

Effects of Starch Acetylation on Application of Thermoplastic Starch as Packaging Material: Degree of substitution, Gelatinization and Biodegradability

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Abstract Thermoplastic starch (TPS), despite its environmental advantages, suffers from brittleness and poor processability due to extensive hydrogen bonding in native starch. This study aimed to characterize the relationship between gelatinization behavior and plasticization of acetylated starch and to evaluate the biodegradability of acetylated-TPS (A-TPS). Native corn starch was acetylated to three different degrees of substitution (DS = 0.020, 0.087, 0.117). FT-IR spectra confirmed successful acetylation with the appearance of C=O stretching peaks at 1726 cm^{-1} , and XRD patterns showed decreased crystallinity as DS increased. Water solubility and swelling power increased from 4.985% and 4.935 g/g (Native starch) to 10.054% and 9.983 g/g (DS 0.117) respectively, indicating enhanced water accessibility. Differential scanning calorimetry revealed a reduction in gelatinization temperature from 71.49°C to 64.80°C and gelatinization enthalpy from 2.925 J/g to 1.777 J/g . These changes suggest improved plasticizer penetration during thermal processing. In disintegration tests under home composting conditions, A-TPS (AS 0.117) film showed greatly faster degradation rate than that of TPS and reached over 90% of degradation within one week. The results indicate that starch acetylation effectively enhances the plasticization level of A-TPS, and its improved biodegradability confirms the potential to broaden the applicability of A-TPS in sustainable packaging materials.

Keywords Acetylated starch, Thermoplastic starch, Degree of substitution, Gelatinization, Biodegradable plastic

Introduction

Bioplastics are emerging as essential materials for achieving carbon neutrality in the packaging industry due to their use of renewable resources, low carbon footprint, and high energy efficiency¹⁾. With the increasing interest in bioplastics, starch is particularly gaining attention as a key resource in the packaging industry due to its abundance and renewability. Thermoplastic starch (TPS) is a representative starch-based material produced by adding plasticizers and heat to starch. During this process, amylose and amylopectin are separated, and plasticizers penetrate between polymer chains, increasing amorphous regions through gelatinization, resulting in a semi-crystalline thermoplastic material²⁾. TPS is being spotlighted as a sustainable packaging material due to its high biomass content, economic viability, and rapid biodegradation rate, which has been reported to fully degrade within two weeks

under typical soil conditions (25°C , 55% RH)³⁾. Thanks to these advantages, TPS is commonly blended with other biodegradable polymers in the form of composites. Palai et al.⁴⁾ and Pokhrel et al.⁵⁾ demonstrated that blending TPS with polycaprolactone (PCL) or poly(butylene adipate-co-terephthalate) (PBAT), enhanced the overall biodegradability by more than eight times. Nonetheless, due to the abundance of hydroxyl groups in starch, TPS tends to exhibit strong hydrogen bonding, making it difficult for plasticizers to fully penetrate. As a result, TPS exhibits poor mechanical strength and high brittleness, and tendency for self-aggregation and incompatibility within composite materials causing serious deterioration in the overall mechanical properties of the composites.

To address these limitations, chemically modified starches—such as octenyl succinylated, hydroxypropylated, and acetylated starch—have been explored, in which hydroxyl groups are substituted with other functional groups. Among them, acetylated starch is one of the most commonly used esterified starches, widely applied in the biomedical, textile, and food industries. It can be synthesized using acetic anhydride, acetic acid, or vinyl acetate, typically in the presence of alkaline catalysts such as NaOH, KOH, or $\text{Ca}(\text{OH})_2$ ^{6,7)}. Notably, acetylation reactions can use not only organic solvents like pyridine

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or dimethyl sulfoxide but also water, making the process environmentally friendly. The physicochemical properties of acetylated starch, including solubility, water absorption, and swelling power, vary depending on the degree of substitution (DS). The reduction in hydroxyl groups weakens hydrogen bonding between amylose and amylopectin chains, while the introduction of acetyl groups increases the intermolecular distance within the structure. Consequently, starch granules swell more readily, and amylose leaches out more easily, resulting in enhanced solubility. The reduction in crystallinity also increases the amorphous region, allowing greater water uptake⁸⁾. Trela et al.⁹⁾ reported that when cassava starch was acetylated with varied DS values, the swelling power at 70°C—within the gelatinization range—increased with DS up to 0.2. Such changes can improve the plasticization efficiency during TPS processing by promoting plasticizer penetration, thus reducing brittleness and aggregation, and enhancing the usability of TPS in packaging applications.

