Modification of functional properties of pullulan–whey protein bionanocomposite films with nanoclay

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Abstract In this study, biodegradable nanocomposite film composed of pullulan – whey protein isolate (WPI) – montmorillonite (MMT) were developed and characterized as a function of incorporating various amounts of MMT nanoparticles (0, 1, 3 and 5 % wt). Results showed that the water-vapor permeability, moisture content, moisture absorption and water solubility decreased when the nano-MMT content was increased. Tensile strength improved and elongation at break simultaneously decreased with increasing MMT content. The glass transition temperature (T_g) and melting-point temperature (T_m) increased with increasing nano-MMT content. Scanning electron microscope (SEM) and X-ray diffraction (XRD) analysis revealed uniform distribution of MMT into the polymer matrix. Atomic force microscopy (AFM) showed enhancement of films' roughness with increasing MMT content.

Keywords Whey protein · Pullulan · Nano clay · Nanocomposite · Film

Introduction

In recent years studies have increasingly focused on biodegradable films due to growing environmental pollution and the waste of petroleum-based polymers. Renewable natural compounds such as animal and plant-based polysaccharides and proteins are considered the best alternatives to synthetic polymers (Boredes et al. 2009). Biopolymers' sensitivity to water and mechanical weakness are the biggest obstacles against replacing synthetic polymers with biopolymers. Mixing biopolymers with proteins (Zolfi et al. 2014), carbohydrates (Motedayen et al. 2013; Koch et al. 2010), various combinations of several proteins and carbohydrates (Guerrero et al. 2013; Arvanitoyannis et al. 1998), lipids (Kowalczyk and Baraniak 2014; Shih et al. 2011) and nano fillers (Almasi et al. 2010; Shahabi-Ghahfarrokhi et al. 2015b) are the common methods to overcome the drawbacks of biopolymers.

Whey protein is a byproduct of the cheese industry. Whey protein isolate (WPI), with over 80 % protein, has extensive applications in preparing emulsions, foams, gels, and biofilms. WPI film is transparent, flexible and odorless and increases the shelf life and nutrition value of the food. It can be acted as an excellent oxygen barrier at low and intermediate relative humidity (RH), and it is high water vapor permeability due to hydrophilic amino acids in its structure (Osés et al. 2009). These properties make it a good candidate for biodegradable films (Zhou et al. 2009; Fairley et al. 1996).

Pullulan (PUL) is an extracellular microbial polysaccharide with a repeating unit: maltotriose trime $\alpha-(1\rightarrow4)\text{Glup}-\alpha-(1\rightarrow4)\text{Glup}-\alpha-(1\rightarrow6)\text{Glup}$. It is produced by a fungus-like yeast, dubbed *Aureobasidium pullulans*. PUL film is colorless, tasteless, odorless, transparent, flexible, a good barrier to oil and oxygen, and heat-sealable (Singh et al. 2008; Xiao et al. 2012).

Previous researchers have used combinations of polysaccharides and proteins in biocomposite films (Ciesla et al.

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2006; Jiang et al. 2010; Kristo and Biliaderis 2006). However, the resulting biopolymers have had poor mechanical and barrier properties. Nanocomposites are one promising material to improve these properties (Avella et al. 2005).

Montmorillonite (MMT) is a group of negatively charged silicate layers with a high aspect ratio (the ratio of longer side to shorter side) and high surface area. Its characteristics are appropriate for reinforcement agents. It consists of 10 Å thin layers with an octahedral aluminum sheet sandwiched between two tetrahedral silica sheets (Cyras et al. 2008). It is abundant, nontoxic, inexpensive, and ecofriendly, and can enhance the mechanical and barrier properties of bionanocomposites (Sinha Ray and Bousmina 2005; Pavlidou and Papaspyrides 2008; Zolfi et al. 2014).

