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Biodegradability of Chemically Modified Starch (RS4)/PVA Blend Films: Part 2

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Abstract Biodegradable films were successfully prepared by using cornstarch (CS), chemically modified starch (RS4), polyvinyl alcohol (PVA), glycerol (GL), and citric acid (CA). The physical properties and biodegradability of the films using CS, RS4, and additives were investigated. The results of the investigation revealed that the RS4-added film was better than the CS-added film in tensile strength (TS), elongation at break (*%E*), swelling behavior (SB) and solubility (*S*). Especially, the RS4/PVA blend film with CA as an additive showed physical properties superior to other films. Furthermore, when the film was dried at low temperature, the properties of the films clearly improved because the hydrogen bonding was activated at low temperature. The biodegradation of films was carried out using the enzymatic, microbiological and soil burial test. The enzyme used in this study was amyloglucosidase (AMG), α-amylase (α-AM) and *β*-amylase (*β*-AM). At the enzymatic degradation test, the GL-added films had an approximately 60% degradation, while the CA-added films were degraded about 25%. The low degradation value on CA-added film is attributed to low pH of film added CA that deactivated the enzymatic reaction. The microbiological degradation teat was performed by using *Bacillus subtilis* and *Aspergillus niger*.

Keywords Chemically modified starch (RS4) · Physical properties · Biodegradability

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Introduction

Biodegradable materials derived from renewable resources have been the center of public interest for environmental protection and sustainable development [1–3]. Biodegradable polymers are, however, suitable in many commodities and medical applications such as packaging, surgical implants, controlled release, and drug delivery systems although their use is still limited because of their high cost or low performances [4].

Natural biopolymers including starch, cellulose, and chitosan were tested, alone or combined with synthetic polymers, for the possibility of forming a fully or partially biodegradable film [5]. Of these materials, starch has been considered the most attractive candidate because of its low cost, easy availability and high production from annually renewable resources [6]. However, the low water resistance and high brittleness of starch films have limited their extensive application [7–9]. Therefore, many attempts have been made to overcome these problems by blending starch with synthetic polymers. However, the biodegradability of starch film decreases with the addition of non-degradable synthetic polymers. Therefore, much interest lies in blending starch with biodegradable synthetic polymers [10].

Starch-based blends present the potential to be widely used in the biomedical and the environmental fields, as they are totally biodegradable, inexpensive (when compared to other biodegradable polymers) and available in large quantities [11]. Furthermore, starch-based polymers can also be converted into complex geometries using standard equipment developed for the processing of synthetic polymers. The properties, applications, and the processing procedures of biodegradable starch-based thermoplastic blends such as starch/poly-caprolactone, starch/cellulose acetate and starch/(ethylene-vinyl alcohol copolymer), have already been reported in the literature [2, 3, 6, 12]. However, new areas are emerging including drug delivery systems, hydrogels, bone cements and bone replacement/fixation devices [13].

The techniques for increasing the compatibility between starch and synthetic polymers include adding a compatibiliser to the blends [14], chemical modifications of the synthetic polymers [15] and starch. They have been proved as effective measures to improve the properties of film. A number of methods are available in starch modification including esterification [16], oxidation [8], etherification and cross-linking [8, 17].

In this work, we report on biodegradability of the starch/PVA blend films reported in our previous work [17]. The biodegradation of films was carried out by using the enzymatic, microbiological and soil burial test.

Experimental

Materials

Starch (cornstarch: CS) was obtained from Doosan Corn Products Korea, Inc. (Korea). Polyvinyl alcohol (PVA), reagent grade glycerol (GL), citric acid (CA), sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), and sodium sulfate were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). α-Amylase (α-AM) (250,000 U/g), β-amylase (β-AM) (50,000 U/g) and amyloglucosidase (AMG) (20,300 U/mg from Rhizopus mold) were purchased from Sigma (St. Louis, MO, USA). *Bacillus subtilis* (RKY3 KCTC 10412BP), which was isolated from soil, was maintained in our laboratory. *Aspergillus niger* (KCTC6910) was purchased from Korean Collection for Type Cultures. PVA was 99% hydrolyzed with a molecular weight average of 89,000–98,000. The water used to prepare starch/PVA blend films was redistilled after deionization.

