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The enzymatic hydrolysis of starch-based PVOH and polyol plasticised blends

Melissa A.L. Russo [a,b,*], Rowan Truss [a], Peter J. Halley [a,b]

[a] The Centre for High Performance Polymers, University of Queensland, Queensland 4072, Australia
[b] The Australian Institute of Bioengineering and Nanotechnology (AIBN), University of Queensland, Brisbane, Qld 4072, Australia

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ABSTRACT

Thermoplastic starch (TPS) materials present several advantages to the plastic industry and when blended with other materials they can exhibit improved mechanical and moisture sensitivity properties compared to pure TPS materials. However, the biodegradability of these blends, through such processes as enzymatic degradation, needs to be characterised to ensure the beneficial properties of TPS are not compromised. The aims of the study were to investigate the effect of varying polyvinyl alcohol (PVOH) content and polyol type within the TPS blends on the rate and extent of starch enzymatic hydrolysis using enzymes α-amylase and amyloglucosidase. The results of this study have revealed that TPS:PVOH blends with a PVOH content at 50 wt% exhibited a significantly reduced rate and extent of starch hydrolysis. The results suggest that this may have been attributed to interactions between starch and PVOH that further prevented enzymatic attack on the remaining starch phases within the blend. The extent of starch hydrolysis was not significantly affected by polyol type, however, the rate of starch hydrolysis from the maltitol blend was significantly reduced compared to sorbitol and glycerol substrates.

1. Introduction

Thermoplastic starch (TPS) materials present several advantages to the plastic industry, due to their renewability, biodegradability, low cost and wide availability (Avérous, 2004; Avérous, Moro, Dole, & Fringant, 2000). However, the applications of TPS materials are limited by poor mechanical strength properties and high moisture sensitivity (Avérous, 2004; Avérous et al., 2000). Researchers have overcome these challenges by successfully blending starch with other synthetic polymers and plasticisers (Follain, Joly, Doleb, & Bliarda, 2005; Follain, Joly, Doleb, Rogeb, & Mathlouthib, 2006; Huneault & Li, 2007; Martin & Avérous, 2001; Matzinos, Tserki, Gianikouris, & Pavlidou, 2002; Talja, Helen, Roosb, & Jouppilaa, 2008). It is imperative to experimentally determine the effect of these blended materials on the biodegradability of TPS when designing these innovative blends to ensure the beneficial properties of TPS are not compromised.

Starch has been successfully blended with other synthetic polymers to improve the overall material properties and performance, such as polylactide (PLA) (Huneault & Li, 2007; Martin & Avérous, 2001), polycaprolactone (PCL) (Matzinos et al., 2002), and polyvinyl alcohol (PVOH) (Follain et al., 2005). Further investigations on TPS:PVOH blends are of particular interest due to their excellent compatibility and improved properties such as tensile strength, elongation, toughness (Chaleat, Halley, & Truss, 2008) and processability, predominantly due to an improvement in melt strength (Fishman, Coffin, Willett, & Onwulata, 2006; Mao, Imam, Gordon, Cinelli, & Chiellini, 2000), compared to pure TPS materials. Additionally, plasticisers such as maltitol, glycerol, and sorbitol, can be added to starch formulations to further improve the properties of the material by:

- Increasing polymer chain mobility and free volume by reducing hydrogen bonding between polymer chains.
- Improve processability (Mathew & Dufresne, 2002).
- Reduction in the T_g (Chaleat et al., 2008).

Starch is a homopolymer mixture of two α-types of polymer: amylose, a mostly linear α-D-(1,4)-glucan, and amylopectin, a highly branched α-D-(1,4)-glucan which has α-D-(1,6) linkages at the branch point (Amass, Amass, Skiads, Angelopoulos, & Lyberatos, 1998). Amylose typically has a molecular weight that ranges from 10^5 to 10^6 g/mol (Buleon, Colonna, Planchot, & Ball, 1998; Roger, Tran, Lesec, & Colonna, 1996) and due to its linear nature, can form alignments that result in strong hydrogen bonding. Amylopectin

* Corresponding author. Address: The Australian Institute of Bioengineering and Nanotechnology (AIBN), University of Queensland, Brisbane, Qld 4072, Australia. Tel.: +61 7 3346 3525; fax: +61 7 3346 3973.
E-mail address: m.russo@uq.edu.au (M.A.L. Russo).

