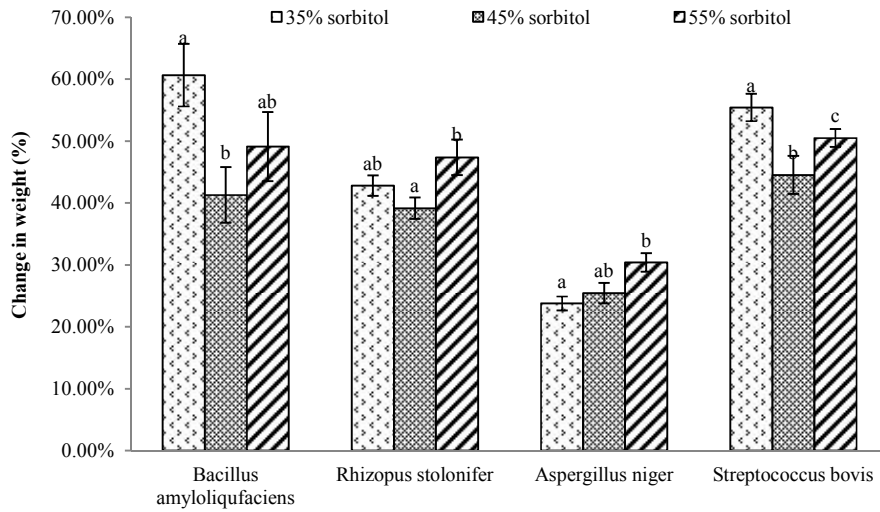


(b)

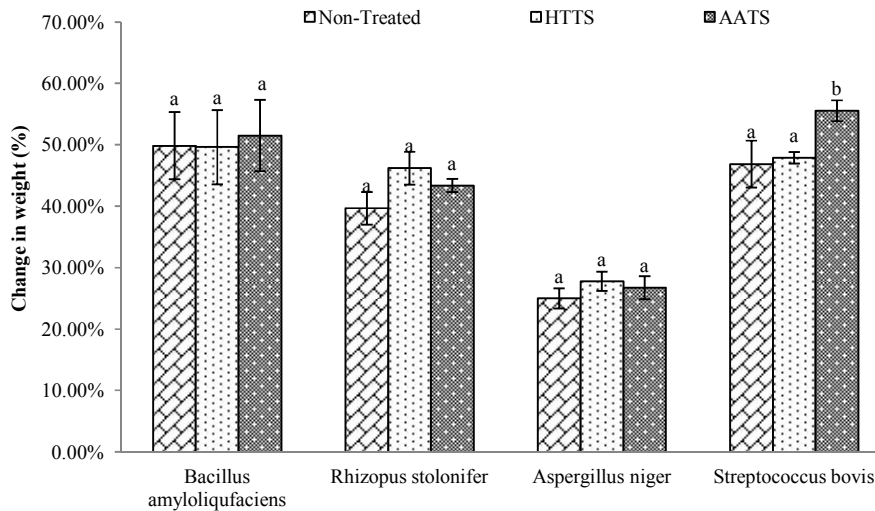


(c)

**Fig. 4. Effect in bio-degradability of films (% change in weight) due to microbes used, concentration and modifications by use of glycerol respectively (a), (b) and (c)**



(a)



(b)

**Fig. 5. Effect in bio-degradability of films (%change in weight) due to concentration and modifications by use of sorbitol respectively (a) and (b)**

#### 4. CONCLUSION

Modifications have improved the physical properties of starch based films. Solubility of the prepared films was higher in AA treatments and it was found to be increased when glycerol was used as plasticizer at higher concentration. Films prepared with hydrothermal treated starch showed highest tensile strength. Further, glycerol concentration decreased the tensile strength of the biopolymer. The percentage elongations of

films were also decreased by increase in glycerol concentration, and HTT starch films had highest percentage elongation. Glycerol concentration increased the WVTR of the starch films while HTT showed the decrease in the WVTR. Similarly, WVTR increased at higher RH. Among four microorganisms viz, *Bacillus licheniformis*, *Streptococcus bovis*, *Aspergillus niger* and *Rhizopus stolonifer*, the first organism showed the highest biodegradation with all types of the polymers followed by *Streptococcus bovis* and

*Aspergillus niger* which showed the least biodegradation. Although the starch of different potato varieties in the waste was not distinguished, possibility of extraction of starch from potato waste and its peels to prepare bio degradable film by using glycerol and sorbitol can be clinched through this research.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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