INTRODUCTION

Cellulose as the most widespread polymer in plants is the most plentiful organic material on the earth (Payne et al., 2015). Due to the fibers’ properties such as eco-friendly, low cost, and biodegradable nature, it is suitable for development of biofilms as food packaging materials (De Azeredo, 2009). However, the use of bio-polymers is limited because of weak and poor mechanical and barrier characteristics. Therefore, one of the research priorities of scientists is to improve four basic functions of biopolymer food packages including protection and preservation, containment, convenience, and marketing (Petersen et al., 1999; Radusin et al., 2016; Sorrentino et al., 2007).

Considering the effect of the moisture content and water activity on food deterioration (Vermeiren et al., 1999), one of the key properties of food packages is their water activity and water vapor.
2 | MATERIALS AND METHODS

2.1 | Materials

Two different types of fibers including NC and NW were provided by Nano Novin Polymer Co. (Sari, Mazandaran, Iran). NC fiber (gel 2.5 wt %) prepared from commercial pure cellulose fibers of softwoods and NW fiber (gel 2.5 wt %) was made from Paulownia fortunei wood. They were then prepared according to the methods described by Yousefi et al. (2018), Yousefi et al. (2018). The NW was composed of 50% cellulose, 30% lignin, 13% hemicellulose, and 7% extractives.

Calcium chloride anhydrous was obtained from Duksan Co., Korea. Magnesium nitrate and glycerol were purchased from Fluka Co. (Buchs, Switzerland) and Merck Co. (Darmstadt, Germany), respectively. Corn starch containing 73% amylopectin and 27% amyllose was purchased from Sigma-Aldrich (S4126, EC: 232-679-6), USA.

2.2 | Biofilm preparation

The biofilms of NC and NW were prepared using Chaichi et al. (2017) method, with some modifications. For each g of filler (corn starch), 0.05 g nanobiofibers and 0.75 g glycerol were applied. In brief, the NC and NW fibers (0.15 g) were dispersed in distilled water (40 ml) and stirred at room temperature for 30 min at 1,000 rpm by heater-stirrer (Heidolf, Standard Hei, Germany). After complete dissolution, glycerol (2.25 g) was added and stirred again for 30 min. In parallel, corn starch (3 g) was dissolved in distilled water (50 ml) by stirring at room temperature. The solutions were then mixed, the volume was adjusted to 100 ml with distilled water and stirred again at 85°C at 1,000 rpm for 30 min. Then, the nanosuspensions were sonicated for 30 min at 80% amplitude and 24 kHz using ultrasound equipment (Hielscher, Model UP 200-240H, Germany). The sonicated solutions were finally poured into petri dishes (diameter of 9 cm) and allowed to dry for 3–4 days at room temperature. The dried biofilms were removed from the plates, and placed in a desiccator containing saturated magnesium nitrate solution at 25°C and 52.8% relative humidity (RH) for at least 48 hr. The following equation was used to prepare the different thicknesses of biofilms, which was obtained from the results of multiple measurements.

\[ S = 0.105B + 0.20 \quad \left( R^2 = 0.997 \right) \]  

where S is the amounts of solution (g) and B is the biofilm thickness (µm)

2.3 | Biofilm characterization

2.3.1 | Thickness

The thickness of biofilms was measured at 5 random positions using a digital micrometer (Mitutoyo Co., Japan), with an accuracy of 1 µm.

2.3.2 | Swelling degree (SD)

The 2 cm × 2 cm pieces of biofilms were weighed and immersed in distilled water. The SD of the samples was measured using Equation 2

\[ SD = \frac{W_f - W_i}{W_i} \]

where \( W_f \) and \( W_i \) are the final and initial weights (g) of the samples, respectively.

