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Influence of the acid type in the production of chitosan films reinforced with bacterial nanocellulose

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ABSTRACT

Chitosan films reinforced with bacterial cellulose (BC) nanoribbons were studied to understand the influence of acid (acetic and lactic acids) on the reinforcing effect. For both acids, the maximum concentration of the reinforcing constituent was 5 wt% with respect to the dry weight of chitosan. The infrared spectra, mechanical properties, morphology and antimicrobial activity of the films were analyzed. The results showed a difference between the acids in their behavior and effect on the reinforcement, with a tensile strength of 12.3 MPa for the acetic acid films and 3.3 MPa for the lactic acid films. Additionally, the bacterial inhibition tests were shown to be positive for the lactic acid films and negative for the acetic acid films. Therefore, exchanging the acid used in these films may be desirable for certain applications.
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1. Introduction

Chitosan is a linear copolymer composed of β-(1-4) linked 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose. It is produced by deacetylation of chitin (β-(1-4) 2-acetamido-2-deoxy-β-D-glucopyranose) [1], which is extracted from crustacean shells, some insects and fungi. Chitosan exhibits some interesting properties, such as film-forming capacity [2,3], antimicrobial activity [4,5] and good barrier properties [6,7]. The possibility of the application of chitosan as a food coating has also been discussed [8,9], making it an ideal candidate for the reduction of environmental and health problems often caused by synthetic polymers used in the food industry [10,11].

Chitosan is soluble in dilute organic acid solutions, typically acetic acid [1–4,7]; however, lactic acid has also been employed in certain applications, such as food packaging [8]. From chitosan solutions, it is possible to form hydrogels [12], films [13] and coatings [9]. The effect of the acid concentration or type on the non-reinforced film properties has been described by some authors, in which the bioadhesion [14], mechanical properties [15] or oxygen and water vapor permeability [16] of the films were analyzed.

Some reinforcements, namely polylactic acid (PLA) and cellulose, were added to the films to improve their mechanical and/or chemical properties [2,7,17]. Cellulose is the most abundant polymer in nature, and its chemical structure resembles that of chitosan in that it is composed of β-(1-4)-D-glucopyranose. This polymer is also an effective option because of its mechanical properties, chemical structure [18] and reinforcing effect [19]. Moreover, cellulose can be obtained from agricultural wastes, such as banana rachis [20], or by fermentation by bacteria of the *Gluconacetobacter* genus [21]. Bacterial cellulose (BC) may be of interest as a reinforcement of the chitosan matrix because it occurs in the form of a pellicle made of highly pure ribbon-shaped cellulose nanofibrils devoid of the hemicelluloses and lignin [18], which are common in lignocellulosic materials. Thus, BC nanoribbons may be a suitable material for reinforcement of the chitosan matrix.

To our knowledge, the effect of the acid type on a reinforced chitosan matrix has not been previously reported in the literature. The interaction between the reinforcement and the solvent is unknown; therefore, the behavior of the films remains to be studied when a simultaneous change of solvent and reinforcement concentration occurs.

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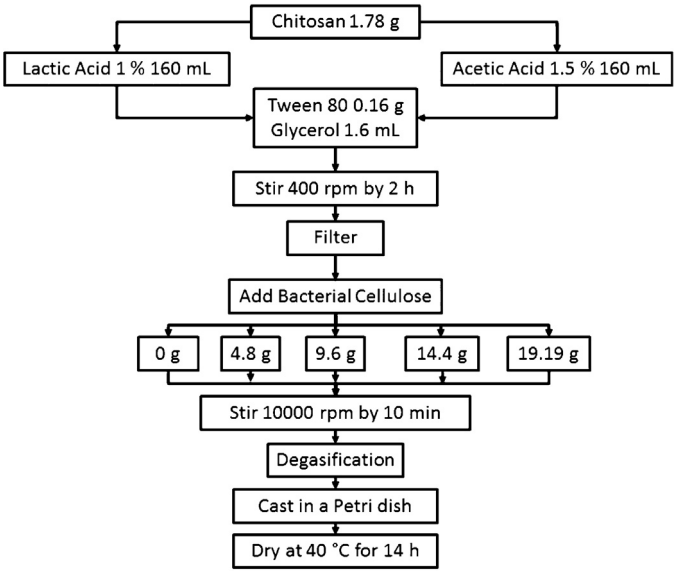

Fig. 1. Flowchart of the film-forming process.

This study analyzes the influence of acetic and lactic acids on BC nanoribbon-reinforced chitosan matrices, which induced changes in the chemical interaction, morphology and mechanical and antimicrobial properties of the films. This study helps to explain the polymer interaction with the solvent and the reinforcement, which is the first stage in the possible development of new materials for the food packaging industry.