In this study, physicochemical properties, gelatinization behavior and the plasticization effects of acetylated starches with varying DS were investigated, focusing primarily on starch-level characterization to establish fundamental structural mechanisms. Acetylated-TPS (A-TPS) films were prepared to examine how chemical modification of starch molecules influences the degradation performance. Ultimately, these findings demonstrate the great potential of acetylated starch to overcome the limitations of conventional starch-based materials and enhance its applicability as an eco-friendly packaging material.

Materials & Methods

1. Materials

Food-grade starch was provided by Samyang Corporation (Corn starch, Seoul, Republic of Korea). Acetic anhydride (ACS reagent, 98.0+%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Phenolphthalein (2% solution in 95% ethanol), sodium hydroxide pellets (98%), sodium hydroxide solution (0.5N standardize solution), hydrochloric acid solution (0.5N standardize solution), glycerol (ACS reagent, 99.5+%), and other reagents used for characterization were obtained from Thermo Fisher Scientific (Waltham, MA, USA).

2. Starch acetylation

The starch suspension was prepared by stirring 100 g of corn starch in 225 mL of DI water at 600 rpm for 30 minutes. While maintaining stirring at the same speed, the pH of the suspension was adjusted to 8.0 by adding 3.0% (w/w) NaOH solution. Acetic anhydride was then added dropwise in amounts of 2 g, 10 g, and 20 g at constant pH range of 8.0–8.4. After the complete addition of acetic anhydride, the mix-

ture was stirred for an additional 30 minutes, and the reaction was terminated by adjusting the pH to 4.5 using 0.5N HCl solution. The suspension was then centrifuged at 2,000 rpm for 30 minutes to separate the precipitate from the supernatant. The recovered precipitate was sequentially washed with water and 94–96% ethanol followed by centrifugation to remove the washing solution. The purified precipitate was then dried at 40°C in dry oven and subsequently ground to obtain acetylated starch powder. The acetyl content and DS of the acetylated starch were determined by titration analysis. 1.0 g of as-prepared acetylated starch was mixed with 25 mL of 0.5N NaOH solution and stirred at 200 rpm for 72 hours. After adding 3–5 drops of 2% phenolphthalein, the mixture was back-titrated to the endpoint using 0.5N HCl solution, and the acetyl content and DS were calculated using the following Equations (1) and (2):

$$\text{Acetyl content (A)} = \frac{(V_1 - V_2) \times 10^{-3} \times N \times 43}{W} \times 100(\%) \quad (1)$$

$$\text{Degree of substitution (DS)} = \frac{162A}{[43 \times 100 - (43 - 1) \times A]} \quad (2)$$

where V_1 represents the volume of HCl solution used for the titration of native starch, V_2 represents the volume of HCl solution used for the titration of acetylated starch, N denotes the normality of the HCl solution, and W refers to the mass of acetylated starch mixed with the NaOH solution. The acetyl content and DS for each sample are summarized in Table 1, where four types of samples were prepared: native starch (NS), acetylated starch (AS) with DS values of 0.020, 0.087, and 0.117.

3. Characterization of acetylated starch

The chemical structures of native and acetylated starch were analyzed using a Fourier-transform infrared spectrometer (FT-IR) (Spectrum 65, PerkinElmer, Inc., MA, USA). The FT-IR spectra were measured in attenuated total reflectance (ATR) mode, with each spectrum obtained by 64 scans in the wavenumber range of 400–4000 cm^{-1} at a resolution of 2 cm^{-1} .