2.3.3 | WVP

The WVP values were determined according to the ASTM Method E96-00 (ASTM, 2007a) as described by Chaichi et al. (2017). At
first, glass permeation cups were filled with 8 g anhydrous calcium chloride desiccant to create a 0% RH storage condition and the surfaces of the cups were covered with films and sealed with molten paraffin and weighted. Then the cups were placed in a desiccator containing magnesium nitrate to create 52.8% RH at 25°C. The RH difference between two sides of the films creates a vapor pressure equal to 1706.57 Pa. The cups were weighted at 2 hr intervals during at least 3 days by a digital balance (0.0001 g accuracy). The slope of the weight gain versus time was obtained by linear regression. Water vapor transition rate (WVTR) and water vapor permeability were calculated using Equations 3 and 4, respectively:

\[ VTR = \frac{\text{Curve slope}}{\text{Film area}} \]  
\[ WVP = \frac{\text{Thickness} \times \text{WVTR}}{\text{Pressure difference}} \]

2.4 | Mechanical properties

The biofilms were cut into 4 cm × 1 cm rectangular strips and were conditioned at 25°C and 52.8% relative humidity. Then they analyzed using a texture analyzer (Hounsfield, Model H5KS, UK) with 500 N load cell (ASTM, 2007a). The initial grip spacing and crosshead speed were, respectively, set at 20 mm and 10 mm/min. Elongation at break (EB) and tensile strength (TS) were calculated using Equations 5 and 6, respectively:

\[ \text{EB\%} = \frac{\text{Final length} - \text{Initial length}}{\text{Initial length}} \times 100 \]  
\[ \text{TS (MPa)} = \frac{\text{Maximum force}}{\text{Film thickness} \times \text{Film width}} \]

2.5 | Structural properties

2.5.1 | X-ray diffraction (XRD)

The XRD patterns were observed in the angular range of 5–80° (2θ) using a Philips X’Pert-MPD diffractometer (Panalytical, Netherlands) with a Cu Kα radiation (λ = 0.154 nm) operating at 40 kV and 40 mA. The interplanar spacing was calculated using Bragg’s Law: 2d sin θ = nλ. Where d is the interplanar spacing (Å), θ is the angle of diffraction (°), λ is the wavelength (nm), and n is the reflection order (Koo, 2006).

2.5.2 | Scanning electron microscopy (SEM)

The surface and cross-section structure of biofilms were observed following freezing under liquid nitrogen, fracturing, mounting, and coating with gold (2 min on a sputter coater) using a scanning electron microscope (VEGA\, TESCAN, Czech Republic) operating at an accelerating voltage of 10 kV and different magnification (1,000, 5,000, and 15,000 x). Energy Dispersive X-ray Spectroscopy (EDXS) (INCA- Oxford instruments- England) was performed in conjunction with SEM for the elemental analysis of the samples.

2.6 | Statistical analysis

A completely randomized design with factorial arrangement was used for statistical analysis. The analysis of variance was performed using a general linear model (GLM) within SAS package (SAS, 2013).

3 | RESULTS AND DISCUSSION

3.1 | SD

The SD of biofilms at different thicknesses and time intervals are shown in Table 1. Water uptake by biofilms was found to be reversely dependent on the thickness. As the highest SDs were obtained at thicknesses lower than 100 µm (p < .05). It can be at least partly attributed to the lower surface area, and in turn, lower interaction with water molecules at higher thicknesses (Cordeiro et al., 2011).

### Table 1

| Biofilms | Time intervals | Thickness (µm) | 60 | 90 | 140 | 180 | 240 |
|----------|---------------|---------------|----|----|-----|-----|-----|
| Nanocellulose | 2 min | 3.63 ± 0.19a | 4.21 ± 0.35a | 3.10 ± 0.11ab | 1.86 ± 0.56bc | 1.13 ± 0.51c |
| | 30 min | 4.13 ± 0.17a | 4.55 ± 0.06a | 3.87 ± 0.18a | 2.27 ± 0.53b | 1.53 ± 0.32b |
| | 60 min | 4.60 ± 0.59a | 4.80 ± 0.46a | 3.28 ± 0.01ab | 2.65 ± 0.35bc | 1.68 ± 0.39c |
| | 24 hr | 5.57 ± 0.85a | 4.82 ± 0.01ab | 3.35 ± 0.27bc | 2.31 ± 0.24c | 1.98 ± 0.54c |
| Nanowood | 2 min | 5.50 ± 1.04a | 2.61 ± 0.62b | 2.67 ± 0.06b | 2.09 ± 0.09b | 1.52 ± 0.40b |
| | 30 min | 4.55 ± 0.66c | 4.23 ± 0.80a | 3.16 ± 0.04ab | 2.12 ± 0.23b | 2.20 ± 0.16b |
| | 60 min | 4.88 ± 0.29b | 3.97 ± 0.66bc | 3.09 ± 0.11bc | 2.33 ± 0.16c | 2.09 ± 0.31c |
| | 24 hr | 3.78 ± 0.70a | 3.47 ± 0.36ab | 3.16 ± 0.34ab | 2.27 ± 0.13ab | 2.03 ± 0.36b |