The addition of low percentages of nanoclay to polymer may lead to increased mechanical strength, and improve heat resistance and barrier properties in food packaging material in comparison to traditional composites (Almasi et al. 2010).

The main purpose of the current study was to determine the ways in which adding MMT to a WPI-PUL composite improves the composite's mechanical and barrier properties. The effects of MMT on WPI-PUL's thermal, crystallinity, and surface properties and microstructure were also investigated.

Materials and methods

Material

WPI was purchased from Arla Food Ingredient (Denmark). PUL was provided from Hayashibara Co. Ltd. (Japan). Analytical-grade chemicals including sodium chloride (NaCl) and calcium chloride (CaCl₂) were provided from Dr. Mojalali Co. (Iran). Calcium nitrite (Ca(NO₂)₂) was purchased from Merck Co. (Germany). Unmodified natural MMT (Cloisite Na⁺) with a density of 2.6 g ml⁻¹ was purchased from Southern Clay Products (Gonzalez, TX).

Film preparation

5 g of WPI was dissolved in 100 ml distilled water and heated at 90 °C for 30 min, then stirred with a magnetic stirrer. Next, 5 g PUL was dissolved in 100 ml distilled water and mixed with the WPI solution at 1:1 w/w, and 30 % w/w (dry base) glycerol was added to the solution as a plasticizer. The MMT was dispersed in distilled water by ultrasonic bath at ambient temperature for 1 h. The MMT dispersion was added to the aqueous dispersion of WPI-PUL at clay concentrations of 1, 3 and 5 % w/w (dry base), and stirring was continued for 1 h. Then, about 70 ml of the sample was poured onto flat, level, non-stick Teflon plates, which were held at room temperature and room relative humidity for approximately 18 h to set. The

dried films were peeled from the plates and stored in plastic bags inside desiccators at 25±1 °C until used. All samples were made in triplicate.

Mechanical properties

The films' tensile strength (TS) and elongation at break (EB) was evaluated using a Testometric Machine M350-10CT (Testometric Co., Ltd., Rochdale, Lancs., England) according to the standard method of ASTM D882 (ASTM 2001). At least three rectangular ribbons 1 cm wide and 10 cm long were cut and fixed at a grip distance of 50 mm. All film specimens were evaluated at a 10 mm/min head speed. TS and EB were calculated using Eq. 1 and 2, respectively.

$$TS = \frac{F_{Max}}{A_{min}} \quad (1)$$

$$EB = \frac{L_{Max}}{L_0} \times 100 \quad (2)$$

where F_{max} is the maximum load, A_{min} is the minimum cross-section area, L_{max} is the extension at the moment of rupture, and L_0 is the initial length of the specimen.

Moisture content

The films' moisture content (MC) was determined (in three replicates) by measuring the weight loss of the film specimens at 105 °C. MC was calculated using Eq. 3.

$$MC = \frac{m_1 - m_2}{m_2} \times 100 \quad (3)$$

where m_1 is the weight of sample before drying and m_2 is the weight of dried sample at 105 °C.

Moisture absorption

Moisture absorption (MA) was measured according to the method of Almasi et al. (2010). In brief, the dried sheets of 20×20 mm² were first conditioned at 0 % RH (prepared using dried calcium sulphate for 24 h). After weighing, they were conditioned in a desiccator containing a saturated calcium-nitrite solution at 25 °C to ensure a relative humidity of 55 %. The specimen was weighed at desired intervals until an equilibrium state was reached. The moisture absorption of the specimen was calculated using Eq. 4.

$$MA = \frac{m_1 - m_2}{m_2} \times 100 \quad (4)$$

where m_1 is the weight of equilibrated film at 0 % and m_2 is the weight of equilibrated film at 55 % relative humidity.

