Morphology and Enzymatic Degradation of Thermoplastic Starch–Polycaprolactone Blends

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ABSTRACT: This study's aim was to evaluate the effect of processing conditions on the morphology and enzymatic degradation of 50/50 (w/w) thermoplastic starch–polycaprolactone blends. The blends, produced from native potato starch, glycerol, and polycaprolactone in a melt mixer using different mixing speeds and temperatures, were cocontinuous, and the blends were very homogeneous. Enzymatic hydrolysis was performed using *Bacillus licheniformis* alpha-amylase and *Aspergillus niger* glucoamylase on both milled and intact samples. The thin layer of polycaprolactone ($\approx 5\ \mu$m) formed on the surface of the thermoplastic starch–polycaprolactone blends during compression molding strongly reduced the rate of enzymatic hydrolysis. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 74: 2594–2604, 1999

Key words: biodegradation; enzymatic hydrolysis; thermoplastic starch; polycaprolactone

INTRODUCTION

Environmental awareness of the nonbiodegradability of the petrochemical-based plastics and the increasing use of composting in waste management has created a need for biodegradable materials. Starch is a nonexpensive, naturally occurring renewable polymer that is a potential raw material for the manufacturing of plastic like materials.

Blends manufactured from granular starch and polyethylene[1,2] became commercially available already in the '70s. However, although the granular starch was easily biodegraded, the residual material consisted of nonbiodegradable polyolefin particles. Since then, granular starch has been blended with other biodegradable polymers, e.g. poly(hydroxybutyrate-*co*-hydroxyvaleriate) and polycaprolactone (PCL), to reduce the cost of these materials.[3–6] The problem with using granular starch is that mechanical properties like tensile strength are significantly reduced when higher amounts of starch are used.

Otey *et al*[7,8] have prepared composite materials containing starch and several hydrophilic synthetic polymers, such as poly(ethylene-*co*-acrylic acid). In these materials the starch granules were disrupted, and starch formed a continuous phase.[9] The amount of starch in these materials can be as high as 90%,[10] but a smaller amount of starch should be used to obtain acceptable mechanical properties. One major problem with these composites is their limited biodegradability.

Starch can be processed under the influence of heat and mechanical energy in the presence of plasticizers into a thermoplastic material.[11,12] During processing the starch granules melt, and an amorphous mass is formed.[13] Poor water resistance and low strength are limiting factors for the use of materials manufactured only from ther-

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Table I TPS/PCL Blends Prepared with Different Mixing Speeds and Temperatures

Sample	Mixing Speed (rpm)	Set Temperature (°C)	Melt Temperature (°C)	Torque (N m)
Blend 1	50	+110	+126	53
Blend 2	50	+120	+134	48
Blend 3	50	+140	+150	42
Blend 4	100	+110	+150	57
Blend 5	25	+145	+149	29
Blend 6	50	+155	+166	34

Melt temperature and torque were recorded during processing.

moplastic starch (TPS), and hence it is often blended with other polymers.[14] Polycaprolactone (PCL) is a linear, partially crystalline polyester that is utilizable by microorganisms.[15–18] Bastioli et al.[19] reported that the presence of starch can increase the biodegradation rate of PCL because a greater surface area is available for microbes and the hydrolysis of PCL is promoted. Trademark Mater-Bi of Novamont (Italy) is a commercially available, biodegradable material containing thermoplastic starch and synthetic polymers, such as polycaprolactone.[20]

Enzymatic studies have been used to examine the degradation of different types of material containing starch,[21] PCL,[22] or polyhydroxybutyrate.[23] The advantages of the enzymatic tests are that they are usually easy to perform, the results can be achieved very rapidly, and degradation products can be analyzed quantitatively without interference from microbial growth or metabolic products.[21] The drawback of this kind of testing is that enzymatic degradation can be somewhat limited because of the lack of biosurfactants and the synergistic action of different enzymes produced by microorganisms in natural conditions. However, enzymatic studies are an excellent way of studying the degradation mechanisms of complex materials.

We had earlier studied the processing of native starches into thermoplastic materials and the analysis of their properties and biodegradation.[24–28] The aim of this study was to evaluate the effect of the processing conditions on the morphology and enzymatic degradation of TPS–PCL blends containing 50 wt % of polycaprolactone.

EXPERIMENTAL

Preparation of the TPS–PCL Blends

Native potato starch was obtained from Avebe (The Netherlands) and PCL Tone P787E (molecular weight 80,000 g/mol) from Union Carbide Corporation (United Kingdom). The blends were prepared with a Haake Rheocord 90 system equipped with a mixer using a two-step procedure. The mixing speed and the temperature were controlled during the processing (Table I). The premix containing native potato starch, water, and glycerol (5:3:2) was added to the mixer and the starch gelatinized at 110°C. The temperature and mixing speed were then increased to the set value, PCL was added, and the melt was mixed for 5 min. The melt temperature and torque were recorded during the processing. The final ratio of native potato starch (dw) : glycerol (dw) : PCL (dw) was 3:2:5. After processing, the materials were compression molded at 140°C using a hydraulic PHI press (City of Industry, CA) with a 5-min cure time and a load of 40 tons. The mold consisted of five stainless-steel plates, and the middle part had a rectangular hole whose dimensions were 100 mm × 150 mm × 2 mm. Before compression molding, the materials were preheated in an oven at 90°C in polyethylene (PE) bags.

Part of the sample was milled cryogenically and sieved. The fraction whose size was between 150 and 400 μm was used for further analysis. The remaining intact samples were stored in PE bags at room temperature. The dry weight of the blends was measured using two different methods. The milled samples were kept in a desiccator containing diphosphorus pentoxide (Merck) at room temperature until the weight became constant (gravimetric analysis). In a modified Karl Fischer method,[29] water was titrated with iodine in the presence of sulfur dioxide, methanol, and a base, using a Mettler DL 18 titrator (Switzerland).

