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Structural modification of bacterial cellulose fibrils under ultrasonic irradiation

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ABSTRACT

In the present study we investigated ultrasounds as a pretreatment process for bacterial cellulose (BC) aqueous suspensions. BC suspensions (0.1–1% wt) subjected to an ultrasonic treatment for different time intervals. Untreated BC presented an extensively entangled fibril network. When a sonication time of 1 min was applied BC fibrils appeared less bundled and dropped in width from 110 nm to 60 nm. For a longer treatment (3–5 min) the width of the fibrils increased again to 100 nm attributed to an entanglement of their structure. The water holding capacity (WHC) and ζ-potnential of the suspensions was proportional to the sonication time. Their viscosity and stability were also affected; an increase could be seen at short treatments, while a decrease was obvious at longer ones. Concluding, a long ultrasonic irradiation led to similar BC characteristics as the untreated, but a short treatment may be a pre-handling method for improving BC properties.

1. Introduction

Cellulose is a linear biopolymer of glucose that mainly exists in plants as a structural component of cell walls. Cellulose consists of an amorphous and a crystalline portion. While crystalline cellulose consists of long chains bound together by strong hydrogen bonds, amorphous cellulose is made up of shorter and weaker chains (Türünç & Meier, 2012).

BC and plant derived cellulose have the same chemical structure, but BC is obtained from bacterial species, such as *Komagataeibacter sucrofermentans*, which have the ability to synthesize pellicles of cellulose, when placed in a culture medium (Martinez-Sanz, Lopez-Rubio, & Lagaron, 2011; Okiyama, Shirae, Kano, & Yamanaka, 1992). This pellicle consists of a bundle of fibrils of about 4 μm wide, which are composed of random nanofibrils less than 100 nm wide (Okiyama, Motoki, & Yamanaka, 1993).

BC has unique physicochemical properties such as higher water holding capacity, higher crystallinity and higher purity as it does not associate with lignin and hemicelluloses, in contrast to plant derived cellulose (Iguchi, Yamanaka, & Budhiono, 2000; Martínez-Sanz et al., 2013; Salas, Nypelö, Rodriguez-Abreu, Carrillo, & Rojas, 2014). Thanks to these properties, BC has been receiving increased attention and has been used in various areas such as biomedicine, cosmetics, paper industry and many others (Iguchi et al., 2000). Although not extensively used in food yet, BC has great potential as a food ingredient, changing the rheological profile of a food, as it serves as thickening, stabilizing or gelling agent. Recently, BC has been shown to act as a stabilizer in emulsions (Kalashnikova, Bizot, Cathala, & Capron, 2011; Paximada, Koutinas, Scholten, & Mandala, 2016; Paximada, Tsouko, Kopsahelis, Koutinas, & Mandala, 2016).

One of the reasons why BC is not systematically used in the food industry is its low ability to be dispersed into water (Agoda-Tandjawa et al., 2010; Lowys, Desbrières, & Rinaudo, 2001). In the food industry the thickeners have to be well-dispersed in order to be more acceptable by the consumers (McClements, 2005), while BC suspensions present pronounced particle aggregation due to Van der Waals attractions and hydrogen bonds (Kuijk et al., 2013).

A number of technological approaches have been developed to enhance the physical properties of the colloidal suspensions of polymer fibrils. The most commonly used method is to submit polymer to controlled acid hydrolysis conditions (Hirai, Inui, Horii, & Tsuji, 2009; Martinez-Sanz et al., 2011; Olsson et al., 2010). However, this is of high energy and cost process that causes intense degradation of the polymer and hence the industry would have had benefit from cheaper alternative methods.

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Chemically less aggressive concepts could be the mechanical treatment of cellulose, such as a high pressure homogenization which is used to treat microfibrillated cellulose (MFC) resulting in changes in the microstructure of the cellulose (Agoda-Tandjawa et al., 2010; Saito, Nishiyama, Putaux, Vignon, & Isogai, 2006).

What is more, high-intensity ultrasound (16–100 kHz, 10–1000 W cm^{-2}) has immense potential for structural and functional properties of cellulose modification. By this method, the energy of ultrasound is transferred to the polymer chains through a process called cavitation, which is the formation, growth and violent collapse of cavities in the water. Therefore, the effect of ultrasound is related to cavitation, heating, dynamic agitation, shear stresses, and turbulence (Vilkhu, Mawson, Simons, & Bates, 2008). Recently, structural and functional changes in ultrasound irradiated plant cellulose, have been reported by (Dehnad, Emam-Djomeh, Mirzaei, Jafari, & Dadashi, 2014; Liu & Yang, 2008; Wang & Cheng, 2009). These authors reported that the controlled depolymerization of plant cellulose can be achieved by employing suitable ultrasonication settings.

