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Effect of nano-clay type on the physical and antimicrobial properties of whey protein isolate/clay composite films

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ABSTRACT

Whey protein isolate (WPI)-based composite films with three different types of nano-clays, Cloisite Na$^+$, Cloisite 20A, and Cloisite 30B, were prepared using a solution casting method, and their physical and antimicrobial properties were determined in order to better understand the effect of nano-clay type on film properties. The resulting films exhibited an opaque appearance and haze, and the degree of this effect depended on type of nano-clays added. However, these films displayed a similar gloss and slightly lower transparency relative to transparent neat WPI film. The type of nano-clay used significantly influenced the tensile and the water vapor barrier properties of the composite films with the exception of Cloisite 30B, which had no negative effect. In addition, the WPI/Cloisite 30B composite films showed a beneficially bacteriostatic effect against Gram-positive bacteria, *Listeria monocytogenes*.

1. Introduction

The functional properties of biopolymer-based edible films or coatings have been shown to act as barrier to solute and gas and enhance food quality and shelf life (Krochta et al., 1994; Gennadios, 2002). However, these films do not display good mechanical and water vapor barrier properties due to their hydrophilic characteristics. To overcome these issues, a new approach has been developed, which use hybrid materials consisting of polymers and layered silicates (Giannelis, 1996). Layered silicates, such as montmorillonite (MMT) clay mineral, result from the stacked arrangement of negatively charged silicate layers and contain a platelet thickness of about 1 nm with a high aspect ratio (ratio of length to thickness) (Sorrentino et al., 2007). The layered silicate filled polymer composites exhibit extraordinary enhancement of mechanical, thermal and physicochemical properties at a low level of filler concentration in comparison to pure polymer and conventional microcomposites (Uyama et al., 2003). In particular, these nanocomposites have excellent barrier properties because the presence of clay layers delays the diffusing molecule pathway due to tortuosity (Bharadwaj, 2001; Sorrentino et al., 2006).

However, most work done on polymer/clay nanocomposites has focused mainly on synthetic polymers (Alexandre and Dubois, 2000; Ray and Okamoto, 2003). Biopolymer-based nanocomposites, in contrast, have been examined in only a few studies (Pandey et al., 2005; Rhim and Ng, 2007). Some of the works done with biopolymer-based nanocomposites were based on starch or polysaccharides, such as wheat and maize starch (McGlashan and Halley, 2003), thermoplastic starch (Park et al., 2003), and chitosan (Lin et al., 2005; Xu et al., 2006; Rhim et al., 2006; Gunister et al., 2007). A few studies on protein-based nanocomposites have been published, including soy protein (Dean and Yu, 2005; Rhim et al., 2005), whey protein (Hedenqvist et al., 2006), and wheat gluten (Olabarrieta et al., 2006). Most of the biopolymer-based nanocomposites have shown appreciable improvements in mechanical and barrier properties compared to the counterpart biopolymer films.

Whey protein has received much attention for its potential use as an edible film and coating because it has been shown to make transparent films and coatings that can act as excellent oxygen barriers and provide certain mechanical properties (Sothornvit and Krochta, 2000, 2005). Unlike chitosan film, whey protein films have not shown any antimicrobial activity; therefore, incorporation of antimicrobial agents, such as sorbic acid, *p*-aminobenzoic acid (Cagri et al., 2001), and lysozyme (Min et al., 2008), is needed to impart this property. Recently, Rhim et al. (2006) found that chitosan-based nanocomposite films blended with some organically modified MMT, such as Cloisite 30B, exhibited antimicrobial activity against Gram-positive bacteria. They postulated that the antimicrobial action came from the quaternary ammonium salt

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of the organically modified nano-clay. Therefore, it is of interest to further investigate the effect of other types of nano-clays on the performance of nanocomposites prepared with readily available biopolymers like WPI.

The main objective of this study was to determine the effects of different types of nano-clays on film properties, such as optical, tensile, and water vapor barrier properties, as well as antimicrobial activity against the selected food-borne pathogenic bacteria of WPI-based composite films.

2. Materials and methods

2.1. Materials

BiPro WPI (97.7% protein) was supplied by Davisco Foods International (Le Sueur, MN) and three types of montmorillonite (MMT) nano-clays including a unmodified MMT (Cloisite Na^+) and two organically modified MMTs (Cloisite 20A and Cloisite 30B) were obtained from Southern Clay Co. (Gonzales, TX). The organic modifiers of the Cloisite 20A and 30B are reportedly dimethyl dehydrogenated tallow quaternary ammonium and methyl tallow bis-2-hydroxyethyl quaternary ammonium, respectively. The general characteristics of these MMT products are listed in Table 1. Glycerol, a plasticizer, was purchased from Aldrich, Milwaukee, WI.