X-ray Diffraction

Diffractograms of milled TPS–PCL blends were recorded on a Philips PW 3710 diffractometer

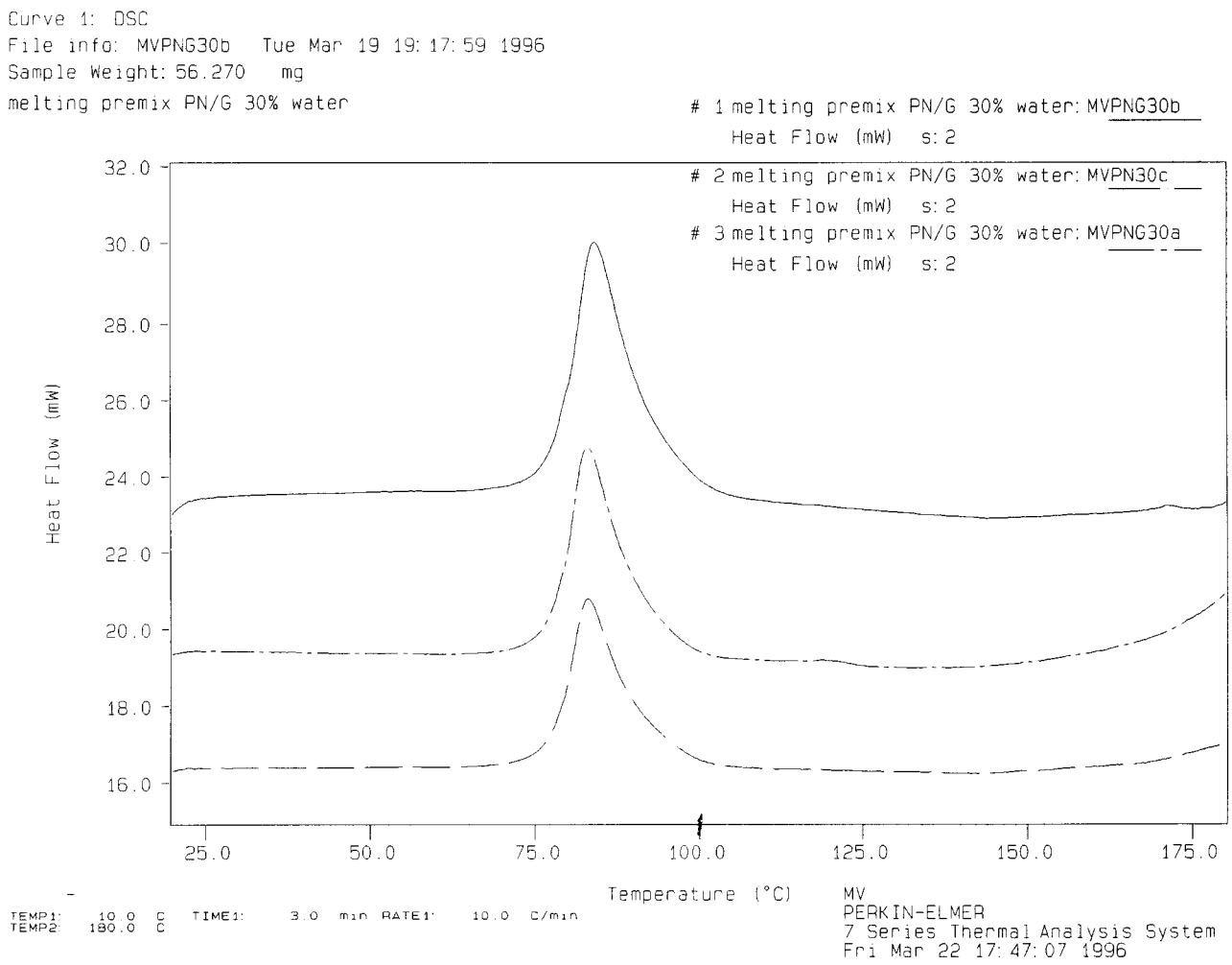

Figure 1a Thermogram of native potato starch–glycerol–water premix.

(The Netherlands). CuKα-radiation, generated at 40 mA and 50 kV, was filtered using a 15μm nickel filter. The X-ray beam was aligned using a 1° divergence slit, a 15-mm beam mask, a 0.2-mm receiving slit, and a 1° scatter slit. The diffractometer was equipped with an Anton Paar TTK temperature chamber. A proportional detector found scattered radiation in the range 4–40° (2θ) using.

Differential Scanning Calorymetry

The melting endotherms of the TPS–PCL blends were recorded on a Perkin-Elmer DSC-7 (Norwalk, CT) using a temperature range of 0–140°C and a heating rate of 10°C/min. The instrument calibration was done with indium (ΔH = 28.6 J/g, T(melt) = 156.6°C) and gallium (ΔH = 79.9 J/g, T(melt) = 29.8°C). An empty pan was used as a reference. The milled samples (≈30 mg) were weighed into stainless-steel pans, which were then sealed hermetically.

Molecular Weight Distribution of Starch

The molecular weight of starch in the milled TPS–PCL blends were determined by size-exclusion chromatography (SEC).[30] A 400-mg sample was moistened for 1 h with 10 mL water, and then 10 ml *2N* NaOH solution was added. The samples were stirred overnight and diluted with 1*N* NaOH. All the solutions were carefully deaerated, and all sample manipulations was carried out under an argon blanket. The GPC instrument consisted of μHydrogel 2,000, 500, and 250 columns and a refractive index detector and dual

