Studies on Glass Transition Temperature of Mono and Bilayer Protein Films Plasticized by Glycerol and Olive oil

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ABSTRACT: Thermomechanical and thermal properties of whey protein, maize prolamin protein (zein), and the laminated whey protein–zein films were studied. The dynamic mechanical (thermal) analysis (DMTA) results showed that the single zein film had higher T_g than single whey protein and zein–whey laminated films. The shift in the T_g values of films from 31.2°C in whey protein film and 88.5°C in the zein film to 82.8°C in the laminated whey protein–zein films may be implied some interaction formation between the two polymers. The small tan δ peaks were observed at −50°C in zein–glycerol films and at −22.37°C in the whey protein films and can be related to β-relaxation phenomena or presence of glycerol rich region in polymer matrix. Zein-olive oil and zein–whey protein–olive oil films showed tan δ peaks corresponded the T_g values at 113.8, and 92.4°C, respectively. Thus, replacing of glycerol with olive oil in film composition increased T_g. A good correspondence was obtained when DSC results were compared with the tan δ peaks in DMTA measurements. DSC thermograms suggested that plasticizers and biopolymers remained a homogeneous material throughout the cooling and heating cycle. The results showed that T_g of zein–glycerol films predicted by Couchman and Karasz equation is very close to value obtained by DSC experiments. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 2848–2854, 2008

Key words: glass transition; maize prolamin protein film; whey powder film

INTRODUCTION

Increased consumer demand for both higher quality and longer shelf life foods in combination with environmental needs for reduction of disposable packaging amounts have led to increased interest for edible film research.[1] Edible films offer potential solutions to these concerns, by serving as a barrier to water, oxygen, carbon dioxide, and lipid transfer in food systems. Edible films can also improve food system mechanical properties and control the loss of volatile flavors and aromas.[2] Furthermore, biodegradable packaging produced from food protein offer the greatest opportunities since their biodegradability and environmental compatibility are assured.[2] Many food proteins such as corn zein, wheat gluten, soy protein isolate, whey protein isolate, and caseins have been formulated into edible films or coatings.[3] The highly hydrophilic nature of these proteins limits their ability to provide desired edible film functions. To improve the moisture barrier properties of these films, waxes and fatty acids were added to film formulations.[3,4]

The addition of plasticizing agents to edible films (added at concentrations ranging 10–60 g/100 g dry matter depending upon polymer rigidity) is often required to overcome film brittleness caused by extensive intermolecular forces.[3] Plasticizers reduce these forces and increase flexibility and extensibility of the films. This avoids cracking of the film during subsecuent handling and storage. The plasticizers must be compatible with the polymer matrix.

Whey, the byproduct of cheese-making, is one of the biggest reservoirs of food protein remaining largely outside human consumption channels.[1] Hence, great efforts are made to find new uses for whey protein, for example, as edible and biodegradable films. The use of pure whey protein can be rather expensive for commercial purposes. On the other hand, whey powder is low in cost and readily available. Because of the high concentration of lactose (∼ 50%, w/w), whey powder has poor film-forming abilities despite its low material cost.[4]

Zein (prolamin fraction of corn protein) is produced commercially from corn gluten meal (CGM). CGM is a coproduct material that is obtained during starch production. It has low price and used mainely for animal feeding.[5] Zein has high proportion of

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hydrophobic aminoacids so that the zein films show lower water vapor permeability in comparision with other proteins such as whey protein and gelatin. Zein film has also higher mechanical strenght than whey protein film and desirable heat seal property. In contrast, whey protein film has higher flexbility and clarity than the zein film.[5] Furthermore, zein can be used as a binder to fatty acids, because both are alcohol soluble. The latter is very importent in relatively hydrophobic film production.[4]

