Kasetsart J. (Nat. Sci.) 34 : 254 - 261 (2000)

Effect of Particle Size on Physical Properties and Biodegradability of Cassava Starch / Polymer Blend

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ABSTRACT

Blending starch with a biodegradable polymer is one method to produce a bioplastic. Due to the high cost of biopolymers, techniques have been developed to enable a high concentration of starch in the blend. The study was conducted in order to determine the effect of particle size (9 to 15 μm) of cassava starch on the physical and biodegradable properties of polycaprolactone (PCL)/cassava starch blend. It was found that particles of cassava starch, after ball milling (0, 60, 90 and 120 minutes), lost their crystallinity as observed by DSC and birefringency. With less crystallinity, particles observed by SEM. easily formed clumps during the blending process. The tensile strength and %elongation decreased from 12.21 MPa and 302.89 of PCL/native starch to 4.48 MPa and 25.63 for the 120 minutes ball milled starch/PCL blend (15 : 85 w/w). Addition of glycerol (25%w/w) into the blend could support only %elongation but not tensile strength. Excellent susceptibility to α-amylase and glucoamylase hydrolysis were evident when small particles were incorporated in the blend, compared to native starch. Blends with 60, 90, 120 minutes ball milled (both with and without addition of glycerol) showed very high biodegradability as determined by total organic carbon (TOC) released after subject to amylase digestion.

Key words : cassava starch, biodegradable plastic, polycaprolactone

INTRODUCTION

Solid waste management is increasingly seen as a major issue in Thailand due to high rates of urbanization and increasing per capita solid waste generation associated with greater affluence. A major component of solid waste is resin based plastic; there are many forms all petroleum based polymers. Plastics possess many properties, such as water resistance and long life, that make them perfect candidates for packaging. It is therefore not surprising that plastics are used for both consumer and industrial products. Unfortunately, the properties that make this product suitable for packaging also are the major cause of the environmental problems related to packaging. Given the wide use of plastics the magnitude of the environmental problem is large and long term. The choices for dealing with this waste are limited as incineration of plastic polymers invariably results in the generation of toxic emissions; the only viable solution is to bury the material in landfills. However, this approach is also limited because of the resistant nature of plastic to biological degradation. In an attempt to ease the environmental burden from plastic, a new generation of products has been developed, known as biodegradable plastic. These plastics are produced from natural or synthetic

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biodegradable polymers that are used individually or as a blend. Commonly used synthetic aliphatic biodegradable polymers include, polyvinyl alcohol (PVA), polylactic acid (PLA), polyethylene succinate (PES), poly (3-hydroxybutyrate) (PHB), polycaprolactone (PCL) (Kaplan *et al*., 1993). These polymers, while possessing the correct technical attributes are also expensive. The production cost of biodegradable plastic is about three to five times more than that of conventional plastic. Therefore, in an attempt to make biodegradable plastics commercially attractive, the use of natural material, which is also highly biodegradable, has been investigated. Starch is identified as a key natural product for the production of biodegradable plastic. Lowering the amount of synthetic polymer by blending with starch is a strategy widely adopted for research (Pranamuda *et al.*, 1996; Koenig and Huang, 1995; Lawton and Fanta, 1994; Takagi *et al*., 1994, Swanson *et al*., 1993) patents (Narayan *et al*., 1996; Tokiwa *et al*., 1995; Tokiwa *et al*., 1993).

In Thailand, about 2 million tons of cassava starch are produced annually at a competitive price. Given the local availability of this abundant starch source, biodegradable plastic production from cassava starch should be developed. Previous work has shown that polycaprolactone (PCL) is a good polymer for blending with cassava starch; the advantages are mainly derived from the low melting point of the synthetic polymer. Cassava starch can be blended either in the granular or gelatinized form and inclusion of this starch can be between 10 to 50%w/w (Pranamuda *et al.*, 1996). The objective of formulating blends of synthetic polymer with starch is that for a desired plastic property, the highest amount of starch be included and therefore less PCL required. The main disadvantage of using starch is that its inclusion weakens tensile strength and elongation. In an attempt to lower the negative impact of starch inclusion, a number of different blending techniques have been investigated. To date, none have proven to be successful; techniques include blending of cassava chip to PCL (Chollakup *et al.*, 1997), incorporating sucrose ester (Sriroth *et al.*, 1999). Inclusion of sucrose ester has a positive effect in increasing the mass of the blend, but does not improve tensile strength or elongation of the blend. The study reported on the effect of particle size of cassava starch granule after reduction by ball milling on the mechanical properties and biodegradability of PCL/cassava starch blend at the level of 85 : 15.