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can have a molecular weight ranging from 10^6 to 10^8 g/mol (Buleon et al., 1998).

Enzymatic degradation, using α-amylase and amyloglucosidase, is one of a number of possible methods that can be employed to hydrolyse starch (Vikman, Itaevaara, & Poutanen, 1995a, 1995b). The degradation of starch has been performed by previous researchers to produce useful biofuels such as ethanol (Antonopoulou, Gavala, Loannis, Angelopoulos, & Gerasimos, 2007; Bai, Anderson, & Moo-Young, 2008). Amylases are the class of glucosidase hydrolysing enzymes that cleave the α-1,4 and/or α-1,6 glucosidic linkages in starch and other polysaccharides (Lehninger, 2005). α-Amylase (EC 3.2.1.1, 1,4-α-D-glucan glucanohydrolase) is a metalloenzyme unable to function in the absence of calcium. It hydrolyses the α-1,4 glucosidic bonds in amylose, amylopectin and glycogen in a random endo-fashion yielding glucose, maltose and some dextrins of higher molecular weight (Colonna, Leloup, & Buleon, 1992). However, it is not capable of hydrolysing α-1,6 glycosidic bonds that exist in amylopectin (Lascaris, 1999). The α-amylases have a molecular weight ranging from 5×10^4 to 6×10^4 g/mol and the optimum pH and temperature for activity is dependent on the enzyme origin. Amyloglucosidase (EC 3.2.1.3 Exo-1,4-α-glucosidase) hydrolyses the last α-1,4 glucosidic linkages at the nonreducing end of amylose and amylopectin as well as the α-1,6 linkages in amylopectin, however the rate of hydrolysis of α-1,4 bonds is an order of magnitude higher than that of α-1,6 bonds (Lascaris, 1999).

ASTM D6400-04 standard describes the method for determining the degradation of plastics in municipal and industrial aerobic composting facilities. The purpose of this standard is to identify products and materials that will compost in a satisfactory manner in commercial and municipal composting facilities (ASTM International, 2004). According to European and American standards, the biodegradation of a plastic under aerobic conditions is measured by the production of carbon dioxide.

Previous investigations on the enzymatic hydrolysis of TPS blends have been performed using α-amylase (Araujo, Cunha Antonio, & Mota, 2004a, 2004b; Bravo Bravo Rodriguez, Jurado Alameda, Martinez Gallegos, Reyes Requena, & Garcia Lopez, 2006) and amyloglucosidase (Vikman, Hulleman, Van der zee, Myllärinen, & Feil, 1995a, 1995b). The use of starch specific enzymes, α-amylase and amyloglucosidase, has proven to be a rapid and efficient method, for obtaining preliminary information about the degradability of starch-based blends (Vikman et al., 1995a, 1995b). In comparison, conventional methods used to degrade starch-based plastics, such as composting, can take weeks to months to gather the required data that is necessary to describe the same result as calculated using the enzymatic degradation method (Ganjyal, Weber, &Hanna, 2007).

The enzymatic hydrolysis of solid starch substrates has been described previously by Colonna et al. (1992). The first phase of hydrolysis commences with the diffusion of enzyme towards the solid–liquid interface. Once adsorbed, forces between the enzymes and binding sites result in the formation of an enzyme-substrate complex with the number of adsorption sites largely dependent on the porosity of the substrate.

Colonna et al.(1992) identified three main factors that can limit the hydrolysis of starch in solid substrates:

- diffusion of enzyme molecules into the solid substrate
- porosity in solid substrates
- adsorption of enzymes onto solid substrates.

