

Biodegradation Behaviour of Thermoplastic Starch: The Roles of Carboxylic Acids on Cassava Starch

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Abstract The process of biodegradation upon modified thermoplastic starch with various types of carboxylic acids was performed via soil burial testing. As such, modification of thermoplastic starch via esterification with citric acid and ascorbic acid was made varied within the range of 1–4 by weight percentage (% w/w). As a result, after citric acid modification and ascorbic acid modification displayed higher moisture resistivity (15.04 and 20.46%) compared to thermoplastic starch, which resulted in 43.79% respectively. In addition, environmental degradation was determined by performing a biodegradation process by using compost soil. Besides, Fourier transform infrared spectroscopy and high performance liquid chromatography tests were carried out to assess the biodegradability attribute of the starch film. The findings showed that the both citric acid and ascorbic acid modifications exhibited lower rates of weight loss, consequently due to the action demonstrated by of carboxylic acids that inhibited the invasion of microbial after exposure to environmental degradation. Furthermore, the optical analysis revealed that both citric acid and ascorbic acid possessed capability to retard the invasion of microbial upon modifications observed from its rather limited formation of microbial colonies. Nevertheless, the carbonyl

index portrayed lower growth rate for citric acid and ascorbic acid, in comparison to unmodified thermoplastic starch. Hence, the study concludes that the addition of carboxylic acid had reduced the rate of degradation for the thermoplastic starch, besides improving moisture resistivity.

Keyword Biodegradation · Chemical modification · Moisture resistivity

Introduction

The ever-increasing concern pertaining to the deleterious effects of synthetic plastic materials upon the environment has promoted continuous researches and studies, as well as intense investigations concerning the production of both biodegradable and compostable materials. Besides, various natural materials have been used in conjunction to the production of biodegradable polymers, such as gluten, zein, lignin, cellulose, chitosan and starch [1]. Among these materials, starch has grabbed more attention than the rest due to its unique attributes, which are being completely biodegradable and its availability from renewable sources at a low cost. On top of that, native starch has been found to acquire several thermoplastic-like properties when processed with a plasticizer under controlled shear and processing temperatures. In fact, starch consists of two components, namely amylose and amylopectin. Amylose refers to a linear polysaccharide with α -1,4-linked D-glucose, whereas amylopectin is comprised of a branched polysaccharide with α -1,6-linked branch points [2].

Despite of its wide range of potential, starch is not commonly used for industrial applications due to its brittleness, aging and hydrophilicity factors. Moreover, plasticized starch fail in satisfying several essential requirements

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meant for general applications because, plasticized starch is sensitive to water. Hence, increase in water content eventually leads, to higher mobility of starch molecules, which also increases the crystallinity attribute of starch due to retrogradation formation. As a result, plasticized starch becomes highly brittle and exhibits almost no elongation at break. Besides, moisture that is absorbed by the plasticized starch further enhances the process of biodegradation, thus promoting microbial attack [3, 4]. Subsequently, plasticized starch can be degraded completely within a short time, and this limits the lifetime of plasticized starch in biopolymer applications. As such, it is important to incorporate hydrophobic properties into starch-based materials. In fact, several modification have been initiated to reduce these limitations [2]. One such chemical modification is the addition of carboxylic acid in the midst of processing of thermoplastic starch (TPS). The addition of carboxylic acid enhances both mechanical and thermal properties, aging, as well as moisture resistivity, in the modified TPS. Thus, substitution of the hydrophobic group induces inhibition of starch retrogradation, thus resulting in lower moisture absorption and aging properties [5]. In view of this, several biodegradation studies have been carried out to validate their improved properties.

Furthermore, studies related to biodegradation that involve the use of soil or compost, especially to stimulate the degradation process of materials have been mainstreamed. In addition, biological environments that permit the growth of various microbial species, increase the rate of degradation. As such, the degradation of starch can be performed either in aerobic or anaerobic conditions. In fact, three metabolic pathways may occur under aerobic conditions, which are (1) oxidation of a terminal methyl group leading to the formation of a carboxylic acid derivative group, (2) hydration of double bonds to tertiary alcohol (aldehyde and ketones), as well as (3) oxygenase-catalyzed cleavage of the internal bonds resulting in polymeric molecular chain breakage [6]. On top of that, the Fourier transform infrared (FTIR) spectroscopy was used to confirm the activities.

Apart from that, the effects of the decomposition of both TPS and modified-TPS film were assessed in terms of the rate of moisture absorption, as well as the mass loss of the tested films. Since the modification was performed via esterification of TPS, the degree of substitution (DS) was determined. Additionally, the intensification of the hydroxyl group, which represented the hydrolysis of starch, has been observed by using FTIR. Moreover, optical images were retrieved to analyzed the formation of microbial colonies upon biodegradation. Besides, the reduction in both the amylose and the amylopectin chains, which had been due to the degradation of the starch structure, had been examined via high performance liquid chromatography (HPLC).