MATERIALS AND METHODS

Cassava starch was obtained from Cholcharoen Co., Ltd. (Thailand). α-Amylase (345 U/mg) from *Bacillus subtilis* and lipase (4,000,000 unit) from *Rhizopus arrhizus* were purchased from Fluka (Switzerland) and Sigma Chemical Co. (USA), respectively. Polycaprolactone (PCL) (Tone-P-767, Mw = 40,000, Tm = 62°C) was from Union Carbide Co., Ltd. (U.S.A.).

Preparation of small particles (ball mill) cassava starch (Marffy and Kula, 1974)

A 20% w/w suspension of cassava starch (100 gm) in ethanol (99.8%v/v absolute ethyl alcohol, MERCK) was poured into a ball mill disintegrator (Dyno-Mill type KDL, Willey A. Bachofen Maschinenfabrik, Denmark) containing 450 ml of glass beads (400 mm diameter). The mill was operated at 3,000 rpm and the temperature control by an outer jacket containing circulated cooling water at 14°C. Samples after milling for 0, 60, 90 or 120 minutes, were dried by evaporation at 35°C in rotary evaporator. Dry mill starch was ground in blender.

Preparation of cassava starch/PCL blend

Sixty grams of dry ball milled cassava starch/ PCL blend (proportion of starch : PCL of 15 : 85)

was prepared using a single screw extruder (Custom Scientific Instrument, Atlas Electric Devices Co., Ltd., U.S.A.) at 95°C, 180 rpm. To ensure homogeneity the blend was re-mixed under the same conditions. To some samples, prior to mixing, glycerol was added at 25% percentage by weight. The viscosity behavior of the blends (after 7 days in room temperature storage) was measured using a flow tester-CFT 500D (Shimadzu, Japan) according to Takagi *et al.* (1994) at 95°C with constant pressure at 40 kgf/cm^2.

Proximate analysis and granule size distribution

Moisture was determined by drying at 105° C to constant weight (AOAC, 1995). The content of protein (total N by Kjeldahl method x 6.25), crude fiber (by acid and alkali treatment), fat (by Soxhlet with petroleum ether extraction) and ash (temperature 600°C for 3 h) were quantified as described by AOAC methods (AOAC, 1990). Starch content was estimated by Polarimetry method (AOAC, 1995). Granule size distribution was determined by image analysis (Carl Zeiss, KS400 v2) recorded directly from a Carl Zeiss Axiophol 2 microscope. Starch samples were suspended in 80% sucrose solution to minimize the effects of refractive index and to provide a sharper image of the granules (Baldwin, 1994).

Degree of hydrolysis of starch granules

Hydrolysis of starch granules was completed using a reaction mixture containing 2.0% starch granules in 0.1M acetic acid-sodium acetate buffer solution (pH 5.0) and 40 ppm $CaCl_2$. Hydrolysis was initiated following addition of a mixture containing 1% (volume of enzyme/weight of starch) of each of the enzymes - glucoamylase (AMG, 300 units g^{-1}: Novo Nordisk) and alpha amylase (BAN, 480 units g^{-1}: Novo Nordisk). Samples were incubated at 35°C with constant shaking. Degree of hydrolysis was determined as outlined by Wang *et al.* (1995).

Differential scanning calorimetry (DSC)

Thermal properties of the blends were measured using DSC (Perkin Elmer DSC7, Norwalk, USA) calibrated using an indium standard. Samples of blends (4mg) were sealed in steel pans. The heating rate was 10°C/min. Enthalpy changes were integrated using DSC-7 standard software, and were calibrated based on the melting enthalpy of indium metal.

Mechanical properties

Pellets of blends were compressed and molded into plaques at 95°C with a pressure load of 150 kgf/cm^2 using a heat press (Toyoseiki Seisakusho Co., Ltd., Japan) employing a 120x120 mm window frame mold of 0.5 mm thickness. After cooling the mold, the plaques were cut into dumbbell-shaped pieces based on ASTM-D638-95 (Anon, 1983) with a Toyoseiki cutter (Toyoseiki Seisakusho Co., Ltd., Japan). They were conditioned in a humidity incubator (WTBbinder, KBF240, Germany) at 50±5% relative humidity and 23±2°C for 12 hours. Tensile strength and %elongation were measured following the method of ASTM-D638-95 (Anon, 1983) using the AGS-5kND-type autograph (Shimadzu Co., Japan).