The molecular mechanism behind the adsorption of an enzyme onto a substrate is not well understood and is the key limiting factor for the hydrolysis of solid carbohydrate substrates (Leloup, Colonna, & Ring, 1990). Additionally, researchers have observed that chemically modified starch, even at low degrees of substitution as is the case for the starch investigated in the present study, can exhibit a lower susceptibility to enzyme attack. Glucosidic products from the degradation of starch such as maltose and maltotriose may competitively inhibit the action of α-amylase (Elodi, Mora,& Milka, 1972). However, amyloglucosidase hydrolyses maltose and maltotriose into glucose and this avoids any inhibition of continued starch hydrolysis from the substrate.

Although research into the enzymatic degradation of starch has been conducted by various authors (Araujo et al., 2004a, 2004b; Vikman, Hulleman, Van der zee, Myllärinen, & Feil, 1999; Vikman et al., 1995a, 1995b), no research has been conducted on the enzymatic degradation of TPS:PVOH and polyol:TPS:PVOH blends. Therefore the aims of this study were defined to investigate the hydrolysis of starch from a series of TPS:PVOH and polyol:TPS:PVOH blends.

2. Materials and methods

2.1. Processing of substrate

2.1.1. TPS:PVOH blends

Five different mixtures were prepared with varying TPS:PVOH w/w%: 100:0, 90:10, 75:25,50:50, and 0:100. The starch was purchased from Penford Australia and is a hydroxypropylated high-amylose starch sourced from maize. The PVOH was purchased from DuPont Australia. These mixtures were extruded using water as a plasticiser, then processed into films of 1 mm thickness using a compression moulder. Compression moulding was conducted using platens of dimensions 150 × 150 mm at a force of 100 kN at temperatures from 125–160 °C for a time of 5–7 min, depending on the PVOH content, before the films were rapidly cooled to room temperature.

2.1.2. Polyol:TPS:PVOH blends

Three plasticised blends were prepared using three different polyols types; maltitol, glycerol, and sorbitol. The different polyol blends are denoted as polyol:TPS:PVOH during the course of the present study. The three different mixtures, also using water as a plasticiser, were prepared with a uniform amount of polyol plasticiser at 30 w/w%, starch at 60 w/w% and PVOH at 10 w/w%, then processed into 1 mm thick films using a compression moulder at the same conditions as previously discussed for the TPS:PVOH blends.

2.2. Preparation of substrate

After compression moulding, all substrate films were cut into small squares of dimensions 5 × 5 × 1 mm and then placed into a relative humidity (RH) cabinet set to 50% RH at 25 °C for 2 weeks to allow for moisture content equilibration. Equilibration of samples was performed to maintain each film at uniform storage conditions. The moisture content within each sample remained unchanged after equilibration at these conditions, therefore the moisture content within each blend had reached equilibrium.

2.3. Preparation of enzyme solution

Commercial grades of α-amylase (sourced from *Bacillus licheniformis*) and amyloglucosidase (sourced from *Aspergillus niger*) purchased from Sigma Aldrich were prepared as a stock solution in 0.1 M PBS (phosphate buffered saline), pH 6.8, with 5×10^{-3} M $CaCl_2$ at 500 and 80 U/mL concentrations, respectively. One unit of the α-amylase liberates 1.0 mg of maltose from starch in

3 min at pH 6.9 at 20 °C. One unit of the amyloglucosidase will liberate 1.0 mg of glucose from starch in 3 min at pH 4.5 at 55 °C.

2.4. Enzymatic hydrolysis of starch

Each substrate was removed from the RH cabinet after equilibration and weighed on an analytical balance prior to any enzymatic degradation test then placed into a sterilised glass test tube. The total volume of the reaction mixture used for each test was 10 mL. Table 1 clearly illustrates the experimental layout employed for each enzymatic degradation test, where each test was repeated in triplicate.

After each test tube was prepared as detailed in Table 1, they were mixed using a vortex mixer then placed in a water bath at 50 °C. The concentration of glucose was determined using the Somogyi–Nelson glucose assay (Nelson, 1944; Somogyi, 1952).

2.5. Determination of glucose concentration

Aliquots of 100 μL from each sample after hydrolysis times of: 1, 3, 5, 7, 24, 48, and 72 h. The initial 7 h of incubation was sufficient to calculate the initial rate of glucose production and therefore, after this period aliquot samples could be taken at longer intervals.