Phase transitions are changes in the physical state of matter due to changes in temperature or pressure. First-order transitions show a step change in enthalpy, entropy, and volume at the temperature of the transition.[6] Melting, crystallization, vaporization, condensation, sublimation, transitions between polymorphic states in fats, starch gelatinization, and protein denaturation are first-order transitions in foods.[6] Materials with amorphous or partially amorphous structures undergo a transition from a brittle glassy solid state to a rubbery or highly viscous state at a material-specific temperature called the glass transition temperature (T_g). Glass transition in amorphous food materials is a second-order phase transition and generally occurs over a range of temperature rather than at a single temperature.[7] Restriction of movement at the T_g results in a very high apparent viscosity ($>10^{12}$ Pa.s) and shear modulus (>109 Pa).[6] The glass transition temperature (T_g) has been an important parameter in the study of synthetic and edible biodegradable polymers, because it influences the use of these materials. For example, it is expected that the permeation of gas and vapor molecules through a film will be higher above T_g, where polymer chains are more mobile.[1] Above T_g, polymeric materials exist in a soft, rubbery state, which impairs barrier properties, whereas below T_g, polymers are as glassy, low-permeable state.[8] The T_g values are also important for determination of compression molding and extrusion temperatures. Generally, T_g is increased by increasing the amount of stiff chains and bonds, bulky side groups, crosslinking between chains, and the degree of crystallinity, whereas T_g is decreased when the amount of low molecular plasticizers is increased. In the case of edible and biodegradable films, water acts as a plasticizer depressing T_g.[9] Water, which is a low molecular weight component, increases free volume and thereby allow increased backbone chain segmental mobility, which in turn produces structural relaxation at a decreased temperature. The depression of T_g, due to plasticization of amorphous components by water or other plasticizers may have significant effect on the shelf-life and stability of foods.

Water can also exert an antiplasticizing effect particularly in the low moisture region by causing an increase in puncture or tensile strength.[10] Moreover, addition of low molecular weight diluents other than water (e.g., polyoles) to glassy polymers, on one hand lowers T_g but at the same time exerts an antiplasticizing effect on the mechanical properties of the polymer. At glass transition, physical properties such as the thermal expansion coefficient, the dielectric constant (for polar materials), and the heat capacity exhibit a discontinuity. Thus, techniques measuring such property changes have been developed for experimental determination of T_g (e.g., dilatometry, calorimetry). Differential scanning calorimetry (DSC) is probably the most commonly used technique for determining T_g.[10] Besides DSC, the utility and advantages of other techniques such as the dynamic mechanical (thermal) analysis (DMA or DMTA) the nuclear magnetic resonance (NMR), and the electron spin resonance spectroscopy (ESR) have been increasingly investigated.[9,10]

The manufacture of multilayer flexible packaging is known as converting. Converting processes include lamination and coating.[5] Lamination is used to improve the performance of polymeric films by combining the properties of several types of films into one sheet. Lamination may be accomplished by hot-pressing heat-sealable films together or by applying an adhesive to the surface of single layers.[5] Lamination of similar materials may be useful to decrease leakage through film poresand and improve hydrophobicity and mechnaical properties. The main goals of this research work was study of thermal and thermomechanical properties of bilayer protein films plasticized by olive oil and glycerol.

EXPERIMENTAL

Material

Zein was obtained from Acros Organics Inc (Reagent Lane, Fair Lawn, NJ 07410). Commercial whey protein concentrate (WPC) was obtained from Optimum Nutrition Inc (305 Steelhead Way, Boise, ID 83704 USA). Glycerol and ethanol 99.8% were purchased from Merck Corp. (Whitehouse Station, NJ 08889-0100).Olive oil was purchase from Gilvan zeitoon (Gilan, Iran).

Preparation of monolayer and bilayer films

Zein dispersions were obtained by dissolving zein (10% w/v) in warm (80°C) aqueous ethanol 80%. Two type plasticizers (glycerol or olive oil) were used for zein plasticizaton, because zein has relatively hydrophobic property and can bind to fatty acids. Glycerol and olive oil were added to the zein solutions at 0.3 and 0.7 g/g of zein, respectively, and then were stirred in mixer at 250 rpm for 30 min.