Preparation of Starch/PVA Blend Films

Chemically modified starch (RS4) was prepared using the method of Shin et al. [17, 18]. Briefly, RS4 starches were prepared from starch (50 g, dry basis, db), cross-linked with sodium trimetaphosphate (STMP, 5.94 g) and sodium tripolyphosphate (STPP, 0.06 g) under alkaline condition (pH 11.5) at 45 °C for 3 h. Cross-linked RS4 starch was ground and passed through a 100 mesh sieve (under Φ 150 μm).

Films were obtained by the casting method [2]. First, PVA solution was prepared by dissolving PVA in hot water (95 °C). Cornstarch (CS), chemically modified starch (RS4), and the additives (GL and CA) were mixed together with water using a Kitchen aid mixer for 10 min. Formulations contained 40% GL and CA (weight basis). PVA solution and mixed CS or RS4/additives were kept at 95 °C for 5 min. Then, the mixture was blended to form homogeneously gel-like solution with a mechanical stirrer (1,200 rpm) at room temperature for 50 min. The total polymer amount was 100 g. CS, RS4, and PVA had the same mass ratio, and the content of additives was expressed as mass percent ratio of additives to total CS, RS4 and PVA weight, respectively. Bubbles, the by-product of preparation, were removed by using an aspirator. The mixing composition is shown in Table 1. The gel-like solution thus prepared was poured on a pre-warmed 70 °C Teflon mould (200 × 200 × 2 mm). Water was evaporated from the moulds in a ventilated oven at 50 °C for 12 h and in a cold laboratory chamber at 5 °C for 72 h. Dried films were put in open polyethylene bags and stored at 25 °C and at RH 55% for 1 week before the measurements were performed.

Table 1 Composition of starch/PVA blend films

Sample name	CS (%)	RS4 (%)	PVA (%)	Glycerol (wt.%)	Citric acid (wt.%)	Drying method[a]
CSP-1	5	–	5	–	–	H
CSP-2	5	–	5	–	–	L
RS4P-1	–	5	5	–	–	H
RS4P-2	–	5	5	–	–	L
CSPGL40-1	5	–	5	40	–	H
CSPGL40-2	5	–	5	40	–	L
RS4PGL40-1	–	5	5	40	–	H
RS4PGL40-2	–	5	5	40	–	L
CSPCA40-1	5	–	5	–	40	H
CSPCA40-2	5	–	5	–	40	L
RS4PCA40-1	–	5	5	–	40	H
RS4PCA40-2	–	5	5	–	40	L

The total polymer amount was 100 g and water is added to 90% of total polymer

[a] H: Film dried in a ventilated oven at 50 °C for 12 h; L: Film dried in a cold laboratory chamber at 5 °C for 72 h

Physical Properties of Films

Tensile strength (TS) and elongation (%*E*) were evaluated for each film using the Instron 6012 testing machine. Three dumbbell shaped specimens (ASTM D-412) were cut from each film. Each specimen had a width of 12 mm. The average thickness of the specimen was about 0.15 mm. The thickness of the films was measured with a mechanical scanner (digital thickness gauge 'Mitutoyo', Tokyo, Japan) at 15 random positions around the film. The mean standard deviation within the film was about 5% of the average thickness. The gauge length and grip distance were both 50.0 mm. Crosshead speed was 20 mm/min and load cell was 250 kg_f. The tests were carried out at 25 °C and 54% RH in a constant temperature and humidity room.

The swelling behavior (SB) and solubility (*S*) of films were measured by the following method. Dried starch/PVA blend films were immersed in distilled water at room temperature (25 °C). After the equilibrium (24 h), moisture on the surface of the film was removed, and the weight of the films was measured. The swelling behavior (SB) in starch/PVA blend film was calculated as:

$$\mathrm{SB} = \frac{(W_e - W_0)}{W_0} \quad (1)$$

where W_e is the weight of starch/PVA blend film at the adsorbing equilibrium, and W_0 is the first dry weight of starch/PVA blend film.