Solubility in water

Solubility in water (SW) was defined as the ratio of the water-soluble dry matter of film that is dissolved after immersion in distilled water. The SW of the film specimens was measured according to the method of Shahabi-Ghahfarrokhi et al. 2015a, 2015b (Shahabi-Ghahfarrokhi et al. 2015b). The 20×20 mm² film specimens were dried at 105 °C and weighed (m_1); the dried samples were then immersed into 50 ml of distilled water for 6 h. The remains of the films were dried at 105 °C and weighed (m_2). SW was calculated using Eq. 5.

$$SW = \frac{m_1 - m_2}{m_2} \times 100 \quad (5)$$

Water-vapor permeability

The films' water-vapor permeability (WVP) was measured gravimetrically according to ASTM E96 standard (ASTM 1995) and corrected for the stagnant air gap inside the test cups. Special glass vials with a diameter of 12.62 mm and a volume of 10 ml were used as test cups. The area of the vial mouth was 1.25×10⁻⁴ m², and the depth was 43 mm. The vials contained calcium-chloride desiccant (0 % RH, assay cup) or nothing (control cup). Each vial was covered with a film specimen, which was sealed to the vial mouth using paraffin. Each vial was placed in a desiccator maintained at 75 % RH with a saturated solution of sodium chloride. The difference in RH corresponded to a driving force of 1753.55 Pa, expressed as water-vapor partial pressure (Shahabi-Ghahfarrokhi et al. 2015b; Zolfi et al. 2014). After the films were mounted, the weight gain of the whole assembly was periodically recorded (with an accuracy of 0.0001 g) every 1 h during the first 10 h and finally after 25 h. The slope (S) of the weight-versus-time plot ($R^2 \geq 0.986$) was divided by the effective film area (A) to obtain the water-vapor transmission rate (WVTR), as shown in Eq. 6 (gm⁻² s⁻¹). This was multiplied by the thickness of the film and divided by the pressure difference between the inner and outer surfaces to obtain the WVP, as shown in Eq. 7 (gm⁻¹ s⁻¹ Pa⁻¹).

$$WVTR = \frac{S}{A} \quad (6)$$

$$WVP = \frac{WVTR \times X}{\Delta P} \quad (7)$$

where x is the average film thickness (m) and ΔP is the driving force (1753.55 Pa).

Film color

The color of the film specimens was measured using a colorimeter (Labscan XE, Hunterlab, USA). A white standard color plate ($L^* = 96.9$, $a^* = -0.33$, $b^* = 0.16$) was used as the background of

all samples and samples color reported according the lab system, in which L , a , and b describe (white–black), (red–green), and (yellow–blue), respectively. All measurements were performed in five replicates. The total color difference (ΔE), yellow index (YI), and white index (WI) were calculated using Eqs. 8–10 (Ahmadi, et al. 2012; Shahabi-Ghahfarrokhi, et al. 2015a).

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (8)$$

$$YI = \frac{142.86 \times b}{L} \quad (9)$$

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (10)$$

where L^* , a^* , and b^* are the color-parameter values of the standard and L , a , and b are the color-parameter values of the specimen.

Differential scanning calorimetry

The films' thermal properties were determined using differential scanning calorimetry (DSC) equipment (Metler Toledo, Switzerland) according to ASTM standard method D 3418–08 (ASTM 2004). A film specimen of approximately 6 mg was cut and placed in a pan of the DSC equipment. Each specimen was then scanned at a heating rate of 20 °C/min between a temperature range of –50 and 250 °C. Nitrogen was used as the purge gas at a flow rate of 20 ml/min. The glass-transition temperatures (T_g) of the different films were determined from the resulting thermograms as the midpoint temperature of a step-down shift in baseline, due to the discontinuity of the specific heat of the specimen. The melting point (T_m) was calculated as the temperature where the peak of the endotherm occurred. All these properties were determined in duplicate and the results averaged.

Scanning electron microscopy

The morphology of the surface and the cross-section of films were observed by field-emission scanning electron microscopy (FE-SEM) KYKY-EM3200 (KYKY, China) with the accelerating beam at 25 kV. The film specimens were sputtered with a thin layer of gold using a KYKY-SBC-12 sputter coater (KYKY, China).