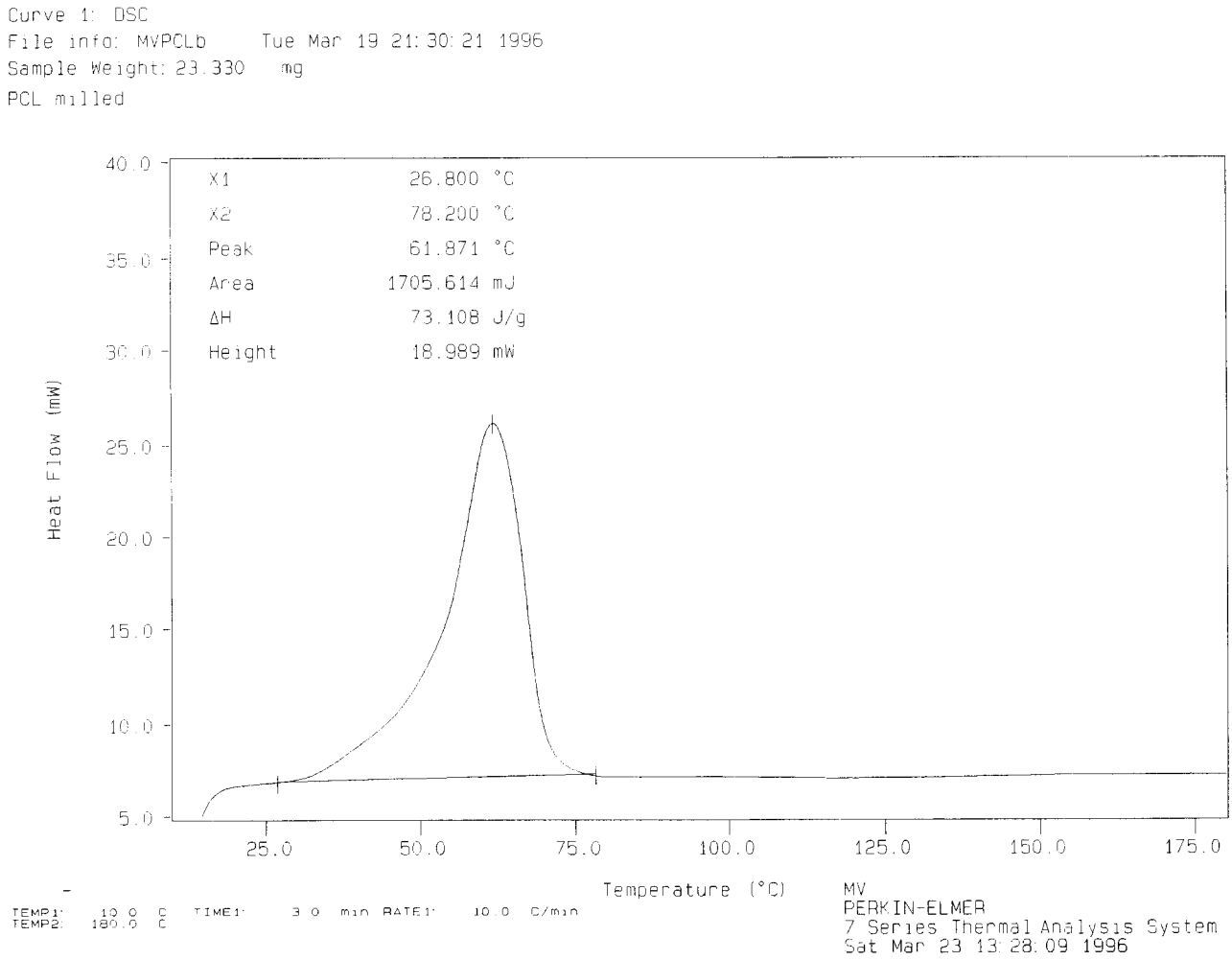

Figure 1b Thermogram of PCL.

angle laser-light scattering detector (15° and 90° measuring angles) (PDI2000, Precision detectors, Amherst, MA). The eluent was 50 m*M* NaOH at a flow rate of 0.5 mL/min.

Enzymatic Hydrolysis

Enzymatic hydrolysis was performed using *Bacillus licheniformis* (Sigma, Germany) alpha-amylase, and *Aspergillus niger* (Fluka, Germany) glucoamylase. Incubations were carried out at 37°C in 15-mL 0.1 *M* acetate buffer (pH 5.0) containing 0.2% sodium azide to prevent microbial growth. The samples were tested both as a piece, with a size of 2 mm × 15 mm × 15 mm, and as a milled fraction, with a particle size of 150–400 μm. The concentration of the enzymes with the pieces of sample was 7.5 U/mL alpha-amylase and 15 U/mL glucoamylase, and with the milled samples 1.5 U/mL alpha-amylase and 3 U/mL glucoamylase. Native granular potato starch (Avebe, The Netherlands) was used as a reference. The rate of enzymatic hydrolysis was measured by analyzing the reducing sugars[31] during incubation, and the depth of enzyme penetration at the end of the experiment was measured using scanning electron microscopy (SEM).

Scanning Electron Microscopy

The morphology of the TPS–PCL blends and the starch removal during enzymatic hydrolysis were observed under a scanning electron microscope (Philips 515, The Netherlands) with an acceleration voltage of 10–11 kV. Cross sections were obtained by freeze-fracturing the samples under a nitrogen flush using a cryo system (Emscope

