

Thermal and Kinetic Properties of Poly(lactic acid) and Transglutaminase-Crosslinked Wheat Gluten Blends

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ABSTRACT: Wheat gluten (10 g) was crosslinked (XL) using 10 units of transglutaminase. Different blends of XL gluten and poly(lactic acid) (PLA) were mixed in a Brabender mixer at 180°C for 10 min. Neat PLA and blends were analyzed using modulated DSC (MDSC). Neat PLA displayed a glass transition (T_g) and exothermic (Cry) followed by endothermic (Mel) transitions. The profile showed a T_g of 0.46 J/g/°C, Cry with 29.9 J/g, whereas Mel exhibited 28.7 J/g. XL wheat gluten displayed one T_g with 0.45 J/g/°C. Samples were subjected to repeated heating and cooling cycles to show the level of compatibility between the two polymers. The activation energy (E_a) and pre-exponential factor (Z) were

determined according to Borchardt and Daniels (B/D) kinetics approach. The blends showed increased E_a values with an increase in the amount of XL gluten. In the presence of 5 and 20% XL gluten, the E_a of PLA increased from 150 to 200 kJ/mol, respectively. A higher number of cycles caused an increase in E_a . The T_g temperature of different PLA/XL gluten blends can be predicted by Gordon–Taylor equation and its modified forms. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 106: 214–219, 2007

Key words: crosslinked; gluten; poly(lactic) acid; biodegradable blend; kinetics; Gordon–Taylor; Fox

INTRODUCTION

There is an ongoing appeal and need for developing new uses for agricultural excess commodities, products, and byproducts. For environmental protection, there is also a demand to develop low cost biodegradable plastics. Cao et al.¹ reported a blend of poly(lactic acid) PLA and poly(hydroxy ester ether) PHEE and cornstarch as a promising blend. PHEE polymer is derived from diglycidyl ether bisphenol A and adipic acid. This thermoplastic material has an amorphous structure with glass transition at 45°C.² PHEE was used for its good adhesion properties. Starch was used to reduce cost. Wheat gluten is a byproduct of wheat starch industry with viscoelastic properties. The mechanical properties of PLA, PHEE, and starch blends have been well studied. Cao et al.¹ also reported that PHEE intrahydrogen bonding was increased at 50% PLA, as shown by solid state FTIR. At 60% PLA and higher PLA, carbonyl groups started forming

interhydrogen bonding with PHEE's hydroxyl groups. Starch–PLA blends were prepared by extrusion. The two polymers were found immiscible and the presence of starch did not change the thermal properties of PLA, whereas the crystallinity, the tensile strength, and elongation of the blend decreased as the starch content increased.³

Transglutaminase (TG) is an enzyme that catalyzes protein crosslinking (XL) through inter- or intramolecular $\epsilon(\gamma\text{-glutamyl})\text{lysine}$ isopeptidic bonds formation. These bonds cause the polymerization of proteins.^{4,5} TG-catalyzed reactions have been used extensively to modify the functional properties of various food proteins, including beef actomyosin, oat globulin, pea legumin, and oat, rice, and maize prolamines.^{6–11} The effects of TG XL on the physicochemical properties of milk proteins have been investigated. These studies have focused on the modification of both whey and casein fractions of milk protein.¹² TG has also been used to crosslink milk proteins with other proteins to form films^{13,14} and other biopolymers.

A major focus of the TG-crosslinked reactions has been the modification of cereal proteins. The effects of TG upon both native and modified soy proteins have been examined. Ikura et al.^{15,16} studied the XL of the 7S and 11S fractions of soy protein. Chanyongvorakul et al.¹⁷ found that 11S globulin gels formed using TG were more rigid and elastic than thermally-induced gels. The XL of soy protein with TG has also been shown to remove the allergenic properties of soy.