The swelled starch/PVA blend films were dried again for 24 h at 60 °C, and its solubility (*S*) was calculated by the following equation:

$$S = \frac{(W_0 - W_d)}{W_d} \quad (2)$$

where W_0 is the first dry weight of starch/PVA blend film, and W_d is the dry weight of swelled starch/PVA blend film.

Degradation Test

Enzymatic Degradation Test

The enzymatic reaction mixture of two solutions, one comprising of 100 mL of distilled water containing 0.1 g of α-amylase (α-AM) and 0.5 g of β-amylase (β-AM) and the other solution comprising of 100 mL of distilled water containing 1 g of amyloglucosidase (AMG), was placed in the clean conical flasks.

The dried samples were cut into 3 × 3 cm square specimens, weighed, and immersed in the conical flasks. The flasks were placed in a shaking incubator with a rate of 120 rpm for 60 h at 30 °C. The samples were removed and rinsed with distilled water to remove the enzyme, and dried in oven for 24 h at 50 °C before they were weighed. The degree of enzymatic degradation (DED) was calculated by the following equation:

$$\mathrm{DED}\% = \frac{(W_I - W_H)}{W_I} \times 100 \quad (3)$$

where W_H is the dry weight of the specimen after enzymatic treatment and W_I is the initial dry weight of the specimen.

Microbiological Degradation Test

Bacteria (*B. subtilis*) and fungi (*A. niger*) were used for the microbiological degradation test. The bacterial strain used in this study was *B. subtilis*, which was isolated from the traditional Korean soybean paste and used as an inoculum for the degradation study. Lactobacilli MRS broth (Difco, Detroit, MI, USA) was used for microorganism. The broth was composed of the following components (per liter); 10 g peptone, 10 g beef extract, 5 g yeast extract, 20 g glucose, 1 g polysorbate 80, 5 g ammonium citrate, 5 g sodium acetate, 0.1 g $MgSO_4$, and 2 g K_2HPO_4. Two % (v/v) *B. subtilis* and *A. niger* culture broth was inoculated in 100 mL MRS broth with the clean conical flasks. The dried samples were cut into 3 × 3 cm square specimens, weighed, and immersed in the conical flasks. The flasks were placed in a shaking incubator with a rate of 120 rpm for 30 days at 30 °C. The samples were removed and rinsed with distilled water to remove the bacteria and fungi, and dried in an oven for 24 h at 50 °C before they were weighed. The degree of microbiological degradation (DMD) was calculated by the following equation:

$$\mathrm{DMD}\% = \frac{(W_I - W_M)}{W_I} \times 100 \quad (4)$$

where W_M is the dry weight of the specimen after microbiological treatment and W_I is the initial dry weight of the specimen.

Soil Burial Degradation Test

Soil burial degradation was performed as described by Devi and coworkers [19] with a slight modification. The garden pots with an approximate capacity of 10 L were filled with soil taken from a culture field in Naju City (Korea). The plastic samples were cut into 3 × 3 cm pieces and buried in the soil at the depth of 10 cm. The pots were placed in an uncovered gazebo. The soil was kept moist by sprinkling water at a regular time interval to maintain 20–40% humidity. The excess water was drained through the hole at the bottom of the pot. The degradation

of the specimen was determined at a regular time interval (15 days) by taking the specimen carefully from the soil and washing it gently with distilled water to remove the soil. The specimen was dried in an oven until a constant weight was obtained. Weight loss of the specimen with time was used to indicate the degradation rate in the soil burial test. The soil burial degradation test started on November 10, 2005 and ended on April 10, 2006.