WPC solutions were made by dissolving 10 g of WPC in 100 mL distilled water. Plasticizer (Glycerol) was added to WPC film solutions at 0.5 g/g of protein. Solutions were mixed at 250 rpm on a magnetic stirrer for 30 min and heated at 90°C.

After stirring, a vacuum with a rotary pump was applied to remove any dissolved air from solutions. Then the solutions were casted at 1-mm thickness onto Teflon plate and dried overnight in an oven. These films were laminated by using a hydraulic cold press at pressure of 100 kg/cm^2 for 5 min.

Thermomechanical properties

The small deformation analysis of the films was performed in tension in a dynamic mechanical thermal analyzer (DMTA, Triton Technology, UK). The tested filmstrips were 35-mm long and 4-mm wide and clamped in the instrument with the initial grip separation 5.5 mm. The films were subjected to a sinusoidal strain on top of a static deformation. The testing was conducted at a constant frequency of 1 Hz and a strain of 0.02% and over a temperature range of −128.1–249.5°C, at a heating rate of 5°C/min. The measurements of each experimental point were done at least in triplicates. When dynamic mechanical spectroscopy is employed within the linear viscoelastic regime to determine T_g, the storage and loss modulus (E' and E'') and loss tangent (tan δ = $\Delta E''/E'$) are measured as a function of temperature at a constant frequency and a selected heating or cooling rate. The glass transition was defined as the midpoint between the onset of the drop in the $\Delta E'$ (obtained from the intercept of the 'glassy' baseline and the tangent to the point of the steepest drop in modulus) and the peak in tan δ.

Thermal properties

The DSC measurements were carried out in a DSC PL Polymer Laboratories, UK. Calibration was based on pure indium and sapphire. An empty Aluminum pan was used as reference. Samples (~ 0.03 g) were scanned at a rate of 10°C/min between temperature ranges of −150 to 320°C. The glass transition temperatures were determined from the resulting thermograms as the midpoint between onset and end temperatures of step changes in heat flow observed during heating and identified as second-order transitions.

RESULTS AND DISCUSSION

The thermomechanical properties of the whey protein, zein and laminated of the whey protein–zein films were determined by DMTA (Figs. 1 and 2). Two tan δ peaks in zein and laminated films and

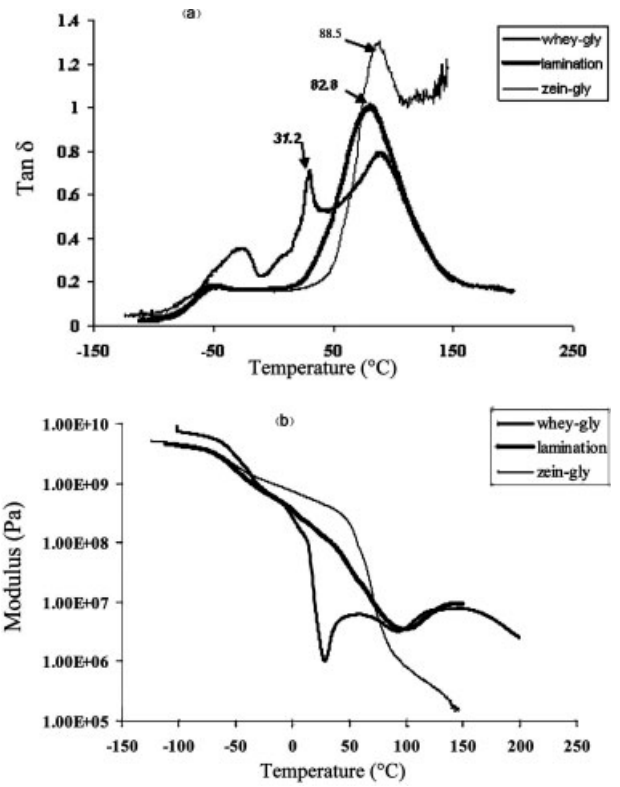

Figure 1 Effect of lamination on loss tangent and storage modulus of films (plasticized by glycerol) in DMTA analysis.

