



# Dry Bacterial Cellulose and Carboxymethyl Cellulose formulations with interfacial-active performance: processing conditions and redispersion

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**Abstract** Dry or powdered formulations of food additives facilitate transportation, storage, preservation and handling. In this work, dry formulations of bacterial cellulose and carboxymethyl cellulose (BC:CMC), easily redispersible and preserving the functionality of the never-dried dispersions are reported. Different processing parameters and their effect on the materials properties were evaluated, namely: (i) wet-grinding of BC (Hand-blender, Microcut Head Impeller, High-pressure

Homogenizer), (ii) drying of BC:CMC mixtures (fast drying at  $\approx 130$  °C and slow drying at 80 °C) and subsequent (iii) comminution to different particle sizes. The dispersibility of the obtained BC:CMC powders was evaluated, and their functionality after redispersion was assessed by measuring the dynamic viscosity, the effect in oil/water interfacial tension (liquid–liquid system) and the stabilization of cocoa in milk (solid–liquid system). The size of BC fibre bundles was of paramount relevance to its stabilizing ability in multiphasic systems. A more extensive wet-grinding of the BC fibres was accompanied by a loss in the BC:CMC functionality, related to the increasingly smaller size of the BC bundles. Indeed, as the  $D_v(50)$  of the wet BC bundles was reduced from 1228 to 55  $\mu\text{m}$ , the BC:CMC viscosity profile dropped and the effect on interfacial tension decreased. This effect was observed both on the never-dried and dry BC:CMC formulations. On the other hand, the drying method did not play a major effect in the materials' properties. In a benchmarking study, the BC:CMC formulations, at a low concentration (0.15%), had better stabilizing ability of the cocoa particles than several commercial cellulose products.

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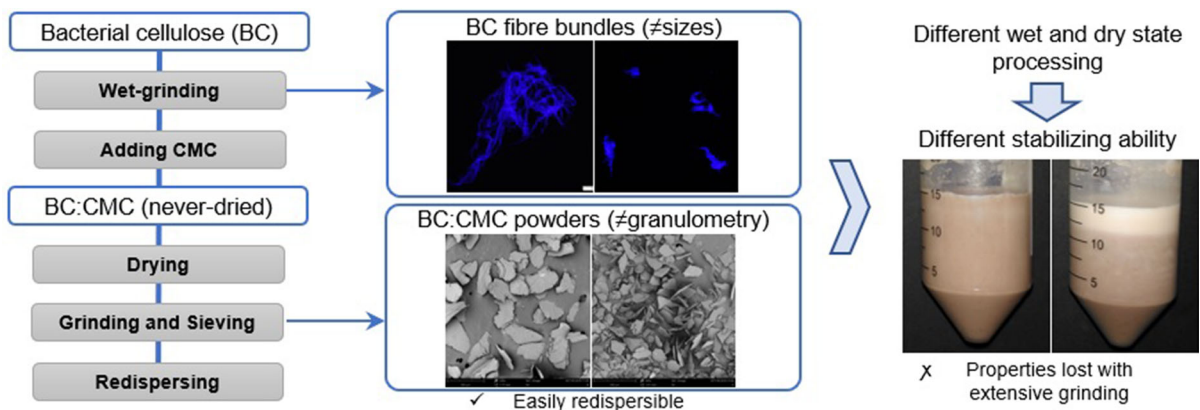
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## Graphic abstract



**Keywords** Bacterial cellulose · Powder formulations · Drying · Comminution · Dispersibility · Solid-in-liquid stabilization

## Introduction

Bacterial cellulose (BC) is an exopolysaccharide synthesized by certain acetic acid bacteria, the *Komagataeibacter* genus being the most important due to the high cellulose yield. As *Komagataeibacter* microorganisms are mandatory aerobes, under static conditions BC is synthesized at the air/liquid interface of the culture medium in the form of a fibrous membrane comprising 99% water and 1% cellulose. Morphologically, this biopolymer is organized in elementary nanofibrils with a lateral size of 6 nm to 7 nm, which assemble into ribbon-shaped fibrils less than 100 nm wide and several micrometres in length (Keshk 2014; Klemm et al. 2011; Kondo et al. 2016; Lee et al. 2014). This high aspect ratio, nanoscale thickness, good mechanical properties and the ability to arrange into structured three-dimensional networks even at low concentrations (Martins et al. 2019) account for the good performance of BC fibres as a texture/rheology modifier and stabilizer of heterogeneous systems, including food products (Ougiya et al. 1997; Paximada et al. 2016; Yaginuma and Kijima 2006; Zhai et al. 2018).

