Mechanical and Thermal Behavior of Canola Protein Isolate Films As Improved by Cellulose Nanocrystals

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ABSTRACT: The effects of cellulose nanocrystals (CNCs) (12, 24, and 36% w/w) on the microstructure and mechanical and thermal properties of canola protein isolate films were evaluated. The incorporation of cellulose nanocrystals led to homogeneous films, and new Fourier transform infrared peaks appeared at 1055 cm⁻¹, indicating the presence and the interaction of CNCs with proteins and glycerol. The addition of CNCs also improved the thermal stability of the films, since higher temperatures were required for their thermal decomposition. In addition, CNC addition resulted in an increase in tensile strength and a decrease in elongation at break values due to strong interactions between the OH groups in proteins, glycerol, and CNCs.

INTRODUCTION

Synthetic plastic materials have slow degradation rates and are produced from nonrenewable, petroleum-based resources. One alternative to synthetic materials is bio-based polymers. These are derived from renewable natural resources and degrade faster than petroleum-based ones. Consequently, bio-based polymers can be incorporated into the soil and act as carbon sources for plant growth.¹,² Another advantage of bio-based polymers is their widespread availability, since they can be derived from nonedible byproducts of domestic and food industries.

Proteins are widely available bio-based polymers with potential applications in the food packaging industry.³ Many proteins can be used to form films.⁴ One of them is canola protein, which is obtained from the cake obtained after pressing canola seeds (Brassica napus) L. for oil extraction and is considered a byproduct of the canola oil industry. The proteins can be in the form of canola protein concentrate and canola protein isolates (CPIs).⁵,⁶,⁷

Although canola is the second most cultivated oilseed in the world, there have been few studies examining its potential as an alternative to synthetic plastic materials.⁸ Protein films have several disadvantages compared to synthetic ones. They have weak mechanical properties and poor barrier properties at high relative humidity.⁴,⁸ One method to improve the film properties involves the addition of cellulose nanocrystals (CNCs). CNCs are crystalline particles up to several micrometers in length with diameters between 1 and 100 nm. These nanoparticles can be obtained from cellulose, which is the most abundant plant material on earth.⁹,¹⁰ CNCs are stabilized by numerous hydrogen bridges, which allows them to be highly ordered and crystalline, resulting in improved mechanical properties of bio-based polymer films.¹¹,¹² In addition, CNCs are able to improve the thermal stability of materials due to their more thermally stable crystalline structure.¹³

The objective of this work was to evaluate the effects of CNC addition on the mechanical and thermal behavior of CPIs films. Prior to preparation of the films, the stability of CNC suspensions and their relationship with the obtained microstructures were also determined.

RESULTS AND DISCUSSION

Zeta-Potential (ζP), Particle Size (Z-Average) and Particle Size Distribution (PDI) of the Protein Suspensions. From Table 1, it can be observed that protein suspensions were highly stable (ζP < 39 mV) and were able

| Film | ζP (mV) | Z-average (nm) | PDI |
|------|--------|----------------|-----|
| CNC-0.0 | −39.0 | 184.0 | 0.818 |
| CNC-12 | −40.1 | 228.4 | 0.520 |
| CNC-24 | −41.6 | 205.5 | 0.510 |
| CNC-36 | −43.1 | 216.9 | 0.483 |

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to remain dispersed in aqueous medium.\textsuperscript{14} Also, the addition of CNCs further improved the stability of the suspensions, with samples containing higher CNC concentrations having higher $\zeta$ values. Since pH is the most influential factor in the stability of aqueous dispersions, the results could be explained in terms of the pH at which the suspensions were prepared (9.5). This pH was far from the isoelectric point of the proteins (pH 4.0–4.5) and allowed them to acquire electrical charges to remain dispersed in the suspension.\textsuperscript{15} On the other hand, CNC particles are negatively charged due to the presence of sulfate groups. This facilitates their dispersion in aqueous media and improves the stability of protein suspensions.