Results and Discussion

Physical Properties of Films

The mechanical properties of cornstarch (CS)/PVA films and the chemically modified starch (RS4)/PVA films are shown in Fig. 1a, b. Tensile strength (TS) and elongation (*%E*) represent the force per unit area required to tear the film and the ability of the film to stretch [8]. Both TS and *%E* of RS4/PVA films were greater than CS/PVA films [17]. It was also observed that when the film was dried at low temperature, the properties of the films were clearly improved. Compared to the starch/PVA blend film to which additives of the same weight percent were added, TS and *%E* of the film dried at 5 °C were higher than those of the film dried at 50 °C, probably because hydrogen bonding was activated at the lower temperature [2].

The water absorption capacity and the degradability are the most important properties for biodegradable materials [20]. The starch/PVA blend films were degraded by surface absorption of moisture and microorganisms, such as fungi and bacteria. The water absorbed on the materials allowed the microorganisms to grow and to utilize the material as an energy source.

Figure 2a, b shows the swelling behavior (SB) and solubility (*S*) of CS/PVA films and RS4/PVA films. In our previous report [17], we have verified that SB or *S* value of the films using RS4 was lower than those using CS. And, in

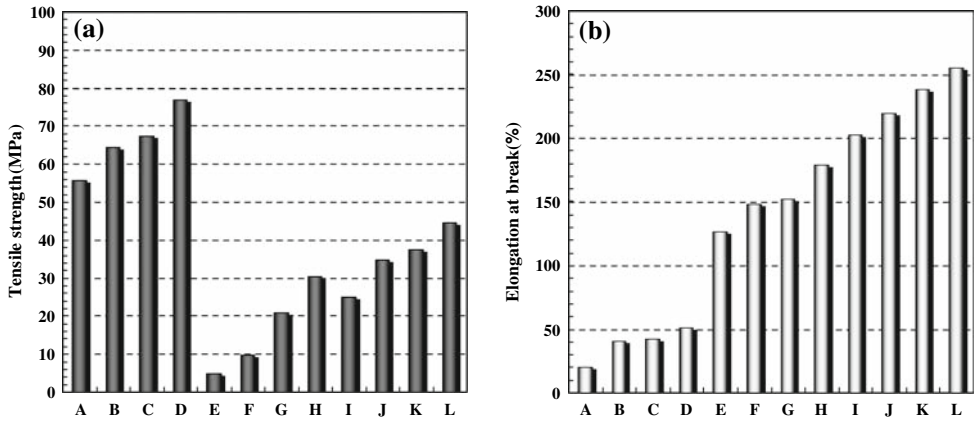

Fig. 1 Tensile strength (TS) and elongation (*%E*) of CS/PVA blend films and RS4/PVA blend films. Where films were stored at 25 °C at RH 52% for 7 days. (**a**) TS of CS or RS4/PVA blend films added glycerol (GL) and citric acid (CA). (**b**) *%E* of CS or RS4/PVA blend films added glycerol (GL) and citric acid (CA) (A: CSP-1, B: CSP-2, C: RS4P-1, D: RS4P-2, E: CSPGL40-1, F: CSPGL40-2, G: RS4PGL40-1, H: RS4PGL40-2, I: CSPCA40-1, J: CSPCA40-2, K: RS4PCA40-1 and L: RS4PCA40-2)