three peaks in the whey protein films can be observed in the DMTA results. The zein–glycerol, whey protein–glycerol and zein–whey protein–glycerol films showed tan δ peak at 88.5, 31.2, and 82.8°C, respectively, (Table I). The most representative parameter of glass transition is T_g and in this temperature, the storage modulus (E') of biological and synthetic polymers exhibits a sharp drop with temperature (α-relaxation), whereas the loss modulus (E'') or tan δ shows a characteristic peak. The tan δ peaks of different films were found at a temperature higher than the onset or midpoint temperature of the E' drop The loss observed in modulus of amorphous synthetic polymers is typically about three orders of magnitude, whereas that decrease observed in biopolymers is about one order of magnitude.[10] This phenomenon reflects motions of fierily long chain segments in the amorphous domains of the polymers (Glassy state is characterized by the freezing out of long-range motions in the case of small molecules and wriggling motion of chains in the case of polymers).[11] The results showed that the single zein film had higher T_g than the single whey protein and zein–whey composite films. The lower T_g of the whey protein films in comparison with zein film may be due to more hydrophilic nature and presence of lactose in whey powder. Lactose is

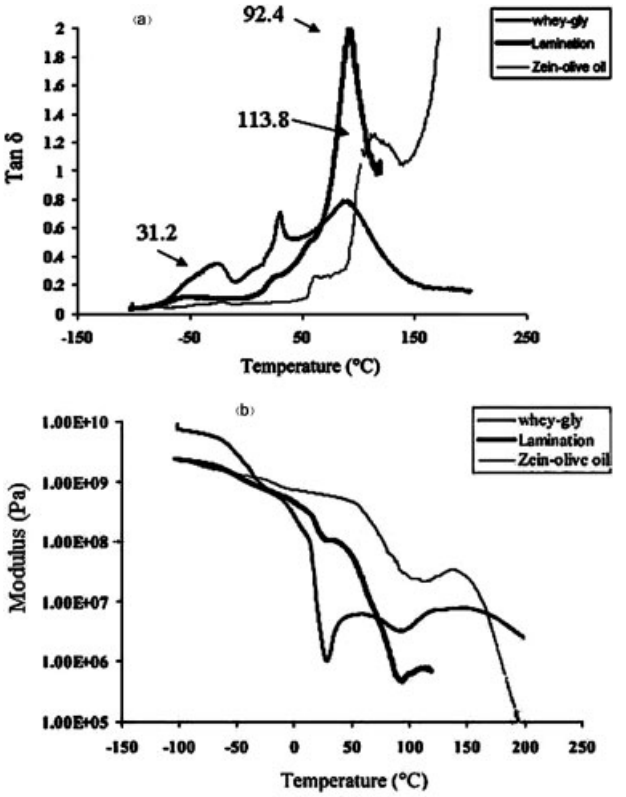

Figure 2 Effect of lamination on loss tangent and storage modulus of films (plasticized by glycerol and olive oil) in DMTA analysis.

a sugar and has relatively low molecular weight and exerts plasticizing effect on protein polymer. The T_g value of pure zein coccurs at 170–180°C and decreases to ~ 60–80°C in the zein films plasticized by polyoles (e.g., glycerol, sorbitol and polyethylen glycol) or fatty acids (oleic acid, palmetic and stearic acid).[5,11] As well as, blending of zein with hydrophilic polymers (e.g., polycaperolacton) can diminish T_g of resulting composite films.[12] The shift in the T_g values of films from 31.2°C in whey protein film and 88.5°C in the zein film to 82.8°C in the laminated whey protein–zein films may be implied some interaction formation between the two polymers.