The 100 μL aliquots were transferred into sterilised test tubes then digested using the Somogyi–Nelson glucose assay, as described by Somogyi (1952) and Nelson (1944), with no alterations made to the procedure. The concentration of glucose in each substrate aliquot was determined by correlating the absorbance of a test sample to a predetermined glucose standard curve. The concentration of glucose produced over time was used as a measure of the degradation of starch from the substrate.

2.6. Determination of remaining starch

The total starch assay kit was purchased from Megazyme International Ireland Limited and the standard procedure for samples containing resistant starch contained in this kit was followed with no alterations. Determination of glucose content in each substrate sample was determined from a pre-calibrated glucose standard curve. The amount of sorbed vapour within each film initially was measured and taken into account for the final starch content calculations.

3. Results and discussion

3.1. Rate and extent of glucose production

3.1.1. TPS:PVOH blends

The rate and extent of starch hydrolysis by the action of α-amylase and amyloglucosidase was measured using the Somogyi–Nelson glucose assay of five TPS:PVOH blends of varying starch concentrations. The production of glucose was used as a measure of starch hydrolysis. The glucose produced together by both the substrate and buffer control was subtracted from the glucose produced together from the substrate, enzyme and buffer result. This was performed to ensure that the production of glucose, reported in the present study, was solely produced by the enzymatic hydrolysis of the starch. Fig. 1 shows the extent of glucose production over a 72 h hydrolysis time for each TPS:PVOH substrate. The experimental data was fitted to an exponential function.

Fig. 2 illustrates the initial rate of glucose production by each substrate up to a hydrolysis time of 7 h. The rate of glucose production was calculated; refer to Table 2, by assuming a linear relationship between the concentration of glucose and time for the first 7 h of hydrolysis. The rate determined for the 100:0 substrate was calculated over the first 5 h due to an earlier plateau in the glucose production compared to the other TPS:PVOH substrates investigated in this study, as shown in Fig. 1.

The results of the Somogyi–Nelson glucose assay demonstrated that the use of enzymes α-amylase and amyloglucosidase successfully hydrolysed the starch within the TPS:PVOH blends to glucose. The rate of starch hydrolysis was most rapid for the pure starch

Table 1
Experimental plan used to hydrolyse TPS:PVOH blends. Each test was performed in triplicate at 50 °C.

Test	PBS (mL) pH 6.8	Enzyme solution (mL)	Substrate addition (yes –y/ no – n)
No substrate	10	0	n
Control	10	0	y
Substrate (TPS:PVOH)	9	1	y
Standard (90:10)	9	1	y

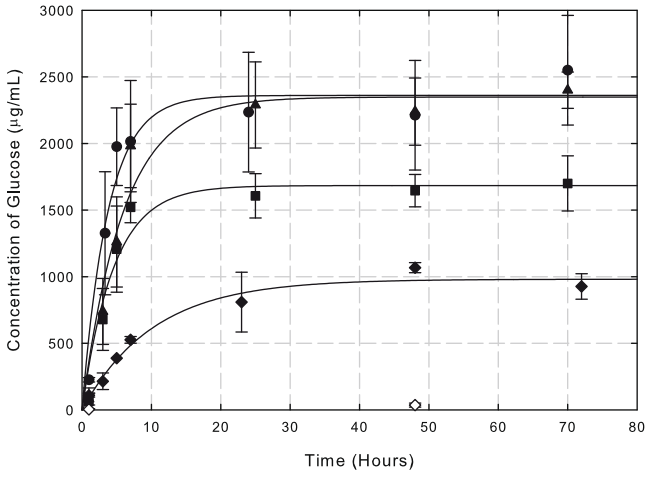

Fig. 1. Concentration of glucose produced for a series of TPS:PVOH blends due to the enzymatic attack by α-amylase and amyloglucosidase; ●, 100:0; ▲, 90:10; ■, 75:25; ♦, 50:50; and ◇, 0:100. Experimental data was fitted to an exponential function and the error bars denote the standard deviation in the data.

