Effect of Acetic Acid and Citric Acid Modification on Biodegradability of Cassava starch Nanocomposite Films

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Abstract: The effect of acetic acid and citric acid modification of cassava starch on the biodegradability of their films reinforced with lignin and nanofiber was determined. Cassava starch was first modified with acetic anhydride and citric acid, characterized, filled with lignin and nanofiber to produce the films. Biodegradation studies were based on determinations of water absorption capacity, soil burial, enzyme degradation and reducing sugar (glucose). Results showed that great variations in water absorption capacities, weight loss in soil, enzyme degradation and reducing sugar were observed with TPS (Thermoplastic starch) having the fastest and highest water uptake, weight loss in soil, enzyme degradation and glucose equivalents while the modified starches had the slowest and least values compared with the native starch. Thus, acetic anhydride and citric acid modification improved the cross linking of the native starch thereby making it less biodegradable but not affecting its overall biodegradability.

Key words: Biodegradation, water absorption, reducing sugar, modification, cassava starch, lignin, TPS (Thermoplastic starch).

1. Introduction

The blooming use of synthetic polymer materials has brought about a great deal of environmental problems despite their merits. Solid waste originating from these materials is a major contributor to environmental pollution because it is non-biodegradable. Moreover, these synthetic polymers usually originate from products of petroleum and are non-biodegradable. In order to overcome this grave problem, more attention is now being shifted to alternative polymer materials that are biodegradable.

Natural polymers have attracted greater attention because they are inherently biodegradable [1]. Among the natural polymers, starch and cellulose are of prime interest due to their complete biodegradability [2], availability from many plants [3], cost effectiveness and renewability [4]. Likewise, cellulose has substantial stiffness, desired mechanical features and thermal recyclability by combustion. Besides, the low density of cellulose results in particular mechanical properties [5].

If cellulose fibers combine with thermoplastic matrix, immense difficulty regarding incompatibility between polar and hydrophilic fibers and non-polar and hydrophobic matrix will result. Thus, in order to improve matrix reinforcement adhesion in composites, starch or the surface of cellulose can be physically or chemically modified. The modification can be carried out by acetylation and alkali treatment [6].

In this study, native cassava starch was modified with acetic anhydride and citric acid.

2. Methodology

2.1 Materials

Cassava tubers (\textit{Manihot esculenta}) were purchased from the local farm of Minna, Niger State while bamboo stems (\textit{Bambusa vulgaris})
were collected from the Izom forest in Gurara, Niger State. Analytical grades of glycerol, ethanol, acetic anhydride (CH$_3$CO)$_2$O), citric acid, sodium hydroxide, hydrochloric acid, sulphuric acid, hydrogen peroxide, glucose, dinitrosalicylic acid, sodium acetate, acetic acid, α-amylase and cellulase were purchased.

2.2 Starch Modification

Cassava starch was modified with acetic anhydride, citric acid and dual modification with acetic anhydride and citric acid as described by Aning [7], Chowdary and Enturi [8] and Veerreddy et al. [9].

3. Characerization of Modified Starch

3.1 Starch PH

Two grams (2 g) of starch was dispersed in 20 ml of distilled water in a 50 ml beaker for 1 h at room temperature. The pH was, thereafter, measured using a pH meter.

3.2 Determination of Acetyl/Carboxyl Content and Degree of Substitution (DS)

The percent acetylation/carboxylation and DS were determined titrimetrically as described by Aning [7]. One gram of acetylated cassava starch sample/or starch citrate sample were suspended in 50 ml of a 75% ethanol solution. The slurry was kept in a water bath at 50 ºC for 30 min with constant stirring. The slurry was then cooled at room temperature and 40 mL of 0.5 M potassium hydroxide was added. The slurry was allowed to stand for 72 h at room temperature with occasional swirling. The excess alkali was titrated with 0.5 M hydrochloric acid using phenolphthalein as an indicator. The solution was allowed to stand for another 2 h. Any additional alkali that might leach from the sample was titrated. Blank with native starch was analysed concurrently. The sample volume, the hydrochloric acid normality and the volume of hydrochloric acid required to titrate the blank and sample were recorded. Duplicate measurements were performed for each starch sample [7].

$$\text{% Acetylation/carboxylation} = \frac{[\text{ml (blank)} – \text{ml (sample)}] \times \text{normality of acid} \times 0.043 \times 100}{\text{Weight of the sample, g (dry basis)}}$$

3.3 Film Production

Nanocomposite films containing fillers (bamboo lignin and bamboo nanofiber) were prepared by casting method. Then nanocomposites were prepared from 100 wt% native and modified cassava starch, containing 5 wt% fillers and 50 wt% plasticizer (glycerol) and 50% (w/v) water. The solutions were prepared by heating the material over a magnetic stirrer at 70 ± 2 ºC and medium stirring rate for 15 min. The mixtures were then poured into petri dishes and oven-dried at 50 ºC for 24 h to obtain the films.

