

## Production of Starch Acetate Films with Addition of Bacterial Cellulose Nanofibers

Vivian C. R. Schmidt<sup>a</sup>, Fernanda Berti<sup>b</sup>, Luismar M. Porto<sup>b</sup>, João B. Laurindo<sup>b</sup>

<sup>a</sup>FEQUI, Universidade Federal de Uberlândia, Caixa Postal 593 Uberlândia, MG, Brasil

<sup>b</sup>EQA, Universidade Federal de Santa Catarina, Caixa Postal 476 Florianópolis, SC, Brasil  
 vivian@feq.ufu.br

Natural fibers have attracted much attention due to their applications as reinforcements in thermoplastic and biodegradable polymers, and improvements in the mechanical and barrier properties of the biofilms. In literature it is reported that the addition of cellulose fibers considerably increased the tensile strength and Young modulus of the films, and reduced their elongation capacity. Nanofibers may have properties similar to traditional fibers. In this context, the objective of this study was to incorporate bacterial cellulose nanofibers in starch acetate films and characterize those films by mechanical and barrier properties. The films were obtained from suspensions prepared with 3 g of starch acetate (produced in laboratory), 100 g distilled water, glycerol (0.30 g.g<sup>-1</sup> starch acetate), guar gum (0.06 g.g<sup>-1</sup> starch acetate) and bacterial cellulose fibers (0.025, 0.075 e 0.125 g.g<sup>-1</sup> starch acetate), dispersed in a mechanical shaker, heated to 85°C and stirred for 30 min to dissolve the starch acetate. While still heated, the suspensions were spread on acrylic plates and dried at 35 °C for 7 h in a forced air circulation oven until the moisture equilibrium was reached (11.5 ± 0.8 %) ("casting" method). After drying, samples were cut from the films and conditioned at 58 %, for 4 d, before testing. Starch acetate films were used as control. The results of tensile trials showed that the acetylated films with addition of bacterial cellulose increased 2, 3 and 7 times the values of stress at break and 2, 3 and 11 times, approximately, the values of Young modulus when comparing to the results of the control films. These results indicated that the addition of nanofibers is an alternative to improve the mechanical resistance of the films. The reductions in the values of hygroscopicity were small.

### 1. Introduction

The production of edible and biodegradable films of carbohydrates and proteins adds value to low-cost raw materials and can play an important role in food preservation (Garcia et al., 2006). However, the resistance of films made with acetylated starch is a limitation to the development of these materials.

In order to improve starch-based films characteristics, many researchers have reported findings on the addition of natural fibers as suitable reinforcements for thermoplastic materials. Most of these studies, focused on the mechanical properties of the films have showed that the fibers incorporation increases the tensile strength and elasticity modulus and decreases the elongation capacity as well as influences some film barrier properties (Follain et al. 2006). Literature has reported that both tensile strength and Young's modulus increased and elongation capacity decreased with increasing fiber concentration of biodegradable materials.

Therefore, natural fibers are elongated structures with round cross sections, obtained from numerous sources such as natural cotton, hemp, sisal, jute, flax, bamboo, palm, sugarcane bagasse, coconut and ramie (Takagi and Asano; 2008). Apart from plant cellulose, cellulose may be also produced by bacteria, known as bacterial cellulose (BC) or microbial cellulose (MC). Although similar in chemical composition, the mechanical properties and structure of bacterial cellulose differ from plant cellulose. Compared with the plant cellulose, bacterial cellulose (BC) has better mechanical properties such as tensile strength and Young's modulus, higher water-holding capacity, higher crystallinity and a fine interlaced network (Rambo et al. 2008). Thus, the bacterial cellulose (BC) is a kind of extracellular polysaccharide present in the

biofilm produced by some bacteria, such as *Gluconacetobacter xylinus* and is classified as nanofibers by some authors (Tischer et al., 2010). According to Rambo et al. (2008), bacterial cellulose exhibited a wide range of dimensions ranging from 1 to 25 nm in width, which corresponds to 10-250 polyglucan chains, and from 1 to 9  $\mu\text{m}$  in length (composed by 2000-18,000 glucose residues). This polymer is highly crystalline and the degree of crystallinity depends on the source and method of chemical treatment (George et al. 2011). The degree of crystallinity of cellulose influences some of its physicochemical properties, such as swelling and water-holding capacity.

