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Characterization of chitosan–nanoclay bionanocomposite active films containing milk thistle extract

Sara Beigzadeh Ghelejlu, Mohsen Esmaiili*, Hadi Almasi

Department of Food Science and Technology, Faculty of Agricultural Engineering, Urmia University, Urmia, Iran

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ABSTRACT

Nowadays, bio-based and antioxidant active packaging is attracting significant attention as one of the preferred emerging technologies to prevent sensitive oxidation of foods. In this study, chitosan/nanoclay nanocomposite active films containing three different levels of sodium montmorillonite (MMT) (1, 3 and 5% w/w based on chitosan) and *Silybum marianum* L. extract (SME) (0.5, 1 and 1.5% v/v) were prepared. The obtained films were characterized in terms of structural, thermal, mechanical, and barrier properties as well as antioxidant behavior. X-ray diffraction patterns confirmed the exfoliated dispersion form of MMT nanolayers. Scanning electron microscopy images showed an increase in films' surface roughness by the addition of MMT. The results indicated that water vapor permeability and solubility of films reduced significantly ($p < 0.05$) by incorporation of MMT and SME. The mechanical and optical properties of films were significantly affected by the content of MMT and SME ($p < 0.05$). Antioxidant properties of the films also were improved by SME incorporation, suggesting that the formulated bionanocomposites could be considered as a promising antioxidant active packaging material.

1. Introduction

The growing demand for high-quality ready-to-eat food products with long shelf-life, along with increasing environmental awareness about substantial waste disposal problems, and decline in fossil fuel resources have encouraged the development of innovative bio-based packaging materials [1,2]. Furthermore, incorporation of biologically active substances such as antioxidants, antifungal agents, antimicrobials, and other nutrients into packaging materials provides extra bio-functional properties for biopolymer-based composites and consequently, increases their application potential in food packaging industry to improve food quality and extending shelf life [1,3].

Chitin, the second most abundant natural biopolymer after cellulose, and its deacetylated form, chitosan, offer a wide range of unique applications in food industry [4]. Chitosan has been considered as an applicable biopolymer-based packaging material, due to its polyelectrolytic nature plus distinguishing features, including biocompatibility, biodegradability, and solubility. In spite of that, low water resistance, poor mechanical, and barrier properties that are attributed to the hydrophilic nature of chitosan limit its usage in functional films [5,6]. Development of nanocomposite films is one of the effective approaches to remedy inherent shortcomings of biopolymer-based packaging materials [2,7]. Dispersion of nanometer-size particles, such as montmorillonite (MMT), in the polymeric matrix at a minimal loading level was reported to enhance thermal, mechanical, functional, and barrier properties of biopolymer-based composites while maintaining their biodegradability [8]. MMT is an abundant nontoxic natural layered silicate clay that has been considered to be a promising nanoreinforcement for chitosan films due to suitable chitosan–nanoclay interactions attributed to its high aspect ratio and large surface area [9]. Formation of intercalated or exfoliated structures, two desirable morphologies, resulting from nanoscale dispersion of nanoclay layers within the polymeric matrix brings about some substantial improvements in nanocomposites [10,11]. There are various reports on the effect of MMT on physical properties of chitosan based nanocomposites [11–13].

Natural biopolymer packaging holds the privilege of inducing innovative product developments, such as individual packaging of particular foods, carrier of plant-based bio-functional compounds and nutritional supplements [1]. Inasmuch as oxidation is one of the main factors affecting the shelf-life of food susceptible to lipid deterioration, incorporation of natural antioxidants into packag-

* Corresponding author at: Department of Food Science and Technology, Urmia University, P.O. Box 165, Urmia 57159-44931, Iran. Fax: +98 443 277 9558.
E-mail addresses: sarabeigzadeh@gamil.com (S. Beigzadeh Ghelejlu), esmaiilim@yahoo.ie, m.esmaiili@urmia.ac.ir (M. Esmaiili), Almasi1985@yahoo.com (H. Almasi).

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ing material can cause prolonged release of active compounds to operate more effectively in preserving quality, safety and sensory properties of food than adding high levels of additives directly into the food [14].

The milk thistle plant, *Silybum marianum* (L.) Gaertn. (Asteraceae) (SME) is an annual or biennial plant mostly known for its medicinal and antioxidant activity that has been among the most investigated plant extracts with known mechanism of action, and is introduced as potential new source of natural antioxidants to replace synthetic ones [15–17]. Silymarin, standardized extract from *S. marianum* contains flavonolignans along with mainly silibin and other phenolic compounds that have been marketed as dietary supplement in the United States without any side effects [18,19]. To the best of our knowledge, there are no reports on the fabrication of antioxidant active films by using SME.

The objectives of this study were to develop an active chitosan based nanocomposite film with satisfactory physical properties and antioxidant activity by incorporating MMT and SME, and to assess its application potentiality via evaluating physical, structural, and antioxidant properties of fabricated bionanocomposite.

2. Materials and methods

2.1. Materials

Chitosan powder of medium molecular weight (MW: 190–310 kDa) with a deacetylation degree of 75–85% was purchased from Sigma–Aldrich (Germany). An unmodified natural sodium montmorillonite (Cloisite Na^+) was provided by Iranian Nanomaterials Pioneers Company, NANOSANY (Mashhad, Iran). Water extract of aerial parts of *S. marianum* (SME) was purchased from Zardbant Pharmaceutical Co., Iran. All other reagents used were of analytical grade.