Regarding the particle size, in the suspension of the control film (CNC-0.0), an average size of 184 nm was observed, while this value exceeded 200 nm in the protein suspensions with CNCs. Likewise, the addition of CNCs caused canola protein suspensions to move from a polydisperse to near monodisperse behavior, which means that CNCs created interactions with the functional groups of proteins and glycerol, increasing and uniformizing the average size of its molecules and making the sizes more uniform. According to Agrawal and Patel, 2011,\textsuperscript{14} the PDI of a suspension is related to the stability of the suspension, with samples having PDI values closer to 0 being more stable over time.

Microstructure of Protein Films. The colloidal stability of CPI suspensions with CNCs resulted in films with a homogeneous distribution of CNC particles (Figure 1). The control film without CNCs (Figure 1A-B) had a homogeneous surface without cracks, similar to those reported for other protein films.\textsuperscript{16,17} Ogale et al., 2000,\textsuperscript{17} reported a smooth and soft microstructure for cross-sectional views in soy protein isolate films, while the valleys and ridges were related to a strong and ductile material.

From Table 2, the stability of protein suspensions led to the development of homogeneous films with no agglomerated particles or structural defects such as pores and cracks. This indicated good compatibility between the different components in the film (CPI, glycerol, and CNC), which agrees with other studies of bio-based polymer films reinforced with CNCs.\textsuperscript{12,18,19} Also, as the CNC content increased, more irregular cross-sections (Figure 1E) were observed, which suggested that films with added CNCs were stabilized by strong interactions between the various functional groups in proteins, glycerol, and CNCs, and therefore, more compact and tougher films were obtained.

Fourier Transform Infrared (FTIR) Spectroscopy Analysis of the CPI Films. Figure 2A shows the FTIR spectra of all of the films. Canola protein films with added CNCs showed five main peaks. The vibrations of the first peak at 3274 cm$^{-1}$ were characteristics of the N–H group in proteins. The second peak at 2920 cm$^{-1}$ corresponded to the stretching of the C–H group. The third (1648 cm$^{-1}$), fourth (1537 cm$^{-1}$), and fifth peaks (1233 cm$^{-1}$) were related to C=O (amide I), N–H (amide II), and C–N (amide III) groups in proteins, respectively.\textsuperscript{20,21} In addition, all films had a peak at about 1039 cm$^{-1}$ that corresponded to the vibrations of the C=O group in carbon number 3 of the glycerol molecule.\textsuperscript{22} For this peak, the films containing CNCs were wider and higher than that of the control film (CNC-0.0). In fact, this peak contained overlapped peaks and these were deconvoluted by a Lorentzian line function (Figure 2B) to observe their corresponding FTIR vibrations.

Two peaks were observed in the deconvoluted FTIR spectra. The first one at 1033 cm$^{-1}$ corresponded to the glycerol molecule present in all of the films. The second peak at 1055 cm$^{-1}$ was due to vibrations of the C−O–C group in the glucopyranose molecule present in the CNC.\textsuperscript{23,24} The displacement of the glycerol peak toward lower wavenumbers in the films with CNCs indicated some intermolecular interactions between OH groups in CNCs, OH groups in glycerol, and the functional groups in the protein.\textsuperscript{25,26} In addition, films containing higher CNC concentrations had higher peaks at 1055 cm$^{-1}$, indicating greater distances of interactions between CNCs and the protein matrix.

Table 2. Thermogravimetric Profile of CPI Films\textsuperscript{a}

| Film      | $T_{max1}$ (°C) | $T_{max2}$ (°C) | $T_{max3}$ (°C) | Res$_{600}$ (%) |
|-----------|-----------------|-----------------|-----------------|----------------|
| CNC-0.0   | 76.46           | 205.01          | 319.37          | 17.53          |
| CNC-12    | 76.47           | 206.54          | 319.43          | 20.57          |
| CNC-24    | 76.51           | 209.78          | 329.15          | 20.72          |
| CNC-36    | 76.59           | 214.01          | 332.63          | 22.34          |

\textsuperscript{a}$T_{max1−3}$ = maximum temperature at each stage of decomposition; Res$_{600}$ = residual decomposition at 600 °C.