Experimental

Materials

Cassava starch was purchased from Thyé Huat Chan Sdn Bhd, whereas glycerol was bought from HmBG Co. Inc. Meanwhile, citric acid and ascorbic acid were purchased from Aldrich Chemical Co. Inc.

Methods

Plasticization

The water content of cassava starch was reduced by drying it in an oven at 80 °C for 30 min. Next, thermoplastic starch was prepared by mixing 65% (w/w) cassava starch with 35% (w/w) glycerol. The mixture was then sealed and stored overnight. On the other hand, both the citric acid and ascorbic acids were first dissolved in additional distilled water. The abbreviations that functioned as labels for each sample and compositions are listed in Table 1. Besides, plasticized TPS, citric acid modification and ascorbic acid modification were prepared as given in the following -: The mixtures were manually fed into a heated two-roll mill machine at 150 °C for 10 min.

Determination of the Degree of Substitution

The degree of substitution was determined by employing the titration method, as proposed by Sudarat et al. [7] (2006) and Silaket et al. [8] (2014). Next, 5 g of starch sample was mixed with 50 mL of distilled water. The mixture was saponified with 0.5 N of sodium hydroxide (NaOH) solution and the excess was then back titrated with 0.5 N of sulfuric acid (H₂SO₄) by using

Table 1 Used symbols and corresponding sample compositions

Symbols	Samples (% wt)			
	Cassava starch	Glycerol	Citric acid	Ascorbic acid
Thermoplastic starch	65	35	0	0
1% Citric acid	65	35	1	0
2% Citric acid	65	35	2	0
3% Citric acid	65	35	3	0
4% Citric acid	65	35	4	0
1% Ascorbic acid	65	35	0	1
2% Ascorbic acid	65	35	0	2
3% Ascorbic acid	65	35	0	3
4% Ascorbic acid	65	35	0	4

phenolphthalein as an indicator. The value of degree of substitution was calculated as follows;

$$\text{Degree of Substitution} = [162 \times \% \text{ substitution}] / 100 \times \text{Mw} - [43] \times \% \text{ substitution} \quad (1)$$

Meanwhile, the percentage of esterification was calculated as follows;

$$\% \text{ substitution} = (25 - V_a) \times (N_b) \times [(M_w)/1000] \times [100/W_s] \quad (2)$$

where V_a refers to the volume of H_2SO_4 , N_b is denoted to the normality of NaOH. M_w represents to the molecular weight of ester group and W_s is the weight of the sample.

Moisture Absorption Test

The moisture absorption measurements were carried out below 100% of relative humidity at room temperature. All the specimens with dimensions of 30 x 10 mm, which had been used for the moisture absorption experiment, were cut from compression sheets. Prior to the absorption experiment, all the specimens were vacuum dried until a constant weight was obtained. The moisture uptake at any point in time, which had been due to moisture absorption was determined by using the following equation;

$$\text{Moisture uptake} = (W_n - W_o) / W_o \times 100\% \quad (3)$$

where W_n refers to the weight of the humid specimens, while W_o represents the original dry value (the initial weight of the material prior to exposure to moisture absorption).

Biodegradation Test of Starch Films

The samples were cut into pieces of 5 x 5 cm. Next, the compost, which was used as the soil medium, was poured into a plastic tray up to a thickness of approximately 18 cm. The samples were buried under 10 cm of soil, at an ambient temperature (~25 °C) which humidity conditions (70–80%). After that, water was sprayed twice a day to sustain the moisture in the compost. The films later were removed at different times and dried in a vacuum oven at 50 °C for 24 h. Besides, the degradation of each sample was determined by the following equation for weight loss;

$$\text{Weight loss} = (W_o - W_a) / W_o \times 100\% \quad (4)$$

where W_o denotes the weight of the original sample before undergoing the degradation test, while W_a refers to the weight of the sample after the degradation test.

Optical Analysis

The changes that took place at the surface of the specimens were examined with an optical microscopy using a Dino-Lite AM4815TL series microscope at $\times 100$ magnification.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectra were collected from the thermoplastic starch, citric acid modification and ascorbic acid modification samples before and after the degradation test with a Perkin Elmer IR spectrum scanner. The thermoplastic starch strips were sliced with a thickness of around 0.2 mm. Besides, 64 scans were signal-averaged to reduce the spectral noise. The degree of degradation was determined by using the carbonyl index.

High Performance Liquid Chromatogram (HPLC)

The changes that were observed in the molecular weight upon the degradation samples were analyzed with the HPLC Shimadzu FRC- 10 A. The HPLC apparatus used was the refractive index detector type, Shimadzu RID-10 A, which was operated at a flow rate of 1 mm/min. Moreover, the column and the detector were maintained at room temperature. After that, 0.1 g of TPS eluent was prepared in 0.5 M of sodium hydroxide (2 ml), as earlier described by Kahar et al. [9]. (2011), with slight modification. Later, a certain amount of the aliquot was diluted in 100 ml of sodium hydroxide and filtered through a 0.45- μm filter to remove any insoluble particles. In addition, mixture of acetonitrile and water (85:15 wt/wt) was used as the mobile phase. Furthermore, samples of 10 μm were injected to analyzed the viscosity of the TPS prior to the changes observed in its molecular weight. The relative increase or decrease in the molecular weight of the TPS was examined based on the shift in the peak position of amylose and amylopectin.