Enzymatic degradation and water resistance of the blends

- **α-Amylase** A plaque of cassava starch/PCL blend prepared as previously described was sliced into a film of 100 μm thick using a microtome (Histoslide 2000 Reichert-Jung, Leica Instrument, Germany). 150 mg of film substrate was mixed with 2.0 ml of 2.0 M phosphate buffer (pH 7.0), 1.0 ml of 0.1% plysurf, 1.0 ml of α-amylase (4,950 U) (omitted from the reaction mixture in control substrate) and 16.0 ml distilled water. The reaction mixture was incubated at 30°C for 16 hours, shaking

at 180 rpm with a gyratory shaker. An aliquot, 5 ml, was withdrawn and filtered through a 0.5 μm filter (Millipore, U.S.A.). The total dissolved carbon of the film, after degradation, was measured with a total organic carbon analyzer (TOC-5000 Analyzer, Shimadzu Co., Ltd., Japan). The total dissolved organic carbon value of each blend was recorded after subtraction with the control.

- **Lipase** Degradation by lipase was as for amylase reactivity using 150 mg of film substrate, 2.0 ml of 0.2 M phosphate buffer (pH 7), 1.0 ml of 0.1% plysurf, 1.0 ml of lipase (2,121 U/ml) and 16.0 ml distilled water.

Scanning electron micrographs (SEM)

Phase structure of cassava starch/PCL blend was observed under a JEOL scanning electron microscope (JSM-35CF, England) at 15-kV acceleration and magnification of 1,000X. The sample was immersed in liquid nitrogen for drying and prepared by sprinkling on double-sided adhesive tape attached to a circular specimen stub and coating with a thin gold layer using Ion Sputter (JFC-1100, England).

RESULTS AND DISCUSSIONS

Cassava starch used in this study was pure (98% starch content, polarimetric method) (Table 1). Starch granule was 15 μm, which is agreeable to that of Thai cassava starch (Sriroth *et al*., 1999). Granule size was reduced after ball milling, following treatment of 60, 90 and 120 minutes, average granule size was reduced to 14.8, 13.8 and 9.1 μm respectively. The distribution of granule size is shown in Figure 1. Size reduction of the granule was proportional to the milling time. The longest treatment time, 120 minutes, resulted in very fine starch particles, but milling for long periods also carried the risk of inducing granule degradation, such as gelatinization. Microscopic observation of the starch granules revealed that many lost their birefringence. This may be due to frictional heat generated during the milling process (in spite of cooling at 18°C).

Endothermic heat flow determined by DSC

Table 1 Composition of cassava starch.

Composition	% (dry basis)
Starch	98.28±0.10
Crude protein	0.10±0.00
Crude fat	0.12±0.07
Ash	0.13±0.00
Fiber	0.04±0.01

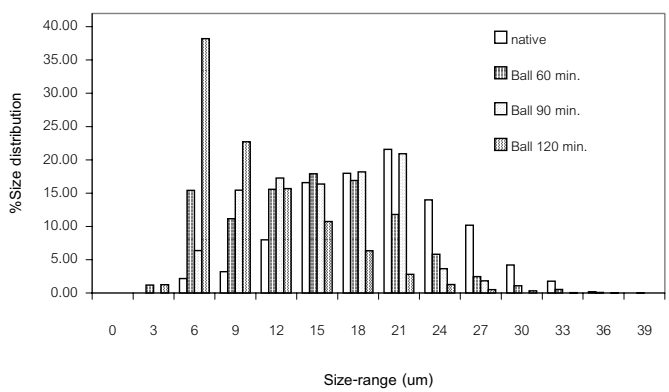


Figure 1 The size distribution of native starch and ball-milled starch treated at various times.

confirmed that the granules were gelatinized (Table 2). Peak temperature (T_p) and enthalpy of the samples after milling decreased. Peak temperature and enthalpy of native starch were 65.7°C and 14.5 J/g, respectively; these values decreased after milling for 120 minutes to 57.2°C and 2 J/g. This suggests loss of granule crystallinity, and greater water absorption and solubilisation. The starch particles formed by milling are also more susceptible to hydrolysis. Results showed that milled samples are significantly more susceptible to hydrolysis by alpha amylase (Figure 2). Ball milled starch samples were 80% hydrolyzed within 50 minutes at 35°C compared to only 40% hydrolysis under similar conditions for non-milled starch.