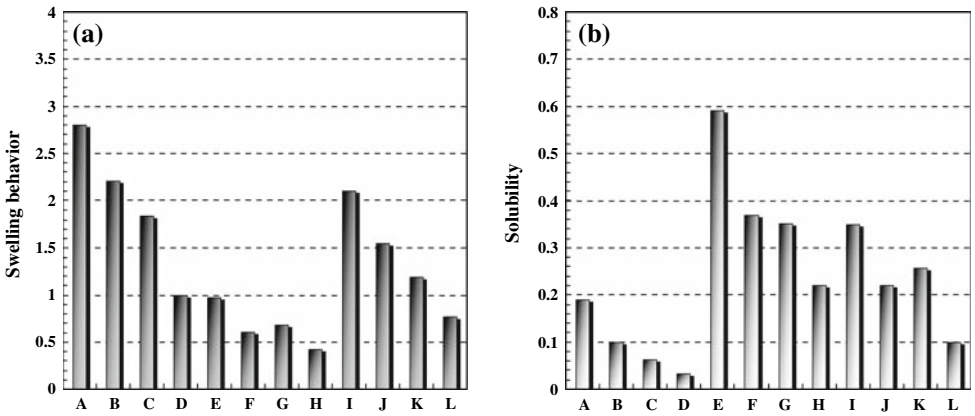

Fig. 2 Swelling behavior (SB) and solubility (*S*) of CS/PVA blend films and RS4/PVA blend films. (**a**) SB of CS or RS4/PVA blend films added glycerol (GL) and citric acid (CA). (**b**) *S* of CS or RS4/PVA blend films added glycerol (GL) and citric acid (CA) (A: CSP-1, B: CSP-2, C: RS4P-1, D: RS4P-2, E: CSPGL40-1, F: CSPGL40-2, G: RS4PGL40-1, H: RS4PGL40-2, I: CSPCA40-1, J: CSPCA40-2, K: RS4PCA40-1 and L: RS4PCA40-2)

this paper, we were able to confirm that SB and *S* value of films using RS4 and dried low temperature were the lowest. From these results, we may expect that the degree of degradation of films using RS4 would be low, but from the perspective of the physical properties, SB and *S*, in particular, the RS4-added film was found to be better than the CS-added film.

Degradation of Films

Degradability of polymers is a critical functionality in their application. Currently, no official standard method has been established in determining the biodegradability of polymers. The enzyme method [21], the microbiological method [22], and the soil burial method [23, 24] have been used by different researchers. Moreover, the biodegradability was also recorded through diverse indexes even in the same method [25].

In order to get an overall biodegradability, the enzyme method, the microbiological method, and the soil burial method were performed simultaneously in the present study.

Amyloglucosidase (AMG) is an exoenzyme cleaving α-(1-4) bound from the non-reducing end of the polysaccharides chain with liberation of glucose. This enzyme hydrolyzes α-(1-6) as well, but at a much lower rate. β-Amylase (β-AM) and α-amylase (α-AM) function in different ways to hydrolyze the acetal bonds of starch. β-AM generally hydrolyzes only the main chain acetal bonds, and does not affect the branch points, whereas α-AM can cleave the bonds either in the main chain or in the branch. Considering a possible enzymatic degradation pattern, the effects of AMG and amylases on the films were investigated in a pure enzymatic suspension.

As seen in Figs. 3 and 4, the PVA film could not be degraded by AMG and α-,β-AM, while the CSP-1 and RS4P-1 are prone to degradation induced by AMG and α-,β-AM.

Figure 3 shows the degree of enzymatic degradation (DED) on CSP-1 and RS4P-1 using AMG and α-,β-AM. The film degraded by AMG was better than by α-,β-AM because AMG cleaved α-(1-4) bounds and α-(1-6) bounds simultaneously during the difference enzymatic reaction. The RS4P-1 had a degradation rate slower than the starch in the counterpart matrix. The degradation rate of the RS4 in the blend film was retarded with crosslink of STMP and STTP in the starch molecule.

Figure 4 represents DED of the film with GL and CA as additives. The GL-added films had approximately 60% degradation, while CA-added films were degraded about 25% presumably because low pH of the CA-added film has deactivated the enzymatic reaction.