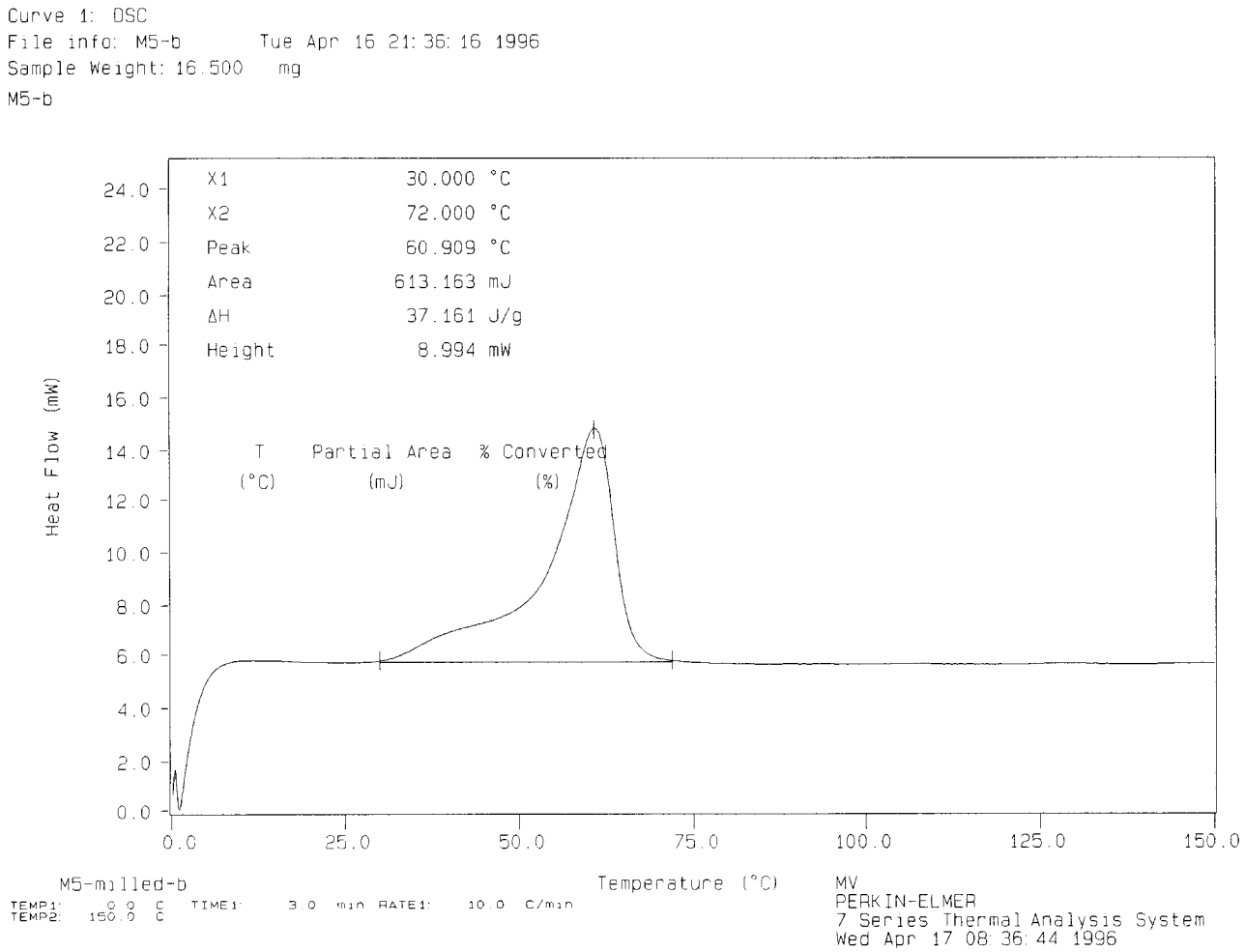

Figure 1c Thermogram of TPS–PCL blend.

SP2000A). The samples were coated with a thin layer of gold.

RESULTS AND DISCUSSION

Properties of the TPS–PCL Blends

The dry weights of the milled TPS–PCL blends varied from 97.0% to 98.2% as measured by both gravimetric analysis and by Karl Fischer titration. The water contents of the blends were low, indicating that almost all the water had evaporated during the melt mixing.

Thermograms of premix (native potato starch + water + glycerol), PCL, and TPS–PCL are shown in Figure 1. According to the results, the starch granules in the premix were completely molten at 100°C [(Fig. 1(a)]. The ratio of TPS and PCL can be established using the melting enthalpy of PCL (75.0 J/g). The melting enthalpies and melting temperatures of the TPS–PCL blends varied from 37.2 J/g to 39.1 J/g and from 60.8°C to 62.3°C, respectively. As a result, the amount of PCL in the TPS–PCL blends was confirmed to be 48–50 wt %. The melting temperature of the milled PCL granules was 61.5°C, and increased to 63.5°C after compression molding.

After plasticization the potato starch was completely amorphous, and no residual or processing-induced crystallinity originating from potato starch was detected using X-ray diffraction (Fig. 2). Native potato starch showed the typical B-type diffraction pattern, as shown in Figure 2.

GPC analysis determines the average molecular weight of amylopectin and amylose. After plasticization of the starch, the typical amylopectin peak present in native starch was not detected (Fig. 3), and the average molecular weight of the

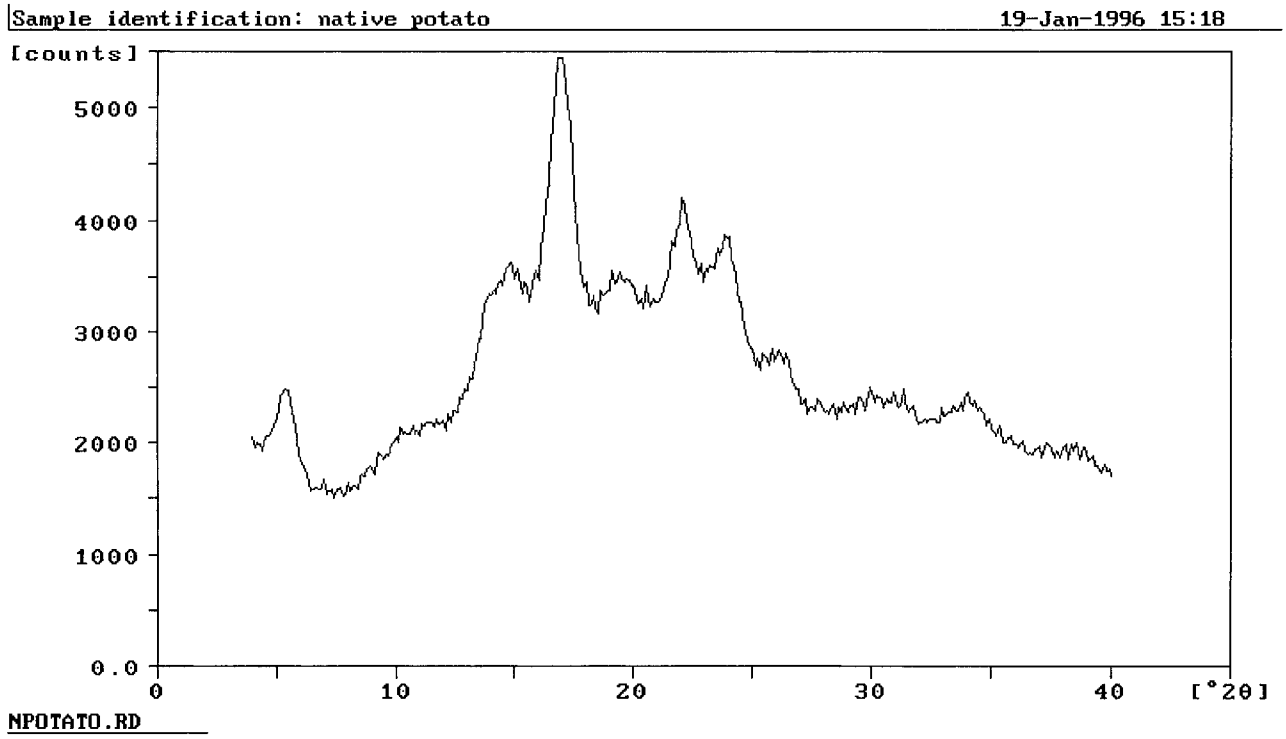

Figure 2a Diffractogram of native potato starch.