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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In the case of gluten, this enzyme induces the formation of high molecular weight polymers despite the low lysine content in gluten proteins.¹⁸ This modification induces drastic changes in the physicochemical properties and rheological behavior of gluten, which can be useful in the food industry. Gluten proteins contain gliadins (viscous), which are monomeric, and glutenin (elastic) subunits, which are linked together through disulfide bridges to form high molecular weight polymers. These viscoelastic properties influence the formation of the flour dough. TG has been found to increase dough-forming qualities of wheat flour and improve the quality of baked goods such as bread and pastries.¹⁹

A modified polycaprolactone resin (PCL) blend with soy protein and polyesters was prepared using extrusion. PCL was modified by adding a maleic anhydride group to act as a compatibilizer. Anhydride groups were found effective in compatibilizing immiscible blends of soy proteins and polyesters.²⁰ Up to 75% (W/W) gluten extruded with PCL produced a blend without significant change in the tensile properties of PCL. The elongation, however, decreased. Both, G' and G'' were a magnitude higher than the PCL.²¹ The objectives of this work were to develop blends using wheat gluten for its viscoelastic properties as a filling material and PLA for its semicrystalline structure as a continuous phase. The use of wheat gluten is consistent with our effort to develop new uses for agricultural excess commodities and for environmental protection.

EXPERIMENTAL

TG (EC 2.3.2.13) from guinea pig liver (Factor XIIIa, lyophilized powder, 1.5–3 units/mg protein) was obtained from Sigma (St. Louis, MO). Vital wheat gluten (75% protein content) was obtained from Midwest Grain Products (Pekin, IL). This gluten was treated to remove remaining starch by heating 10% gluten/water slurry at 95°C for 20 min and centrifuging for 20 min ($2000 \times g$) three times separately. The precipitate was freeze-dried and ground to pass through an 80-mesh sieve. This purified gluten which contained 91% protein dry basis, as measured by LECO (LECO CHN-2000 instrument (Feuerwerkerstr, 39 Ch-3602 Thun, Switzerland), was used for the TG XL reaction.

TG crosslinking reaction

TG (10 units) was dissolved in 2L 0.1M TRIS-HCl buffer, pH 7.5, at ambient temperature. With moderate stirring, purified vital gluten (10 g) was slowly added to the buffer/TG solution (gluten was added slowly to avoid formation of globules). The reaction continued with gentle stirring for 24 h at 40°C using

a stirplate/heater with a temperature probe. After 24 h, the suspension was placed in dialysis tubes, dialyzed against distilled cold water for 24 h, freeze-dried, and milled to pass through an 80-mesh sieve.

Blending

Different ratios of PLA : XL gluten blends were prepared, 95 : 5; 80:20; 73 : 30; or 50 : 50 and blended in a Brabender mixer (South Hackensack, NJ) mixing bowl. The mixing temperature was set at 180°C, and the mixing bowl was torque-calibrated before sample addition. The mixer was preheated to 180°C and the PLA pellets were blended first for 2 min at 10 rpm. After PLA pellets melted, gluten (8% moisture) was added and the speed of blending was increased to 50 rpm. Total mixing time was 15 min. Total weight of each sample was 50 g. The blends were cryogenically pulverized to fine powder for further analysis.

Differential scanning calorimetry

MDSC analysis was performed using a TA Instruments 2920 MDSC (TA Instruments, New Castle, DE). Freeze-dried XL gluten with 3% moisture content (25–30 mg) was placed in stainless steel pan and sealed. While in the DSC, samples were subjected to heating and cooling cycles to determine the ability of PLA to crystallize in the presence of XL gluten. The reduction in thermal parameters, such as ΔH and peak temperature will indicate some form of interaction between the blend components (gluten and PLA), which can be a topic of another article. Samples were heated from 0 to 200°C at a heating rate of 7°C/min, held at 200°C for 1 min, cooled (7°C/min) to 0°C, and held for 1 min. This cycle of heating and cooling was applied to each sample until disappearance of the transitions. As controls, purified vital wheat gluten and neat PLA were also analyzed on the MDSC using the same conditions. The kinetics data was collected by heating each blend at different heating rates (2, 5, 7, and 9°C/min). The activation energy was obtained by analyzing the data using the soft ware provided by TA Instrument.

