# A Research Note

# Stability of Cornstarch-containing Polyethylene Films to Starch-degrading Enzymes

## A. A. STRANTZ and E. A. ZOTTOLA*

*Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Avenue, St. Paul, Minnesota 55108*

## ABSTRACT

Processes have been developed to incorporate cornstarch into plastics with the intent of increasing the rate of plastic biodegradation. The effect of starch-degrading enzymes on food-grade polyethylene film that contained 6% cornstarch (CSPE) was examined. Control polyethylene film with no added starch, CSPE and laboratory grade soluble starch were treated with α-amylase, β-amylase, or amyloglucosidase. Samples were removed periodically and were subjected to the Nelson-Somogyi method for the determination of reducing sugar content. Treatment with α-amylase and β-amylase released over 30% of the soluble starch as glucose, while less than 1% of the starch in CSPE was released. Amyloglucosidase activity released up to 50% of the soluble starch as reducing sugar. However, less than 4% of the CSPE starch was liberated. Image analysis of iodine-stained films showed that enzymatic treatment did not remove surface granules. These results indicated that breakdown of CSPE by starch degrading enzymes was limited.

Increasing public concern over dwindling landfill space and accumulation of surface litter has prompted the development of degradable plastics (4,7). Plastics degrade as the result of mechanical damage, photodegradation, and chemodegradation. Both photo- and chemodegradation involve the generation of free radicals which shorten the plastic polymer chains. Shorter chains result in reduced physical strength. The result is that large plastic pieces become small plastic pieces (5,8).

Biodegradation requires the action of microorganisms. Theoretically, biodegradation of plastic polymers could be enhanced by the incorporation of starch into the plastic polymer network. The action of microbial starch degrading enzymes would create gaps in the plastic. This would decrease the physical strength of the plastic and increase the surface area available for attack by plastic-degrading

microorganisms. A significant amount of plastic is used to package food products. If cornstarch-containing plastic film has no adverse effects on food quality or food safety, its use in food packaging could lead to decreased petrochemical usage and increased corn utilization.

Microorganisms responsible for the biodegradation of cornstarch-containing film must produce enzymes that are capable of breaking down the starch. Unfortunately, there are food-spoilage and pathogenic organisms that produce these same enzymes. The objective of this study was to examine the effect of starch-degrading enzymes on food-grade, cornstarch-containing plastic film.

## MATERIALS AND METHODS

### Film preparation

Rexene 320 (Rexene Corp., Odessa, TX), a low-density, virgin polyethylene (PE) plastic film that contained food-grade slip and antiblock agents, was extruded by a local plastic manufacturer (Polar Plastics, Oakdale, MN) and used as a control. Experimental film was prepared by combining the same polymer with a master-blend concentrate that contained 40% cornstarch and 60% polyethylene to result in a final cornstarch concentration of 6% in the film (CSPE). The cornstarch-containing concentrate did contain pro-oxidant. Both film types had nominal thickness of 2 mil (0.05 mm).

Films were cut into 2.5 x 7.5-cm rectangles which weighed approximately 0.1 g. Samples used to evaluate changes in the physical properties of films were 2.5 x 15-cm in size. The increased length was required for Instron analysis. Care was taken not to stretch the film while cutting. A 0.6% (wt/vol) solution of laboratory-grade soluble starch (SS) (Fischer Scientific Co., Fairlawn, NJ) was prepared. One ml of this solution contained the same amount of starch present in the CSPE film samples.

### Enzyme preparation

Amyloglucosidase (AG) from *Aspergillus niger*, α-amylase (AA) from *Bacillus* sp., and β-amylase (BA) from sweet potato (Sigma Chemical Co., St. Louis, MO) were rehydrated and diluted in distilled water. Appropriate dilutions were prepared so that more enzyme than would be required to degrade the amount of starch in each sample would be available; 570, 280, and 52 IU per sample of AA, BA, and AG were used, respectively.

### Sample treatment

Samples were suspended in 25 x 150-mm screw-capped test tubes that contained a balanced salt solution (BSS). A modification of Hank's solution, with no phenol red, was used because it contained calcium and other minerals required for amylase activity but did not contain any added carbon source (6). The pH of the solution was adjusted to 6.9 when AA was used, or to 4.6 for BA and AG. Enzyme was added to tubes that contained PE film, CSPE film, SS solution, and to tubes containing no substrate. Tubes that held samples for Instron testing received twice the volume of enzyme since these film pieces were twice as large as those tested for starch breakdown. Control tubes with no added enzyme were also prepared. The amount of BSS used was adjusted so that the final volume of each reaction mixture was constant. Tubes were incubated at 50°C in a circulating water bath. Portions of supernatant were removed periodically and reducing sugar content was determined (9). Absorbance values were converted to $\mu$ moles of reducing sugar by comparison to standard curves generated with either glucose or maltose. A minimum of three separate trials were performed with each enzyme. Within each trial, analyses were performed in triplicate.

### Visual examination of films

After incubation in the BSS, with or without enzyme, films were stained for 3 min in Gram's iodine solution. Stained films were examined using image analysis (40-10 Image Analyzer; Analytical Measuring Systems, LTD., Essex, United Kingdom) for the presence of starch granules. Stained starch granules present in 25 fields were counted on control films and on those treated with each enzyme. Duplicate films from each treatment were analyzed.

