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Green bionanocomposite based on kefiran and cellulose nanocrystals produced from beer industrial residues

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ABSTRACT

Environmental concern about synthetic polymers and nanoparticles bring about development of the green bionanocomposite. Nanocellulose (NC) as safe nanofiller was prepared from beer industrial residues by acid hydrolysis in this study. ATR–FTIR spectrum showed no change in chemical structure of kefiran and NC after mixing. However, mechanical, visual, and WVP properties of kefiran/NC films improved with NC, but thermal properties and water sensitivity of them declined, simultaneously.

1. Introduction

Environmental concerns, finitude of petroleum resources, and increasing the oil price during the recent years have been three main reasons for paying considerable attention to biodegradable polymers from renewable resources. Hence, now there is an increasingly growing attention to biodegradable polymer as a gas and moisture barrier to increasing foods shelf-life. Although biodegradable polymers have several advantages, there are some restrictions in their application, e.g. low mechanical and weak moisture barrier properties [1,2].

Kefiran, as a microbial polysaccharide, produced during kefir production, has attracted much attention as an interesting biopolymer over the recent years [1–5]. This biopolymer is extracted from kefir grain as a byproduct of kefir production process. Kefir has functional properties such as antimutagenic and antimicrobial activities that can be affected by kefiran [6]. Previous researches showed that the mechanical properties and the water vapor permeability (WVP) of kefiran film were comparable with some synthetic polymers [3], but our results showed that kefiran had considerable mechanical and visual properties. However, the WVP of kefiran is not as good as synthetic polymers; consequently, it needs some modifications [2,7].

Different nanoparticles have been mixed with biopolymers to give them more strength and barrier properties [1,2,8,9], but there are some concerns about ecological pollution and toxic properties of nanoparticles [10]. In contrast, green nanoparticles like nanocellulose (NC) are safe and biodegradable reinforcing agents [11,12].

Acid hydrolysis is the most frequently used method to prepare cellulose nanostructure in which rod-like cellulose nanocrystals are isolated [13]. If the hydrolysis intensity is high enough, the aspect ratio of cellulose nanowhiskers decreases to less than two [12]. Such produced cellulose nanocrystals are commonly named spherical cellulose nanocrystals, which were prepared in our previous study using beer industrial residues (BIR) as raw material [13]. The cellulose nanocrystal is hereafter called nanocellulose (NC).

The main scope of the current research is to reinforce kefiran biopolymer by produced NC using BIR. Moreover, the physical, thermal, mechanical and chemical properties of kefiran/NC nanocomposite are characterized and discussed.

2. Materials and methods

2.1. Material

BIR was collected from Behnoosh malt industrial Co., Iran. The analytical grade chemicals, including sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium chloride ($CaCl_2$), sodium

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chloride (NaCl), acetic acid (CH_3COOH), and hydrochloric acid (HCl) were purchased from Dr. Mojallai Co., Iran. Sodium chlorite ($NaClO_2$) was also provided from Fluka Chemical Co., Germany.

2.2. Preparation of NC

NC was prepared according to our previous study [13]. BIR (1:10) was boiled for 1 h and dried at 105 °C. The dried BIR (1:10) was then soaked in NaOH (2%) overnight. The soaked BIR was washed with water and then was treated by 12% NaOH at 121 °C for 45 min (three times) to produce BIR pulp. The BIR pulp was washed and subsequently dried at 105 °C. To remove residual lignin, the pulp slurry (1:20) was treated twice by a solution containing 3% $NaClO_2$ and 1.5% CH_3COOH at 75 °C for 1 h. To remove hemicelluloses and residual starch, the treated pulp was soaked in 3% KOH solution overnight, followed by treating at 80 °C for 1 h. The pulp was further bleached using 3% $NaClO_2$ and 1.5% CH_3COOH at 75 °C for 1 h. Finally, the purified cellulose of BIR was dried at 105 °C [13].

The slurry of purified BIR cellulose (1:10) was hydrolyzed by 10% HCl at 80 °C for 4 h. The hydrolyzed cellulose was washed 3 times by centrifugation (Mikro 200, Hettich, Germany) at 6000 rpm, 4 °C for 30 min. Next, for removing residual acid and neutralizing pH, dialysis tube (Sigma Aldrich, Germany) soaked in distilled water was used. To disperse coagulated neutralized cellulose particles, ultrasonic treatment (Hielscher UP200S, Germany) for 15 min resulted in NC. The average diameter of NC was 97 ± 18 nm [13].