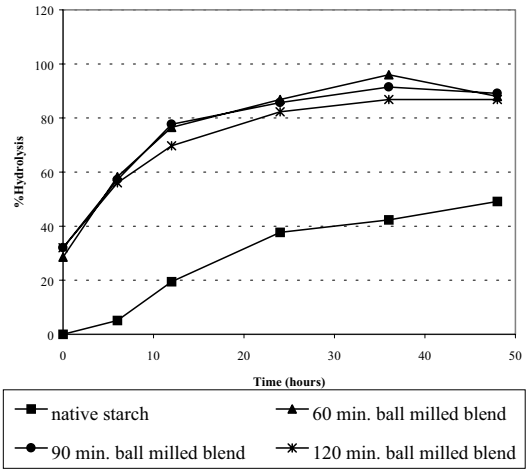


Figure 2 Hydrolysis of ball milled starch by alpha amylase (BAN 480 units/g) and glucoamylase (300 units/g).

Homogeneity of a starch-polymer blend is considered an important factor for processing. Fine particles of ball milled starch tended to form clumps during the blending process. The clumps could be observed by scanning electron microscope. Clump formation was related to the mechanical disruption of the granules. Untreated starch granules blended with PCL showed good distribution in the continuous phrase of PCL (Figure 3a). Possibly, the lower hygroscopic nature of the starch granules was responsible for preventing the formation of clumps. Addition of glycerol in the starch – polymer blend aided the distribution of starch and strengthened the granule (Figure 3b). This confirmed a previous observation that starch granules were stabilized by a cross linking effect of glycerol inside the granule (Van Soest *et al*., 1995). After ball milling to fine particles, clump formation was unavoidable, even for the samples milled for a short period (Figure 4a and 4b). These clumps were not as strong as native starch granules because of the absence of crystalline zones. Following addition of glycerol to the blend, distribution of particles in the continuous phase of PCL was improved but some clumping still occurred. This phenomenon was probably the cause for the decrease in mechanical properties. The tensile strength and %elongation of PCL/native starch at 85/15 ratio were 12.21 MPa and 302.89 respectively. The values decreased to 4.48 MPa and 25.63 in the blend of 120 minutes ball milled starch (Table 3). Milling time did not seem to be important with

Table 2 Thermal properties of native and ball-milled cassava starch.

	Peak temperature (T_p)	Enthalpy (J/g)
Native starch	65.74±0.11	14.54±1.71
60 min ball milled	59.60±4.82	5.60±1.84
90 min ball milled	58.78±5.13	4.05±0.35
120 min ball milled	57.18±2.86	2.11±0.58

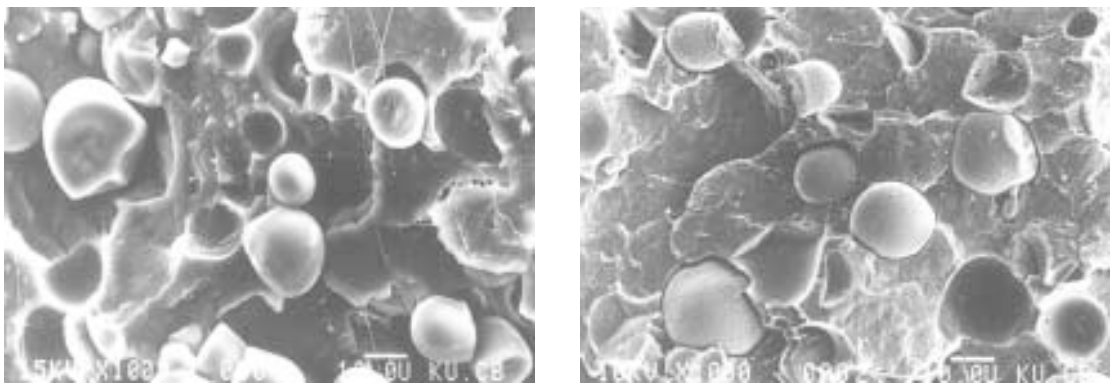


Figure 3 Scanning electron micrographs of PCL films containing 15% native cassava starch (a) and with addition of 25% glycerol (b).