RESULTS AND DISCUSSION

PLA used in this work is a semicrystalline polymer with $M_w = 2 \times 10^5$ g/mol and glass transition (T_g) temperature at 61.8°C with 0.46 J/g/°C. In addition to the T_g , PLA displayed an exothermic (crystallization) at 127°C followed by an endothermic (melting) at 157°C transitions, wheat vital gluten used in this blend displayed a glass transition at 45.6°C with 0.46 J/g/°C. The T_g values of the neat PLA or wheat gluten and the blends are shown in

TABLE I
 T_g Values of 100% PLA and Crosslinked (XL) Gluten and Their Blends

% PLA/XL gluten	T_g ($^{\circ}\text{C}$)
100 PLA	61.8
95	59.2
80	55
70	52.2
60	50.2
50	47.1
100 XL gluten	45.6

Table I. Heating and cooling cycling is one of the techniques used to study interactions between polymer blends.¹ The ΔH value of the blends decreased as the number of heating-cooling cycles increased, indicating lower presence of crystalline state in the blend either because of degradation or interaction between gluten and PLA. Although PLA did not transform to 100% amorphous structure due to heating-cooling cycling, as shown by X-ray diffraction, cycling reduced its level of crystallinity.^{1,22} The number of cycles needed to transform PLA crystallinity to a level undetectable by MDSC varied based on the amount of PLA in the blend. Higher PLA level in the blend required longer cycling. The 95 : 5, 80 : 20, 70 : 30, and 50 : 50 required 130, 8, 7, and 4 cycles to show no detectable crystallinity, respectively. The MDSC data in Figure 1(a,b) showed the gradual decrease in ΔH of both exo- and endothermic transitions as a function of cycle number. The diminishing of the ΔH value indicates loss of the PLA crystallinity caused by the heating-cooling cycles. The endothermic and the exothermic transitions of the 80 : 20 blend of uncrosslinked gluten lasted for 35 cycles before vanishing, indicating loss of crystallinity, while neat PLA lasted for 250 cycles.²² It is clear from the number of the required cycles of the 80 : 20 blend of both XL or uncrosslinked that the XL gluten sample reduced the number of cycles by 77% (from 35 to 8), indicating better interaction with PLA and less phase separation. Low number of cycles means that PLA transformed into mostly amorphous state or generally, noncrystalline state. The effectiveness of the XL gluten could be in its ability to form a network before it goes through deamination as the number of cycles increases. The onset and peak temperatures of all blends decreased as the number of cycles increase. That is another indication of PLA crystals transformation to an amorphous state along with the above-mentioned reduction in the ΔH values. The onset and peak temperatures of the PLA : XL gluten, 95 : 5 and 80 : 20 blends are shown in Figure 2(a,b). The first 30 cycles exhibited a drop in the onset and

peak temperatures during both melting and crystallization, which indicates change in PLA crystallization kinetics. The glass transition also exhibited drop in temperature signifying the effect of cycling on the amorphous alongside changes in the crystalline structure [Fig. 3(a,b)].

The Borchardt and Daniels (B/D) kinetics approach was used to calculate the activation energy (E_a) and the pre-exponential factor (Z). This method permits the calculation of E_a , Z value, ΔH , reaction order, and rate constant (K) from a single DSC scan.²³ The method was originally prescribed for solutions and was further modified for solids by other researchers.²⁴ TA Instrument provided the software necessary to apply the B/D kinetics method. The B/D method assumes that reactions follows n th order, obeys the general rate equation, and presuppose Arrhenius behavior. The E_a and Z values were obtained from the slope of the straight line of the plot of $\ln \{k(T)\}$ versus $1/T$ (Arrhenius plot). The E_a and Z values of each cycle of PLA/gluten and PLA/XL gluten blends are shown in Figure 4(a,b) respec-