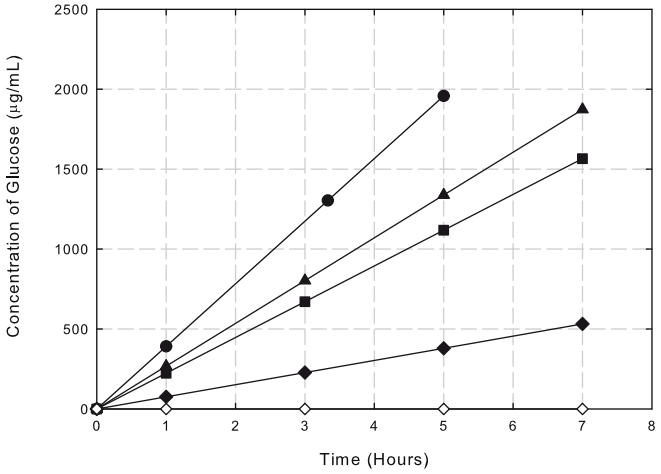

Fig. 2. Concentration of glucose produced for a series of TPS:PVOH blends in the first 7 h of enzymatic degradation due to the action of α-amylase and amyloglucosidase on the TPS:PVOH substrates; ●, 100:0; ▲, 90:10; ■, 75:25; ♦, 50:50; and ◇, 0:100.

Table 2
A summary of the rates of glucose production from each TPS:PVOH substrate.

Substrate	Rate (μg/mL h)	R^2
100:0	392	0.98
90:10	268	0.97
75:25	224	0.97
50:50	76	0.99
0:100	0	0.99

substrate (100:0) and decreased with the addition of PVOH. The same trend was observed for the extent of starch hydrolysis. The rate of starch hydrolysis determined in the present study for the 100:0 substrate (392 μg/mL h) correlated well with the literature value reported by Li, Corke, and Beta (2007) (473 μg/mL h), considering the differing structure of the TPS used in this study and the high maize starch substrate used in the comparative study. Li et al. studied a semicrystalline starch that was ≈65 wt% amylose and measured the rate of starch hydrolysis over an incubation period of 1–3 days (Li et al., 2007). In the present study the structure of the starch was amorphous, ⩾90 wt% amylose and the rate of starch hydrolysis was calculated over an incubation period of 5–7 h.

3.1.2. Polyol:TPS:PVOH blends

The glucose production from a series of polyol plasticised blends (polyol:TPS:PVOH) was measured using the same procedure as for the TPS:PVOH blends discussed previously. The enzymatic degradation of sole maltitol, glycerol and sorbitol using α-amylase and amyloglucosidase was investigated, however no glucose was produced. Therefore, the total concentration of glucose produced from the substrate was only due to the enzymatic hydrolysis of the starch within the substrate.

The extent of glucose production for each polyol:TPS:PVOH substrate, refer to Fig. 3, was measured over a 72 h incubation period. The experimental data was fitted to an exponential function. It was noted that the extent of glucose production for each polyol:TPS:PVOH blend after 72 h of incubation, refer to Fig. 3, were not significantly different from each other. However, the shape of the glucose production curve for the glycerol and sorbitol blends varied in comparison to the maltitol blend during the initial stages of the incubation. The data for glycerol and sorbitol containing substrates displayed a sharp incline until 7 h and then immediately reached a plateau until the end of the incubation period. In the case for maltitol, the production of glucose peaked 17 h after the glycerol and sorbitol substrates and could be a result of the inhibition of α-amylase by maltitol.