In this context, the aim of this study was to incorporate bacterial cellulose nanofibers in starch acetate films and evaluate barrier and mechanical properties of the films.

## 2. Materials and methods

### 2.1 Obtaining the bacterial cellulose

The bacterium *Gluconacetobacter hansenii*, strain ATCC 23769, obtained from the "Collection of Tropical Culture" (CCT) (Fundação André Tosello, Campinas – SP) was used for the production of bacterial cellulose using the methodology adapted from the procedure described by Recouvreux et al. (2010).

The inoculum was cultivated in 500 mL of culture medium composed of mannitol (25 g/L), yeast extract (5.0 g/L) and bactopectone (3.0 g/L). The compounds were diluted in water and the pH was adjusted to 6.5 with HCl and NaOH solutions, followed by sterilization in an autoclave at 121 °C for 20 min. Then, 10 % (v/v) inoculum from a stock culture of *G. hansenii* was added to the sterilized culture medium and maintained at 25 °C under static conditions for 30 days.

Immediately after this period, the cellulose membranes were treated with 0.01 M NaOH solution for 24 h, washed with distilled water and the pH was adjusted to 7. After pH adjustment, the membranes were processed as they were obtained and still moist were dispersed in water for incorporation in the starch acetate films.

### 2.2 Preparing starch acetate films with bacterial cellulose

A suspension was prepared with 3 g starch, 100 g distilled water, glycerol (0.30 g/g starch acetate), guar gum (0.06 g/g starch acetate) and bacterial cellulose (0.025; 0.05; and 0.125 g/g starch acetate). The suspension was dispersed into a Turrax homogenizer (IKA, Germany) under heating up to 85 °C. Then, it was stirred for 30 minutes to solubilize the starch acetate, and while still heated, the suspension was spread on acrylic plate and subsequently dried at 35 °C for 7 hours in a forced air circulation oven (Tecnal model TE 394/2, Brazil) until the equilibrium moisture content was achieved ( $11.5 \pm 0.8$  %). After drying, samples were cut and conditioned at two different relative humidity levels (58 % and 75 %) for two days before being tested. Figure 1 shows the flow diagram of the preparation of the starch acetate films by both methods. The need of incorporating guar gum (Microm Química Ltda. Brasil) to the film-forming solutions is to avoid fibers sedimentation during the gelatinization process (Müller, 2009).

### 2.3 Film characterization

Prior to determinations, the samples were conditioned at 58 % relative humidity for four days. The mechanical properties were determined by tensile tests (tensile strength-TS, and elongation at break-ELO) using a TA-XT2i texturometer Stable Micro Systems (SMS, Surrey, England). The mechanical properties were determined by way of the tensile strength (tensile strength, TS, elongation at break,  $\epsilon$ ) using the TA-XT2i Stable Micro System texture analyzer (SMS, Surrey, England). The methodology was based on the work of Gontard et al. (1992) and carried out according to the ASTM D828–97 standard test methods (ASTM, 1997). The samples were cut into a rectangular shape with 100 mm  $\times$  25 mm. The distance between the claws was 50 mm and a test speed of 0.8 mm/s was used. Ten samples were evaluated for each test. The scanning electron microscopy of the films was performed by a scanning electron microscope JEOL JSM, model 6390LV, with tungsten-filament electron source and secondary and back-scattered electron detector. The water vapor permeability was determined in diffusion cells specific for this purpose (Sarantopoulos and Oliveira, 2002), using gradients of relative humidity (2-3 to 75 %). Each film sample (54 mm diameter disk) was sealed over the circular opening of a permeation cell containing silica gel, and placed in a desiccator containing distilled water at 25 °C. The cells were weighed every hour for 12 h using an analytical balance (AM5500, Mars) and then every 24 h. The amount of water vapor transferred through the sample area was determined from the weight gain versus time. All the tests were performed in triplicate.