Atomic-force microscopy (AFM)

Surface topography of the sample investigated using an Atomic Force Microscope Dualscope/ Rastroscope C26 (DME, Denmark) in noncontact mode. Film specimens were cut into thin pieces and fitted by double-sided tape onto the sample stage. Samples measuring 5×5 μm² were scanned. All image analysis and surface parameters were conducted using

Dualscope/Rasterscope SPM software (Version 2.1.1.2). The mean surface roughness (S_a) and mean square roughness (S_q) of the films were calculated by Eqs. 11 and 12, respectively.

$$S_a = \iint_a |Z(x, y)| dx dy \quad (11)$$

$$S_q = \sqrt{\iint_a (Z(x, y))^2 dx dy} \quad (12)$$

X-ray diffraction (XRD)

The XRD profiles of the film specimens were taken using a Bruker Advance D8 (Karlsruhe, Germany). The specimens were irradiated by copper Cu K α X-ray beam ($\lambda = 0.15418$ nm) generated with a 40 kV accelerating voltage and a resulting electric current of 30 mA. Sample were scanned by a symmetric reflection geometry in the range of $2\theta = 5^\circ$ – 40° with a scanning rate of 0.02° at room temperature.

D-spacing between the crystalline layers of the polymer chain was estimated using the Bragg diffraction equation Eq. 13.

$$d = \frac{\lambda}{2 \sin \theta} \quad (13)$$

where d is the d-spacing between layers.

The crystallinity degree (CD) of the film specimens was calculated using Eq. 14.

$$CD = \frac{CA}{TA} \times 100 \quad (14)$$

where CA is the crystalline peak area and TA is the total area of the XRD pattern.

Statistical analysis

Statistics were analyzed on a completely randomized design with the analysis of variance (ANOVA) procedure using SPSS software (Version 20; SPSS Inc., USA). Duncan's multiple range tests were used to compare the difference among mean values of film specimens' properties at the 0.05 level of significance.

Results and discussion

SEM micrographs

Figure 1 shows the surface and cross-section of the WPI-PUL and its nanocomposite films. A good affinity between the nanoparticles and the polymer matrix causes homogenous distribution of nanoparticles in the polymer matrix (Rhim et al. 2006; Casariego et al. 2009). Hence, all WPI-PUL-MMT nanocomposites except WPI-PUL-MMT (5 % MMT) had a smooth surface (Fig. 1 (S-1, 2, 3, and 4)); this confirms the compatibility and miscibility of MMT and polymeric matrices. Cross-sections of the film specimens (Fig. 1 (C-1, 2, 3, and 4)) showed increasing the tortuosity of structure with increasing MMT content.

AFM

As shown in previous studies, AFM analysis is a powerful tool for surface analysis. Figure 2 (S, and P (1, 2, 3, and 4)) shows the 2D AFM micrographs of WPI-PUL and WPI-PUL-MMT nanocomposite films. As shown, some cavities were observed

Fig. 1 SEM micrographs of (S) surface, and (C) cross-section of 1) WPI-PUL, 2) WPI-PUL-MMT (1 %), 3) WPI-PUL-MMT (3 %), and 4) WPI-PUL-MMT (5 %)

Fig. 2 AFM micrograph; (S) Non-contact micrographs, (P) Phase micrograph, and (T) Topography of 1) WPI-PUL, 2) WPI-PUL-MMT (1 %), 3) WPI-PUL-MMT (3 %), and 4) WPI-PUL-MMT (5 %)

at the surface of the WPI-PUL film. The result is consistent with the films' water-vapor permeability and barrier properties. Increasing the MMT content decreased the cavity. The cavities disappeared completely at 3 % MMT. Some shiny spots appeared on the phase micrograph of the films with increasing MMT content in the WPI-PUL-MMT nanocomposite (Fig. 2-P (1, 2, 3, and 4)). The shiny spots are clearly recognizable in WPI-PUL-MMT 5 %, suggesting that the shiny spots were MMT particles.