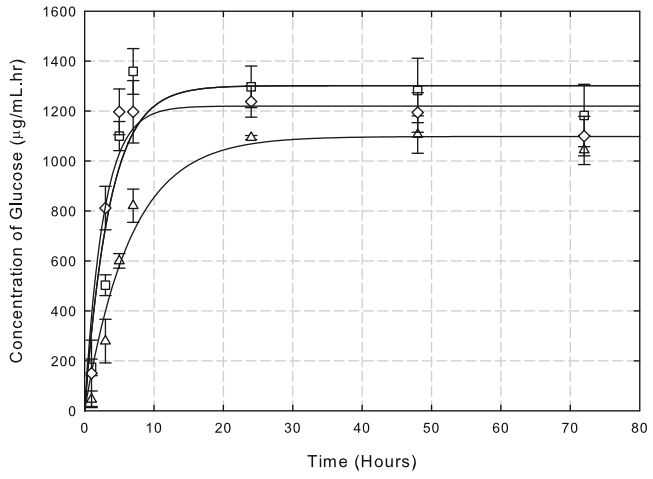

Fig. 3. The extent of glucose production for a series of polyol plasticised TPS:PVOH blends over a 72 h incubation period; Δ maltitol:TPS:PVOH, □ glycerol:TPS:PVOH, ◇ sorbitol:TPS:PVOH. Experimental data was fitted to an exponential function and the error bars denote the standard deviation in the data.

Fig. 4 shows the initial rate of glucose production achieved for each polyol:TPS:PVOH substrate. The rate and extent of starch hydrolysis, refer to Table 3, for each polyol:TPS:PVOH was calculated using the same method used previously for TPS:PVOH blends. The rate of glucose production for the sorbitol substrate was calculated over the first 5 h due to an earlier plateau in the glucose production compared to the remaining polyol:starch substrates.

The results of this study have shown that the type of plasticiser blended with TPS:PVOH blends impacted on the rate of starch hydrolysis. However, the extent of starch hydrolysis for each blend was not significantly different after 72 h of incubation. Maltitol has been reported previously as an effective inhibitor of α-amylase, attributing to the reduced rate of glucose production observed in this present study (Banks, Haxell, Lunn, Pacey, & Roberts, 2001). The rate of glucose production decreased in the following order according to polyol type: sorbitol, glycerol then maltitol.

In the sorbitol blend investigated in this present study, the sorbitol migrated to the film surface. This behaviour was observed by Krogars et al. for proportions of sorbitol over 33 wt% (Krogars et al., 2003). Migration prevented the sorbitol from acting as a plasticiser in the film and consequently dissolves from the film surface once immersed in water. Additionally, phase separation of glycerol in glycerol-starch blends has been reported at glycerol contents of 40 wt% and higher (Talja, Helen, Roosb, & Jouppilaa, 2007), however blends of these compositions did not form part of the scope of the present study.

3.2. Quantification of remaining starch within TPS blends

The amount of starch within each blend was quantified before and after enzymatic degradation using the Megazyme Total Starch Assay Kit.

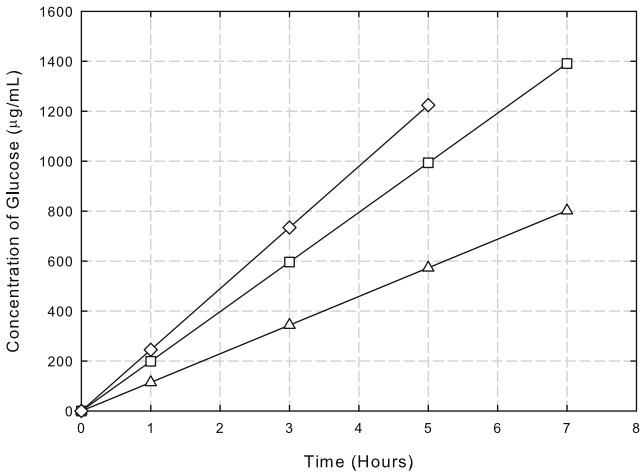

Fig. 4. Concentration of glucose produced from a series of polyol:TPS:PVOH blends in the first 7 h of incubation; Δ maltitol:TPS:PVOH, □ glycerol:TPS:PVOH, ◇ sorbitol:TPS:PVOH.

Table 3
A summary of the rates of glucose production from each polyol plasticised starch-based substrate.

Substrate	Rate (μg/mL h)	R^2
Maltitol:TPS:PVOH	115	0.98
Glycerol:TPS:PVOH	199	0.98
Sorbitol:TPS:PVOH	245	0.98

3.2.1. TPS:PVOH blends

The data in Table 4 summarises the amount of starch within each TPS:PVOH substrate initially, after a hydrolysis time of 72 h and the overall percentage of starch hydrolysis on a w/w basis from the blend.