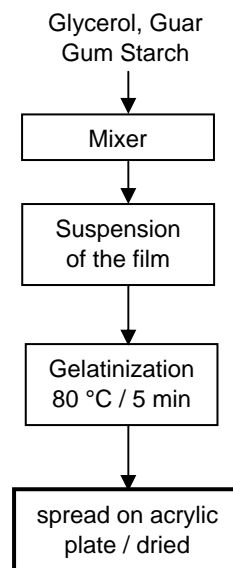


Figure 1. Flowchart of the preparation of the bacterial cellulose

### 3. Results

The starch acetate films with bacterial cellulose presented phase separation, once the dispersed fibers have formed domains. In the films with concentrations above 15 g fibers bacterial cellulose/100 g starch acetate, domains were easily noticed, as shown in Figure 2. Considering the domains formed by the cellulose fibers and bacterial cellulose nanofibers, we chose to study low concentrations of bacterial cellulose (2.5, 7.5 and 12.5 g/100 g starch acetate).

To find the benefits of the addition of bacterial cellulose, starch acetate films with 0.6 DS and 0.30 g glycerol/g starch were used as control. The results of stress and elongation at break and Young's modulus are shown in Table 1.

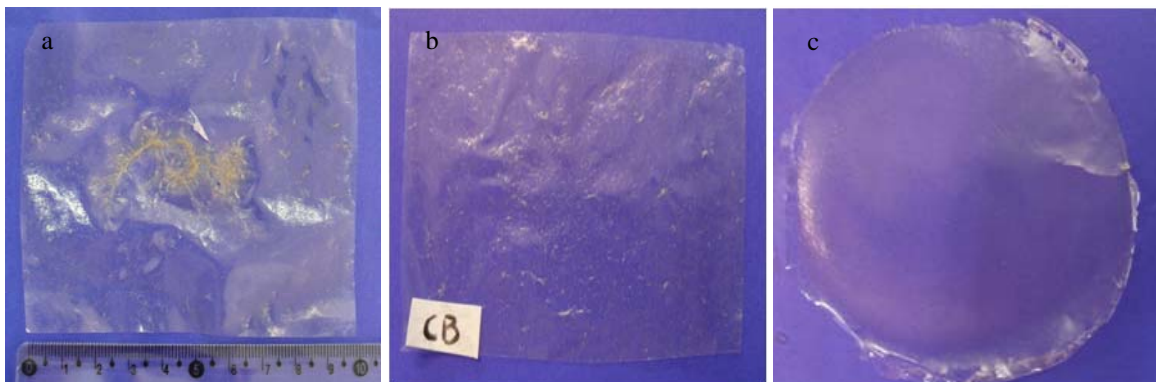


Figure 2. Films of the starch acetate (SA) with (a) e (b) high concentration of the bacterial cellulose of 20 g fibers/100g SA (c) low concentration of the bacterial cellulose of 12,5 g fibers/100 g SA.

Increasing the concentration of fiber in the starch acetate films decreased the elongation at break and increased tensile strength and Young's modulus. Similar results were reported in studies with starch and cellulose fiber (MA et al, 2005). These results can be explained by the chemical and structural compatibility between the starch and cellulose chains (Averous and Boquillon, 2004). The glycerol partition between the starch matrix and fibers is also reported as a phenomenon that influences the elasticity modulus of the films due to the unplasticized starch matrix (Kunanopparat et al. 2008). Improving the film properties depends on the kinds of application to which these materials are designed.

Table 1. Results of the tensile strength, elongation at break and Young's modulus for the films of the starch with bacterial cellulose.