starch was strongly reduced, from 10^8 g/mol to below 2×10^7 g/mol (Table II). The significant reduction in molecular weight was especially clear in the case of blend 6, which was prepared at the highest melt temperature (166°C). It should be noted, however, that starch depolymerization can also be partially caused by compression molding.[24]

Morphology and Enzymatic Degradation of the TPS–PCL Blends

The removal of starch by incubation with alpha-amylase and glucoamylase resulted in a porous structure that was easily visible by SEM (Fig. 4). According to the SEM studies both phases were cocontinuous with the TPS–PCL ratio used, but thermoplastic starch had a particlelike, interconnected structure. The size of the pores in the PCL matrix was small, varying from 0.5 μm to 5 μm [Figs. 4(b) and 4(c)]. In general, the phase structure of the TPS–PCL blends depends on the composition and melt viscosities of both polymers. When the melt viscosities are close to identical, a better phase structure and smaller pore size will be expected. According to the SEM micrographs shown in Figure 4, blends 1, 2, and 3 may have a slightly finer phase structure (smaller pore size) compared to blends 4, 5, and 6.

During processing the melt temperature increased from the set value due to internal friction (Table I). The increase in mixing speed led to the higher melt temperature. When the mixing speed was kept constant during processing (blends 1, 2, 3, and 6), a higher melt temperature resulted in lower torque [(Fig. 5(a)]. Low torque could be the reason for the coarser phase structure, as in the case of blend 6. With the same melt temperature of 149–150°C (blends 3, 4, and 5), an increase in mixing speed resulted in a higher torque [Fig. 5(b)]. With the same torque during processing (blends 1 and 4), a higher melt temperature was obtained with a higher mixing speed.

As expected, the enzymatic hydrolysis of the blends proceeded from surface of the material inward [Fig. 4(d)]. The borderline between the porous area and the intact area was very distinct. When the original upper surface of the TPS–PCL test piece was examined by SEM after enzymatic hydrolysis [Fig. 4(e)], only a few holes in a continuous filmlike structure were detected. The reason for this was the thin layer of PCL ($\approx$5 μm) formed on the upper surface of the plates during compres-

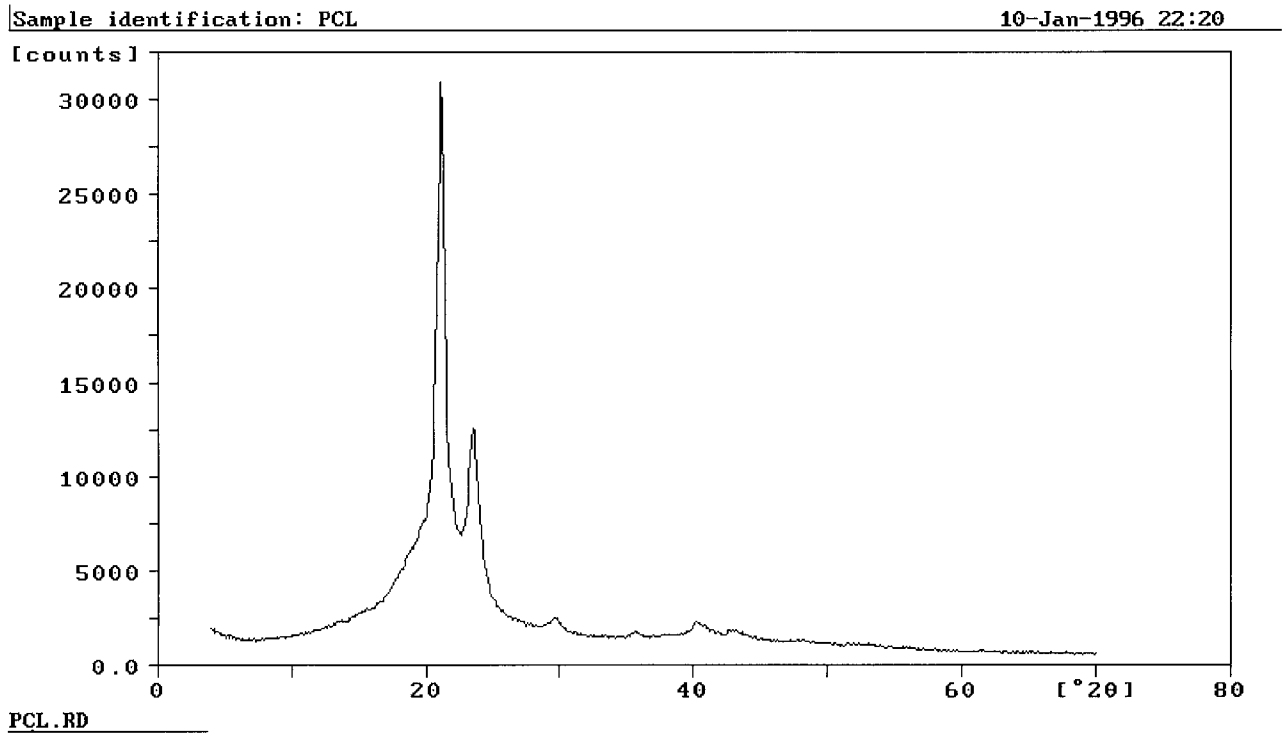

Figure 2b Diffractogram of PCL.

sion molding [Fig. 4(f)]. Because of this layer of PCL, the rate of enzymatic hydrolysis in the original surface was reduced, and the enzyme penetration depth was smaller compared to the cut surface. When the depth of enzyme penetration in the original surface and in the cut surface is known, the volume of the hydrolyzed part in the TPS–PCL pieces can be calculated as a percentage (Table III).