The quantification of the total amount of starch within the substrate, after a hydrolysis time of 72 h, suggests that starch hydrolysis was significantly affected by PVOH content. The 50:50 substrate only achieved 79% starch hydrolysis, whereas the remaining TPS:PVOH blends achieved extents of starch hydrolysis from 92% to 100%. Chaleat et al. observed that for the 50:50 blend, compared to the remaining TPS:PVOH blends investigated in the preset study, the following properties were prominent: lower moisture content, higher modulus and the possibility of complexing between starch and PVOH as a single helix (Chaleat, 2008). According to the enzymatic diffusion results published by Wool, Raghavan, Wagner, and Billieux (2000), for starch–polyethylene composites with a starch fraction of 0.57, only 42% of the overall starch was degraded within a solution of a constant concentration of enzymes at long times (1400 time steps). Lower initial moisture content impacts on the rate of water and enzyme diffusion into the 50:50 blend, resulting in a slower rate of enzymatic hydrolysis of the starch within the blend. Adequate plasticisation of the blend is required for the enzyme to diffuse towards the substrate (Colonna et al., 1992).

Therefore, the authors of this present study have hypothesised that more than one mechanism is valid to explain the reduced enzymatic hydrolysis of starch for the 50:50 blend:

(1) A "barrier" mechanism, refer to Fig. 5a, may be occurring where the PVOH encapsulates the starch and this further prevents enzymatic attack on remaining starch. Araujo et al. have postulated the same effect in their work on the enzymatic starch hydrolysis of a starch:poly(ethylene-vinyl alcohol) blend that experienced significantly reduced starch hydrolysis (Araujo et al., 2004a, 2004b). Furthermore, it is possible that after the rate of glucose production reached a plateau, the PVOH phase inverts and becomes the continuous phase and "protects" remaining starch from enzymatic attack. Ke et al. have reported that PVOH, at proportions greater than 30 wt%, formed a continuous phase with starch (Ke & Sun, 2003).

(2) Starch molecules become physically inaccessible to enzymes due to complexing between starch and protein molecules. Nehmer et al. have noted this effect for extruded starch materials (Nehmer, Nobes, & Yackel, 2006).

(3) Gelatinised starch molecules have reassociated themselves (conformation change) during and/or after the extrusion process, making them resistant to enzymes after processing (Nehmer et al., 2006).

(4) The interaction between the starch and PVOH at the interface may alter the conformation of the starch, refer to Fig. 5b and prevent the interaction between the starch and enzyme. Chaleat et al. have hypothesised that a strong interaction may exist between starch and PVOH at the interface between the two phases (Chaleat, 2008).

(5) The formation of a starch-PVOH complex may have formed that rendered the complexed starch completely resistant to enzymatic attack. Shogren, Thompson, Greene, Gordon, and Cote (1991) and Imam, Gordon, Thompson, Harry-O'Kuru, and Greene (1993) have found that starch can form v-type complexes with synthetic polymers.

To test the validity of the "barrier" proposed mechanism for the 50:50 blend, starch assays were conducted using the Megazyme Total Starch Assay with the recommended particle size (0.5 mm) and with cryogenically milled film (particle size 40–60 μm). No significant difference was found between the measured wt% of starch (38 wt%) for both particle sizes. Therefore, if PVOH acts as a barrier against enzymatic attack, a significant increase in the liberation of starch, due to milling, should have been evident for the initial measured wt% of starch in the blend. Due to the lower than expected value of starch within the 50:50 blend, interactions between the remaining 12 wt% starch and PVOH may be forming an intermediate phase, or the starch molecules become physically inaccessible to enzymes due to complexing or reassociating themselves (conformation changes).

The absorption of water by each blend is important for successful enzymatic attack on the substrate, especially for solid substrates. It has been reported by Finch et al. that the degree of hydrolysis of PVOH affected its solubility properties (Finch, 1973). It was shown that PVOH grades with degrees of hydrolysis above 98.5% exhibit reduced solubility. The PVOH used in this study has a degree of hydrolysis above 99%, therefore enzyme diffusion into the PVOH rich blends may be significantly slower.