Statistical Method

The analytical determination for all samples had been carried out by using the Minitab Ver. 17. The analysis of variance (ANOVA) and the Tukey's test were employed to compare the differences between the mean values retrieved at 5% level of significance.

Results and Discussion

Degree of Substitution

The indication on the amount of ester group formed on the backbone of the starch that resulted from the esterification of the thermoplastic starch, citric acid modification and ascorbic acid modification had been determined by the degree of substitution. The degree of substitution value for modified-TPS were in the range of 0.027–0.054 and 0.031–0.052 for citric acid and ascorbic acid modification, respectively. Thus, the esterified TPS obtained in this study was the low degree of substitution type [10]. In fact, Sudarat et al. [7] (2006) stated that the increased

amount of esterifying agents induced the increase in the degree of substitution of starch esters due to the substitution of carboxyl group onto the hydroxyl group within the starch backbones. Furthermore, based on Table 2, the thermoplastic starch portrayed no degree of substitution value because no esterification process had taken place. However, after the modification, both modified-TPS demonstrated degree substitution values based on the type of carboxylic acid added. 2% of citric acid modification showed higher degree of substitution value, which was 0.042, in comparison to 2% of ascorbic acid modification, which resulted in higher acidity of citric acid than ascorbic acid. Moreover, the variances in degree of substitution and % of substitution can be attributed to numerous factors, for instances, the type of esterifying agent, pH value, botanical origin as well as structure and size of starch granules [10]. In addition, higher degree of substitution value points towards higher resistivity of modified-TPS to water absorptivity and moisture absorption mainly due to the substitution of hydrophobic ester group with hydrophilic hydroxyl group of starch molecules.

Table 2 Degree of substitution (DS) and percent of esterification (% substitution) of thermoplastic starch, citric acid modification and ascorbic acid modification

Type of starch	% substitution	Degree of substitution
Thermoplastic starch	–	–
1% Citric acid	0.722b	0.027b
2% Citric acid	1.131a	0.042a
3% Citric acid	1.276a	0.048a
4% Citric acid	1.439a	0.054a
1% Ascorbic acid	0.823c	0.031c
2% Ascorbic acid	0.955c	0.036c
3% Ascorbic acid	1.263c	0.047c
4% Ascorbic acid	1.395c	0.052c

Results are expressed as the means of three determinations. Values with different superscript are significantly different ($p < 0.05$) by ANOVA and Tukey's test

Moisture Absorption Test

On top of that, moisture sensitivity is another important criterion for numerous practical applications that involve starch products. Figure 1 illustrates the correlation between the moisture content in plasticized starch with various type of carboxylic acids and storage times at 100% of relative humidity. As time increased, the rate of absorption among the samples increased until an equilibrium was attained, whereby the water uptake displayed by the samples had been at a maximum. Furthermore, thermoplastic starch (43.79%) demonstrated a higher rate of

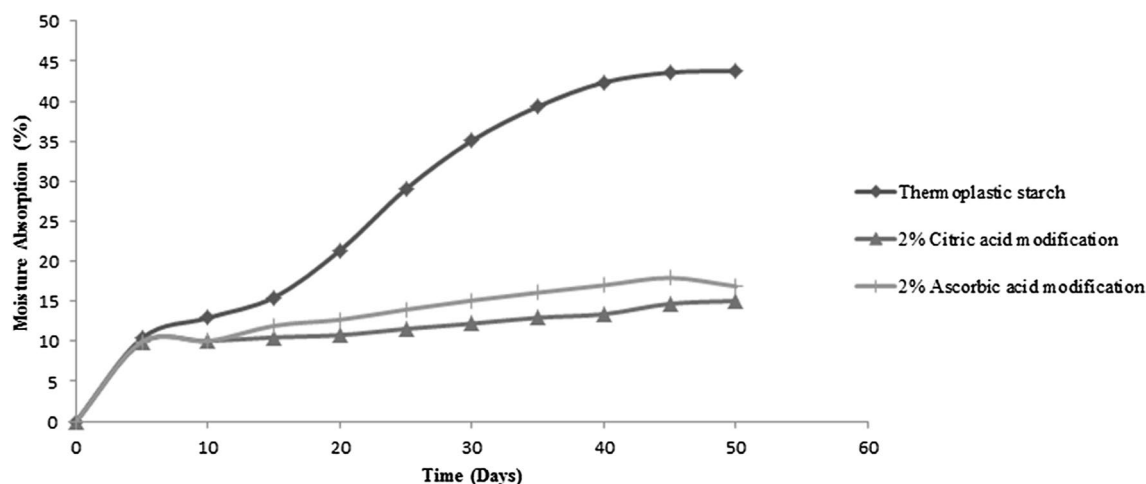


Fig. 1 Rate of moisture absorption of Thermoplastic starch, 2% of citric acid modification and 2% of ascorbic acid modification under 100% of relative humidity