Figure 2-T (1, 2, 3, and 4) shows the 3D topographies of WPI-PUL and WPI-PUL-MMT films. The height of the peaks increased with increasing MMT content. The roughness parameters, such as S_a and S_q (explained in Table 2), confirm an increasing roughness parameter with increasing MMT content. Rhim similarly found that increasing MMT content in an agar-based nanocomposite increased S_a and S_q , which then leveled off. He attributed this to the aggregation of clay embedded in the polymer matrix above a certain level of MMT (Rhim 2011).

Fig. 3 XRD pattern of MMT, WPI-PUL, and WPI-PUL-MMT nanocomposites (values in parentheses are crystallinity degree according to the main peak around $2\theta=14.5$)

XRD

Figure 3 shows the X-ray diffraction pattern of MMT, WPI-PUL, and WPI-PUL-MMT films. MMT showed diffraction peaks at $2\theta=8.22, 11.39, 19.84, 28.17$, and 35.09 . The peak at 8.22 was used to study the behavior of MMT silicate layers and polymer chains. XRD pattern revealed that at WPI-PUL-MMT 1 %, the peak at $2\theta=8.22$ disappeared due to formation of an exfoliated structure of MMT. The result confirms the extensive diffusion of polymer chain inside galleries of MMT and the complete exfoliation of polymers in the WPI-PUL-MMT nanocomposite films (Almasi et al. 2010). But at WPI-PUL-MMT 3 % and 5 % weak peaks observed at 8.56 ($d=0.518$ nm) and 8.62 ($d=0.514$ nm), respectively. These results indicate that either the WPI the PUL polymer chains or both entered into the silicate layers, forming intercalated WPI-PUL-MMT bionanocomposites, without reaching complete exfoliation.

Both the molecular weight of the polymer and polar interactions between the MMT and the polymer could influence polymer intercalation (Almasi et al. 2010). Strong polar interactions between the hydroxyl groups present in the WPI and PUL chains and in the silicate layers probably caused the intercalation of biopolymer chains into the MMT layers' galleries.

The peak around $2\theta, 14.5$, was used for calculating the crystallinity of the film specimens. As shown in Fig. 3 WPI-PUL film was amorphous and the CD of the film specimens increased at 3 % MMT, but at 5 % MMT the CD decreased drastically. This may be due to the aggregation of MMT particles at high MMT contents (Rhim 2011). The roughest cross-section at WPI-PUL-MMT 5 % (Fig. 1-C- 4) and shiny spots in the AFM phase micrograph of WPI-PUL-MMT 5 % confirm this result.

Moisture content, moisture absorption, and solubility

Table 1 shows the effect of MMT on the MC, MA, and SW of WPI-PUL and its nanocomposites. The MA and MC of the film specimens decreased with increasing MMT content up to 3 %. MC is related to the total void volume occupied by water molecules in the polymer matrix (Li et al. 2011). A good

Table 2 Mechanical properties and calculated roughness parameters of WPI-PUL film and WPI-PUL-MMT incorporating various concentrations of MMT^{*†}

MMT content (%)	Tensile Strength (MPa)	Elongation at break (%)	Mean surface roughness (S_a)	mean square roughness (S_q)
0	2.07 ± 0.1^c	179.90 ± 30.82^a	1.81 ± 0.33^c	2.27 ± 0.36^d
1	4.66 ± 0.57^b	67.58 ± 7.76^b	2.38 ± 0.28^c	3.23 ± 0.36^c
3	8.6 ± 1.71^a	33.97 ± 7.76^c	3.49 ± 0.23^b	4.47 ± 0.40^b
5	6.37 ± 1.16^b	17.44 ± 6.43^c	4.57 ± 0.56^a	5.91 ± 0.74^a