For bulk commercial applications, dry formulations offer several advantages over aqueous suspensions, such as a decrease in the size and mass, thus lower

storage space and transportation costs, improved storage stability and lower risk of contamination. As with plant cellulose, the properties of BC are mostly lost upon drying due to irreversible structural changes—a phenomenon called hornification. One of the most accepted mechanisms for hornification is cocrystallization of the cellulose fibres. As water evaporates, the thin fibres come closer and aggregate, establishing hydrogen bonds between adjacent crystalline domains. These bonds are not broken upon resuspension in water without a significant energy input, resulting in a so called hornified material (Klemm et al. 2011; Newman 2004).

One of the most usual approaches to prevent hornification is to mix cellulose with an additive to create a steric barrier prior to drying, such as carbohydrates or biopolymers. These polymers might bear a charge, as in the case of the water-soluble polyanionic Carboxymethyl Cellulose (CMC), one of the most commonly used additives in hydrocolloidal cellulose formulations (Klemm et al. 2011; Zhao et al. 2011). CMC is generally recognized as safe (GRAS) for food consumption by the American Food and Drug Administration and approved by the European Food Safety Authority, often found in food products as Cellulose Gum or E466. It is used as thickener, stabilizer, gelling agent and texture modifier, alone or in combination with non-modified plant-derived celluloses. Being a modified cellulose, CMC shows a selective adsorption towards non-modified cellulose fibres, since it has chemically similar backbone segments, facilitating cooperative hydrogen bonding (Kargl et al. 2012; Zemljic et al. 2006). Bearing

negatively charged carboxylic groups, CMC imparts a surface charge to cellulose structures upon adsorption. The electrostatic repulsion forces increase the swelling and dispersion of cellulose in aqueous media, improving its colloidal stability and contributing to the three-dimensional network formation (Martins et al. 2019). On the other hand, the presence of a charged drying-aid prevents cooperative hydrogen bonding between adjacent cellulose polymer chains during water evaporation, avoiding hornification. In this type of formulations CMC plays a dual role as co-drying agent and stability enhancer. This methodology has been used for plant cellulose fibres and crystals, in order to obtain dry materials with improved dispersion (Butchosa and Zhou 2014; Naderi et al. 2015). When redispersed in water, the CMC layer coating the cellulose particles rehydrates, freeing the particles. The material then recovers its initial gel-like form with a microscopic network of interconnected fibres or crystals, preserving, in theory, the functional properties as those of the never-dried material (Agarwal et al. 2018; Zhao et al. 2011).

Only a few works have yet focused on obtaining rehydratable or redispersible cellulose from bacterial sources (Amin et al. 2014; Pa'E et al. 2014); in fact, most of the reports on dry BC formulations are published as patents, which suggests the industrial interest in the use of such materials for areas ranging from food and cosmetics to reinforcement materials and composites (Tammarate 1999; Watanabe et al. 2000; Yang et al. 2001, 2011). Some of these works claim the restoration of the BC fibres properties, as compared to those of the never-dried versions. However, the redispersion methods involve the use of high energy mixing, high temperature, long dispersion times or combinations of these, as well as the use of additives in excess amounts. These energy and time-intensive processes represent high capital and operating costs when considering industrial applications. Furthermore, most of the reports are mainly focused on demonstrating the BC's redispersibility, not clearly demonstrating the recovery of the BC technological properties upon redispersion, as compared to the never-dried material.