X-ray diffraction patterns were examined using an X-ray

Table 1. Acetyl content and degree of substitution of as-prepared acetylated starch

Samples	Acetic anhydride (g/100 g starch)	Acetyl content (%)	Degree of substitution
NS	0	0	0
AS 0.020	2	0.54	0.020
AS 0.087	10	2.26	0.087
AS 0.117	20	3.01	0.117

diffractometer (Smart Lab SE, Rigaku, Tokyo, Japan) used a Cu K α source ($\lambda = 1.54 \text{ \AA}$) at 2°/min of scanning speed within a diffraction angle range of $2\theta = 5\text{--}35^\circ$ in room temperature.

To evaluate the accessibility of plasticizers to starch chains during the preparation of A-TPS from acetylated starch, water solubility and swelling power were measured. The analysis was conducted at 70°C, which is the typical gelatinization temperature of corn starch. The water solubility and swelling power of the acetylated starch were measured with slight modifications to the method proposed by Colussi et al.¹⁰⁾. The mixture of 1.0 g of acetylated starch and 50 mL of DI water was prepared in a conical tube and heated in a 70°C water bath for 30 minutes. The conical tube was then cooled to room temperature and centrifuged at 4,000 rpm for 30 minutes to separate the precipitate and supernatant. The precipitate was collected, and the supernatant was dried at 103°C until a constant weight was reached. The weights of both the precipitate after centrifugation and the dried supernatant were then measured. Water solubility and swelling power were calculated using the following Equations (3) and (4):

$$\text{Water solubility (WS)} = \frac{W_d}{W_i} \times 100(\%) \quad (3)$$

$$\text{Swelling power (SP)} = \frac{W_p}{W_i - W_d} \quad (4)$$

where W_i represents the mass of acetylated starch mixed with DI water, W_d refers to the mass of the dried supernatant, and W_p denotes the mass of the precipitate after centrifugation.

The thermal behaviors of acetylated starch during the gelatinization were assessed using a differential scanning calorimetry (DSC) (Q20, TA Instruments, New Castle, USA). The mixture of acetylated starch and DI water at a 3:7 ratio (11–12 mg) was sealed in an aluminum pan and stabilized for 1 hour. The analysis was conducted under a nitrogen atmosphere (50 mL/min), with the temperature range set from 20°C to 100°C with a heating rate of 10°C/min. The peak temperature (T_{gel}) and enthalpy (ΔH_{gel}) of gelatinization were determined from the DSC thermogram.

4. Assessment of disintegration behavior of acetylated-TPS

To investigate the effect of acetylated starch on the disintegration properties of A-TPS, casting films of native and acetylated starch were prepared as follows; The casting solution was prepared by adding 8.0 g of acetylated starch and 2.4 g of glycerol to 200 mL of DI water, followed by mixing at 500 rpm for 10 minutes. The solution was heated at 85°C in water bath with stirring at 600 rpm using a mechanical stirrer for 30 minutes. Subsequently, the solution was sonicated for 1 minute using an ultrasonic processor (750 W, 20 kHz), completing the preparation of the casting solution. The casting solution was cast onto 150 mm diameter petri dishes (55 g per

dish) and dried in an incubator at 45°C with constant relative humidity (RH) kept at 50%.

The disintegration of TPS casting films in home compost was conducted according to ISO 20200:2015 with minor modification. The synthetic waste was composed of 40% sawdust (Oak tree sawdust, Jaehyeong Energy Corp., Sejong, Republic of Korea), 30% alfalfa hay (>14% of protein content, <20% of cellulose content, Petco, Incheon, Republic of Korea), 10% starch (Corn starch, Samyang Corp., Seoul, Republic of Korea), 5% sucrose (ACS reagent, Thermo Fisher Scientific, Waltham, MA, USA), 4% corn-seed oil (CJ Cheiljedang, Incheon, Republic of Korea), and 1% urea (ACS reagent, 99.0–100.5%, Thermo Fisher Scientific, Waltham, MA, USA). Each material was sieved to ensure a particle size of <5 mm and dried in a 60°C dry oven for over 12 hours before being mixed. Distilled water was added to the synthetic waste to achieve a moisture content of 55%, and any water loss during the experiment was replenished according to the standard requirements. The composting container (Polypropylene, 305 mm $\times$ 234 mm $\times$ 134 mm) with a lid was used for this study, and circular holes with diameter of 5 mm were drilled on both side walls of the container, 65 mm above the bottom. TPS film samples, cut into 25 mm $\times$ 25 mm pieces, were fixed in stainless steel mesh and buried in the prepared synthetic waste. The degradation was carried out in a humidity chamber at 25°C, 50% RH for 4 weeks. Each week, samples were retrieved, and their appearances were assessed visually and taken photos.

