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Development of whey protein isolate bio-nanocomposites: Effect of montmorillonite and citric acid on structural, thermal, morphological and mechanical properties

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ABSTRACT

The use of bio-nanocomposites for liberation of active substances is one of the popular alternatives for safely preserving food. In this context, the objective of this paper was to evaluate the interaction between montmorillonite sodium clay nanoparticles (MMT) and citric acid (CA) on whey protein isolate (WPI) bio-nanocomposites, developed by *casting*. CA was added to aid in the dispersion of MMT layers. The thermal analysis revealed that the addition of MMT, alone, increased the thermal stability of the bio-nanocomposites, but there was a reduction when combined with CA. The elastic modulus (EM), tensile strength (TS), elongation (E) and puncture strength (PS) increased with increasing MMT content from 0 to 3% by weight, with the consequent reduction of the puncture deformation. The combination of MMT and CA caused a decrease in TS and EM and increase in elongation making the films less rigid, weaker and more extensive due to the plasticizing effect. The improvement of these properties can be attributed to good dispersion/exfoliation of MMT in the WPI matrix, as seen in X-ray diffraction and transmission electron microscopy. The interaction between MMT and CA enabled to obtain bio-nanocomposites with thermal, structural, morphological and mechanical properties for use as packaging materials.

1. Introduction

The application of biodegradable polymers derived from renewable resources, replacing the synthetic polymers, has become a promising way to develop new packaging in order to solve environmental pollution problems caused by synthetic polymer residues (Dias et al., 2014; Tunç & Duman, 2010; Wang et al., 2005; Zhang, Yu, Xie, Naito, & Kagawa, 2007). Interest in whey for the preparation of edible and biodegradable films has recently attracted interest as a way to add value and contribute to reducing its environmental impact for being a dairy product waste.

The whey protein isolate (WPI) is the purest form of whey protein. Films based on whey protein isolate (WPI) have been studied due to their edible and/or biodegradable film forming properties and for presenting good mechanical resistance and being excellent barriers to oxygen, lipids and aromas (Mulvihill & Ennis, 2003; Ramos et al., 2012). However, like other protein-based films, due to their hydrophilic nature, they have a low moisture barrier that can be improved with the incorporation of hydrophobic substances (Kim & Ustunol, 2001; McHugh, Huxsoll, & Krochta, 1996; Ramos et al., 2012).

Biodegradable packaging from natural materials has low barrier, mechanical and thermal properties. Nanocomposite films based on layered silicates can improve these material packaging properties. Sodium montmorillonite (MMT-Na^+) is the most widely used clay in natural polymer nanocomposites. It can be used as a component in food packaging as a reinforcing, natural, non-toxic, chemically and thermally stable material (Kaczmarek & Podgórski, 2007; Tunç et al., 2010; Wang, Chen, Kotaki, & He, 2007). Due to its lamellar structure and high aspect ratio the nanoclays can effectively increase the tortuosity of the path of molecules in diffusion. Thus, a significant improvement in barrier properties can be achieved with the addition of relatively small amounts of montmorillonite clays (Vartiainen, Tammelin, Pere, Tapper, & Harlin, 2010). Furthermore,

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being hydrophilic, the MMT-Na^+ can form stable suspensions in water, which facilitates their dispersion in water-soluble polymers such as whey protein isolate.

Citric acid (CA) is a natural organic acid found in citrus foods. It is used as a food additive in various food production processes for its antibacterial and acidulant effect; reinforces antioxidant and anti-darkening action of other substances and flavor and aroma improvement (Park, Chough, Yun, & Yoon, 2005). Citric acid is added in packaging films to increase plasticizing and dispersion montmorillonite on nanocomposites besides promote improvements in the mechanical properties and water vapor permeability (Cagri, Ustunol, & Ryser, 2004; Shi et al., 2007; Wang, Zhang, Han, & Bai, 2009).

The use of MMT to develop composite films made from WPI has been reported in the literature (Sothornvit, Hong, An, & Rhim, 2010; Sothornvit, Rhim, & Hong, 2009; Wakai & Almenar, 2015). Research has been conducted evaluating the effect of the interaction between citric acid and montmorillonite clay on the thermal, structural, morphological and mechanical properties of bio-nanocomposites, but little has been reported about this interaction for whey protein isolate. Thus, the objective of this paper was to evaluate the interaction between MMT and CA on whey protein isolate bio-nanocomposites, developed by *casting*. This study focused on patterns of X-ray diffraction, microstructural characteristics, glass transition temperature, thermal stability, tensile and puncture properties.