It has been shown[28] that native starches plasticized with glycerol are completely hydrolyzed by amylolytic enzymes. When the TPS–PCL pieces were hydrolyzed with amylolytic enzymes, 47% to 69% of the original starch was removed after 11 days of incubation (264 h) [Fig. 6(a), Table IV] measured as reducing sugars. The addition of fresh enzymes did not increase the rate of enzymatic hydrolysis. Native potato starch was used as a comparison, and after 11 days 80% of the original starch had been hydrolyzed. The volume of the hydrolyzed part of the sample shown in Table III correlated well with the amount of starch hydrolyzed from the TPS–PCL pieces shown in Table IV. This indicates that the thermoplastic starch is exposed to the amylolytic enzymes and not entrapped inside the PCL matrix. Due to the increased surface area, enzymatic degradation of the milled TPS–PCL blends was very rapid, although a lower concentration of amylolytic enzymes was used compared to the degradation TPS–PCL pieces. Enzymatic degradation of milled samples reached the level of 36–53% in 3 h [Fig. 6(b), Table IV]. After the addition of fresh enzymes (48 h), the rate of enzymatic hydrolysis increased, 93–97% of the original starch was finally hydrolyzed after 172 h.

Because of the thin layer of PCL on the surface of blends, the rate of enzymatic hydrolysis should be evaluated by determining the enzyme penetration depth in the cut surface (no PCL covering) and the amount of starch hydrolyzed from the milled samples. The enzymatic hydrolysis of the milled samples was more rapid when the blends were prepared at higher melt temperatures (Table IV). The enzymatic hydrolysis of the TPS–PCL pieces was fastest in the case of blend 5 (Table III). One reason for the better degradation of blends 4, 5, and 6 could be the depolymerization of starch at the higher melt temperatures; however, there were no significant differences in the average molecular weight (Table II). On the other hand, blends that were prepared at a higher molding temperature also had a coarser structure, as shown in the SEM micrographs (Fig. 4).

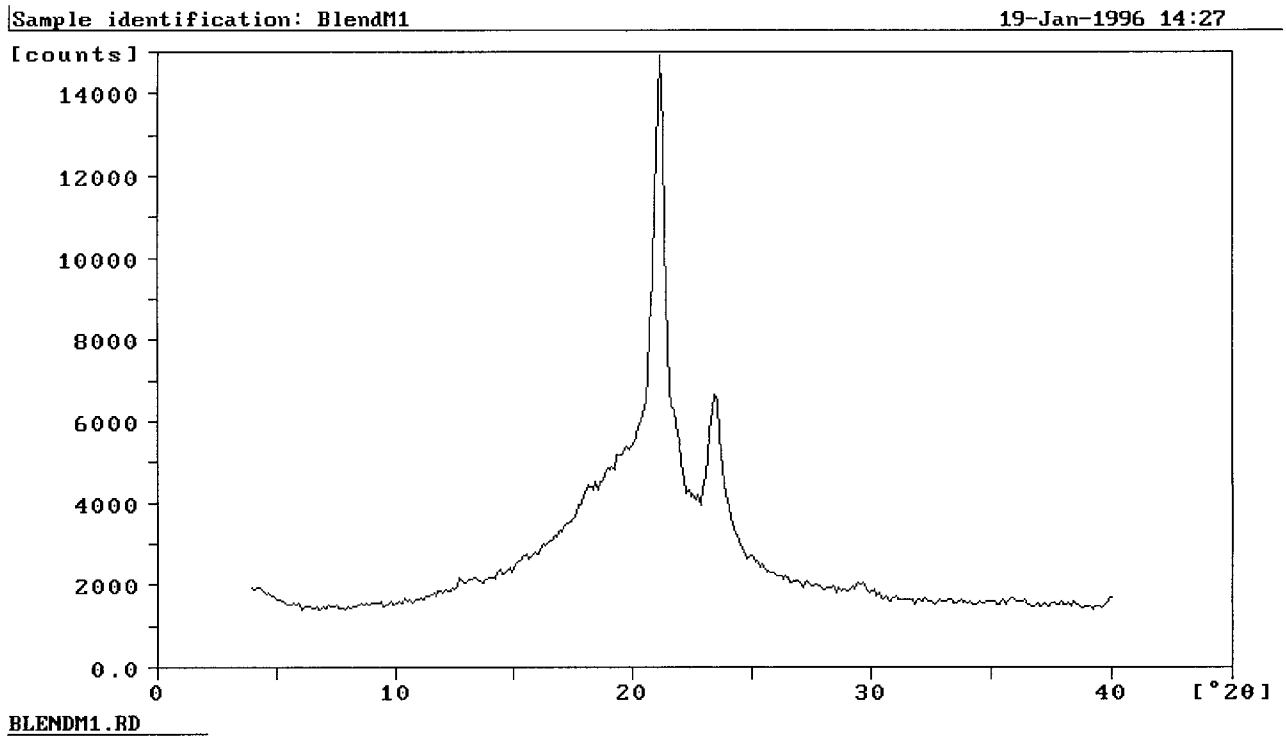

Figure 2c Diffractogram of TPS–PCL blend.

According to Iwamoto and Tokiwa,[22] the higher the miscibility of PCL and the conventional plastics, the slower was the degradation of PCL in their blends by the lipase enzyme. However, more studies are needed to evaluate the correlation between processing conditions and the enzymatic degradation of TPS–PCL blends.

