Food Packaging and Shelf Life 10 (2016) 87–96

Contents lists available at ScienceDirect

Food Packaging and Shelf Life

journal homepage: http://www.elsevier.com/locate/fpsl

Gelatin-based films reinforced with montmorillonite and activated with nanoemulsion of ginger essential oil for food packaging applications

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ARTICLE INFO

Article history:
Received 5 April 2016
Received in revised form 1 September 2016
Accepted 20 October 2016
Available online xxx

Keywords:
Edible films
Active packaging
Nanocomposite
Emulsion
Physical properties
Antioxidant activity

ABSTRACT

Montmorillonite (MMT) and nanoemulsioned ginger essential oil (GEO) were incorporated into gelatin-based films to produce activated films with improved physical properties, with potential for food packaging applications. Nanoemulsions prepared with 1, 3 or 5% of GEO presented similar characteristics. Rheological properties of film-forming solutions were slightly affected by the concentration of GEO used. The incorporation of MMT and GEO into gelatin-based films increased the thickness and decreased the solubility in water, moisture content and superficial hydrophobicity of films (only with MMT). However, the combined effect of MMT and GEO improved significantly ($p < 0.05$) the elongation at break, puncture force and puncture deformation of gelatin-based films. The presence of MMT increased the roughness of the films and slightly reduced their crystallinity. The nanocomposite and activated films presented antioxidant activity but not antimicrobial activity.

1. Introduction

Gelatin is a biopolymer with excellent film-forming properties (Langton & Hermansson, 1993; Sobral, Menegalli, Hubinger, & Roques, 2001). In general, the gelatin-based films present good mechanical resistance and high elasticity but also low water vapor permeability and high sensitivity to ambient humidity (Rao, 2007). Recently, improvements in the properties of these films have been studied by reinforcement of the biopolymer matrix with nanoparticles load, producing a material often called bionanocomposites or only nanocomposites (Jorge et al., 2014; Vanin et al., 2014; Flaker, Lourenço, Bittante, & Sobral, 2015). These films can also be carrier of bioactive compounds conferring antimicrobial and/or antioxidant activities, for instance, to the film (Ibarguren et al., 2015) and, in this case, an active nanocomposite film can be produced (Vanin et al., 2014).

The montmorillonite (MMT) is one of the nanomaterials most commonly used in biopolymers-based films technology. MMT is characterized by a moderate negative surface charge that displays a perfect crystalline structure, formed by a two-dimensional layer having a central octahedral sheet of aluminum oxide and magnesium oxide linked with two external silica tetrahedrons (Majdzadeh-Ardakani, Navarchian, & Sadeghi, 2010; Slavutsky, Bertuzzi, Armada, García, & Ochoa, 2014). MMT can be potentially used to control the release of antimicrobial compounds from film to the packed product, and also controlling the inside atmosphere of the packaging, depending of the gas and water vapor permeability improvement (Fabra, Jiménez, Atarés, Talens, & Chiralt, 2009).

Among the bioactive compounds used to activate biopolymer-based films, the essential oils (EO) are very interesting because they have high potential activities, such as antimicrobial, insecticidal and antioxidant activities, but they are difficulty to incorporate in the film formulation due to their sparing solubility in water, usually used as the solvent. Thus, a potential solution for that problem is to disperse the EO in water as a nanoemulsion (Otoni et al., 2014; Acevedo-Fani, Salvia-Trujillo, Rojas-Graü, & Martín-Belloso, 2015). Nanoemulsions can be produced using low

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http://dx.doi.org/10.1016/j.fpsl.2016.10.004
2214-2894/

energy methods, or by using high pressure devices, such as a Microfluidizer, once it is scalable for industrial production (Lee & Norton, 2013).

The incorporation of EO nanoemulsions in films formulation is a recently adopted technique. Few studies on this subject were found (Acevedo-Fani et al., 2015; Otoni et al., 2014). But, no publications were found in the literature on films charged with nanoparticles and activated with EO nanoemulsions. Thus, the main objective of this research was to develop gelatin-based films reinforced with MMT and activated with the ginger essential oil nanoemulsioned in water. The main interest was to study the impact of loads on physical, optical, mechanical, thermal and structural properties of gelatin-based films. Antioxidant activity was determined and antibacterial activities against *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella enteritidis* were also verified to a possible application in food packing sector.

2. Material and methods

2.1. Material

The biopolymer used was a pigskin gelatin (bloom 242–248; molecular weight $\approx 5.2 \times 10^4$ Da; moisture content = 9.3%) that was kindly provided by Gelita South America (São Paulo, Brazil). Glycerol (Synth) was used as plasticizer and MMT nanoparticles (Nanomer® clay, Aldrich, reference number 688,659-500G) was used as the filler. Ginger essential oil (GEO), Tween 20 and Span 80 were purchased from Sigma–Aldrich Chemicals Company and canola oil was acquired in a local market. Distilled water was used as solvent to prepare film-forming solutions and nanoemulsions, as well.