Relaxations at lower temperatures (labeled β, γ, δ,... with decreasing transition temperature) are generally due to local movements of the main chain, or rotational and vibrations of terminal groups or other side chains (short-range motions).[3] The magnitude of these transitions is much smaller than the main α-relaxation. The small tan δ peaks (low temperature transition) were observed at −50°C in zein–glycerol films and at −22.37°C in the whey protein films and can be related to β-relaxation phenomena or presence of glycerol rich region in polymer matrix. The T_g at −50°C was higher than the reported value of −93°C for pure glycerol.[13] The higher values were probably caused by zein domains that were miscible with glycerol, resulting in an increased T_g due to increase in the average molecular weight. In the whey protein films, third peak at 92.88°C could be associated to some disulfide bridge break downing.

Zein–olive oil and zein–whey protein–olive oil films showed tan δ peaks corresponded the T_g values at 113.8, and 92.4°C, respectively, (Table I). So that, replacing of glycerol with olive oil in film composition, increased T_g and also it would be expected improvement in film barrier property. This could be attributed to larger molecular weight and more hydrophobic nature of olive oil constituents in comparision with glycerol.

The measurements of the glass transition temperature of a polymer mixture are often claimed as a criterion to establish its miscibility.[2,14] A single α-relaxation (T_g) was observed in whey protein–zein laminated films and separate transition was not detectable. However, it seems this behavior does not necessarily imply miscibility between whey protein and zein at a molecular level. Nevertheless, the manifestation of a single glass transition in the DMTA curves of the laminated films may be attributed to a close proximity of the T_gs of the individual polymeric components and a similar plasticization behavior.

The T_gs of various films were also determined by DSC (Figs. 3 and 4). DSC measurements confirmed glass transition phenomena in whey protein, zein and laminated films. Thermograms show only a change of the baseline toward a higher value, indicating that whey protein and zein is highly amorphous polymer undergoing a glass transition phenomenon. The results of DSC measurements are compared with DMTA measurements in Table I. A good correspondence was obtained when DSC results were compared with the tan δ peaks in DMTA measurements. However, relatively high discrepancy at T_g (obtained by different methods) was observed in laminated films plasticized by glycerol. A simple comparison between mechanical and calorimetric determinations of T_g is not easy. Techniques are sensitive to different degrees of molecular mobility. The T_g measured by DMTA is dependent on fre-

TABLE I
T_g (°C) of Different Films Obtained by DSC and DMTA

Film type	DSC	DMTA (Tan δ)
Zein-glycerol	61.4	88.5
Whey protein-glycerol	43.65	31.2
Zein-olive oil	91.85	113.8
whey protein-zein-glycerol	58.85	82.8
whey protein-zein-olive oil	85.91	92.4