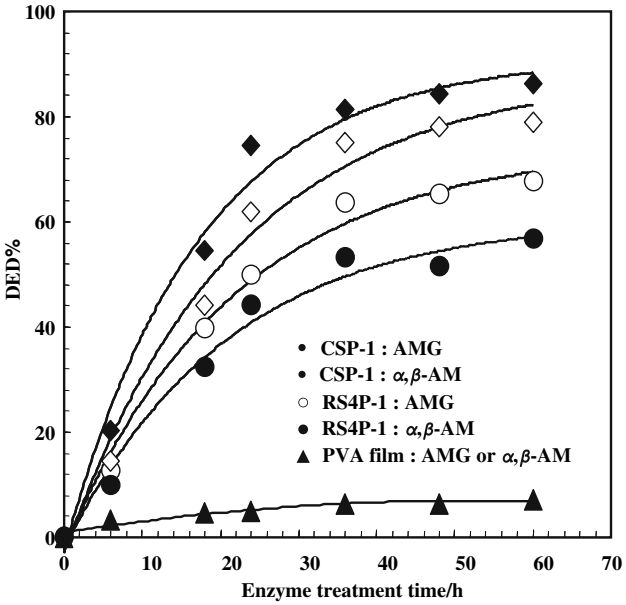

Fig. 3 Enzymatic degradation of CSP-1 and RS4P-1 using amyloglucosidase (AMG), α-amylase (α-AM) and β-amylase (β-AM)

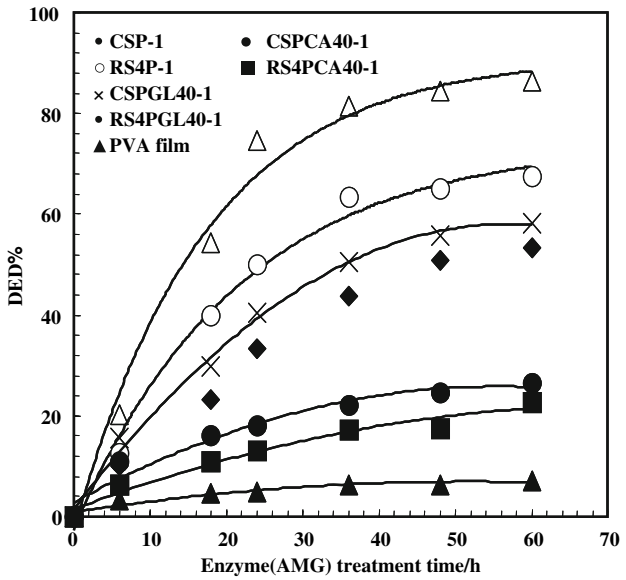

Fig. 4 Enzymatic degradation of films using Amyloglucosidase (AMG)

The effects of microorganic treatment on the starch/PVA films were studied to further investigate the biodegradability. *B. subtilis* and *A. niger* were used because they are amylase producing microorganisms.

Figure 5a, b shows the degree of microbiological degradation (DMD) of each film. Compared to DMD of CSP-1 and RS4P-1, DMD of CSP-1 was higher than RS4P-1. The DMD of CA-added films was higher than GL-added films because CA was used to the nutritive elements of

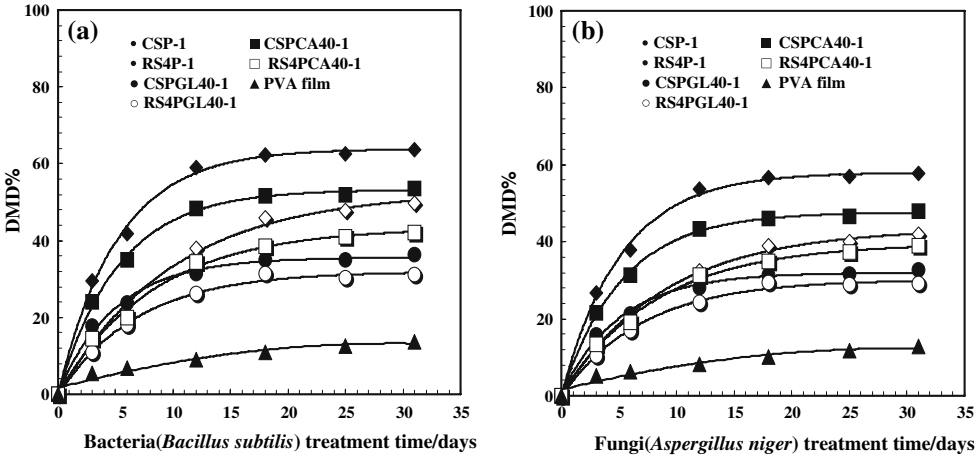

Fig. 5 Microbiological degradation of starch/PVA films. (**a**) DMD of films using Bacteria (*Bacillus subtilis*). (**b**) DMD of films using Fungi (*Aspergillus niger*)