Note: The different letters indicate significant differences in row (p < .05).
Regardless of thickness, the hydration ability of biofilms increased over time. The highest SD for NC and NW biofilms was recorded after 24 hr (Table 1). The greater surface area of nanoparticles compared with their relevant micro-structures may vindicate their higher interaction with filler improving functional properties of the resultant material (Arora & Padua, 2010; De Azeredo, 2009; Lagaron et al., 2005; Radusin et al., 2016). The most rapid swelling rate was recorded for NW biofilm (5.50 ± 1.04 g/g after 2 min). The high swelling capacity of NW biofilms makes it possible to incorporate water-soluble active agents into their network during swelling. This characteristic is notable for the pad fabrication which is expected to be used in food active packaging.

### 3.2 | WVP

Considering the central role of water in food deterioration, the WVP is one of the most important properties of biofilms. As shown in Figure 1, the film thickness affected the WVP properties. However, a significant effect was only found in the NW group \((p < 0.05)\) where the highest WVP value was found at lower thicknesses \((2.96 \times 10^{-10} \text{g/m/sPa at the thickness of } 125 \mu \text{m})\) facilitating their application for development of new pads.

### 3.3 | Mechanical properties

Mechanical properties of biofilms (EB and TS) at different thicknesses are presented in Table 2. The results indicated that the thickness of biofilms could not exert considerable influences on the EB. The highest EB values of NC and NW biofilms were, however, found at the thickness of 175 \(\mu\)m (23.15% and 19.57%, respectively). The TS values of NC and NW were 1.32 and 0.70 MPa, respectively.

The EB and TS seem to have a nonlinear relationship with the thickness of the samples. The greatly improved EB of nano biofilms can be mainly due to the interfacial hydrogen and ion interaction between the polymer matrix and cellulose (Cao et al. 2008). Nevertheless, the mechanical performance of a specific composite is related to fiber traits, length, and production conditions (Cordeiro et al., 2011).

The mechanical properties of nano-biopolymers in this study were comparable to the previously reported results for different types of cellulose nanofibers (Abdollahi et al., 2013; Abdollahi et al., 2013; Agustin et al., 2013; Azeredo et al., 2010; Chaichi et al., 2017; Fairley et al., 1996; Wan et al., 2009). In the investigation by Ljungberg et al. (2005), nano-composite materials showed higher EB % and lower fragility compared to more aggregated samples, which is in accordance with our results.

![Figure 1](image1.png)  
**FIGURE 1**  
Water Vapor Permeability (WVP) of nanocellulose (NC) and nanowood (NW) biofilms at different thicknesses.

![Figure 2](image2.png)  
**FIGURE 2**  
XRD patterns of (a) nanocellulose (NC); (b) nanowood (NW) biofilms.

### TABLE 2  
Elongation at break (EB) and tensile strength (TS) properties of Nanocellulose (NC) and Nanowood (NW) biofilms

| Biofilms   | Thickness (\(\mu\)m) | Elongation at break (%) | Tensile strength (MPa) |
|------------|-----------------------|-------------------------|------------------------|
| Nanocellulose | 125                   | 18.53 ± 3.96\(^a\)     | 1.16 ± 0.15\(^{ab}\)   |
|            | 175                   | 23.15 ± 9.05\(^a\)     | 1.32 ± 0.14\(^a\)      |
|            | 225                   | 17.99 ± 4.73\(^a\)     | 1.11 ± 0.08\(^{ab}\)   |
|            | 275                   | 16.30 ± 2.33\(^a\)     | 0.84 ± 0.09\(^{b}\)    |
| Nanowood   | 125                   | 15.15 ± 2.27\(^a\)     | 0.66 ± 0.02\(^{a}\)    |
|            | 175                   | 19.57 ± 4.15\(^a\)     | 0.70 ± 0.12\(^{a}\)    |
|            | 225                   | 16.35 ± 6.30\(^a\)     | 0.59 ± 0.12\(^{a}\)    |
|            | 275                   | 13.75 ± 5.38\(^a\)     | 0.45 ± 0.13\(^{a}\)    |