absorption, in comparison to 2% of citric acid and 2% of ascorbic acid modification, all due to the hydrophilic and hygroscopic properties of both the starch and the plasticizer. In addition, the polar groups at the end of the molecules had been predicted to provide access to water, thus enhancing moisture uptake. For instance, an analysis performed by Niazi et al. [11] (2015) showed that the exposure of glycerol-TPS films at relatively high humidity stimulated a more rapid starch retrogradation rate compared to that of urea-TPS films, which generated moisture absorption. On the other hand, moisture resistivity was increased due to the additions of carboxylic acids, which led to a lower rate of absorption. Meanwhile, the formation of ester bonds enhanced the hydrophobic properties of the modified-TPS. Besides, Table 3 portrays a hike in the esterifying agents, which further increased the degree of substitution values. These had been due to the substitution that occurred upon the hydroxyl group found in the backbone of the starch. Furthermore, the strong and stable hydrogen bonds that formed between carboxylic acids and starch had managed to suppress the process of retrogradation, thus hindering the water molecules from moving through the samples.

Table 3 Weight loss of thermoplastic starch film during compost test

Sample	% Weight loss during degradation		
	5 days	20 days	30 days
Thermoplastic starch	30.66a	56.99b	72.63c
2% citric acid	15.36d	42.86e	61.48f
2% ascorbic acid	20.59g	49.85h	65.39i

Results are expressed as the means of three determinations. Values with different superscript are significantly different ($p < 0.05$) by ANOVA and Tukey's test

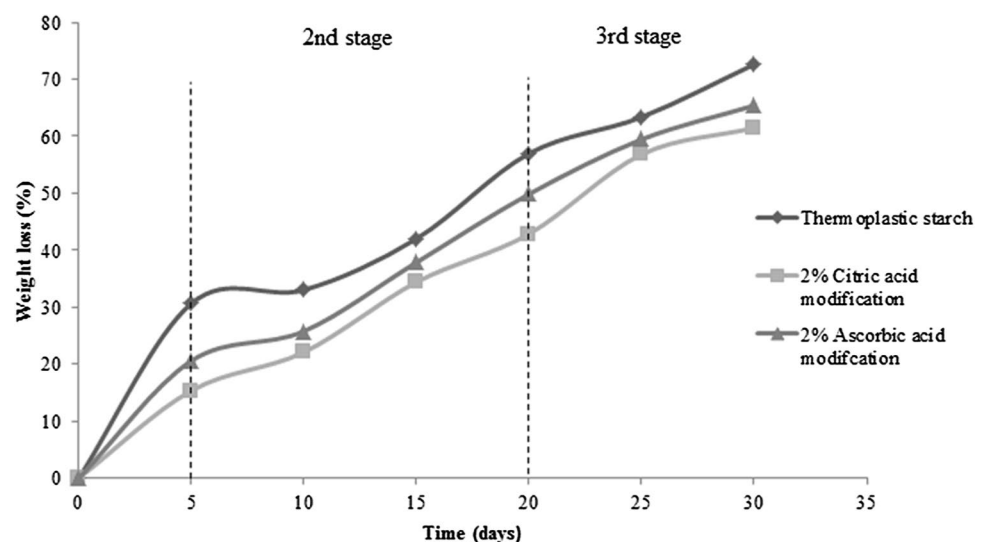
Soil Burial Test

Percentage of Weight Loss

The weight loss observed among the thermoplastic starch films during the degradation process is depicted in Table 3. The weight loss that had been recorded ascertained that the degradation process did take place in the samples due to the presence of moisture and microorganisms in the soil. The weight loss further caused some changes to the surface morphology of the samples. As a result, thermoplastic starch demonstrated the highest percentage of weight loss (72.63%), when compared to modified-TPS.

Figure 2 presents the attributes of the degradation process represented in three stages for of all the starch films that had been tested. In the initial stage, thermoplastic starch recorded 30% of weight loss after 5 days of degradation. On the other hand, weight losses of 15 and 20% were recorded for 2% of citric acid and 2% of ascorbic acid modification, respectively. This primary degradation stage occurred mainly due to the leaching of glycerol from the starch matrix. Besides, more changes took place on the surface of all thermoplastic starch samples, which indicated the beginning of the degradation process that influenced the aspects of tonality and pores with emerging small cracks. Moving on, during the second stage, the weight losses experienced by all the starch samples increased rapidly, in fact, as high as 70% of thermoplastic starch, while 60% for 2% of citric acid and 2% of ascorbic acid modification each, respectively. On top of that, degradation could be associated with the biological activities that took place in the composting vessel at this stage. This phenomenon was further correlated with a lower rate of weight loss. Lastly, in the final stages, the weight loss portrayed by thermoplastic starch was 72%, whereas those of 2% of citric acid

Fig. 2 Representative weight loss curve of a; Thermoplastic starch, 2% of citric acid modification and 2% of ascorbic acid modification for 30 days



modification and 2% of ascorbic acid modification were 61% and 65%, respectively. Although the rate of degradation highlighted a dramatic increase substantial changes had been noted on the appearance of the samples. Moreover, all the starch films were broken into small pieces due to the degradation process. Additionally, the fragmentation of the polymer into lower molecular mass was generated probably due to abiotic reactions, like oxidation and hydrolysis, or biotic reactions, like degradation by microorganisms in the sample [12]. This step was later followed by the bio-assimilation of the starch fragments by microorganisms and their mineralization.