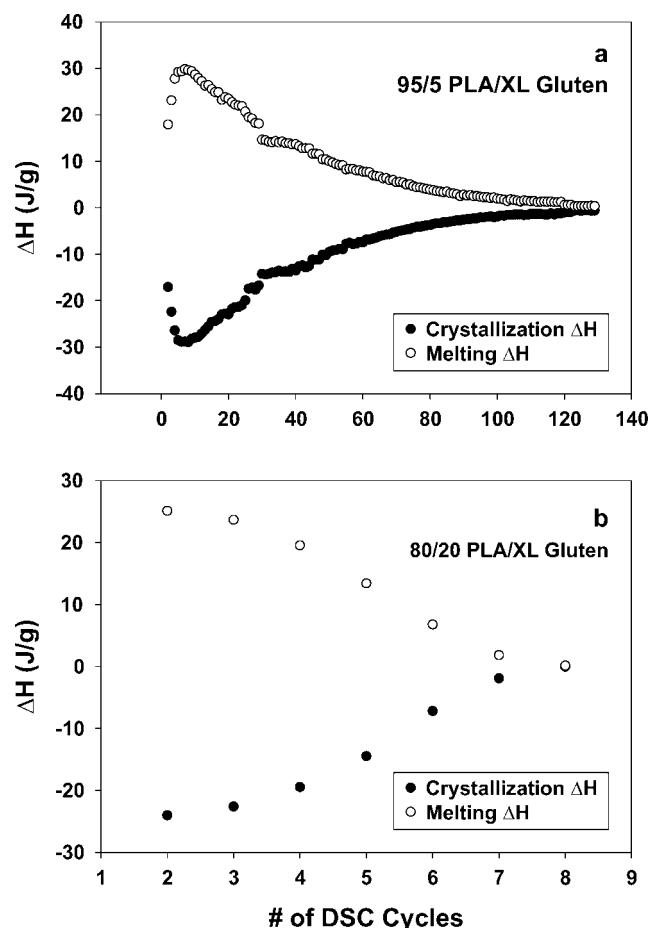


Figure 1 DSC cycling enthalpies (ΔH) of crystallization and melting transitions. (a) 95/5 PLA/XL gluten; (b) 80/20 PLA/XL gluten.

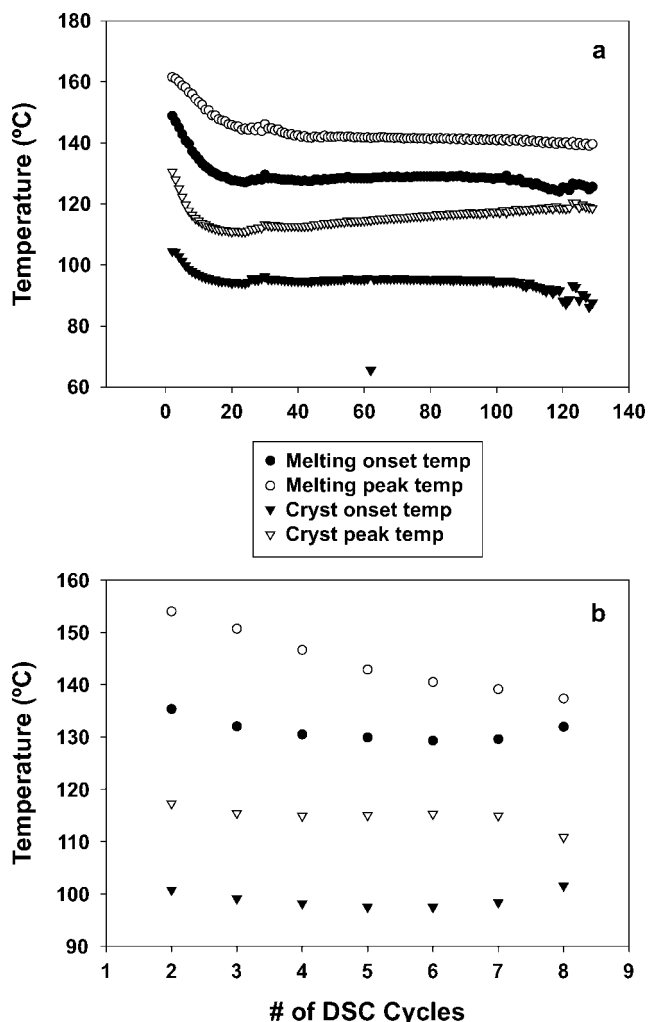


Figure 2 DSC onset and peak temperatures of crystallization and melting transitions. (a) 95/5 PLA/XL gluten; (b) 80/20 PLA/XL gluten.

tively, where the E_a and Z were plotted versus cycle number.