3.4 Water Absorption Test

Pieces of the films were first oven-dried at 50 ºC for 24 h after which, samples were weighed for the dry weight, and then placed in a bath containing distilled water at room temperature. After 1, 3, 5, 7, 9, and 11 h, the samples were removed from distilled water and weighed. The water absorption capability (WAC) was calculated with the equation below [10]:

$$\text{WAC %} = \frac{W_{wet} - W_{dry}}{W_{dry}} \times 100$$

where, $W_{wet}$ represents the weight of the wet specimen and $W_{dry}$ represents the weight of the dry specimen.

3.5 Enzyme Degradation Test

Enzyme degradation test of the films were carried out as described by Zahra et al. [11] with slight
modifications. The enzymatic reaction mixture, comprising 1 ml of α-amylase and cellulase and 25 ml of 0.1 M acetate buffer, was placed in clean beaker. The dried samples of the films were cut into 3 × 3 cm² specimens, weighed, and immersed in the beaker. After 1, 3, 5, 7, 9 and 11 h, the samples were removed, rinsed with distilled water to remove the enzyme, dried, and weighed, respectively. The DED (Degree of enzymatic degradation) was calculated using the Eq. (4) [12]:

$$\text{DED} \% = \frac{W_0 - W_d}{W_0} \times 100$$ (4)

where, $W_0$ and $W_d$ represent the initial and final weights (before and after degradation, respectively) of the blends.

3.6 Detection of Reducing Sugars

Reducing sugars in degradation solutions were quantified by the dinitrosalicylic acid (DNS) method [13]. DNS (1 ml) was added to 1 ml of the sample to be analysed using 1 mg/ml glucose stock solution as a standard. The blank was prepared using 1 ml of control sample. The mixture was heated at 90-100 °C for 10 min, cooled to room temperature, then 5 ml of distilled water was added, and the absorbance at 540 nm was measured. The respective carbohydrate concentrations were obtained by comparison with a standard curve.

3.7 Soil Burial Test

Soil burial test was performed on the films according to the method described by Thakore et al [14]. The films were buried in compost soil that was purchased from a flower nursery; Two different pots were filled to their approximate capacity of 10 L with soil and compost. The samples were cut into 30 × 50 mm² pieces and buried in the soil at a depth of 10 cm. The soil was placed in the laboratory, and the moisture of the soil was maintained by sprinkling water at regular time intervals. Excess water was drained through a hole at the bottom of the pot. The degradation of the samples were determined at regular time intervals (7 days) by carefully removing the sample from the soil and washing it gently with distilled water to remove soil from the film. The sample was dried under vacuum until a constant weight was obtained. Weight loss of the sample over time was used to indicate the degradation rate of the soil burial test. The soil burial test was studied by evaluating the weight loss of the film over time. The weight loss was determined every seven days from the starting day, and was calculated using the equation below:

$$\text{Weight loss} \% = \frac{W_i - W_d}{W_i} \times 100$$ (5)

where, $W_d$ is the dry weight of the film after being washed with distilled water and $W_i$ is the initial dry weight of the specimen.

4. Results and Discussion

The pH, percentage acetylation/carboxylation and DS of modified and native starches are shown in Table 1.

The pH values of the chemically modified starches (5.52, 5.15, 5.38 for starch acetate, starch citrate and starch mixture respectively) were significantly lower (P > 0.05) than that of the native starch (7.43) due to the reaction of starch with acetic anhydride and citric acid. These starches falls within the pH range obtained for most starches employed in the food, cosmetics and pharmaceutical industries therefore,
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they may be employed in the food, pharmaceutical, and cosmetics industries [15]. This result is consistent with that of Omojola et al. [16] and Nnemeka et al. [17] who also obtained a pH value of 4.59 for Icacinia starch citrate [16, 17].

Result also showed that the modified starches are significantly substituted with acetyl group and carboxyl group compared to the native starch. Even though, the Food and Drugs Administration of the United States (FDA), Food and Agriculture Organisation (FAO) and Ricón, Rached, Aragoza & Padilla stated that the maximum acetyl content allowed for food starches utilization in food industry is 2.5% [18, 19]. The value obtained for the acetylated cassava starch in this study is higher than 2.5% thus; it is not suitable for use in the food industry (FDA) but can be used as packaging material. The high values obtained are similar to those reported for acetylated banana starch [20], barley starch [21], corn starch [7], maize starch [22], oat starch 23], pea starch and chick-pea starch [21].

4.1 Water Absorption Capacity (WAC)

The effect of starch modification on water absorption capacities of the films is shown in Fig. 1. Result from this study showed that the water absorption capacity of modified starch films were significantly (p > 0.05) lowered compared to that of TPS (negative control) and native starch film (positive control). Result also showed that the water absorption capacity varied among the modified starches with starch citrate being lowest.