### Instron testing

Changes in the physical properties of the film were evaluated using the Instron (Model 1122, Instron Corp., Canton, MA). Load-elongation to failure tests were conducted to measure elongation and tensile strength of the plastic (1).

### Statistical analysis

Analysis of variance was performed on image analysis and Instron data using the methods of Devore and Peck (3).

## RESULTS AND DISCUSSION

Soluble starch solutions were used to confirm enzyme activity. Treatment with AA released approximately 35% of the starch present as reducing sugar; however, less than 2% of the starch was released from CSPE film (Fig. 1). A similar pattern was seen when AG was used. Although BA was capable of cleaving 30% of the soluble starch, there was no detectable release of reducing sugar from the CSPE. Cone and Wolters (2) observed that less than 13% of the starch present in corn was released after treatment with $\alpha$-amylase for 4 h at 39°C. The conformation of the starch molecules would have affected degradation. Starch in the corn was in its native granular state, whereas SS has been gelatinized, making it more accessible to enzymatic attack.

Microscopic examination of untreated CSPE film showed that intact starch granules were present (Fig. 2A). These granules were roughly circular with dark centers. Treatment with iodine solution stained surface granules, but those embedded in the polymer network remained unstained (Fig. 2B).

After incubation of CSPE films in BSS with and without enzymes, stained starch granules were still present on the surface of the film. Enzyme treatments did not remove granules that were present on the surface of the film (Table 1). It was possible that stained granules were
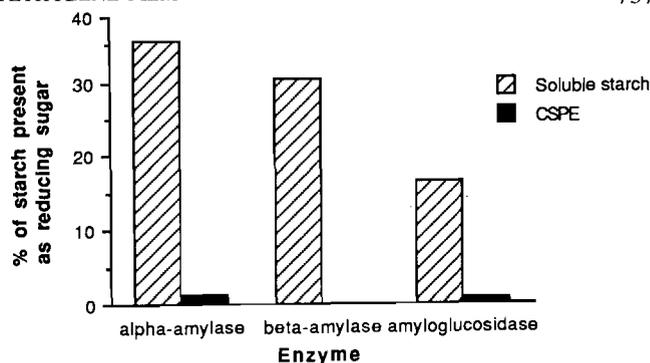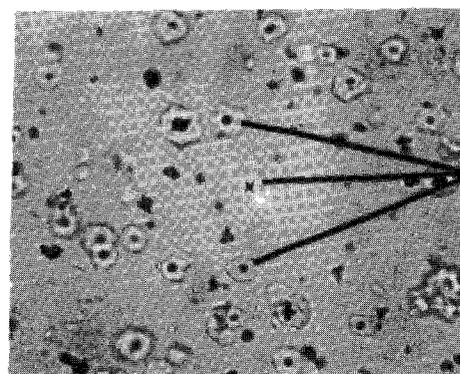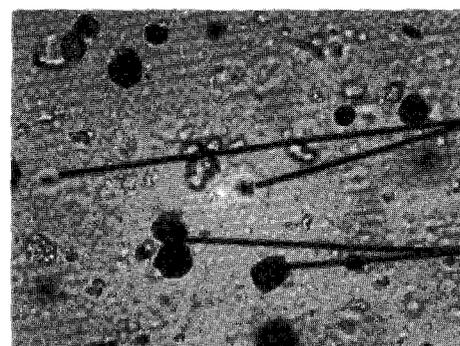


Figure 1. *Percentage of starch enzymatically converted to reducing sugar.*

A. Untreated film



Starch Granule

B. Film stained with Iodine



Starch Granule

Stained Starch Granule

Figure 2. *Microscopic appearance of starch granules in cornstarch-containing polyethylene film (100x magnification).*

partially embedded in the polymer network. Gaps in the plastic could have been large enough to permit penetration of the stain but not large enough to permit contact between enzyme and substrate.

TABLE 1. *Number of starch granules present per field of vision on the surface of cornstarch containing plastic film after incubation with enzymes at 50°C for 24 h.*

| Enzyme | Average No. of granules |
|---|---|
| Control (no enzyme) | 14.26[a] |
| $\alpha$-Amylase | 17.70[a] |
| $\beta$-Amylase | 12.50[a] |
| Amyloglucocidase | 17.30[a] |

Means with different superscripts are significantly different.

Treatment of plastic in BSS at 50°C resulted in a decrease in the ability of the plastic to stretch before breaking, regardless of whether or not enzyme was present (Table 2). Plastic subjected to degradation by AG retained somewhat more ability to elongate. There was no significant difference in the tensile strength of the plastic between treatments, but incubation at 50°C did decrease the tensile strength by approximately 15%.

TABLE 2. *Changes in elongation and tensile strength of corn-starch-containing plastic after incubation with starch degrading enzymes at 50°C for 24 h.*

| Enzyme treatment | % of original elongation | % of original tensile strength |
|---|---|---|
| α-Amylase | 36.2[a] | 82.4[a] |
| β-Amylase | 35.6[a] | 86.6[a] |
| Amyloglucosidase | 55.7[b] | 85.5[a] |
| No enzyme | 34.9[a] | 88.5[a] |

Means with different superscripts are significantly different.

These results indicate that CSPE films are resistant to degradation by starch degrading enzymes. Although this suggests that incorporation of cornstarch into polyethylene does not enhance biodegradability, inaccessibility of starch as a substrate for microbial growth would be advantageous if this type of film is to be used to package food products.

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