Figure 1. Scanning electron microscopy (SEM) micrographs of representative CPI films ± CNC. (A, B) CNC-0.0 film; (C–E) CNC-36 film. (B) and (E) are cross-sectional views of CNC-0.0 and CNC-36 films, respectively.
Thermal Stability of CPI Films. Figure 3 and Table 2 show the thermogravimetric curves (TGA) results of the films with CNC addition. All of the films showed three stages of weight loss (Figure 3A). The first stage occurred between 50 and 125 °C, which corresponded to the evaporation of water from the films. The second stage occurred at 125−270 °C, and this was due to the evaporation of glycerol. Finally, the third stage occurred between 270 and 500 °C, which corresponded to the degradation of proteins and CNCs. For the control film (CNC-0.0), the second stage was longer and began at a lower temperature than those in the films containing CNCs (Figure 3A,B). This indicated that interactions in the control film were weaker than those in films containing CNCs and required a lower temperature to start decomposition.

Figure 3B shows the first derivative of the mass loss curves and presents the thermal stability of the films more clearly. The maximum decomposition temperatures in the derivative curves are summarized in Table 3. Films containing higher CNC concentrations had higher maximum decomposition temperatures ($T_{\text{max}1}$ and $T_{\text{max}3}$), indicating improved thermal stability. Li et al., 2017, found similar thermal behavior in soy protein isolate films plasticized with glycerol and containing microcrystalline cellulose (MCL). They attributed their results to the numerous hydrogen bonds between MCL and the protein molecules.

The CNC-36 film had the highest maximum decomposition temperatures ($T_{\text{max}2}$ and $T_{\text{max}3}$) and residual content at the end of combustion. This was due to the greater number of interactions between CNCs and protein molecules, which improved its thermal stability. These greater number of interactions are consistent with the homogeneous film structure in SEM micrographs (Figure 1C-D) and higher peaks in FTIR spectra (Figure 2B).

Tensile Properties of CPI Films. Figure 4 shows the tensile property results for the CPI films. The addition of CNCs led to an increase in tensile strength (TS) and a decrease in elongation at break (EB) values of the films. The tensile strengths of the 12, 24, and 36% (w/w) CNC films increased significantly ($p<0.05$) by 32, 199, and 296%, respectively, over that of the control film. This could be explained by the stable proteins in suspension ($\zeta P<−39$ mV), which led to the development of films with a homogeneous distribution of CNCs throughout the protein matrix. Also, the numerous molecular interactions between CNCs and the protein molecules resulted in the CNCs being able to support stress transfer from the protein matrix. This led to an increase in tensile strength.
From Figure 4, an increase in CNC concentration led to significant decreases (p < 0.05) in elongation at break values. These results indicated that adhesion between CNCs and the protein matrix was relatively weak. Consequently, this weak adhesion caused debonding to occur at the interface between CNCs and the protein matrix, which resulted in tensile failure at lower elongation values. Since CNCs are crystalline particles and have a rigid structure, they could bridge the connections between glycerol and protein to promote more ordered structures and stronger interactions along the polymer matrix, which led to more resistant but less flexible films. This behavior is typical of bio-based polymer films reinforced with cellulosic nanomaterials. The nanometric dimensions and abundant OH groups in the cellulosic materials led to an increase in contact area and interfacial interactions with other components, while reducing the mobility of polymer chains.13,31

## CONCLUSIONS

The addition of CNCs improved the stability (ζP < −39 mV) of CPI suspensions and reduced their polydispersity. This colloidal stability was reflected in the SEM micrographs of the cast films that showed a homogeneous morphology. FTIR results also indicated good compatibility between CNCs, glycerol, and canola protein molecules. The CPI films containing CNCs showed greater thermal stability than the control film. Also, the addition of CNCs led to an increase in tensile strength and a decrease in elongation at break values of the films. This indicated that CNCs were able to support stress transfer from the protein matrix during tensile testing. These results showed that CPI films containing CNCs might have potential application as a food packaging material.