The E_a value for PLA/gluten blend exhibited an increase when compared to the neat PLA, while the increase in the gluten quantity in the blend showed a sharper increase in E_a [Fig. 4(a)]. The presence of XL gluten showed a rapid increase in the blend E_a relative to the PLA uncrosslinked gluten [Fig. 4(b)]. The increase in E_a value in the presence of gluten is indicative of gluten interference in the structure PLA, resulting in higher E_a . The Z factor exhibited similar behavior as a function of cycle number [Fig. 5(a,b)]. Unlike gluten, XL gluten has higher molecular size, thus slower molecular mobility, which may explain the ability of PLA molecules to move faster, causing higher Z values.

Generally, a single glass transition (T_g) or its shift in a polymer blends is considered an indication of partial or complete miscibility. Based on the data

discussion presented here, the shifting of T_g or E_a values and the results of the cycling study, these blends are thought to be miscible. Blends miscibility has been widely discussed in the literature.^{25,26} A number of model equations were used to predict the T_g dependence of polymer blends, such as Gordon–Taylor, Kwei, and Fox equations. Figure 6 shows the predicted T_g values using the above-mentioned equations and the experimental data. Gordon–Taylor equation is represented as follows.²⁷

$$T_g^b = \frac{W_1 T_{g1} + K W_2 T_{g2}}{W_1 + K W_2} \quad (1)$$

where T_g^b = blends glass transition, W_1 and W_2 , weight fraction of PLA and XL gluten respectively, T_{g1} and T_{g2} , glass transition of PLA and XL gluten respectively, and K , adjustable fitting parameter related to miscibility i.e., the strength of the interaction between the two components. The Gordon–

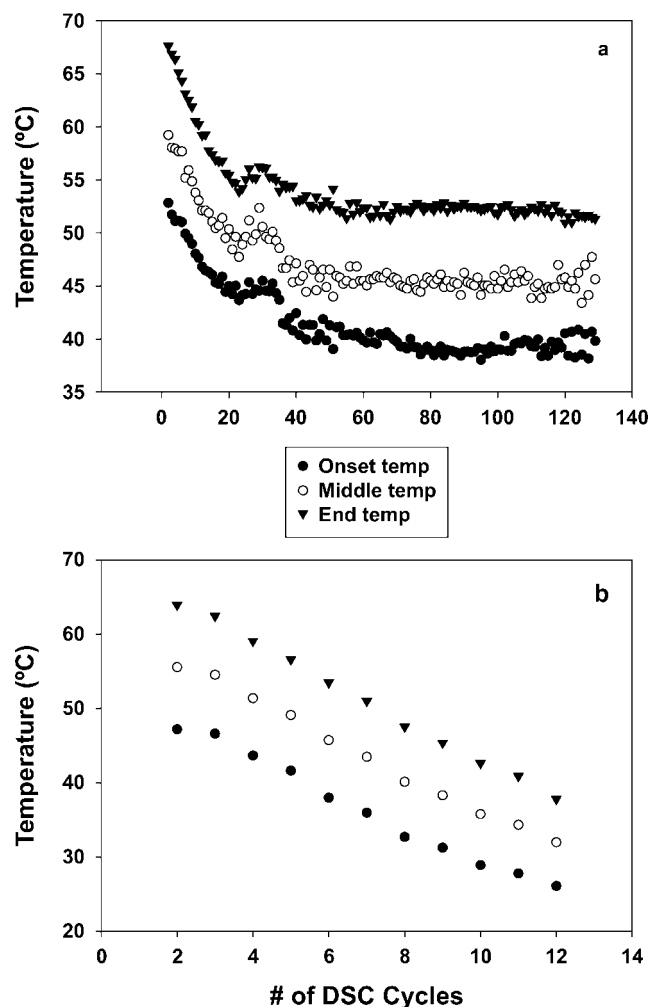


Figure 3 DSC onset, middle and final temperatures of glass transition (T_g). (a) 95/5 PLA/XL gluten; (b) 80/20 PLA/XL gluten.