Modification led to decrease in both weight loss and water uptake. Modification decreased the water solubility of films because the modifiers (acetic anhydride and citric acid) act as a barrier against the diffusion of the water to films. Low water uptake/absorption in the modified starch films can be explained in terms of changes in molecular structure of the starch. A cross-link network is formed in the starch molecules when hydroxyl group (OH) is replaced with acetyl and carboxyl groups, which inhibit the absorption of water as these groups are less hydrophilic than the OH group [24]. This result is similar to that of Khan et al. [25], Lawal [22], Michelle [24], Taghizedeh et al. [26] and Abbasi [27] who also observed low water absorption capacities for modified corn, cocoyam, and tapioca starches . Since most of the former packaging films made out of starch are not suitable for packaging because they absorb water easily, acetic anhydride and citric acid modified cassava starch may be used because water absorption is reduced.

![Fig. 1 Water absorption capacity of TPS, modified and native starch films.](image)
5. Degree of Enzyme Degradation (DED) and Yield of Reducing Sugar Using DNS Method

The effect of starch modification on the degree of enzymatic degradation of the various films and their release of reducing sugar (glucose) are presented in Figs. 2 and 3. The result showed that enzyme degradation of the films is significantly different from one another. Degradation is most rapid in native starch films especially pure TPS film and slower in modified films with starch mixture being the slowest.

Amylase attacks the bonds faster than the modified and native starch films such that about 95.87% weight loss was observed after 9 hours of immersion in enzymatic solution. On the other hand, weight losses of 98.61%, 95.99%, 95.81% and 95.94% were observed for native, acetylated, carboxylated and mixture starch films respectively after 11 hours.

The result also showed that the rate of glucose production was most rapid for the native starch films...
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particularly TPS. However, the modified starch films showed a very much decrease in glucose production such that the amount of glucose produced after 6 hours of immersion into the enzymatic solution were; 4.998 mg/ml, 2.705 mg/ml, 2.511 mg/ml, 2.366 mg/ml and 1.997 mg/ml for TPS, native, starch acetate, starch citrate and starch mixture films respectively; showing the same trend as in enzymatic degradation.

Natural starch is known to be hydrophilic as a result of the free OH group in its structure. The substitution of this group with acetyl and carboxyl groups increases cross linking of the starch, decreases the hydrophilicity thus, stabilizes and slows degradation of the films.

The branched chains are attacked by glucoamylases. This is because α-amylases are endoamylases, which speed up the hydrolysis of the internal α-1, 4-glucosidic linkage in the starch in a random manner. These enzymes break down the starch into smaller sugar units, which in the end are converted to the basic glucose units. Because acetic anhydride and citric acid have replaced some of the α-1,6glycosidic bonds and make them unavailable for amylase activities, amylase action is reduced thereby accounting for the slowness of the film. Hence the hydrolysis of the cassava starch films weakened the interaction between cassava starch alone as is the case of TPS and cassava starch and the fillers as is the case of modified and native starch nanocomposite films thereby resulting to higher weight loss by the TPS film after just 5 hours of immersion into the enzymatic solution followed by that of the native starch nanocomposite film and then the modified starch nanocomposite films. This result is similar to that of Azahari et al. [28] and Zahra et al [11].

6. Soil Burial Test

The result in Fig. 4 shows the effect of starch modification on the weight loss of starch films in the compost soil against time. The result revealed that the weight losses varied among the films with modified starch films having the least weight loss and slowest rate of degradation compared to TPS and native starch films. About 98.11% of TPS film degraded in compost soil in 96 hours of the study. Next is native starch film with degradation rate of 97.37% in compost soil in about 120 h of the study. The modified starch films however, degraded in 144 h with starch mixture film having the least degradation rate (97.09%, 96.29% and 95.93% for starch acetate film, starch citrate

Fig. 4  Weight loss of TPS, modified and native starch films after 144 h in soil.
film and starch mixture film respectively). The weight loss of the modified starch films during composting in this study clearly showed that the modified starch films are biodegradable. However, the weight losses of the modified starch films are slower than TPS and native starch film suggesting that the biodegradability of the modified starch films is lower than native starch film. This result is similar to Lin [29] who reported that the degradation rate of native corn starch is faster than that of modified corn starches. It therefore further shows that the acetic anhydride and citric acid improves the strength of the films leading to slower degradation in the compost soils. However, that these modified starches have increased strength and biodegradation means that it could be a good alternative to synthetic packaging materials used in most developing world.

7. Conclusions

The present study showed that modification with acetic anhydride and citric acid lowered the water absorption capacities of cassava starch films thus, slowing down their degradation rates but not affecting their overall biodegradability. Therefore, the modified starch films with acetic anhydride and citric acid may substitute the non-biodegradable polyethylene mulch film that is seen as a significant environmental pollutant.

Reference


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