2.2. Film preparation

WPI and WPI/clay composite films were prepared using a solution or a solution/intercalation method (Rhim and Ng, 2007). WPI film solutions were prepared by dissolving 10 g of WPI in 100 ml distilled water with 5 g of glycerol. For the preparation of WPI/clay composite film mixtures, 5% (w/w, relative to WPI) of nano-clays, at which level clay content is commonly used for preparation of nanocomposites with biopolymers (Rhim et al., 2006), were used. At first a precisely weighed nano-clay (0.5 g) was dispersed with distilled water (100 ml) and stirred using a magnetic stirrer overnight to reach complete hydration/swelling; however, Cloisite 20A (hydrophobic nano-clay) required additional dispersion using a probe sonicator (VCX-500, Sonics & Materials, Inc., Newtown, CT) for 2 h before stirring overnight. Then 10 g of WPI was added to obtain an aqueous solution, followed by adding 5 g of glycerol. All the film-forming mixtures were heated to 90 °C for 30 min in a water bath, cooled to room temperature, and degassed using a bath-type ultrasound sonicator. The film-forming mixtures were then casted onto leveled Teflon-coated glass plates (24 × 30 cm) framed at four sides. The cast plates were dried at ambient temperature (22 ± 2 °C, 50 ± 5% RH) for 2 days and then the films were peeled off from the glass plates.

2.3. Film thickness and conditioning

Film thickness was measured using a hand-held micrometer (No. 7326, Mitutoyo Manufacturing Co., Ltd., Tokyo, Japan) to the nearest 0.00254 mm (0.0001 in). Five thickness measurements were taken on each testing specimen and the average value was used in tensile strength (TS) and water vapor permeability (WVP) calculations as well as determining transparency property. All film samples were preconditioned for at least 48 h in a constant-temperature humidity chamber at 25 °C and 50% relative humidity (RH) before testing. Three replications were used to determine each film property.

2.4. Optical properties (color, gloss, haze, and transparency)

Color values of WPI-based composite films were measured using a colorimeter (Minolta, CR-200, Tokyo, Japan). A white standard color plate (L^* = 97.75, a^* = −0.49 and b^* = 1.96) was used as the background for color measurements. CIE system (L^*, a^* and b^*) values were averaged from three readings for each sample. The total color difference (ΔE) was calculated as follows:

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

The results were also expressed as ΔE values with the control WPI films as reference.

The gloss of WPI-based composite films was determined at incidence angles of 20° (G^{20}) and 60° (G^{60}) using a reflectance meter (BYK Gardner, micro-TRI-Gloss, Silver Spring, MD) in accordance with ASTM D523, and reported as a gloss unit (GU; % of standard), based on three readings for each sample. A highly polished plane surface of black glass with a refractive index of 1.567 served as the primary gloss standard and was assigned to an arbitrary gloss value of 100, which differed depending on the angle used. The sample was placed on the matte surface of black acrylic plates (TAP Plastics, Sacramento, CA), which have 0.2 and 3.0 GU for the 20° and 60° angles, respectively, at room temperature (22 ± 2 °C, 50 ± 5% RH).

Table 1
Characteristics of different commercial montmorillonite-based nano-clays.

MMT type	Characteristics		
	Cloisite Na^+	Cloisite 20A	Cloisite 30B
Organic modifier	None	Dimethyl dihydrogenated tallow, quaternary ammonium (2M2HT)	Methyl tallow, bis-2-hydroxyethyl, quaternary ammonium (MT2EtOH)
Structural formula	$Na_{0.33}(Al_{1.67}Mg_{0.33})Si_4O_{10}(OH)_2$	CH_3 MMT + CH_3-N^+-HT HT	CH_2CH_2OH MMT + CH_3-N^+-T CH_2CH_2OH
Modifier concentration	–	95 meq/100 g clay	90 meq/100 g clay
Moisture content	4–9%	<2%	<2%
Particle size (90% <)	13 μm	13 μm	13 μm
Color	Off-white	Off-white	Off-white
Density	2.86 g/ml	1.77 g/ml	1.98 g/ml
Relative hydrophobicity	Hydrophilic	Strongly hydrophobic	Less hydrophobic

Data from the manufacturer (Southern Clay Co., Gonzales, TX, USA).
T: beef tallow (~65% C_{18}, ~30% C_{16}, ~5% C_{14}); HT: hydrogenated tallow; M: CH_3.