2.2. Nanoemulsions preparation

Nanoemulsions were prepared always with 5% (total weight) of emulsifiers (1% Tween 20 and 4% Span 80), based on preliminary results to achieved nanoemultions stability, and 10% (total weight) of lipid fraction attaining a constant lipid fraction (Wang et al., 2013). Canola oil was used to maintain the lipid fraction equal in all emulsions, only the concentration of essential oil changed. This fraction was constituted by canola oil and three concentrations of GEO: 1, 3 and 5%.

Thus, the primary emulsification was performed mixing the water with Tween 20 and mixing separately the canola oil with the essential oil and Span 80. To prepare 100 g of a nanoemulsion GEO 1% it was mixed 1 g of Tween 20 with 85 g of water, and 4 g of Span 80 with 1 g of GEO and 9 g of canola oil, for then mix both solutions. In the case of nanoemulsion GEO 3 and 5%, all quantities were the same except for GEO and canola oil that were of 3 and 7 g (3%), and 5 and 5 g (5%), respectively. Both dispersions were heated during 3 min at 50 °C and then mixed using a high speed homogenizer (ultra-turrax, Ika, model T25 basic, Staufen, Germany) at 24000 rpm/5 min in an ice/water bath to maintain the emulsion temperature. Nanoemultions were homogeneized during different times and rotations and 24000 rpm/5 min were selected based on mean particle size determined by the volume-surface mean diameter (D3.2), polidispersity, zeta potential (see 2.2.1) during a storage time of 14 days at 4 °C. Then, the emulsions were homogenized also using a Microfluidizer (M-110Y, Microfluidics Corporation, Westwood, MA, USA) at 1000Psi, by 3, 4 or 6 cycles to reduce the particle size, also operating in an ice/water bath. Nanoemulsions were analyzed and used immediately after their preparation.

2.2.1. Physical and rheological analyses of nanoemulsions

The nanoemulsions were characterized in terms of the mean particle diameter, zeta potential and rheological properties. The mean particle diameter of the samples was determined by photon correlation spectroscopy, using a ZetaPlus equipment (Brookhaven Instruments Company, Holtsville, NY, USA), and the zeta potential of the samples was obtained using the same equipment, through mobility electrophoretic assays at 25 °C (Flaker et al., 2015).

The rheological behavior of nanoemulsions was analyzed, in triplicate, at 25 °C, using a rotational rheometer (AR2000 Advanced Rheometer; TA Instruments, New Castle, DE, USA), with standard size double concentric cylinders (internal radius = 16.0 mm, external radius = 17.5 mm, height = 53 mm and gap = 2000 μm). Steady shear measurements were performed between 0 and 200 s^{-1} (Moraes, Carvalho, Bittante, Solorza-Feria, & Sobral, 2009; Jorge et al., 2014).

2.3. Gelatin-based film-forming suspensions with montmorillonite and ginger essential oil nanoemulsions

Film-forming suspensions (FFS) were prepared from a mixture of gelatin (5 g gelatin/100 g FFS), MMT (5 g MMT/100 g gelatin), nanoemulsion of GEO (2 g pure GEO/100 g gelatin) and glycerol (30 g glycerol/100 g gelatin). To allow comparison of effects, gelatin/MMT and gelatin/GEO-based FFS were also prepared as well as gelatin-based FFS without MMT nor GEO (as control).

The FFS were prepared in three parallel steps: 1) the gelatin was hydrated in distilled water for 30 min at room temperature, and then dissolved at 70 °C/30 min using a thermostatic bath (Marconi, model MA 179, Piracicaba, SP, Brazil); 2) the MMT was hydrated at room temperature in distilled water under high speed agitation using an ultraturrax (Ika, model T25 basic) at 20,000 rpm/min for 30 min and reserved during 24 h before used; 3) nanoemulsions were also prepared as described in Section 2.2. Then, the MMT dispersion, the nanoemulsion and the gelatin solution were mixed under mechanical agitation (Tecnal, model TE 039, Piracicaba, SP, Brazil) at 60 °C/2 min. In order to eliminate the air bubbles, the FFS was also ultrassonicated (Unique, model MaxiClean 1400, Indaiatuba, SP, Brazil) at 60 °C/10 min. Three replicates of each FFS were prepared.

2.3.1. Rheological studies in FFS

Rheological tests of FFS were carried out in the same rheometer described in the Section 2.2.1 using a cone and plate geometry (cone angle 2°, 60 mm diameter). All results were analyzed using the software Rheology Advantage Data Analysis V.5.3.1 (TA Instruments).

For the dynamic tests, samples were submitted to temperature scanning tests in the linear viscoelastic region, under constant frequency (1 Hz) and constant oscillatory stress (3 Pa), and changing the temperature between 5 and 50 °C by heating and cooling, always at a rate of 1 °C/min. The behavior of the storage (G') and loss (G") moduli as a function of temperature allowed to determine gelling (Tsol–gel) and melting (Tgel–sol) temperatures, calculated as the temperature where G' changed drastically as an inflexion point.

The flow curves were also determined for FFS as describe in the Section 2.2.1, but at a 35 °C to guarantee the domain sol to FFS. The measurements were performed in an extended shear rate range (0–200s^{-1}). In these cases, considering the non-Newtonian behavior of FFS, the rheological properties were calculated according to the Power's law model (Eq. (1)).

$$\sigma = K(\dot{\gamma})^n \quad (1)$$