Meanwhile, in the soil, the water absorbed by the samples along with the degradation test, had been expected to cause swelling that could enhance the process of degradation due to increased in microbial attacks. For instance, an experiment conducted by Zuhair and Anwar [3] (2015), revealed that the absorption of moisture from the soil during soil burial and emergence of microorganisms led to a reduction in the mechanical strength of the PP/starch blend. Therefore, organic acids were added to reduce the swelling of the samples, thus impeding microorganism attack in the sample due to improvement in the moisture resistivity. As such, 2% of citric acid modification displayed a lower rate of degradation compared to 2% of ascorbic acid modification with regard to both organic acids. Meanwhile, Henry et al. [13] (2013) discovered that biodegradability did not only depend on environmental degrading conditions, but also on the chemical structure of the polymer. Furthermore, as the citric acid structure formed stronger hydrogen bonds with starch than the ascorbic acid structure did, the moisture resistivity of citric acid had been higher than that of ascorbic acid. Therefore, the microbial attacks on citric acid modification showed slower degradation than those occurred in ascorbic acid modification. Hence, from the analysis of weight loss percentage, one can conclude that the degradation rate displayed by the modified TPS had been improved by the chemical–mechanical hydrolysis process, when compared to that of unmodified TPS, probably due to the strong hydrogen interaction between the hydroxyl groups of the starch molecules and the organic acids.

Optical Analysis

Changes that had been observed in the surface morphology of the samples are presented in Fig. 3. The optical photographs of thermoplastic starch, 2% of citric acid and 2% of ascorbic acid modification after 30 days of soil burial tests exemplified certain changes in their tonality, as well as some formations of voids and cracks. As such, the microbial colonies were represented by the formation of white regions on the surface of the samples. Moreover, Afq and

Azura [6] (2013) confirmed that the white regions noted on the surface of sago starch/NRL indicated the formation of microbial colonies after 4 weeks of composting. In fact, these microorganisms generated enzymes that possessed the capability to break the backbone of the starch. Furthermore, the thermoplastic starch film is illustrated in Fig. 3a, while Fig. 3b presents the formation of a large and wide colony compared to those of modified-TPS (Fig. 3c, d). The variance was attributed to the fact that the hydrogen linkages between starch and glycerol could be easily broken down compared to the hydrogen linkages of starch with carboxylic acids. Furthermore, the thermoplastic starch displayed lower resistivity towards moisture absorption, thus, accelerating microbial attacks. During microbial infection, the microbes formed a colony that spread throughout the entire film, causing hydrolysis of the glycosidic linkages in the starch. This decreased the physical properties, but further increased the mass loss of the plasticized starch film. Besides, more cracks were observed on 2% of ascorbic acid modification, in comparison to those on 2% of citric acid modification, along with the formation of microbial colonies. Hence, ascorbic acid had been found to be more reactive than citric acid, as it can easily oxidize. The oxidation process further caused the formation of voids, which enhanced microbial infection throughout the film [5, 6, 14]. Meanwhile, citric acid functioned as an antimicrobial agent, besides causing plasticizing in the films. Therefore, the citric acid modification developed better resistivity towards microbial attacks than that of ascorbic acid modification. On top of that, since the citric acid modification showed better resistivity towards moisture absorption, it also reduced the formation of voids and infiltration of microorganisms into the film. This result is supported by an experiment conducted by Seligra et al. [15] (2015) that looked into starch/CA, which showed that citric acid decreased microorganisms attacks due to their resistivity towards moisture absorption.

FTIR Analysis

The structural changes that took place upon thermoplastic starch and modified-TPS after soil burial were further analyzed via FTIR. As such, Fig. 4 and Table 4 present the FTIR spectra of thermoplastic starch, 2% of citric acid and 2% of ascorbic acid modification before and after soil burial. The FTIR analysis had been carried out based on the identification of absorption bands resulting from the vibration of the functional groups of TPS before and after the degradation process. Moreover, the wave numbers related to the C–O and C=O groups are depicted in Table 4. As observed after the degradation process, all the samples demonstrated similar trends, except for some slight changes in the intensity of certain absorption peaks, as well as the