2. Material and methods

2.1. Material

Whey protein isolate (WPI 9400) with 90% protein was obtained from *Hilmar Ingredients* (Hilmar CA, USA). Glycerol (GLY) from *Sigma-Aldrich* (Brazil), granular anhydrous citric acid, *Cargill* (Uberlândia, MG, Brazil) and the Cloisite Na^+ (natural montmorillonite nanoparticles) were provided by Southern *Clay Products, Inc* (Gonzales, TX, USA).

2.2. Preparation of whey protein isolate bio-nanocomposites

WPI films were developed. The control film (Control) was prepared by dissolving 6 g (w/v) of WPI and 2.4 g of glycerol (GLY) (g/100 g of WPI) in 44 mL and 56 mL of distilled water, respectively. (WPI) and (GLY) were separately subjected to continuous agitation on a magnetic stirrer for 30 min at room temperature. After stirring, the solutions were poured together and kept under continued agitation for 10 min at room temperature. The pH was then adjusted to 8 with NaOH 2N and the final solution submitted to an ultrasonic homogenizer (*Sonifier Cell Disruptor Branson – Model* 450D, Manchester, UK) for 10 min at an output of 80 W/25 °C. Subsequently, the solution were heated at 90 °C for 30 min in a water bath, cooled to room temperature and poured on glass plates (18 × 30 cm). The other films were developed as was the control film (Table 1). MMT (0; 1; 3 g/100 g of WPI) and CA (0; 5; 10 g/100 g of WPI) being dispersed in glycerol. The thickness control was accomplished by the volume applied to the support, corresponding to 110 mL. The films were then dried at room temperature for 48 h to ensure slow evaporation of the solvent and film formation.

2.3. Film conditioning and thickness

All films were stored at a controlled temperature of 23 ± 2 °C and 50 ± 5% relative humidity for 48 h before analysis, according to the D618-00 method (ASTM, 2000). The average thickness the films was measured by reading at ten distinct points, randomly chosen in each test body, using a *Mitutoyo* digital micrometer (accuracy 0.01 mm Mitutoyo, Suzano, SP, Brazil).

Table 1
Symbols of WPI/MMT/CA bio-nanocomposites.

Code	MMT (g·100 g^{-1} WPI)	CA (g· 100 g^{-1} WPI)
Control	0	0
MMT0CA5	0	5
MMT0CA10	0	10
MMT1CA0	1	0
MMT1CA5	1	5
MMT1CA10	1	10
MMT3CA0	3	0
MMT3CA5	3	5
MMT3CA10	3	10

2.4. X-ray diffraction (XRD)

XRD analysis was performed on a Shimadzu XRD-6000 (Shimadzu, Tokyo, Japan) using a graphite crystal as monochromator with Cu-$k_{\alpha 1}$ (λ = 1.5406 Å) radiation. The samples were fixed in aluminum holder and step-scanned at 0.02° s^{-1}, 2θ from 4° to 60°, speed 1° min^{-1} and 30 kV, 30 mA. The basal spacing (d) in the composites was calculated by the Bragg equation (Equation (1)):

$$sen\theta = \frac{\lambda}{2d} \quad (1)$$

where: λ corresponds to the wavelength used (λ = 1.5406 Å) to generate radiation in the equipment; *d* is the distance to be calculated between the clay lamellae; θ (theta) is the angle where the XRD pattern peak is detected.

2.5. Transmission electron microscopy (TEM)

The montmorillonite nanoparticle dispersion was visualized through TEM (FEI Tecnai G2 Spirit BioTWIN) at 120 kV. The films with a thickness of 60 ± 10 nm were cut by using an Ultramicrotome Leica EM UC6, temperature −115 a −180 °C with a Cryo 35° (Diatome) diamond knife and cutting speed of 1 mm/s. The sections were collected and placed on copper transmission grids coated with Lacey carbon 300 mesh carbon film.

2.6. Differential scanning calorimetry (DSC)

DSC was performed in a DSC TA 60 calorimeter (Shimadzu Corporation, Kyoto, Japan). The detection limit of the apparatus is 0.3 W, the sample weight was 4–6 mg and an empty capsule was used as reference. The heating and cooling ramps were fixed at 10 °C min^{-1} and varied between −50 °C and 200 °C. Firstly, heating from 25 to 200 °C to eliminate the thermal history. Secondly cooling from 200 to −50 °C. Lastly a second heating up to 200 °C (ASTM, 1999, 2003). From the DSC curves, the glass transition temperature (T_g) was measured in the second heating. The initial denaturation temperature (T_d) and maximum denaturation temperature (T_{max}), were evaluated in the first heating curve according to Ryan et al. (2008).

2.7. Thermogravimetric analysis (TG)

The thermal stability of the films was evaluated by thermogravimetric analysis in a DTA-TG Shimadzu 60 H (Shimadzu Corporation, Kyoto, Japan). The analyzes were performed under a nitrogen atmosphere at a flow rate of 50 mL/min with heating from 40 °C to