2.3. Kefiran purification

Kefir grains were dissolved in boiling water (1:10) for 30 min. After the first centrifugation at 10,000 × *g* for 30 min, all of the undissolved portions of grain were almost decanted. Then, polysaccharides were precipitated by mixing with chilled ethanol (1:1) at −18 °C overnight. Purification procedure was followed by centrifuging at 10,000 × *g* for 30 min at 4 °C and three times washing with water for removing water solvable impurities [2]. The white precipitated polysaccharide is hereafter called kefiran. The total polysaccharide content of kefiran was 92% according to the phenol sulfuric method [14].

2.4. Film preparation

The aqueous solution of 2 wt% kefiran was prepared and 40 wt% (dry base) glycerol as plasticizer was added to it. On the other hand, NC was dispersed in distilled water by sonication for 30 min at room temperature. Different concentrations of NC dispersion (1, 2 and 3 wt% (dry base)) were added to the aqueous solution of kefiran and mixed for 10 min. After degassing, the film forming solutions were casted by pouring the mixture onto Teflon plates and dried at 25 °C in oven and room relative humidity. The dried films were peeled off the casting surface. All the film specimens were conditioned inside desiccators containing saturated calcium nitrite (Merck Co., Germany) solution to ensure a relative humidity of 55% at 25 ± 1 °C for 48 h.

2.5. Microstructure

Microstructural analysis of the cross-sections and surface area of the dried films was conducted by SEM (CamScan MV2300, Canada). The films specimens were sputtered with gold using a KYKY-SBC-12 sputter coater (KYKY, China). All cross-sections and surface area specimens were examined using an accelerating voltage of 19.0 kV.

An atomic-force microscope (AFM, DualscopeC26, DME, Denmark) was used to take micrographs of the NC. The specimen was placed onto freshly cleaved mica and left to dry at room temperature prior to scanning.

2.6. Measurements

2.6.1. Thickness

The thickness of specimens was measured by a hand-held micrometer with an accuracy of 0.01 mm at 13 random positions for each film.

2.6.2. Moisture content

The moisture content (MC) of the specimens was determined (three replicates) by measuring the weight loss of films before and after drying in a laboratory oven (Shimaz Co., Iran) at 105 ± 1 °C until constant weight.

2.6.3. Moisture absorption

Moisture absorption (MA) was measured according to the method of Almasi et al. [8]. In brief, the dried sheets of 20 × 20 mm^2 were first conditioned at 0% RH (prepared by dried calcium sulphate) for 24 h. After weighing, they were conditioned in a desiccator containing saturated calcium nitrite solution at 25 °C to ensure a relative humidity of 55%. The specimen was weighed at desired intervals until the equilibrium state was reached. The moisture absorption of the specimen was calculated with Eq. (1):

$$\mathrm{MA} = \frac{w_e - w_0}{w_0} \times 100 \quad (1)$$

where W_e and W_0 are the weights of specimen after equilibration at 55% RH and the initial weight of the specimen, respectively. All measurements were performed in three replicates.

2.6.4. Solubility

Solubility in water (SW) was defined as the ratio of the water-soluble dry matter of film that is dissolved after immersion in distilled water [4]. A 20 × 20 mm^2 specimen was cut from each film, dried at 105 ± 1 °C to constant weight in a laboratory oven (Shimaz, Iran), and weighed to determine the initial dry weight (m_1). The solubility in water of the specimen was measured from immersion assays in 50 ml of distilled water with periodic stirring for 6 h at 25 °C. After that period, the remaining pieces of films were taken out and dried at 105 ± 1 °C until constant weight (m_2). SW of the specimens were calculated using Eq. (2):

$$\mathrm{SW} = \frac{m_1 - m_2}{m_1} \times 100 \quad (2)$$

2.6.5. Color

Film color was determined using a colorimeter (Labscan XE, Hunterlab, USA). Film specimens were placed on a white standard plate and the lightness (L) and chromaticity parameters a (red–green) and b (yellow–blue) were measured. All the colors can be explained by L values ranging from 0 (black) to 100 (white); minus values of a (greenness) to its positive value (redness); and minus values of b (blueness) to its positive value (yellowness). All measurements were performed in 5 replicates. Total color difference (ΔE) and whiteness index (WI) were calculated using Eqs. (3) and (4) [4]:

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

where L^*, a^*, and $b^*$are the color parameter values of standard ($L^* = 93.7$, $a^* = -1.13$ and $b^* = 1.24$) and L, a, and b are the color parameter values of the specimen:

$$\mathrm{WI} = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (4)$$