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Production and characterization of a new biodegradable fenugreek seed gum based active nanocomposite film reinforced with nanoclays

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ABSTRACT

In the present work, fenugreek seed gum (FSG)/clay nanocomposite films were prepared with nanoclays (Na^+ montmorillonite [MMT], halloysite [HNT] and Nanomer® I.44 P [NM]) at different amounts (0, 2.5, 5.0 and 7.5 g clay/100 g FSG) by solution casting method and characterized. Increasing amount of nanoclay significantly ($P < 0.05$) improved oxygen barrier and thermal properties of the biodegradable films. Agar diffusion tests revealed that FSG based nanocomposite films exhibited strong antimicrobial properties against foodborne pathogens namely *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Bacillus cereus* independently of clay type and concentration. In the case of mechanical properties, nanoclay incorporation up to 5% provided higher ($P < 0.05$) tensile strength (TS) properties while elongation at break (EB) values of the films significantly ($P < 0.05$) decreased in the presence of clay in the film matrix. SEM micrographs showed that especially lower levels (up to 5%) of nanoclay reinforcements provided a homogeneous and smooth film structure. In conclusion, FSG based nanocomposite films reinforced with nanoclays up to 5% showed a precious potential to be used in antimicrobial food packaging applications.

1. Introduction

Tremendous increase in the use of petroleum based plastics have caused serious problems due to their non-degradability for long periods in the environment. As a result of growing demand for decreasing their use in food packaging, recent researches have been focused on use of biodegradable alternatives for non-degradable plastics. Biodegradable materials are known as environmentally friendly, nontoxic and are made with low energy consumption [1]. A number of biodegradable natural and synthetic polymers such as chitosan, gelatin, starch, poly(lactic acid), polyesters and/or their blends have been investigated in the literature for food packaging applications [2–7].

In general, carbohydrate and protein based packaging films have favorable oxygen permeability values at low-medium relative humidity levels. On the other hand, their resistance to water vapor is generally poor because of their hydrophilic character [8]. Brittleness and poor mechanical properties are other drawbacks of biodegradable polymers. Therefore, their use in food packaging industry is limited [9]. Nanotechnology has been suggested to improve characteristics of the biodegradable polymers taking into consideration the cost effectiveness [10]. It has been demonstrated by a number of studies that addition of nano-scale fillers into the matrix of biodegradable polymers enhanced their mechanical and barrier properties. In order to success such developments in the packaging materials, uniform dispersion of nanofillers in the polymer matrix is required. Nanoclays have drawn great attention with their high-aspect ratio and high surface area. Preparation of packaging materials using polymer-clay nanocomposites is a cost effective alternative of conventional packaging industry. Montmorillonite (MMT), that is a alumina-silica layered clay, is the most studied type of clay in nanocomposite based packaging materials while studies with halloysite (HNT) that is a two-layered clay with tubular structure as nanofiller for packaging materials are relatively limited [11–14]. Surface of interlayer galleries of layered silicates are covered with interchangeable Ca^{+2} and Na^{+2} cations, giving them hydrophilic behavior and non-compatibility with hydrophobic polymers. Organophilization is given name of the method that is carried out to enable biocompatibility with polymers by expanding the galleries and exchanging the surface cations with various cations [15]. There are many organically modified nanoclays commercially available to be used as polymer nanofillers.

Galactomannans are the main storage polysaccharide molecules present in many leguminous seeds. Seeds of fenugreek (*Trigonella*

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foenum-graecum) that is widely grown in the Mediterranean region are known to have high levels of galactomannans, comprising a linear mannose chain linked by β-(1,4) glycosidic bonds [16,17]. Studies have shown that fenugreek seed gum (FSG) is able to form emulsions better and more stable than those of 11 commercial gums tested [18]. To the best of our knowledge, FSG has not been tested for its film forming properties for food packaging applications. In this study, it was aimed to prepare and characterize FSG based nanocomposite films as affected by reinforcement with different types of nanoclays.

2. Materials and methods

2.1. Materials

Fenugreek (*Trigonella foenum-graecum*) seed was obtained from Doğan Baharat Ltd. (Istanbul, Turkey). Nanoclays named Na^+ montmorillonite (MMT, surface area 250 m^2/g), halloysite, (HNT, surface area 64 m^2/g, 30–70 nm × 1–3 μm nanotube) and Nanomer® I.44 P (NM) were purchased from Sigma (Germany). Glycerol was supplied from Merck (Germany).