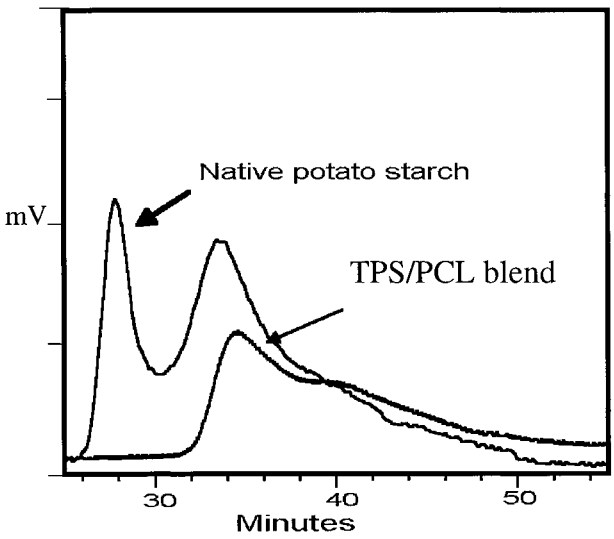

Figure 3 Molecular weight distribution of starch in TPS–PCL blend 6 and native potato starch.

CONCLUSIONS

Enzymatic hydrolysis of starch in 50/50 (w/w) TPS–PCL blends using amylolytic enzymes coupled with analysis using SEM showed that blends were cocontinuous and the thermoplastic starch had a particlelike, interconnected structure. The TPS–PCL blends were very homogeneous, and

Table II The Average Molecular Weight of the Native Potato Starch and Milled TPS/PCL Blends

Sample	Average Molecular Mass $M_w \times 10^6$ (g/mol)
Native potato starch	104
Blend 1	17
Blend 2	17
Blend 3	15
Blend 4	14
Blend 5	16
Blend 6	9

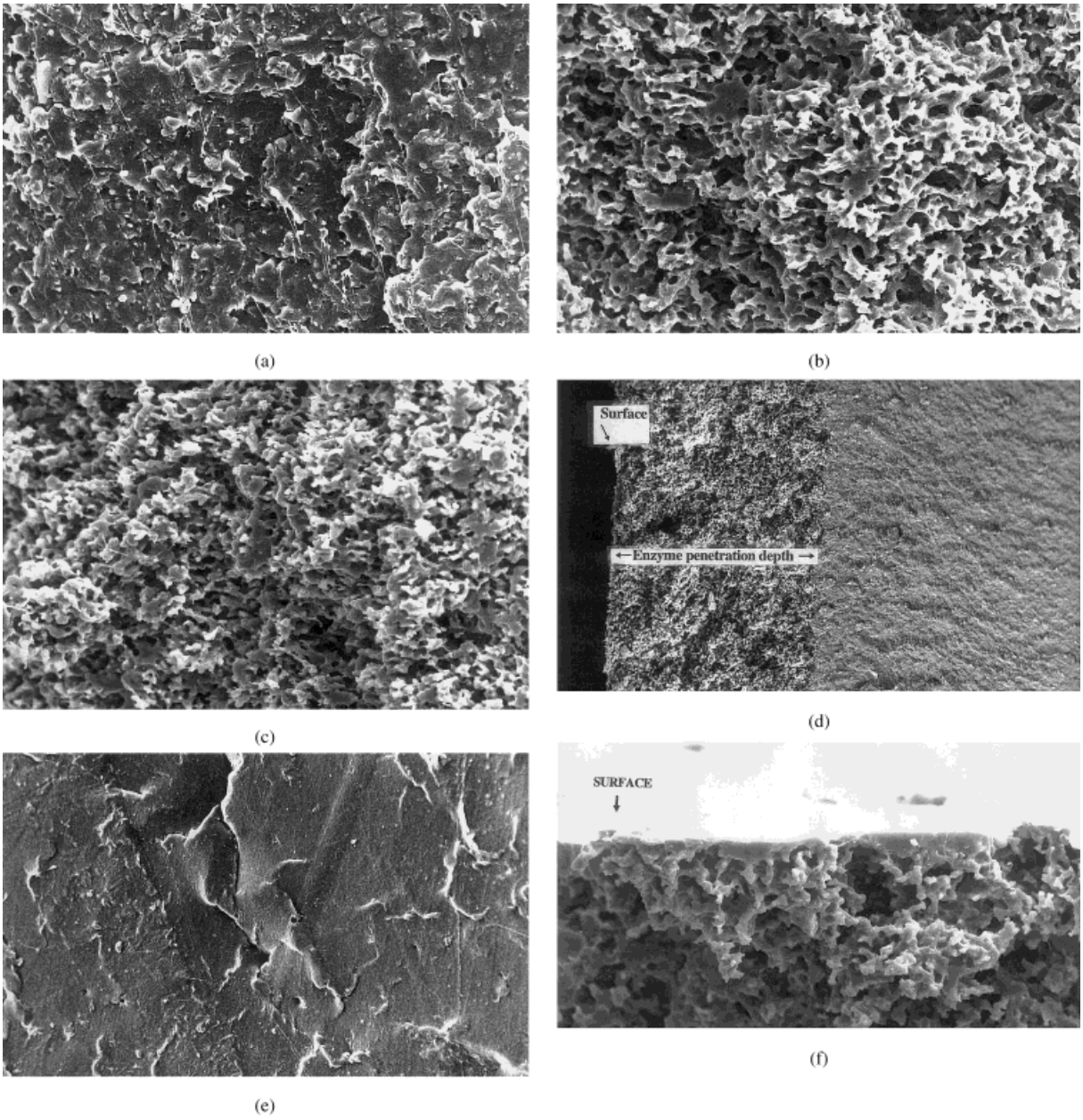

Figure 4 Scanning electron micrographs of TPS–PCL blends after enzymatic hydrolysis: (a) cross section of the TPS–PCL blend 6 before enzymatic hydrolysis; (b) cross section of the TPS–PCL blend 6 with the coarse phase structure after enzymatic hydrolysis; (c) cross section of TPS–PCL blend 3 with the fine phase structure after enzymatic hydrolysis; (d) cross section of TPS–PCL blend 1 after enzymatic hydrolysis showing the hydrolyzed and intact area; (e) original upper surface of the TPS–PCL blend after enzymatic hydrolysis (only a few holes were detected in a continuous filmlike surface); and (f) thin layer of PCL formed on the top of the TPS–PCL plate.

the size of the TPS pores in PCL matrix was very small, varying from 0.5 μm to 5 μm. A thin layer of PCL was formed on the upper surface of the TPS–PCL plates during compression molding. After processing, the starch was completely melted, and no crystallinity originating from potato starch was detected in the TPS–PCL blends. The average molecular weight of potato starch was strongly reduced during processing from 10^8 to below 2×10^7 g/mol.