Results & Discussion

1. Chemical structure

Figure 1 shows the FT-IR spectra of native and acetylated starch with varying DS. Compared to native starch, acetylated starch exhibits the transformation of hydroxyl groups linked to carbon rings into acetyl groups. The FT-IR spectra of acetylated starch reveal the emergence of a new peak at 1726 cm^{-1} , attributed to the C=O bonds present in the acetyl groups. The intensity of this peak increases with higher DS, and the peak around 1240 cm^{-1} , corresponding to the C–O stretching vibrations, is also observed. Meanwhile, the intensity of the peak at 3294 cm^{-1} corresponding to the O–H stretching vibrations decreased due to the lower hydroxyl group content in A-TPS. Similarly, the peak at 1640 cm^{-1} associated with the O–H bending vibrations of water molecules also reduced^{9,11)}. Diop et al.¹²⁾ stated that this peak originates from tightly bound water. It is inferred that the substitution of hydroxyl groups with acetyl groups in acetylated starch reduces the opportunity for water molecules to interact with the starch structure.

2. Crystalline structure

Figure 2. XRD spectra of native and acetylated starch.

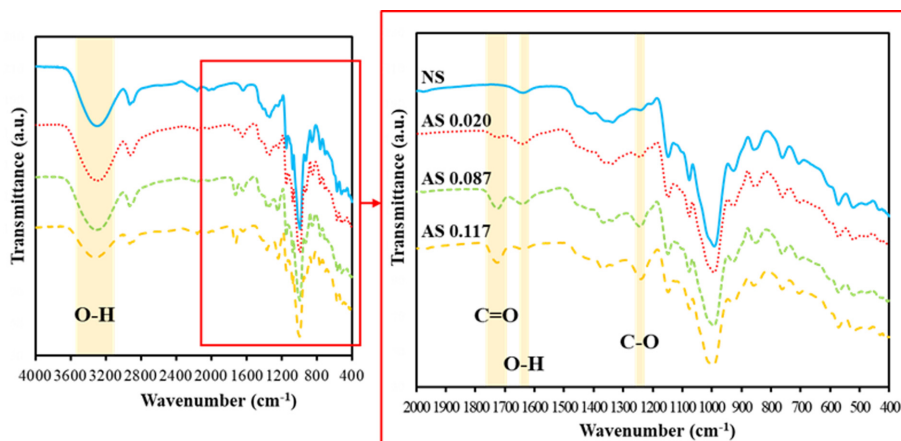


Fig. 1. FT-IR spectra of native and acetylated starch.

The changes in the crystalline structure of starch molecules with acetylation were analyzed using the XRD technique, and the diffraction peaks of native and acetylated starch are shown in Figure 2. Starch granules are semi-crystalline structure comprising crystalline and amorphous regions. The crystalline region is formed by the double helices of amylopectin, while amylose chains and branched segments of amylopectin constitute the amorphous region. Crystalline structure of starch is classified into the A-type (left-handed parallel-stranded double helices packed in a monoclinic unit cell) and B-type (left-handed parallel-stranded double helices packed in a hexagonal unit cell) depending on the configuration of the double helices. These structures are typical of cereal (e.g. corn, wheat) starch and fruit or tuber (e.g. potato, banana) starch, respectively^{13,14}. The XRD pattern of native starch exhibited characteristic diffraction peaks at 2θ around 15.2° , 17.2° , 18° , and 23.2° , which are indicative of the A-type crystalline structure. Acetylated starch shows a diffraction pattern in which the intensity of the corresponding peak slightly decreased as the