Concentration of the (g/100g starch acetate)	Tensile Strength, TS (MPa)	Elongation at break, $\epsilon$ (%)	Young's modulus (MPa/%)
0 (control)	2.13±0.29 <sup>a</sup>	140.52±22.17 <sup>a</sup>	0.53±0.14 <sup>a</sup>
2.5	4.88±0.74 <sup>b</sup>	42.13±0.92 <sup>b</sup>	1.68±0.33 <sup>b</sup>
7.5	6.54±0.90 <sup>c</sup>	15.89±1.22 <sup>c</sup>	2.21±0.43 <sup>c</sup>
12.5	14.90±0.50 <sup>d</sup>	4.05±0.40 <sup>d</sup>	6.20±0.50 <sup>d</sup>

<sup>a,b,c,d</sup> Values with the same letter at the same column are not different statistically ( $p < 0.05$ ).

The water vapor permeability of the starch acetate films with different concentrations of bacterial cellulose were also investigated (2.5, 7.5 and 12.5 g/100 g starch acetate). Figure 3 shows the results of water vapor permeability. The addition of bacterial cellulose in the starch acetate films did not influence the water vapor permeability. Despite the chemical interaction and the structural compatibility between starch and cellulose chains, the rearrangement of the fibers does not influence the starch structure. As can be seen in Figure 4, micrographs of starch acetate films containing bacterial cellulose were performed to investigate how the fibers were dispersed in the starch acetate matrix: a) surface of films of starch acetate ( $\times 5000$ ), b) fracture of films of starch acetate ( $\times 800$ ), c) surface of film of starch acetate with addition of bacterial cellulose (12, 5 g/100 g starch acetate) ( $\times 500$ ) d) fracture of films of starch acetate with addition of bacterial cellulose (12.5 g/100 g starch acetate) ( $\times 1000$ ), e) fracture of films of starch acetate with addition of bacterial cellulose (12.5 g/100 g starch acetate) ( $\times 500$ ), f) fracture of films of starch acetate with addition of bacterial cellulose (12.5 g/100 g starch acetate) ( $\times 2000$ ).

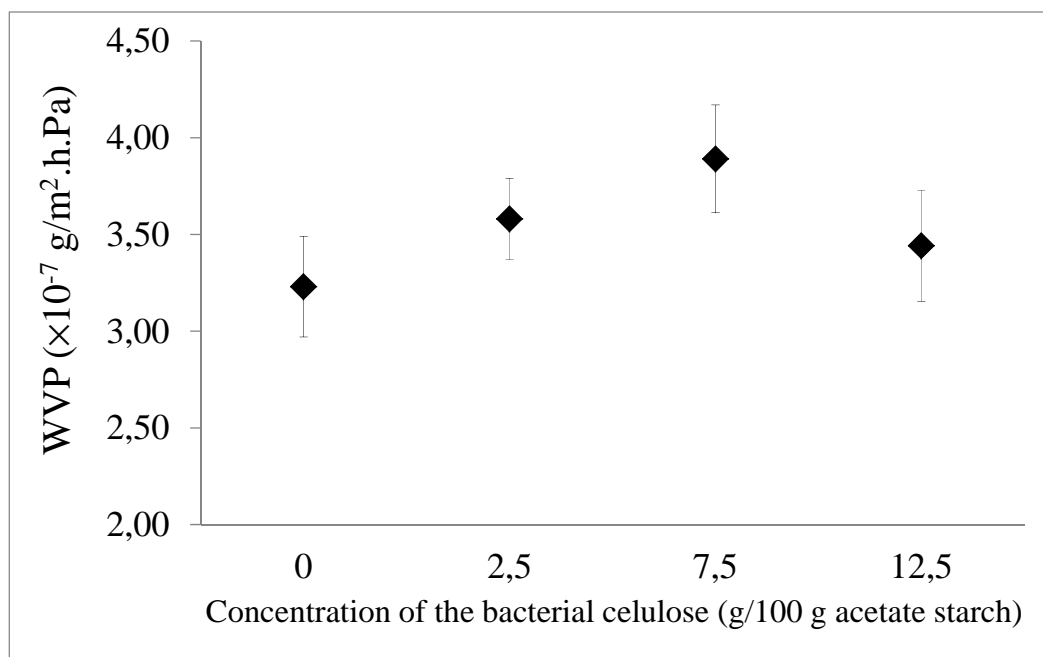


Figure 3. Water vapor permeability (PVA) of the films of the starch acetate added of the addition of different concentrations of bacterial cellulose.