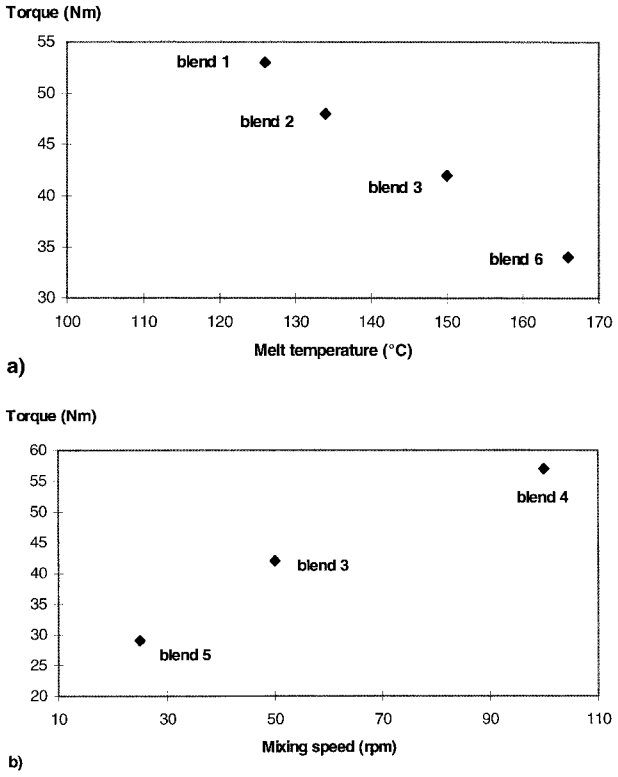

Figure 5 Processing parameters during blending: (a) influence of melt temperature on the torque at constant mixing speed (blends 1, 2, 3, and 6); (b) influence of mixing speed on torque at constant melt temperature (blends 3, 4, and 5).

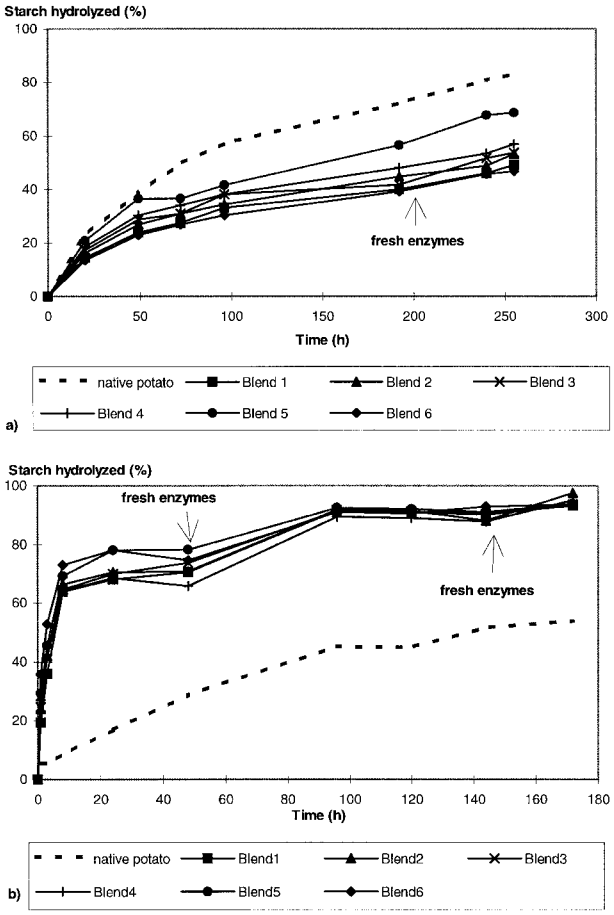

Figure 6 The enzymatic degradation of the blends, measured as reducing sugars: (a) piece of material and (b) milled.

Enzymatic hydrolysis of the milled TPS–PCL samples was more rapid compared to the TPS–PCL pieces, indicating the importance of surface area of the samples in the biodegradation tests. Enzymatic hydrolysis proceeded from the surface of the material inward, and the borderline between the hydrolyzed section and the intact area was very distinct. The PCL layer on the surface of

Table III The Depth of Enzyme Penetration in the Original Upper Surface and in the Cut Surface (No PCL Covering), the Volume of the Hydrolyzed Part, and the Phase Structure of the Blends

Sample	Depth of Enzyme Penetration[a]		Volume of Hydrolyzed Part[b] (% Original)	Phase Structure[a]
	Cut Surface (μm)	Original Surface (μm)		
Blend 1	630	430	44	fine
Blend 2	620	500	51	fine
Blend 3	650	510	52	fine
Blend 4	660	590	60	coarse
Blend 5	770	670	68	coarse
Blend 6	730	430	44	coarse

[a] Estimated on the basis of SEM pictures.
[b] Calculated on the basis of the depth of enzyme penetration.

Table IV The Amount of Starch Hydrolyzed (Percent of the Original) During Enzymatic Hydrolysis, Measured as Reducing Sugars

	Starch Hydrolyzed (%)		
Sample	Piece (264 h)	Milled (1 h)	Milled (3 h)
Blend 1	49	19	36
Blend 2	53	24	42
Blend 3	54	25	41
Blend 4	57	27	42
Blend 5	69	29	46
Blend 6	47	36	53

the TPS–PCL plates reduced the rate of the enzymatic hydrolysis. The enzymatic hydrolysis of the TPS–PCL blends was more rapid at higher blending temperatures, which could be due to a coarser phase structure. According to enzymatic hydrolysis using the liberation of reducing sugars and the enzyme penetration depth assessed with SEM, no starch was entrapped inside the PCL matrix.

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