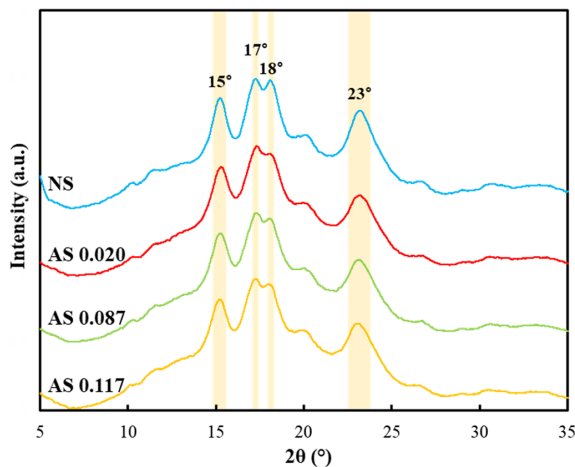


Fig. 2. XRD spectra of native and acetylated starch.

DS increased. Several studies have explained that the acetylation of starch reduces the number of hydroxyl groups and weakens the hydrogen bonding between starch molecules due to the steric hindrance of bulky acetyl groups, leading to a relative decrease in crystallinity^{15,16,17}. On the other hand, Liu et al.¹⁸) demonstrated that the A-type crystalline structure exhibits strong crystallinity, making it resistant to the penetration of acetic anhydride. As a result, acetylation occurs primarily in the amorphous regions, and no significant changes in diffraction peaks are observed at low DS.

3. Water solubility and swelling power

Water solubility and swelling power of starch reflect the interactions between molecular chains in the crystalline and amorphous regions of starch granules¹⁹. These properties were used to explain changes in molecular interactions of acetylated starch. Figure 3 presents water solubility and swelling power of native and as-prepared acetylated starch. As DS increased, both properties exhibited a distinct upward trend with water solubility and swelling power rising from 4.985% to 10.054% and from 4.935 g/g to 9.983 g/g, respectively,

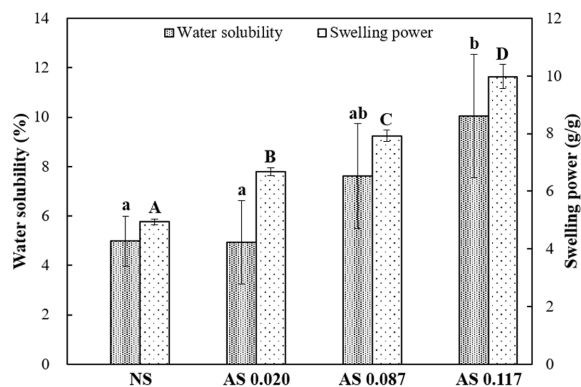


Fig. 3. Water solubility and swelling power of native and acetylated starch at 70°C .

indicating enhanced water accessibility of the acetylated starch. For swelling power, a statistically significant increase was observed across all samples. In the case of water solubility, AS 0.117 was significantly higher than NS and AS 0.020, demonstrating an overall increasing trend as DS increased. Meanwhile, under the 70°C condition, the samples with higher DS are speculated to exhibit larger standard deviations due to their high structural sensitivity. Ayucitra et al.²⁰⁾ reported that the substitution of hydroxyl groups with acetyl groups induces structural reorganization, which can weaken the granular structure of starch. Therefore, water permeation into amorphous regions of the granules is facilitated, and the leaching of amylose becomes easier, leading to increased swelling power and water solubility. This behavior is deeply tied to the inherent amylose/amylopectin ratio of the corn starch. Although acetylation initially targets the accessible amorphous regions as established in Figure 2, the resulting cumulative steric hindrance gradually destabilizes the adjacent crystalline domains, thereby promoting the leaching of amylose chains. This structural transition aligns with recent literature emphasizing that the amylose/amylopectin ratio fundamentally dictates the crystalline disruption and macromolecular behavior of starch-based materials²¹⁾. These findings show that the reduction in intermolecular forces between starch molecules and the increase in amorphous regions lower the energy required to disrupt the strong crystallinity of starch.