However, to the best of our knowledge, the literatures about structural modification of bacterial cellulose under high-intensity ultrasound were limited, and the effects of ultrasound irradiation on the physical properties of bacterial cellulose nanofibrils (BCN) aqueous suspensions of cellulose have not been reported to-date.

2. Materials and methods

2.1. Bacterial cellulose production

Bacterial cellulose was produced as described previously (Tsouko et al., 2015). Briefly, bacterial cultivations (*Komagataeibacter sucrofermentans* DSM 15973) were carried out using a synthetic medium as described by (Hestrin & Schramm, 1954) containing a carbon source (20 g/L), yeast extract (5 g/L), peptone (5 g/L), Na_2HP0_4 (2.7 g/L) and citric acid (1.15 g/L). The inoculum was prepared by growing the microorganism at 30 °C and 100–120 rpm during 2 days, in Hestrin and Schramm liquid medium. Fermentations were carried out in 250 mL Erlenmeyer flasks containing 50 mL of synthetic medium and were inoculated with 10% v/v inoculums volume. All shake flasks were incubated at 30 °C in static mode for 15 days.

After cultivation, bacterial cellulose (BC) was removed from the cultures and rinsed with tap water to remove any residual media. Next, it was treated with 2 M NaOH to eliminate bacterial cells and then washed repeatedly with tap water until the BC dispersions obtained a neutral pH.

2.2. Treatment of BC

The purified BC pellicles were cut into small pieces with scissors and mixed with deionized water to prepare a BC suspension (4% wt concentration). The BC pieces were further disintegrated with a high shear blender for 10 min (13500 RPM, Ultra Turrax T25, IKA, Germany) which led to the formation of a white precipitate. High shear was used as a first step in order to cut off the cellulose matrices into smaller pieces (0.5 cm thickness). However, small fibrils cannot be obtained only with the high shear blender and ultrasonication was further used.

A dilution of the suspensions with deionized water took place leading to a final concentration of 0.1, 0.5 and 1% wt respectively. The suspensions was then submitted to ultrasonic treatment by an ultrasonic homogenizer model Sonopuls 3200 (Bandelin Electronic Gmbh & Co., Berlin) equipped with a 3 mm in diameter microtip (MS 73, 284 μm_{ss} peak-to peak amplitude). Ultrasonication carried out at a frequency of 20 kHz, while the processing time was 1 (BC1), 3 (BC3) and 5 min (BC5) and the final nominal power added to each sample was 82 W. The temperature was maintained at 25 (±1) °C by circulating cold water with a pump. All samples were prepared in triplicate.

2.3. Water holding capacity

To determine the water holding capacity of BCN suspensions, they were centrifuged at 5000 RPM for 15 min. After the removal of the supernatant, the sediment was weighed and dried at 60 °C in order in order to ensure complete drying. WHC was calculated by the following equation:

$$WHC = \frac{W_r}{Wc} \quad (1)$$

Where W_r is the mass of the removed water during drying and W_c is the dry content of cellulose. The results are reported as the average of at least three samples.

2.4. Transmission electron microscopy (TEM)

The microstructure of cellulose aqueous suspensions was determined using a JEOL 100s equipped with an image acquisition system. Samples of freshly prepared suspensions were diluted 20 times with deionized water, freeze-dried, stained with PTA that is commonly used for staining cellulose fibrils (Colvin & Sowden, 1985) and placed on the grid. After drying at room temperature, several pictures were taken from random sample positions representing the overall structure of the suspensions. These pictures were analyzed with an image analysis software (Image-Pro Plus 7.0, Media Cybernetics, Rockville USA) in order to measure the fibrils' width.

2.5. ζ-potential measurements

ζ-potential measurements were carried with Dynamic Laser Light Scattering (ZetasizerNano ZS, Malvern Instruments, Worcestershire, UK) at 25 °C. As the ζ-potential is related to the electrophoretic mobility of the particles, the ζ-potential is calculated from the measured velocity using the Smoluchowski equation. The samples were previously diluted (1:100) with deionized water to avoid multiple scattering effects. The measurements are reported as the mean of at least two differently prepared injections, with five readings per injection.

2.6. Stability of BCN suspensions

The gravitational stability of suspensions upon storage was followed by measuring the backscattering (BS) intensity along the height of an optically transparent tube using a Turbiscan MA 2000 apparatus (FormulAction, Toulouse, France). Suspension samples (approximately 6 mL) were brought into test tubes, sealed with a plastic cap and stored at 20 °C. Measurements were performed with intervals of 24 h and a total period of 20 days. The stability is presented as the phase separation (PS), which is calculated as:

$$PS\% = \frac{H_p}{H_t} \times 100 \quad (2)$$

where H_p is the height of the serum layer and H_e is the total height of the suspension. A lower PS therefore represents a more stable suspension. The results are reported as the average of at least three samples.