Table 4
The wt% of starch within each TPS:PVOH blend at time = 0, time = 72 h and the overall percentage of starch hydrolysis. The error in the data is represented as the standard deviation in the data.

Substrate	Wt% of starch (t = 0)	Wt% of starch (t = 72 h)	% Starch hydrolysis
100:0	100 ± 2.5	0 ± 1	100
90:10	89 ± 3	2 ± 1	98
75:25	71 ± 3	6 ± 3	92
50:50	38 ± 3	8 ± 3	79
0:100	0 ± 1	0 ± 1	0

3.2.2. Polyol:TPS:PVOH blends

After the polyol:TPS:PVOH blends were hydrolysed for 72 h, the overall percentage of starch hydrolysis from the blends were for

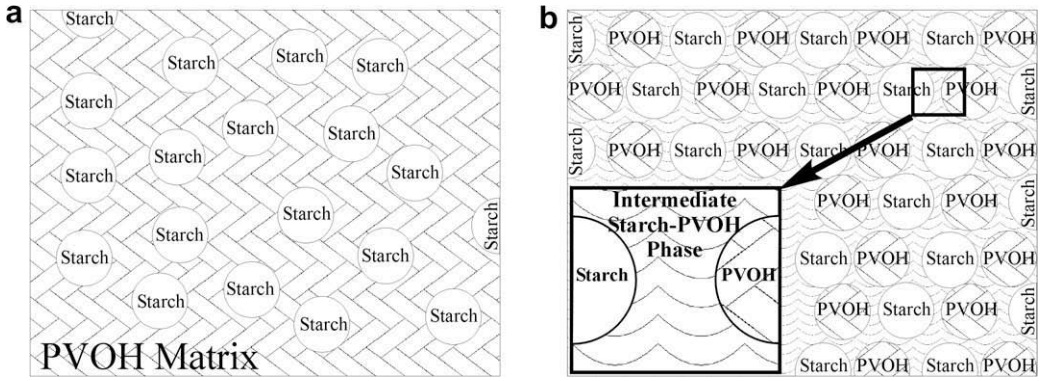

Fig. 5. Proposed mechanisms for the reduced hydrolysis of starch from the 50:50 blend, (a) "barrier" mechanism and (b) formation of an intermediate starch-PVOH phase.

the maltitol:TPS:PVOH blend, 85%; glyercol:TPS:PVOH, 90% and for the sorbitol:TPS:PVOH blend, 88%.

This result shows that the percentage of starch hydrolysis from each polyol blends was uniform and that plasticiser type did not impact on the extent of starch hydrolysis from the polyol:TPS:PVOH blends.

4. Conclusions

The aims of this work were to characterise the rate and extent of starch hydrolysis, using starch degrading enzymes α-amylase and amyloglucosidase, from a series of TPS blends of varying starch content and plasticiser type. The use of these enzymes to enzymatically hydrolyse the starch within each blend has proven to be an excellent method for determining the rate and extent of starch hydrolysis from each blend, providing preliminary information on the biodegradability of the material in a short experimental time.

The results of this study have demonstrated that PVOH significantly impacts on the rate and extent of starch hydrolysis within the blend. The hydrolysis of starch from substrates at 50 wt% PVOH were most affected, where both the rate and extent of glucose production was significantly reduced. Four mechanisms have been proposed to explain the starch hydrolysis behaviour of the 50:50 blend: PVOH acts as a "barrier" to remaining starch; the starch molecules become physically inaccessible to enzymes due to complexation or reassociation to form conformation changes; an intermediate phase occurs between starch and PVOH at the interface. From the results of this study, a combination of starch complexation and the formation of an intermediate phase are believed to be the dominant mechanisms for the reduced starch hydrolysis observed for the 50:50 blend.

Plasticiser type significantly affected the rate of starch hydrolysis, however no significant differences in the extent of starch hydrolysis were noted. The results suggest that maltitol inhibits the action of α-amylase by a reduction in the rate of glucose production whereas no inhibitory effect was observed for glycerol and sorbitol substrates.

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