^{*} Means within each column with same letters are not significantly different ($P<0.05$)

[†] Data are means $\pm$ SD

dispersion of MMT particle in the polymer matrix and good interaction between polymers may have reduced the void volume occupied by water molecules. But increasing MMT content up to 5 % increased MA and MC. However, while this enhancement was not significantly different, as Fig. 1-C- 4 shows, the homogeneity of MMT particles in polymer matrix declined in WPI-PUL-MMT 5 % The SW of WPI-PUL-MMT films decreased with increasing MMT content (Table 1). Almasi et al. (2010) believed that decreasing water absorption using MMT is due to creation of hydrogen bonds between hydroxyl groups of polymer and oxygen atoms in MMT. The occupation of the polymer's hydroxyl groups by MMT decreases the polymer matrix's absorption of water. The hydrogen bond between the polymer matrix and MMT create a strong structure and decrease the SW. In other words, MMT can improve WPI-PUL-MMT composites' sensibility to water. Our results are comparable with previous research (Almasi et al. 2010). Furthermore, XRD patterns confirm the increasing crystallinity of WPI-PUL-MMT film specimens and support the MC, MA, and SW data.

WVP

The effects of MMT content on WVP of WPI/PUL and its nanocomposite is exhibited in Table 1. WVP of the WPI - PUL - MMT nanocomposites were declined up to 32 % with increasing MMT content. The lowest WVP observed at WPI -

Table 1 Physical properties and WVP of WPI-PUL film and WPI-PUL-MMT nanocomposites incorporating various concentrations of MMT^{*†}

Films	Moisture content (%)	Moisture Absorption (%)	Solubility (%)	WVP ($\times 10^{-10} \text{ gm}^{-1} \text{ s}^{-1} \text{ Pa}^{-1}$)
WPI - PUL	16.93 ± 0.70^a	15.48 ± 1.86^a	87.46 ± 2.74^a	1.62 ± 0.01^a
WPI - PUL - 1 % MMT	14.85 ± 0.49^{ab}	13.11 ± 1.82^{ab}	62.84 ± 8.04^b	1.59 ± 0.01^b
WPI - PUL - 3 % MMT	12.99 ± 1.61^b	9.81 ± 1.66^c	61.35 ± 2.69^b	1.1 ± 0.02^c
WPI - PUL - 5 % MMT	14.46 ± 1.56^b	10.68 ± 0.94^{bc}	62.63 ± 4.00^b	1.13 ± 0.05^c

^{*} Means within each column with same letters are not significantly different ($P<0.05$)

[†] Data are means $\pm$ SD

Table 3 Hunter color values (L , a , and b), total color difference (ΔE), yellowness index (YI), and whiteness index (WI) of WPI-PUL and WPI-PUL-MMT films as a function of MMT concentration ^{*,‡}

MMT content (%)	L	a	b	ΔE	YL	WI
0	89.38±0.86 ^a	-0.15±0.37 ^a	3.07±0.64 ^a	9.15±0.99 ^a	4.92±1.04 ^a	88.92±0.92 ^a
1	89.07±0.75 ^a	-0.23±0.43 ^a	3.23±0.43 ^a	8.52±1.21 ^a	5.18±0.70 ^a	88.59±0.74 ^a
3	88.76±0.83 ^a	-0.3±0.48 ^a	3.3±0.63 ^a	8.39±1.10 ^a	5.32±1.00 ^a	88.26±0.79 ^a
5	87.69±1.10 ^b	-0.69±0.48 ^b	3.38±0.65 ^a	8.35±1.60 ^a	5.51±1.06 ^a	87.19±1.08 ^b

* Means within each column with same letters are not significantly different ($P < 0.05$)

‡ Data are means±SD

PUL - MMT 3 % due to layered and tortuous structure of MMT block the transmission of vapor through the film matrix (Park et al. 2003). Furthermore, compact structure of WPI - PUL - MMT3 % (Fig. 1-C-3) enhanced barrier properties of the nanocomposite. This phenomenon is observed in previous researches and high CD proposed as an effective factor on WVP (Almasi et al. 2010; Shahabi-Ghahfarrokhi et al. 2015b; Rhim 2011; Casariego et al. 2009).