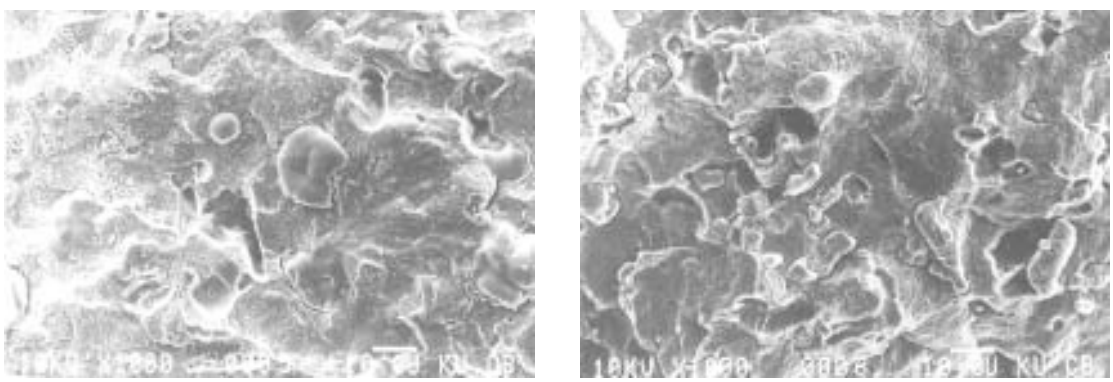


Figure 4 Scanning electron micrograph of PCL film containing 15% of 90 minutes ball milled starch (a) and with addition of 25% glycerol (b).

respect to mechanical properties; the shortest time seemed to induce the changes responsible for the altered effects. Following addition of glycerol to the blend, the tensile strength did not change but the %elongation was improved. However, it is reported that small particles of corn starch (2 μm) when used in a low-density polyethylene (LDPE) blend results in higher elongation and tensile strength compared to big granules (Lim *et al*., 1992). However, it should be noted that the small particles of ball milled starch still possess the birefringence and crystallinity. The granules will be expected to be normally distributed in the continuous polymer phase, and will show less tendency to form clumps. The results of our experiment confirmed that the distribution of starch in PCL and existence of crystalline zones in granules were critical for mechanical properties.

In contrast to the mechanical properties, biodegradability of the blend was improved when ball-milled samples were used. Blends with glycerol showed very high susceptibility to α-amylase hydrolysis but very low activity to lipase. The maximum rate of biodegradability, expressed by total organic carbon (TOC), was found in the samples blended with 60 and 90-minute ball milled

Table 3 Physical properties of PCL/cassava starch at the ratio of 85/15 with and without glycerol.

	Tensile strength (MPa)	% elongation
PCL/ MS		
0 min	12.21±3.2	302.85±45.9
60 min	5.67±2.0	20.22±3.9
90 min	5.07±32	24.30±15.7
125 min	4.48±3.1	25.63±8.5
PCL/MS/Glycerol		
0 min	11.71±4.2	391.24±39.7
60 min	5.81±1.0	27.62±9.8
90 min	6.98±1.8	35.7±21.8
120 min	6.94±3.0	67.61±26.4

starch. For the 120 minutes ball milled sample, less enzyme activity was evident, comparing to the 60 and 90 minute treatment. This would be the effect of homogeneity and clump. In general, blends produced with ball-milled samples were more biodegradable than native starch (Figure 5).

CONCLUSION

Reduction of starch granules to small particles does not improve the mechanical properties of PCL/cassava starch blends. The reasons are that the fine particles loose their crystalline regions during milling, thus tend to form clumps during blending. Addition of glycerol slightly improves elongation of the blend, but not tensile strength. The obvious advantage of using ball-milled starch in a polymer blend is the much-improved biodegradability.

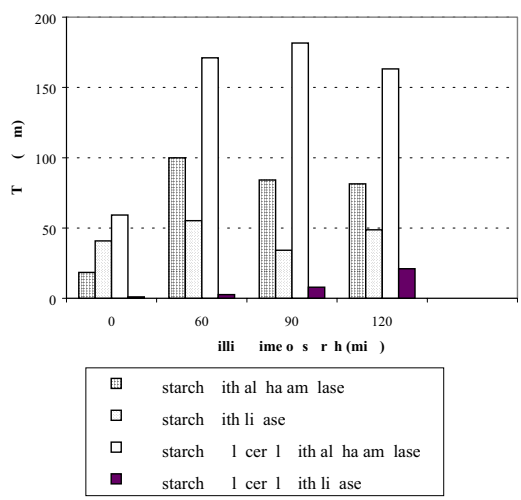


Figure 5 Enzymatic degradability by alpha amylase and lipase of PCL/cassava starch blend with and without glycerol.

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Received date : 15/03/00
Accepted date : 30/06/00