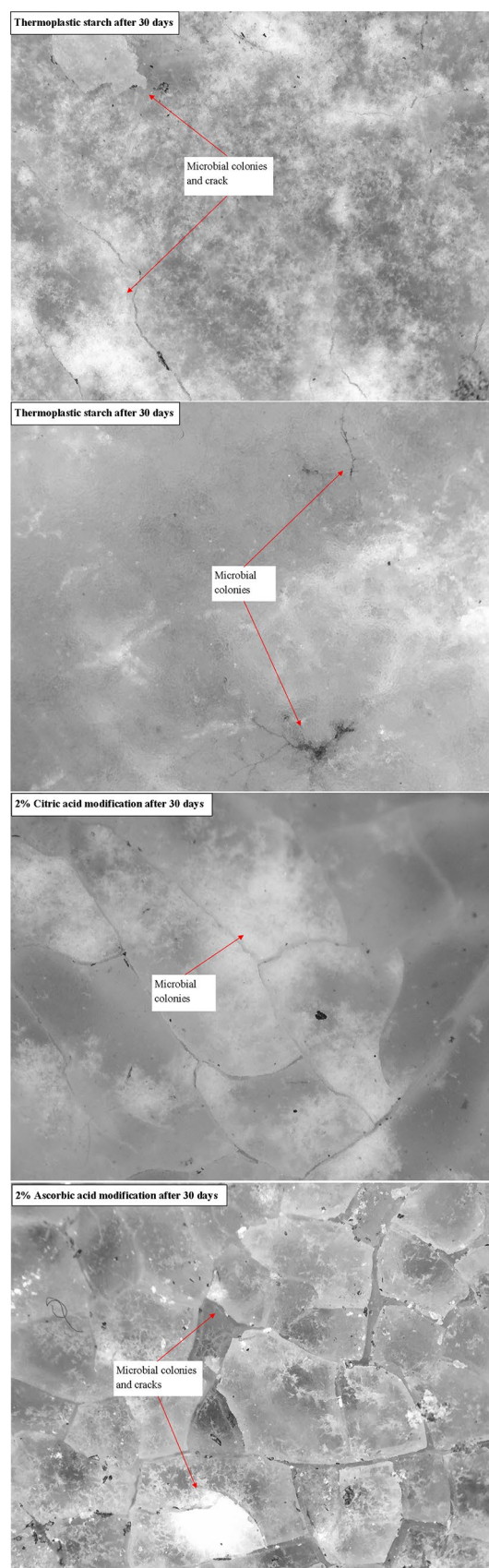
Fig. 3 Biodegradation of; Thermoplastic starch, 2% of citric acid modification and 2% of ascorbic acid modification after 30 days

emergence of new peaks for thermoplastic starch film. Nevertheless, after the degradation process, broader peaks with strong intensities were noticed at the hydroxyl and carbonyl regions. Hence, the intensity of the C=O bands in 2% of citric acid and 2% of ascorbic acid modification increased, indicating that the process of degradation had indeed occurred. These analyses are supported by Yaacob et al. [16] (2016), Afq et al. [6] (2013) and Torres et al. [17] (2011). Furthermore, an additional peak was observed for thermoplastic starch after the degradation process, which reflected the formation of a carbonyl group at 1603.1 cm^{-1} . The formation of carbonyl group on the thermoplastic starch after soil burial confirmed that degradation had actually occurred, thus leading to several changes in the characteristics of the polymer.

Besides, Fig. 4 portrays that 2% of citric acid and 2% of ascorbic acid modification showed less significant changes in the FTIR spectra pattern as degradation took place. This proves that carboxylic acid is capable of protecting TPS from microbial action and retrogradation. On top of that, the carboxyl group in citric acid and ascorbic acid is capable of interacting strongly with the C–O group in glycerol. Hence, the formation of hydrogen bonds is prevented between the hydroxyl groups of TPS, thus reducing the degree of TPS re-crystallization. Therefore, in order to justify the occurrence of biodegradation, the carbonyl index was calculated accordingly. As displayed in Fig. 5, 2% of citric acid and 2% of ascorbic acid modification highlighted lower growth rates for the carbonyl index with respect to thermoplastic starch.

HPLC Analysis

The degree of degradation for thermoplastic starch and modified-TPS had been determined via HPLC analysis. Table 5 presents the data obtained from the HPLC analysis upon molecular weight of starch after 30 days of testing. After 30 days, the samples were found to experience loss of weight due to microbial attacks, which resulted in degradation of starch chain. The microorganisms were infused into the samples through the cracks that appeared on the surface and leached out the plasticizer in the thermoplastic starch, which caused embrittlement. Moreover, several factors, including availability of water, temperature, oxygen usage, minerals, pH, redox potential, as well as carbon and energy sources, influenced the growth of microorganisms that accelerated the microbial attacks. Therefore, the molecular weight of the tested samples reduced as the degradation rate increased. Additionally, the HPLC chromatographs