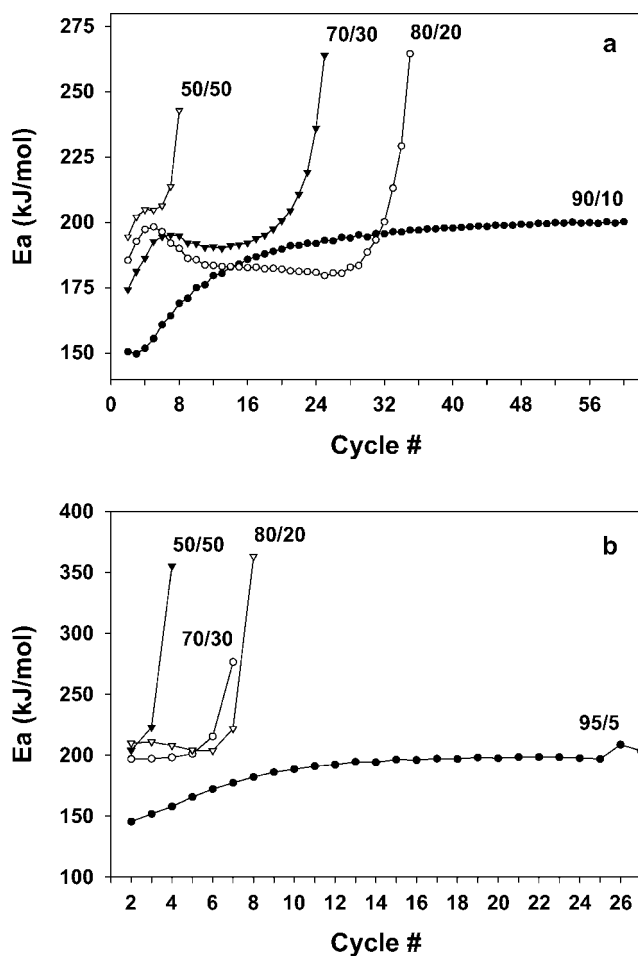


Figure 4 DSC of cycling activation energies (E_a) (assuming reaction rate $n = 1$). (a) PLA/gluten; (b) PLA/XL gluten.

Taylor equation can be rearranged to take the following linear form where K will be the slope of the line.

$$T_g^b = T_{g1} + K \left[\frac{W_2}{W_1} (T_{g2} - T_g^b) \right]$$

where $\frac{W_2}{W_1} (T_{g2} - T_g^b) = X$, and $T_g^b = Y$, and $K = \text{slope}$.

When the experimental data was applied to Eq. (1) of PLA/XLgluten blends a $K = 0.315$ and $R^2 = 0.89$ was obtained (Fig. 6). The value of the parameter K cannot be used for completely different blend ratios.²⁸

The Gordon–Taylor equation was modified by Kwei et al.²⁹ to take the following format.

$$T_g^b = \frac{W_1 T_{g1} + KW_2 T_{g2} + qW_1 W_2}{W_1 + KW_2} \quad (2)$$

where the $qW_1 W_2$ term represents the polymer–polymer interactions, such as hydrogen bonding. The application of Eq. (2) showed a better fit with K

$= 3.6$ and $q = -39$ as shown in Figure 6. The negative q value is attributed to contribution by electronic polarization.¹

The Fox equation was also used to predict the T_g dependence of PLA/XL gluten blends and it is represented as follows.

$$\frac{1}{T_g^b} = \frac{W_1}{T_{g1}} + \frac{W_2}{T_{g2}} \quad (3)$$

Equation 3 can be rearranged to take the following format.

$$T_g^b = \frac{T_{g1} T_{g2}}{W_1 T_{g2} + W_2 T_{g1}}$$

The Fox equation predicted the T_g^b of the PLA/XL gluten blends with an error of 11, 7.8, 6.9, 4.9, 2.5% for the PLA/XL gluten ratios 50/50, 60/40, 70/30, 80/20, 95/05, respectively, which is an average of 6.5%. The Fox fitting data is also shown in Figure 6.