2.2. Preparation of FSG based nanocomposite films

Following sequential procedures were performed to obtain the film forming solutions. Firstly, aqueous FSG solution was prepared by addition of 1.5% (w:v) FSG into distilled water containing 1.5% (w:w) of glycerol and the resulting solution was mixed at 65 °C for 3 h at 1.000 rpm using a magnetic stirrer. The amount of FSG and glycerol used in the solution was selected based on the results of a series of preliminary studies. Then the nanoclay (2.5%, 5% of 7.5% w:w, based on the FSG percent) was slowly added into the mixture and the further mixing was performed for 3 h at the same conditions. The film forming solution was degassed under vacuum for 5 min in order to eliminate the bubbles. Nanocomposite films were prepared by pouring the film forming solutions onto Teflon plates and by drying at 45 °C for 24 h. Dried film samples were stored in desiccators containing saturated calcium nitrate until analyses.

2.3. Characterization of FSG based nanocomposite films

2.3.1. Moisture content

Moisture contents of the FSG based nanocomposite films were determined gravimetrically. For this aim, film sample (2–3 g) was dried at 105 °C for 24 h in a conventional oven and percent moisture was calculated [19].

2.3.2. Film thickness

Thickness of the FSG based nanocomposite films was measured using a digital micrometer (Digimatic Indicator, Mitutoyo Corporation, Japan). At least 5 measurements were done from different points of the films randomly and the average values were calculated.

2.3.3. Color

L^*, a^* and b^* values of the nanocomposite films were measured using a chromameter (CR-400 Konica Minolta Sensing, Inc., Osaka, Japan). Following the calibration of the tool, the film samples were placed on the white standard plate (L^* = 93.49, a^* = 0.25, b^* = 0.09) and the measurement was performed. At least five measurements were done from different points of the film [20].

2.3.4. Antimicrobial activity

Disc diffusion method was used for determination of antimicrobial activity of the films against 4 foodborne pathogenic bacteria namely *Escherichia coli* O157:H7 ATCC 33150, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19118 and *Bacillus cereus* FMC19. Prior to analyses, cryopreserved strains were activated in Nutrient Broth (Merck, Germany) at 37 °C for 24 h twice. The films which were cut into square pieces (1 × 1 cm^2) were placed on petri plates containing Nutrient Agar (Merck, Germany) that were previously seeded with 1% (v:v) of the broth culture of the activated strains. Then the petri plates were incubated at 37 °C for 24 h and the clear (inhibition) zones formed around the films were measured with a caliper [21].

2.3.5. Mechanical properties

Mechanical properties of the nanoclay reinforced nanocomposite films produced with FSG was determined according to ASTM D882 standard method [22] using a texture analyzer (TA.XT Plus Stable Micro Systems, Surrey, UK). Firstly, film samples were cut in rectangular shape (1 × 14 cm) and conditioned at 43% relative humidity for 72 h. Initial grip distance and strain rate were set to 10 cm and 1.25 cm/min, respectively. Tensile strength (TS, MPa) and elongation at break (EB, %) were calculated by the software. A minimum of 5 specimens was analyzed for each film.

2.3.6. Barrier properties

Water vapor permeability (WVP) of the nanocomposite films were determined based on the standard method described by ASTM [23]. Glass cup containing silica gel was heated at 105 °C for 24 h before analysis in order to remove the possible damp and covered with the FSG based nanocomposite film samples. Then the covered cups were kept in desiccators containing distilled water at 25 °C for 24 h. The weight of the cups were measured during the storage at 0th, 5th, 15th, 20th and 24th hour. WVP of each film was calculated using the following equation:

$$WVP = \frac{w}{t}\chi\frac{x}{\Delta P\, x\, A} \quad (1)$$

where x, ΔP and A are mean film thickness (μm), relative pressure difference (kPa) at 25 °C and film area, respectively while w/t was calculated by determination of the absorbed water by the system at the steady state using the linear regression.

Oxygen transmission rate (OTR) of the FSG based nanocomposite films was measured by determination of peroxide value of antioxidant-free sunflower oil (15 mL) present in a conical flask (25 mL) covered with the film samples based on the method of Kurt and Kahyaoglu [7] with slight modifications. Following the storage of the oil containing flasks at 60 °C for 9 days, peroxide value of the oil samples were determined by sodium thiosulfate titration.

2.3.7. Differential scanning calorimetry (DSC)

In order to determine the thermal properties of the FSG based nanocomposite films, DSC analysis was performed using a DSC tool (DSC Q20, TA Instruments, Inc., USA). A 5–10 mg of the film sample that was placed in aluminum pan was heated from −100 °C to 300 °C at a heating rate of 10 °C/min. Melting point (T_m) and melting enthalpy (ΔH) were determined from the DSC thermogram. An empty aluminum pan was used as reference [24].

2.3.8. Fourier transform infrared (FTIR) spectroscopy

Molecular characterization of the film samples was carried out using a FTIR spectrophotometer (Bruker Tensor 27, Bremen, Germany) at room temperature. The measurements were performed in the range of 4000–400 cm^{-1} wavelength.

2.3.9. Scanning electron microscopy (SEM)

Morphological properties and distribution of the nanoclays in the polymer matrix of the nanocomposite films were determined by field-emission SEM (FE-SEM Quanta FEG 250, FEI, USA) equipped