## EXPERIMENTAL SECTION

### Materials

The canola protein isolate (CPI; protein content = 77.95 ± 0.02%) used for the film preparation was previously prepared from a canola cake (Hyola 401 variety) obtained from the Instituto Nacional de Investigaciones Forestales (INIFAP, Mexico). An 8.1% w/w cellulose nanocrystal (CNC; particle length = 44–108 nm and particle diameter = 2.3–4.5 nm evaluated by atomic force microscopy) suspension was used as a reinforcing material and was kindly provided by CelluForce Inc. (Canada). Analytical grade glycerol (J.T. Baker) was used as a plasticizer to avoid cracking in the films.

### Preparation of Films

The films were prepared from two suspensions. Suspension 1 was prepared by dissolving 3.5 g of CPI in 40 mL of distilled water and adjusted to pH 9.5 with 2 N NaOH and mechanically stirred for 30 min at room temperature (25 °C). This suspension was then homogenized for 5 min at 10 000 rpm in a Polytron PT 3000 (Brinkmann). Suspension 2 involved dispersing the CNCs (Table 3) in 30 mL of distilled water. Glycerol was added to suspension 2 at a constant concentration of 50/100 g of CPI, and the mixture was stirred for 30 min at room temperature (25 °C). This suspension was homogenized using the same conditions as those for suspension 1. The next step was mixing both suspensions by mechanical stirring for 30 min at 25 °C and then homogenized as described above. The homogenized suspension was later degassed to remove entrapped air, and 70 mL of the suspension was placed in a square silicone mold (25 × 25 cm²), where the films were casted at room temperature (25 °C) for 24 h.

### Stability, Particle Size, and Size Distribution of CPI Suspensions

The stability and particle size distribution of the CNC suspensions in canola protein were evaluated using a Zetasizer Nano ZS (Malvern Instruments, Ltd., Westborough, MA). The samples were prepared by diluting each suspension in ultrapure water at a solid concentration of 0.5%. Dilutions were placed in a quartz cell, and their ζ-Potential (Pζ) values, particle sizes (Z-average), and particle size distributions (PDIs) were determined at 25 °C.

### Characterization of CPI Films

#### Scanning Electron Microscopy (SEM)

The surface and cross-sectional views of the films were observed in a Hitachi S-4700 scanning electron microscope (Hitachi, Japan) using a voltage of 2 kV and a magnification of 1000×.

#### Fourier Transform Infrared (FTIR) Spectroscopy

The chemical composition of the films was determined using attenuated total reflectance in a Nicolet iS10 FTIR spectrometer (Thermo Scientific, Japan). The samples were scanned over an absorbance range of 885–4000 cm⁻¹. Each infrared (IR) spectrum contained 24 scans with a resolution of 4 cm⁻¹.

#### Thermogravimetric Analysis (TGA)

The thermal behavior of the films was determined using a Pyris TGA 2950 (PerkinElmer, Waltham, MA) according to ASTM E1131-08. Prior to the test, the films were conditioned for at least 48 h at a relative humidity of 43.2% ± 0.4, by using a supersaturated K₂CO₃ saline solution. Each 7–9 mg film was heated from 40 to 600 °C at a rate of 10 °C/min. Nitrogen gas was introduced into the sample chamber at a rate of 20 mL/min. Two replicates were performed for each sample.

#### Mechanical Analysis

The tensile properties of the films were determined using a TAXT2i texture analyzer (Stable, Micro Systems, Survey, U.K.) in accordance with ASTM D882-10. The films were cut into rectangles with 10 mm width and 100 mm length. The dimensions were measured using a digital micrometer (Mitutoyo Cor., Kanagawa, Japan) at five random positions. Before each test, the films were conditioned at a relative humidity of 32% for 5 days at room temperature (26 °C). The distance between clamps was 60 mm, while the strain rate was 10 mm/s. A load cell of 25 kg was used in the tests. The tensile strength (TS) and the elongation at break (EB) values were determined from the obtained data. At least 15 replicates were performed for each sample.
Statistical Analysis. Data were analyzed using one-way analysis of variance, and the statistical differences (p < 0.05) among treatments were obtained by the Tukey test using Minitab version 17.1.0 statistical software (Minitab Inc., State College, PA).

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Notes
The authors declare no competing financial interest.

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