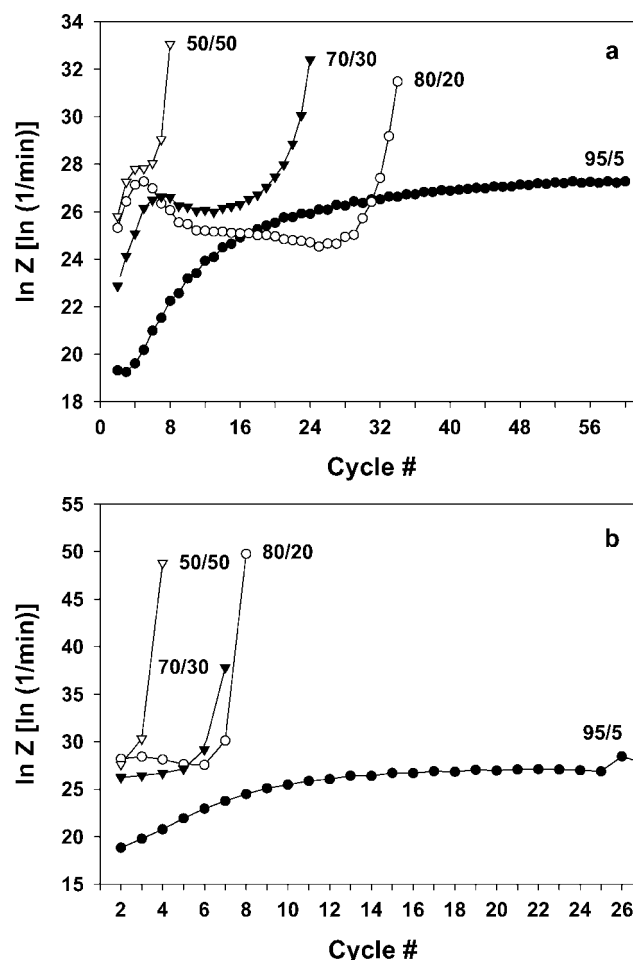


Figure 5 DSC of cycling log Z values (assuming reaction rate $n = 1$). (a) = PLA/gluten; (b) PLA/XL gluten.

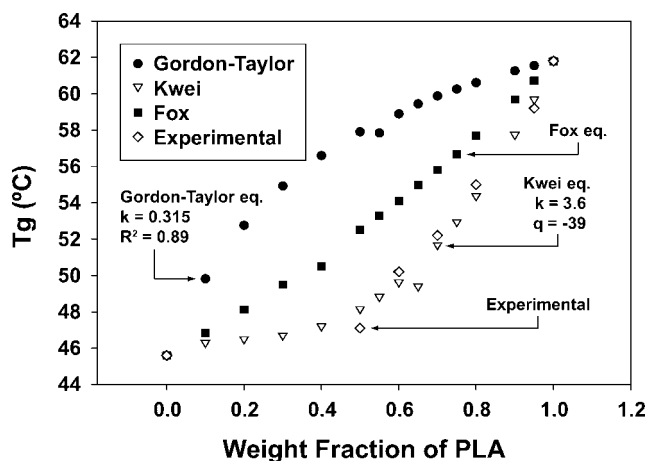


Figure 6 Glass transition (T_g) versus composition in PLA/XL gluten blends.

CONCLUSIONS

Heating and cooling cycling showed a decreasing ΔH value as the number of heating-cooling cycles increased, indicating low presence of crystalline PLA in the blend either because of degradation or interaction between gluten and PLA because degraded PLA molecules are not large enough to form crystals and the interaction of PLA and gluten prevents PLA crystallinity as well. PLA did not transform to 100% amorphous structure because of heating-cooling cycling, as shown by X-ray diffraction. The amount of PLA in the blend was the limiting factor that determines the number of cycles needed to transform PLA crystallinity to a level undetectable by MDSC. The 95 : 5, 80 : 20, 70 : 30, and 50 : 50 ratios required 130, 8, 7, and 4 cycles, respectively, to show no detectable crystallinity. XL gluten showed a 77% decrease in the number of cycles needed versus uncrosslinked gluten. Low number of cycles means that PLA transformed into mostly amorphous state or generally, noncrystalline state. The effectiveness of the XL gluten in reducing the number of cycles indicates stronger physical interaction with PLA compared to uncrosslinked PLA. Based on the data discussion presented here, the shifting of T_g or E_a

values and the results of the cycling study, these blends are thought to be miscible. Gordon-Taylor, Kwei, and Fox equations were found useful to predict T_g temperature of different PLA-gluten blends.

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