On the path of devising methods for drying cellulose particles, the effect of processing conditions and their impact in the redispersibility, rehydration and final properties has also been studied by some authors (Khoshkava and Kamal 2014; Nechita and

Panaitescu 2013; Peng et al. 2013). In particular, some work has also been done with BC alone (Clasen et al. 2006; Pa'E et al. 2014).

In this work, dry powdered and easily rehydratable BC formulations with CMC (BC:CMC) are reported, which can achieve full redispersion in water under low-energy mixing and within a few minutes, contrarily to other dry (plant based) cellulose products available in the market. To produce the dry powder formulations, different grinding, drying and dispersing methods were evaluated for their impact on the final product's properties. This study presents, for the first time, a systematic approach towards the understanding of the relevant processing conditions for the development of dry BC:CMC formulations bearing suitable redispersion ability, while not compromising the functional features. In particular, in this work we analyse the impact of the extension of grinding (both in wet or dry state) on the size of the obtained fibre bundles and on its functional properties. To our knowledge, the relevance of the size of the bacterial cellulose fibre bundles on its performance as a hydrocolloid has never been reported.

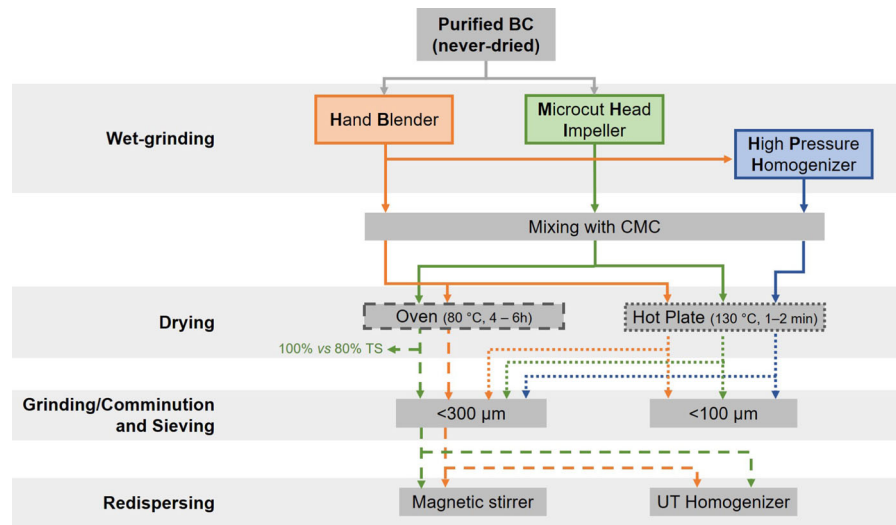
## Materials and methods

### Preparation of the BC:CMC powders

The different process conditions used to prepare the dry BC:CMC samples are outlined in Fig. 1.

### BC production and purification

BC membranes were produced by static culture using *Komagataeibacter xylinus* BPR 2001 (ATCC 700,178), according to the optimized parameters described by Rodrigues et al. (2019). Briefly, the strain was grown in Hestrin–Schramm culture medium (in m/v): 2.0% glucose (Fisher Chemical), 0.5% peptone (OXOID), 0.5% yeast extract (OXOID), 0.27% disodium phosphate (Panreac) and 0.115% citric acid (Panreac), initial pH 5.5 set by using 18% (v/v) HCL (Fisher-Chemical). This culture (inoculum) was incubated for 2 d at 30 °C under static conditions. Afterwards, it was transferred to large square containers with a different culture medium, making up 10% of the final volume. This cultured medium consisted of 0.27% (m/v) disodium phosphate, 0.115% (m/v) citric



**Fig. 1** Scheme of the several steps and processing conditions used in this work to obtain the different dry BC:CMC powders

acid, 5.38% (m/v, sugar basis) molasses (a gift from RAR Refinarias de Açúcar Reunidas, S.A.; Portugal), 1.91% (m/v, protein basis) CSL (a gift from COPAM Companhia Portuguesa de Amidos, S.A.; Portugal), 0.63% (m/v) ammonium sulphate (Panreac) and 1.38% (v/v) ethanol (Fisher-Chemical), pH 5.5 (set with 18% HCl). The culture was incubated in static conditions for 30 days at 30 °C.