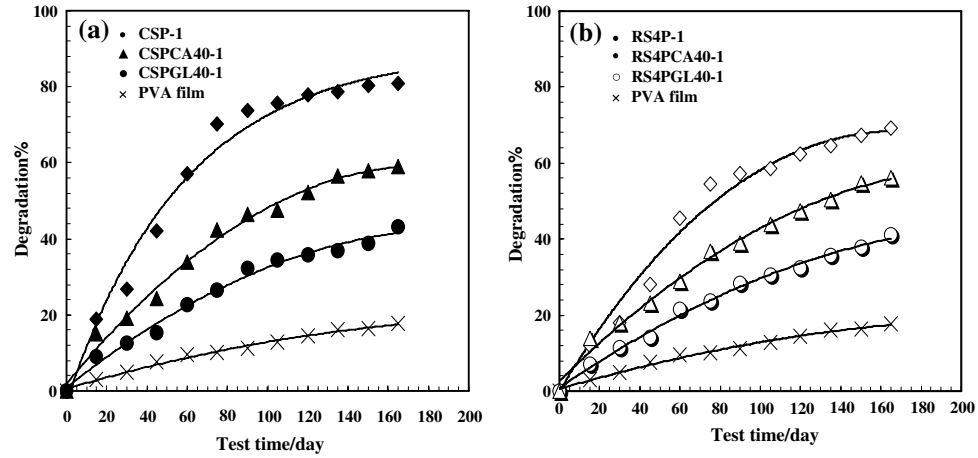

Fig. 6 Degradability of films in the soil burial test. (**a**) Films using cornstarch (CS) and glycerol (GL) and citric acid (CA) as additives. (**b**) Films using chemical modified starch (RS4) and glycerol (GL) and citric acid (CA) as additives

B. subtilis and *A. niger*. Then, the degradation rapidly occurred for about 12 days perhaps because the activation of *B. subtilis* and *A. niger* declined.

The soil burial test provides a realistic environment where soil humidity, temperature and type and the amount of microorganisms are in less control and change with seasons. All the tested specimens had the same shape and size to avoid the effects of the film's shape on its biodegradability. Biodegradability of the samples was studied by evaluating the weight loss of the films over time (Fig. 6a, b).

In all the films, a rapid degradation occurred in the initial 60 days, followed by a slow degradation until the end of the experiment. In contrast, the PVA film exhibited a higher resistance against soil burial biodegradation (18%).

Conclusions

Cornstarch (CS) film and chemically modified starch (RS4)/PVA blend film were synthesized by using the mixing process and the casting method. The calculation results of physical properties tensile strength (TS), elongation at break (*%E*), swelling behavior (SB) and solubility (*S*) of the synthesized films using RS4 added GL and CA as additives demonstrated that the films gained superior physical properties.

In addition, TS, *%E*, SB and *S* values were found to be superior when the film was dried at 5 °C rather than at 50 °C because of the hydrogen bonding occurring at low temperature.

In order to get an overall biodegradability, the enzyme method, the microbiological method and the soil burial method were applied. The PVA film was hardly degraded by enzyme, i.e., amyloglucosidase (AMG), α-amylase (α-AM) and *β*-amylase (*β*-AM), while the CSP-1 and RS4P-1 are prone to degradation when induced by AMG and α-,*β*-AM. GL-added films had approximately 60% degradation, while CA-added films degraded about 25%. The effect of microorganic treatment on the starch/PVA films was studied. Compared to the degree of enzymatic degradation (DED) of CSP-1 and RS4P-1, DMD of CSP-1 was higher than RS4P film. And the DMD of CA-added films were higher than GL-added films. The soil burial test provided a realistic environment where soil humidity,

temperature and type and the amount of microorganisms are in less control and change with season. In all the films, a rapid degradation occurred in the initial 60 days, followed by a slow degradation until the end of the experiment.

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