2.2. Preparation of nanocomposite films

Specific amount of chitosan powder was dissolved into 1% v/v acetic acid solution at 90 °C for 20 min in order to obtain 2% w/v aqueous chitosan solution using a magnetic stirring at 1250 rpm. Non-solubilized chitosan was removed using Whatman no. 3 filter paper and a vacuum pump. Then the solution was cooled to room temperature. Nanoclay (MMT) suspensions with 3 different concentrations (1, 3, and 5% w/w of chitosan) were prepared by dispersing appropriate amounts of MMT into 10 mL of 1% acetic acid solution following 10 min ultrasonic homogenization using probe sonicator (Bandelin UW 2200, Germany). Afterwards, 150 mL of chitosan solutions were added slowly into the pretreated nanoclay suspensions, and all were stirred for 4 h. As a plasticizer, glycerol at a level of 25% w/w of chitosan was added to the mixture, and was stirred at 40 °C for more than 30 min. In order to acquire proportional quantities of SME to the chitosan solution (0.5, 1 and 1.5% v/v), proper amounts of SME were added to the solutions, and homogenized at 20,000 rpm for 2 min by an Ultra-Turrax homogenizer (IKA T10 basic Ultra-Turrax, Staufen, Germany). After removal of bubbles by degassing under vacuum for 5 min, 30 mL of each mixture were cast evenly onto glass petri dishes (12 cm in diameter), and dried in an oven at 30 °C for 24 h.

Chitosan based nanocomposite films containing various amounts of MMT and mixtures of MMT and SME were coded as MMT and MMT/SME, respectively. Pure chitosan film without any nanoreinforcement and extract was used as a control sample. Dried films were then peeled off, and preconditioned at 25 °C and 50% RH for at least 48 h in a desiccator containing saturated magnesium nitrate solution before any evaluation.

2.3. Characterization of films

2.3.1. Film thickness

Thickness of films was measured at ten random positions of each sample using a manual digital micrometer (Mitutoyo, Japan) with an accuracy of 0.01 mm. The calculated mean values were used in calculations of water vapor permeability and mechanical properties.

2.3.2. X-ray diffraction (XRD)

XRD patterns characterizing the micro structure of pristine nanoclays, chitosan, and chitosan/clay nanocomposite films were obtained using a LabX XRD-6000 Shimadzu diffractometer, operated at 40 kV and 30 mA, equipped with CuKα radiation at a wavelength of 0.1546 nm and a curved graphite crystal monochromator [20]. Samples were scanned over the range of diffraction angle $2\theta = 1–12^\circ$, with a scanning rate of 1°/min at room temperature. Inter layer space or d-spacing of nanoclay particles was calculated by Bragg's law ($\lambda = 2d \sin\theta$) (1), where λ is the wavelength of the X-ray radiation used (0.1546 nm), d is the spacing between diffraction lattice planes, and θ is the measured angular position for each peak.

2.3.3. Scanning electron microscopy (SEM)

Surface morphology of films was examined using a Tescan Vega-3 scanning electron microscope under high vacuum condition and at an accelerating voltage of 20.0 and 10.0 kV. Pieces were cut from films, deposited onto aluminum specimen stubs, freeze dried, and coated with gold before examination.

2.3.4. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) measurements were performed with a SETARAM Instrumentation (Caluire, France). 10 mg of preconditioned samples (at 25 °C and 50% RH for 2 days) were sealed in a standard aluminum pans, and scanned through a temperature range of 30–300 °C at a heating rate of 10 °C/min under a nitrogen atmosphere. An empty aluminum pan was used as an inert reference and calibration was performed using an indium standard. The resulting first scan thermograms were used in order to determine glass transition temperature (T_g) and melting point (T_m).

2.3.5. Color measurement

Impact of SME and MMT on color parameters of films was determined by using a colorimeter (Minolta model CR-410, Japan). The colorimeter was standardized with a white calibration plate ($L^* = 93.49$, $a^* = 0.25$, $b^* = 0.09$), afterwards film specimens were placed on white plate, and color values (L^* (lightness), a^* (red/green), and b^* (yellow/blue)) were determined. Three readings at different sites of each film were recorded. The average values were used in calculation of total color difference (ΔE) and whiteness index (WI) according to the following equations [21]:

$$\Delta E = \sqrt{\left(L_i^* - L^*\right)^2 + \left(a_i^* - a^*\right)^2 + \left(b_i^* - b^*\right)^2} \quad (2)$$

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (3)$$

2.3.6. Mechanical properties

Mechanical properties of films including tensile strength (TS) (MPa), elongation at break (EB) (%), and elastic modulus (EM) (or Young's modulus) (MPa) were determined at 25 °C with a TA.XT Plus Texture Analyzer (Stable Microsystems, UK) according to ASTM standard method D882-10 (2010) [22]. Three dumbbell shaped test specimens with dimensions of 8cm × 0.5 cm were prepared from each of the film samples, and conditioned at 50% RH and