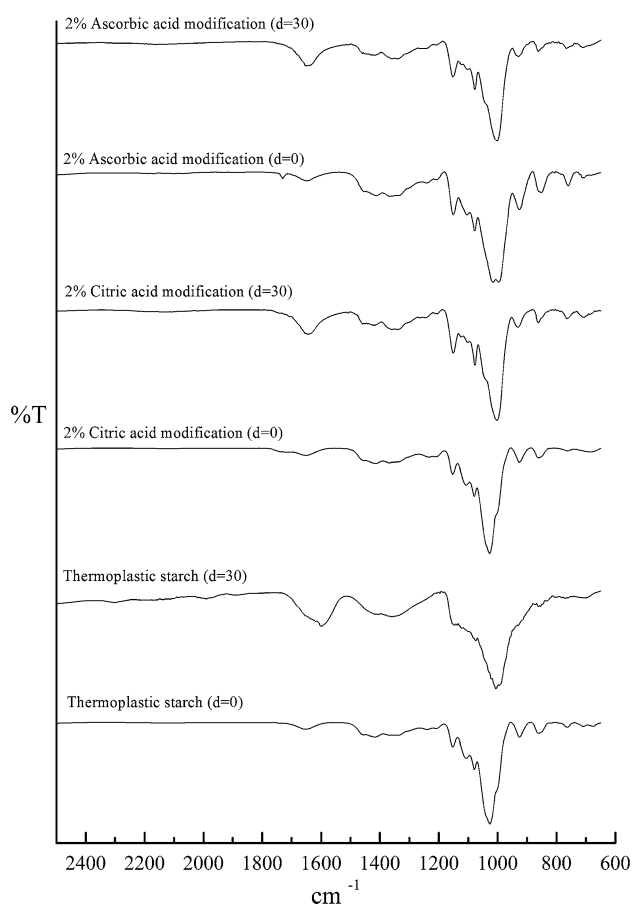


Fig. 4 FTIR spectra for Thermoplastic starch, 2% of citric acid modification and 2% of ascorbic acid modification before and after 30 days of degradation test

Table 4 Influence of soil burial test on the vibration wave number of C–O and C=O groups in FTIR spectrum

Sample	Vibration wave number (cm ⁻¹)	
	0 days	30 days
Thermoplastic starch	1025.8 and 1151.9 925.8	1152.6 and 1008.3 1603.1
2% citric acid	1026.2 and 1152.2 925.4 1740	1146.9 and 1077.6 927.3 1643.6
2% ascorbic acid	1026.5 and 1152.8 924.7 1736	1077.6 and 1146.6 927.3 1649.3

illustrated in Fig. 6a, b, c display the effects of degradation upon thermoplastic starch, 2% of citric acid, and 2% of ascorbic acid modification after 30 days of testing. All the graphs, which exhibited bimodal distribution, portrayed a similar pattern. In fact, both elution profiles ascribed the distribution of amylopectin to the first peak, whereas amylose to the second peak. Nonetheless, after 30 days of

degradation, both the amylose and amylopectin contents were reduced, as shown in Table 5. Besides, the amount of amylose and amylopectin contents of the thermoplastic starch had been evaluated based on the percentage of the area from the chromatogram. In addition, Table 5 shows a greater amount of reduction in amylopectin, in comparison to the amount of amylose after biodegradation. This was generated by the continuous deterioration of the α -1,4-glycosidic bond in amylopectin. Moreover, the continuous cleavage of the amylopectin chain increased the number of amylose (linear) chains as the degradation process took place. Therefore, some reductions were noted in Mw for the degraded starch materials.

Other than that, the chromatogram in Fig. 6 exemplifies that the degraded thermoplastic starch peak eluted later, when compared to that for modified-TPS. Although modified-TPS had lower molecular weight than thermoplastic starch, both displayed lower degradation rate due to their antimicrobial attribute. Furthermore, although both carboxylic acid had been able to deteriorate the starch macromolecule, they continued to function as antimicrobial agents, which impeded the growth of microbes and reduced the microbial attacks on the starch chain. Likewise, Krishna and Srivastava [18] (2010) opined that microorganisms secrete endoenzymes and exoenzymes, attack the substrate and cleave the molecular chain of the polymer into segments. Nonetheless, the addition of organic acids induced the solubility of acids in the microbial cell membrane. These caused leakage across the cell membrane as the interior of the cell was acidified, inducing the inhibition yield to energy metabolism. Moreover, compared to 2% of citric acid modification and 2% of ascorbic acid modification, the degraded 2% of ascorbic acid eluted later than 2% of citric acid modification. Thus, the degradation rate of ascorbic acid modification had been higher than that of citric acid modification. Furthermore, although both carboxylic acid functioned as antimicrobial agents, ascorbic acid alone failed in displaying efficiency in inhibiting microbial growth unless combined with other organic acids. Citric acid, nevertheless, had been observed to act effectively on its own, since 0.3% of citric acid had been capable of deterring the growth of the *Salmonellae*- sp [19].