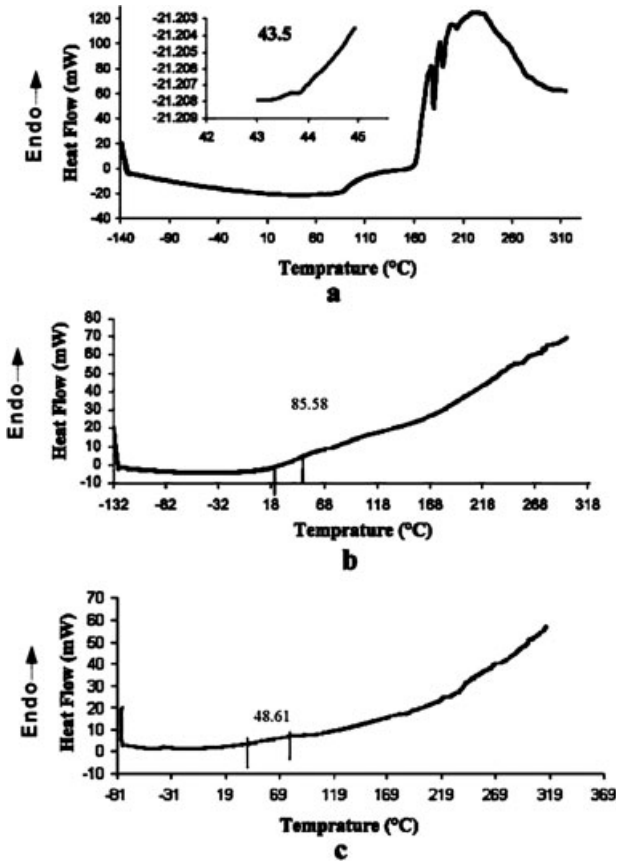

Figure 3 Typical differential scanning calorimetry thermograms of various films plasticized by glycerol: zein films (a), laminated zein–whey protein (b), and whey protein film(C).

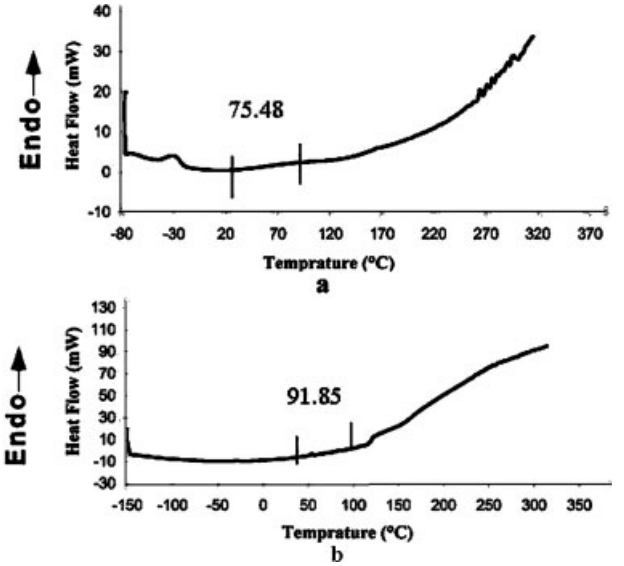

Figure 4 Typical differential scanning calorimetry thermograms of various films plasticized by olive oil: zein films (a) and laminated zein–whey protein (b).

quency, and T_g measured by DSC depends on the heating rate used because it is a kinetically determined transition. However, the DMTA method is a more sensitive method and the glass transitions were easier to detect.[9] These two methods could be considered as complementary test. DMTA results are very reproducible for high water contents, where glass transition occurs at a low temperature avoiding water loss. DSC results could be more reliable at low water content because no water loss occurred.[9]

Protein–protein and protein–plasticizer interactions also can be investigated by differential scanning calorimetry (DSC) (Figs. 3 and 4). DSC thermograms of zein, whey protein, and laminated films showed plasticizers compatibility with biopolymer and effectiveness of plasticization process. Sharp and first-order transition peaks were not found in heating and cooling scans of the whey protein films and laminated films for the temperature range scanned (−150 to 300°C). No peaks related to melting and crystallization of plasticizers were observed and only one phase transition was detected. Thermograms suggested that plasticizers and biopolymers remained a homogeneous material throughout the cooling and heating cycle, because phase separation (separated glass transition temperature or melting and crystallization peaks) between plasticizers and films and between two biopolymers in laminated films was not observed. If polymer and plasticizers blend or two different polymers are immiscible, the mixture will exhibit two T_g corresponding to the two pure phases. In fact, this is a way of checking phase separation in amorphous blends. Melting peaks and flow region was observable in the zein films because zein is thermoplastic biopolymer. The absence of a flow region in the whey protein films may be related to the presence of intermolecular covalent cross bondings, such as disulfide bonds and thereby whey protein is known as a thermosetting biopolymer.