4. Thermal properties

Table 2 presents the gelatinization peak temperature (T_{gel}) and enthalpy of gelatinization (ΔH_{gel}) for native starch and acetylated starch. T_{gel} of native starch was observed at 71.49°C, while it decreased to 67.97°C for AS 0.087 and further to 64.80°C for AS 0.117. Similarly, ΔH_{gel} showed a decreasing trend with an increase in the DS, declining from 2.925 J/g to 1.777 J/g, as illustrated in Figure 4. Wang et al.²²⁾ explained the starch gelatinization process in two major stages: from 20°C to 60°C, water reversibly binds to starch molecules while maintaining the molecular structure. Subsequently, between 60°C and 90°C, hydrogen bonds between starch molecules are disrupted, allowing water molecules to bind directly with starch molecules, leading to the destruction of starch granules. Swelling power of starch and intermolecular forces between them are critical factors determining the gelatinization temperature and enthalpy^{23,24)}. As shown in Figure 3, the acetylation of starch reduces intermolecular

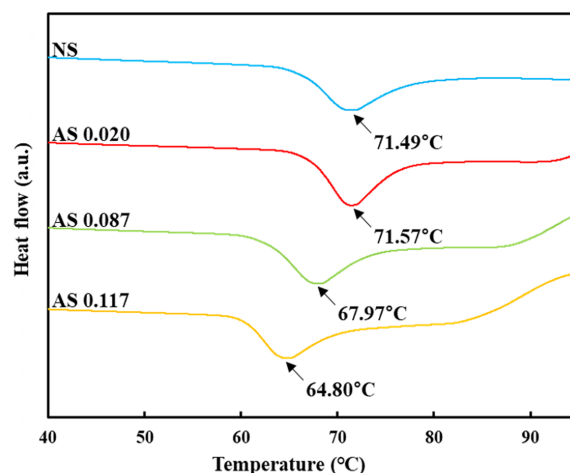


Fig. 4. DSC curves of native and acetylated starch during gelatinization.

forces and increases amorphous regions, which supports the reduction in thermal energy required for starch gelatinization. From a processing perspective, this lower thermal energy requirement allows for reduced temperatures during extrusion or compounding, thereby minimizing the risk of starch thermal degradation and offering distinct advantages in production. This suggests that the reduced intermolecular force of acetylated starch allows easier penetration of plasticizers between molecular chains, potentially resulting in a higher plasticization effect during A-TPS production. A-TPS with a high level of plasticization exhibits reduced brittleness, resulting in improved flexibility and alleviated aggregation. Therefore, the effect of acetylation is expected to overcome the critical drawbacks of conventional TPS.

5. Disintegration of A-TPS casting films in home composting condition

The chemical modification of starch should not adversely affect the inherent biodegradability of TPS. According to ISO 20200:2015, fragments smaller than 2 mm are considered completely disintegrated, and Figure 5 shows disintegration of the samples over a 4-week period. Although all films were initially clean and transparent, they became increasingly opaque as degradation progressed. Similar changes during degradation were also reported by Yap et al.²⁵⁾, such as surface dulling and color shifts. TPS and A-TPS (AS 0.020) exhibited similar disintegration trends, with only small fragments remaining

Table 2. Gelatinization behavior of as-prepared acetylated starch

	NS	AS 0.020	AS 0.087	AS 0.117
T_{gel} (°C)	71.49 ± 0.213 ^A	71.57 ± 0.095 ^A	67.97 ± 0.053 ^B	64.80 ± 0.112 ^C
ΔH_{gel} (J/g)	2.925 ± 0.119 ^a	2.745 ± 0.110 ^{ab}	2.625 ± 0.123 ^b	1.777 ± 0.058 ^c

* Values with different superscripts within the same row are significantly different by Duncan's multiple range test ($p < 0.05$).