2. Materials and methods

2.1. Materials

Chitosan was provided by Colorquimica S.A., located in La Estrella, Colombia. Its deacetylation degree was 82% according to the procedure defined by Shigemasa et al. [22] and its molecular weight was 692 kDa, which was calculated by a viscosimetric method described by Martinez-Camacho et al. [23].

BC nanoribbons were produced using the novel *Gluconacetobacter medellinensis* bacterium [24], which was cultured according to the procedure described by Castro et al. [21]. Acetic and lactic acids were purchased at an analytical grade. Tween 80 and glycerol (99 wt% purity) were obtained from a Protokimica S.A., in Medellín, Colombia.

2.2. Methods

2.2.1. Preparation of reinforced and non-reinforced chitosan films

Chitosan films, matrices and reinforced films were formed following a modification of the procedure defined by Fernandes et al. [2], shown in the flowchart in Fig. 1, by dissolving the biopolymer in lactic or acetic acid at concentrations of 1% (v/v) and 1.5% (v/v), respectively. The solutions were stirred and filtered to remove any impurities.

The solutions were reinforced with BC nanoribbons at concentrations of 1.25, 2.5, 3.75 or 5 wt% with respect to the dry weight of the chitosan. The solutions were subsequently homogenized using an Ultra-turrax unit for 10 min at 10,000 rpm. Finally, the film and matrix solutions were degassed in a vacuum system and casted in Petri dishes to be oven dried and prepared for further characterization.

2.2.2. Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR)

Infrared spectroscopy experiments were performed according to the method described by Zuluaga et al. [20] using an FTIR spectrometer (Nicolet 6700 Series) equipped with a single-reflection ATR and a type IIA diamond crystal mounted in tungsten carbide. The diamond ATR had a sampling area of approximately 0.5 mm^2, where a consistent reproducible pressure was applied to every sample. The infrared spectra were collected at 4 cm^{-1} resolution over 128 scans.

2.2.3. Mechanical properties

The tensile strength and Young's modulus of the samples were measured on an Instron universal Testing Machine (with a 50 N load cell and pneumatic side-action grips) at room temperature with a gauge length of 19 mm and a tensile rate of 25 mm/min, according to ASTM D882 [25]. The specimen dimensions were 80 mm in length and 10 mm in width.

Prior to the test, the samples were conditioned in a controlled chamber with a relative humidity of 50% and a constant temperature of 25 °C.

2.2.4. Transmission electron microscopy (TEM)

Transmission electron microscopy of the BC nanoribbons was performed according to the procedure described by Castro et al. [21]. The nanoribbons were homogenized in a Waring blender, diluted in distilled water and sonicated to achieve good dispersion. Drops of the suspensions were deposited onto glow-discharged, carbon-coated TEM grids and then negatively stained with 2 wt% uranyl acetate. The samples were observed using a Philips CM200 microscope operating at 80 kV. The images were recorded on Kodak SO163 films.

2.2.5. Scanning electron microscopy (SEM)

SEM was used to observe the fracture of the films as well as the dispersion of the BC nanoribbons. After the mechanical tests, fractured samples were coated with gold/palladium using an ion sputter coater and observed using a Jeol JSM 5910 LV microscope operating at 10 kV, as described by Zuluaga et al. [20].

2.2.6. Antimicrobial activity

Staphylococcus aureus is a gram-positive bacterium; present on the human skin, mucous membrane or food. It is estimated that in the United States alone, *S. aureus* was the source of 1.3 million infections and 390,000 hospital admissions per year [26], this microorganism is resistance to some antibiotics used in the food industry [27]. For these reasons it was considered important to assess the antimicrobial activity of the chitosan films against it.

Antimicrobial activity was assessed according to a modification of the method developed by Beverlya et al. [8]. Samples were cut into 2 cm × 3 cm rectangular strips, placed in 15 mL of nutritive agar, which had previously been inoculated with a solution of *Staphylococcus aureus* NCTC 8325, and kept at 37 °C for 24 h. The inhibition radii were then measured for each sample.

3. Results and discussion

The FTIR spectra of the films formed with acetic acid show a peak between 3600 and 3000 cm^{-1}, which is characteristic of the overlap between the OH and NH_2 absorbances (Fig. 2a). Moreover, broadening associated with the polymer–water, water–plasticizer and plasticizer–polymer interactions can be observed in the spectra [23].

Other peaks appearing between 2000 and 700 cm^{-1}, marked by the gray lines in Fig. 2a and detailed in Fig. 2c, can be attributed to the stretching bands of CH_2, the CH_3 in-phase deformation and

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