The depression of T_g by the addition of a diluent or plasticizer may be explained by a number of theoretical approaches such as free volume theories or classical thermodynamic theories (Couchman–Karasz model).[10] A thermodynamic consideration of the entropies of the components at the T_g of the mix-

TABLE II
T_g and ΔC_p of Films Constituents

Constituents	T_g (K)	ΔC_p (J/g K)
Zein	402.92	2.086
Whey protein	349.322	5.834
Glycerol	165.582	1.373
Olive oil	154.562	0.703
Water	138	1.94

TABLE III
T_gs Obtained by Experimental Method (DSC) and Predicted by Couchman-Karasz Model

Film type	Moisture content (%)	Plasticizer content (%)	T_g (K) predicted	T_g (K) experimental
Zein–glycerol	12	23	335.47	334.635
Zein–olive oil	6.52	41	339.90	365.0
Whey protein–glycerol	26	33	304.26	316.8

tures leads to the following equation for a three-component mixture (biopolymer–water–plasticizer):

$$T_g(\text{mixture}) = \frac{X_1\Delta C_{p1}T_{g1} + X_2\Delta C_{p2}T_{g2} + X_3\Delta C_{p3}T_{g3}}{X_1\Delta C_{p1} + X_2\Delta C_{p2} + X_3\Delta C_{p3}} \quad (1)$$

In eq. (1), the subscript 1 refers to water, the subscript 2 refers to zein or whey protein, and the subscript 3 refers to glycerol or olive oil, X is the weight fraction of each component and ΔC_p is the change in heat capacity observed at T_g ($\Delta C_{\text{pi}} = C_{\text{liquid}} - C_{\text{glass}}$).

The Gordon–Taylor equation has been more easily and successfully used to fit T_g data of various biopolymers such as zein–oleic acid.[11,15,16] However, this latter equation cannot be extended to more than bicomponent systems.

For obtaining a better prediction of mixture T_g, the following equation, which is equivalent to eq. (1) but avoids the use of water C_p, was used:

$$T_g(\text{mixture}) = \frac{X_1T_{g1} + X_2K_1T_{g2} + X_3K_2T_{g3}}{X_1 + X_2K_1 + X_3K_2}$$

$$K_1 = \frac{\Delta C_{p2}}{\Delta C_{p1}}$$

and

$$K_2 = \frac{\Delta C_{p3}}{\Delta C_{p1}}.$$

In addition, K is a constant that is proportional to the plasticizing effect of diluents (water for subscript 1 and glycerol or olive oil for subscript 3) on the polymer (zein or whey protein with the subscript 2). Table II shows T_g and ΔC_p values of components of zein and whey protein films which obtained by DSC. The results show that T_g of zein–glycerol films predicted by Couchman and Karasz equation is very close to value obtained by DSC experiments (Table III). However, there is difference between experimental and predicted T_g valuesin zein–olive oil ($\sim$ 3%) and whey protein–glycerol films ($\sim$ 7%). It seems that the incomplete compatibility of plasticizers and proteins is responsible for this phenomenon. Glycerol appears to be more miscible with polymers than olive oil, because of its lower molecular weight, but the compatibility was not complete and could explain the deviation from the Couchman–Karasz theory. Couchman–Karasz equation cannot be used for laminated films, because this theory only can be applied for mixed systems.

CONCLUSIONS

Whey protein and zein films containing glycerol and olive oil were prepared by casting method and laminated by using a cold press. The thermal behavior of the whey protein, zein and whey protein–zein laminated films were investigated by dynamic mechanical thermal analysis (DMTA) and differential scanning calorimetry (DSC). Both techniques showed that the films containing olive oil had higher glass transition temperature (T_g) than those containing glycerol. As well as, laminated films had higher T_g when compared with the whey protein films. The T_g values obtained from two different methods were close. For the laminated films, there was no clear evidence of separate phase transitions of the individual polymeric constituents or a separate plasticizer phase. The results show that T_g of zein–glycerol films predicted by Couchman and Karasz equation was very close to value obtained by DSC experiments.

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