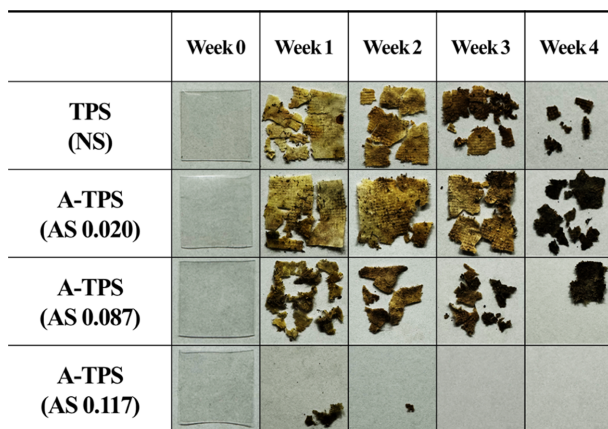


Fig. 5. Visual characteristics of TPS and A-TPS casting films during degradation.

after four weeks. In contrast, A-TPS (AS 0.087) and A-TPS (AS 0.117), showed a faster disintegration rate. Notably, A-TPS (AS 0.117) exhibited the most rapid degradation, as a result, majority of the film disintegrated within just one week. This behavior is likely attributed to the crystallinity of A-TPS, which decreases as the DS increases. According to Díaz et al.²⁶⁾, polymer chains with lower crystallinity allow enzymes easier access to their active sites, thereby enhancing biodegradation. These results indicate that acetylation does not compromise the inherent biodegradability of conventional TPS. On the contrary, the reduced intermolecular forces caused by the introduction of acetyl groups may positively influence biodegradation, suggesting the potential for developing highly biodegradable packaging materials.

Conclusions

This study explored the effect of acetylation on the physicochemical properties of starch for TPS applications. Acetylation was processed by native corn starch and different amounts of acetic anhydride (2, 10, and 20 g) were reacted resulting in DS of 0.020, 0.087, and 0.117. FT-IR analysis confirmed the successful incorporation of acetyl groups into the starch molecules. X-ray diffraction patterns revealed a slight decrease in crystallinity with increasing DS, indicating that the substitution of hydroxyl groups with bulky acetyl groups reduced intermolecular interactions. However, since acetylation occurs primarily in the amorphous regions, no marked changes in the diffraction peaks were observed. At 70°C, water solubility increased from 4.99% (Native starch) to 10.05% (DS 0.117), and swelling power rose from 4.94 g/g to 9.98 g/g, indicating enhanced water accessibility into the starch granules. Differential scanning calorimetry revealed a decline in the gelatinization temperature from 71.49°C to 64.80°C and a reduction in gelatinization enthalpy from 2.93 J/g to 1.78 J/g with increasing DS. These changes demon-

strate that acetylation weakens the hydrogen bonding between starch chains and facilitates plasticizer penetration, thereby improving plasticization efficiency during A-TPS formation.

To evaluate the effect of acetylation on the disintegration behavior of A-TPS, casting films were prepared and subjected to a four-week home composting test. While the TPS (NS) and A-TPS (AS 0.020) films showed gradual disintegration over 3 to 4 weeks, A-TPS films with a AS 0.087 or higher exhibited accelerated degradation. In particular, the A-TPS film with a DS of 0.117 showed nearly complete disintegration within just one week, which is likely attributed to the increased amorphous regions and reduced crystallinity, facilitating faster hydrolysis and improved enzyme accessibility.

Overall, this study demonstrates that acetylation of starch not only enhances its plasticization behavior by modifying its thermal and water interaction properties but also improves its degradation performance. Furthermore, the substituted acetyl groups, even at low levels, are expected to enhance the interfacial compatibility of TPS when blended with other commercial hydrophobic biodegradable polymers, such as PCL or PBAT, which is a key factor for manufacturing flexible composite packaging films. These findings suggest that the application of acetylated starch with a controlled DS contributes to the broader utilization of starch in biodegradable plastics, and that it is a promising material for sustainable packaging.

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