*Note: Values are mean ± SE. Different letters indicate significant differences \((p < 0.05)\) among different thicknesses for each biofilm.*
3.4 | XRD analysis

The XRD patterns of the NC and NW biofilms are shown in Figure 2. While NC showed two sharp peaks at $2\theta = 15.6^\circ$ and $22.51^\circ$, there was no sharp peak in the diffractogram of NW. Three broad halo peaks at $2\theta = 13.59^\circ$, $28.06^\circ$, and $42.90^\circ$ observed in NW indicate an amorphous structure of NW which can, in turn, increase the hydration ability. These results are in accordance with the results of SD evaluation where NW samples had higher levels of SD compared with NC biofilms. The Piassava leaf has also been reported to comprise three broad halo peaks (Cordeiro et al., 2011). In the study conducted by Bodin et al. (2007), the diffractograms of NC biofilms were a combination of crystalline and amorphous peaks indicating their semi-crystalline structures.

3.5 | SEM analysis

SEM allows an area of interest to be examined at different magnifications (1000–15000 x). The SEM image revealed the morphological changes in NW biofilms at different thicknesses. The particle size of NW ranged from 51.94 to 75.27 nm (Figure 3). The NW particles were highly aggregated, with some particles overlapping each other. The feature might be due to the strong hydrogen bonding of the particles and to the preparation of test specimens during the drying step (Habibi et al., 2010). Lu and Hsieh (2010) mentioned that the strong H-bonding among cellulose nanocrystals (CNC) overcomes the repulsion of surface negative charges when CNC is in the dry phase. Scanning electron micrographs of the films showed homogeneous dispersion of NC in the starch matrix without any porosity. The NW biofilms had a denser matrix with good structural integrity at higher thicknesses.

Although the surface morphology of NW biofilms was different from their cross-section structure at lower thicknesses, no differences were found at higher thicknesses.

3.6 | EDXS analysis

EDXS can be applied in conjunction with SEM to explore the elemental composition of biofilms. EDXS spectra of NW biofilms were recorded in the binding energy region of 0–10 kV. Chemical elements...
TABLE 3  Estimation of relative abundance of chemical elements in nanowood (NW) particles and biofilms at different thicknesses

| Elements | Nanowood particles | Nanowood biofilms | Nanowood biofilms |
|----------|--------------------|-------------------|-------------------|
|          | Weight % | Atomic % | Thickness (125 µm) | Surface | Cross-section | Thickness (175 µm) | Surface | Cross-section |
| C        | 50.10  | 57.43  | 45.21 | 53.05 | 50.60 | 59.89 | 45.50 | 53.48 | 45.33 | 53.57 |
| O        | 48.92  | 42.09  | 51.35 | 45.23 | 39.50 | 35.10 | 50.36 | 44.44 | 49.24 | 43.68 |
| Si       | 0.98   | 0.48   | 3.43  | 1.72  | 9.90  | 5.01  | 4.15  | 2.08  | 5.44  | 2.75  |

The elemental composition of NW biofilms was, however, different from that of NW. At lower thicknesses, the surface and cross-section of the films showed different element distribution. The higher O content of the surface of low-thickness NW films can increase the moisture absorption vindicating the higher SD, WVP, and amorphous degree of these biofilms.

4  CONCLUSIONS

Two cellulose fibrils (NC and NW) were used to produce biofilms with various thicknesses. The properties of biofilms were affected by film thickness. Considering the high hydration ability and WVP of low-thickness NW films, they are promising candidates to develop biodegradable films with the potential to be used as moisture absorbent pads in active food packaging. Furthermore, NW is directly produced from natural wood without using any chemical substances; the process is, therefore, environmentally friendly and green.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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