Compared to starch acetate films, the films produced in this study showed roughness and small pores on the surface, as can be seen in Figure 4c. Figure 4d exhibits small holes when using higher magnification (1000x). This may be due to the domains formed by the fibers, i.e., a higher concentration of fibers in some specific regions. Micrographs of films surfaces and fractures exhibited no sedimentation of nanofiber on the plate surface. However, the smooth and uniform structure obtained in the starch acetate films was

changed with the addition of fibers. In the Figure 4f, the arrows indicate small pores possibly formed by bacterial cellulose nanofibers, given that fiber length ranges from 1 to 9  $\mu\text{m}$  approximately. Woehl et al. (2010) reported no difference between the micrographs of starch-based films containing hydrolyzed bacterial cellulose and non-hydrolyzed cellulose films. Muller et al. (2009) observed smooth and homogeneous surface of starch films reinforced with cellulose fibers.

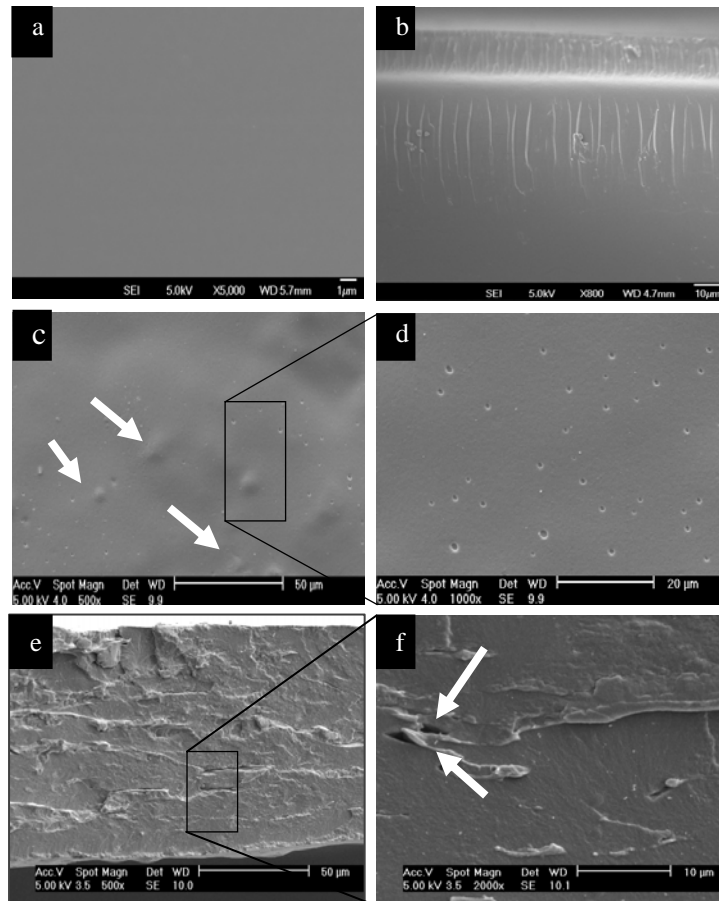


Figure 4. Micrographs of films of starch acetate and of the films of starch acetate with addition of bacterial cellulose (12.5 g/100 g starch acetate).

#### 4. Conclusion

Regarding the addition of bacterial cellulose in starch acetate films, the incorporation of cellulose fibers reinforced mechanically starch films, which had higher tensile strength and lower deformation capacity. The reinforced films showed no difference in water vapor permeability as compared to the starch acetate films without fibers. As bacterial cellulose fiber is biodegradable, its use as reinforcing agent is a viable alternative for improving the properties of the starch-based films.

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