Mechanical properties

Table 2 shows the results for the mechanical properties of WPI-PUL and WPI-PUL-MMT films. The TS of the film specimens increased from 2.027 to 8.60 MPa with increasing MMT content up to 3 %. This is due to strong interfacial interaction between the layered silicate, with its vast surface area, and the polymer matrix. The TS of the MMT 5 % specimen dropped to 6.37 MPa; nevertheless, the TS of all the nanocomposite films were more than that for WPI-PUL alone. As shown in the SEM micrograph (Fig. 1-C, S-4), a non-

uniform distribution of MMT particles in the polymer matrix and the decreasing CD of WPI-PUL-MMT 5 % (Fig. 3) were the occasion of the TS decline in the MMT 5 % nanocomposite.

The EB of the nanocomposites was negatively affected by MMT content, because layered silicates act as reinforcement filler and reduce the flexibility of the films (Rhim 2011). The positive effect of MMT on mechanical properties agrees with the findings of other researchers (Almasi et al. 2010; Huang et al. 2006).

Color

Table 3 gives the color values for WPI-PUL and WPI-PUL-MMT films. Except with 5 % MMT, there were no significant difference between color parameters of the WPI-PUL-MMT nanocomposites and blank film, except for MMT 5 %. There were significant differences between the L , a , WI values of WPI-PUL-MMT 5 % and other specimens, but no significant difference between their ΔE values. (Park et al. 2003) found

Fig. 4 Thermogram and thermal properties of WPI-PUL and WPI-PUL-MMT nanocomposites

that color values are dependent on components' compatibility and surface polarities. Thus, MMT had no side effect on the visual properties of the WPI-PUL-MMT nanocomposite films.

DSC

Figure 4 shows the results for the thermograms and thermal properties of WPI-PUL and WPI-PUL-MMT nanocomposite specimens. The nanocomposites' T_g – the temperature at which the material undergoes a structural transition from an amorphous solid state (glassy state) to a more viscous rubbery state – increased with increasing MMT content up to 1 % MMT, and decreased again with MMT content above that. Below T_g , films are rigid and brittle, whereas above T_g they become flexible and pliable (Ghanbarzadeh and Oromiehi 2009). As mentioned in the XRD analysis, exfoliation of the silicate layers of the MMT and biopolymer chains restrained the movement of the segments and increased the films' T_g (Rimdisit et al. 2008). In contrast, a high nanoparticle content in the polymer matrix could enhance the flexibility of the biopolymer chain like a ball-bearing and decrease the nanocomposite's T_g (Shahabi-Ghahfarrokhi et al. 2015b). As shown in Fig. 4 the T_m of WPI - PUL-MMT 1 % decreased, but it was not significantly different from other specimens except WPI-PUL-MMT 5 %. T_m is dependent on the heat stability of the protein structure (Jiang et al. 2010).

The T_m of WPI-PUL-MMT 5 % was enhanced due to increases in the number and strength of cross-linkages between the MMT and the polymer matrix.

Conclusion

The results of this study showed that MMT could improve the mechanical properties, thermal properties, and water resistance of WPI-PUL film specimens without any visual or color drawbacks. The tortuous structure of MMT in the polymer matrix enhanced the nanocomposite films' water-barrier properties. SEM micrographs of WPI-PUL-MMT nanocomposites revealed a homogenous dispersion of MMT in the polymer matrix. Most characteristics of the nanocomposites could be attributed to the homogenous dispersion and exfoliated structure of MMT.

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