Conclusion

The results showed that the rate of degradation for modified-TPS had been successfully reduced, while improving its moisture resistivity after the process of esterification, which increased the value of the degree of substitution. On the other hand, the weight loss displayed by the thermoplastic starch was reduced with the addition of citric acid

Fig. 5 Carbonyl index of Thermoplastic starch, 2% of citric acid modification and 2% of ascorbic acid modification before and after soil burial test

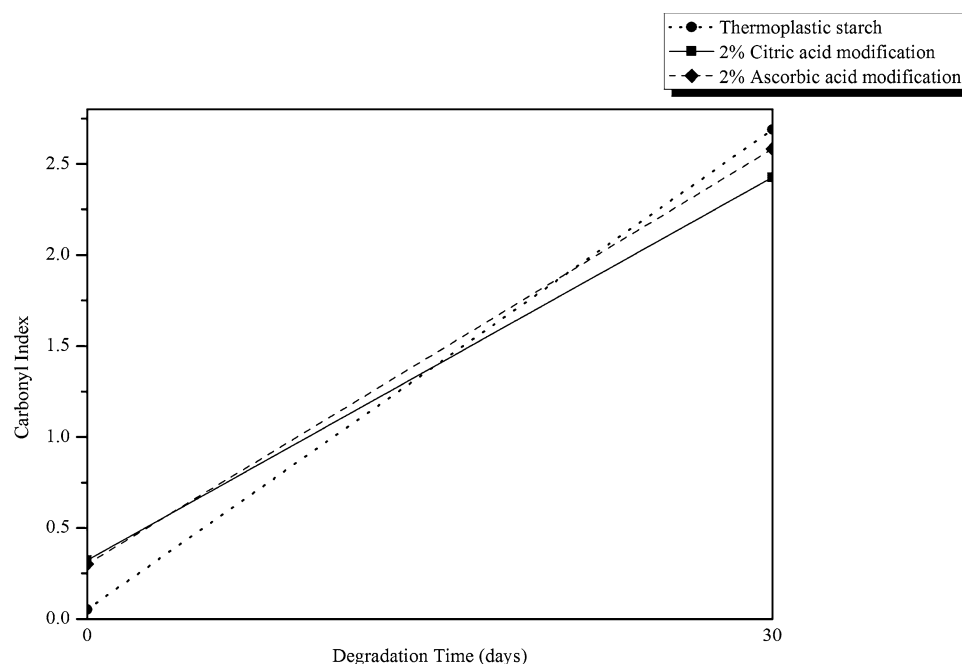


Table 5 Chromatogram data on soil burial test

Sample	Day 0				Day 30			
	Peak 1 ^a		Peak 2 ^b		Peak 1 ^a		Peak 2 ^b	
	Retention time	Area (%)	Retention time	Area (%)	Retention time	Area (%)	Retention time	Area (%)
Thermoplastic starch	2.95	82.25	3.67	15.71	3.159	79.56	3.68	15.51
2% citric acid	3.00	81.49	3.68	16.72	3.08	80.96	3.64	17.83
2% ascorbic acid	3.05	81.63	3.69	16.27	3.112	80.69	3.69	17.29

^aIndicate amylopectin regions

^bIndicate amylose regions

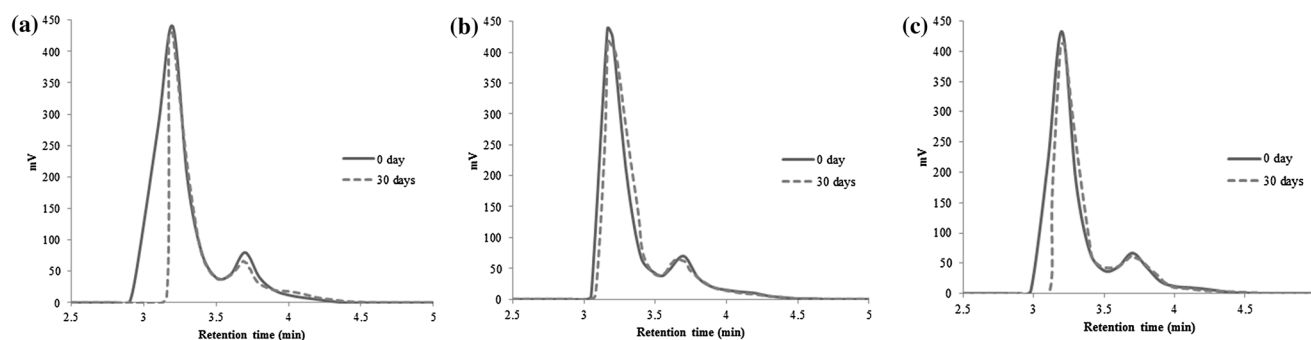


Fig. 6 HPLC chromatogram of **a** Thermoplastic starch, **b** 2% of citric acid modification and **c** 2% of ascorbic acid modification at 0 day and 30 day

and ascorbic acid, which further inhibited retrogradation and microbial growth. Meanwhile, during the process of soil burial process the amorphous fraction of starch, namely

amylopectin, was exposed to microorganism attacks. These results are also supported by observations retrieved from the HPLC analysis.

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