After cultivation, the BC membranes were washed with distilled water, then thoroughly with 0.1 M NaOH (Fisher-Chemical) at room temperature, with changes twice a day, until the membranes turned completely white. Then, they were washed again with distilled water until reaching the same pH as that of distilled water.

#### Wet grinding of BC

Purified bacterial cellulose membranes were wet-ground by three different methods (Fig. 1):

- Using a Sammic fixed speed hand blender (HB), 9000 rpm (model TR250, Samic, S.L.), for about 1 min until a visually homogeneous pulp was obtained;
- Submitting the BC membranes (previously cut in cubes) to 2 passages through a Microcut Head Impeller (MHI), Comitrol® Processor Model 1700 (Urschel Laboratories Inc.). In each passage, grinding of BC was done at 900 Rpm, using a

labyrinth Vericut impeller and a cutting head 160 at 5° inclination;

- The pulp obtained with the hand blender was further processed in a High-Pressure Homogenizer (HPH) using a GEA Niro Soavi, model Panther NS3006L, at 600 Bar. BC was collected after 1 cycle (HPH-1), 2 cycles (HPH-2) and 6 cycles (HPH-6), through the High-Pressure Homogenizer.

#### Mixing Bacterial cellulose with sodium carboxymethyl cellulose

The wet-ground BC pulp obtained by the three different methods described in the previous section, was mixed with a solution of sodium Carboxymethyl Cellulose (CMC, 90 KDa, D.S. = 0.7, Sigma) at a mass ratio of 1:1 (BC:CMC), to a final solids content of 2%. This mixture was left under magnetic stirring overnight at room temperature.

#### Drying of the BC:CMC mixtures

To study the effect of the drying method, wet BC:CMC mixtures obtained from "Mixing bacterial cellulose with sodium carboxymethyl cellulose" Section (HB, MHI and HPH ground BC:CMC) were dried by two different methods:

- Fast drying in a hot plate (HP), an Ariette Crepes Maker, model 183. A thin layer of BC:CMC sample was spread over the hot plate at 130 °C, for only a few minutes (1–2 min). With this process, a completely dry BC:CMC product was obtained (100% total solids (TS)).
- Slow drying in an oven (OV), in aluminium pans for 4 h to 6 h, at 80 °C. MHI ground samples were retrieved at 80% TS and 100% TS, to evaluate the effect of residual moisture on the dispersibility of BC:CMC formulations.

### *Grinding and sieving*

The HP and OV dried materials were ground with a High Power Herb Grain Grinder Cereal Mill Powder Grinding Machine Flour 600G. To assess the effect of particle size on the properties of the BC:CMC formulations, the ground powders were then sieved to different particle size ranges: < 100 µm (Endecotts, Ltd, aperture 106 µm), < 300 µm (Endecotts, Ltd, aperture 300 µm).

### *Evaluation of the dispersibility*

To evaluate the dispersibility of the dry and ground BC:CMC mixtures, samples were prepared at 0.5% (m/v on dry basis, by correcting the amount of residual moisture of BC:CMC) in water, at room temperature, by two methods:

- Low mechanical shearing, using a magnetic stirrer (MS) plate (Stuart SD162), at 900 rpm for 5 min;
- High shear mixing, using an Ultra-turrax Homogenizer (UT) Unidrive X1000D (CAT Scientific) with a T20-F generator dispersing tool, at 15,000 rpm for 5 min.

The dispersed materials were spread over a petri dish and observed at naked eye.

### *Microscopic observations*

For fluorescence optical microscopy, never-dried BC samples (0.01% m/v, dry basis) wet-ground by the above-mentioned methods, were placed on glass slides, stained with 10 µg/mL Calcofluor White stain (Sigma) for 1 min (protected from light), coverslipped and visualized on an Olympus BX51 fluorescence

microscope with a DAPI filter (Excitation wavelength: 365–370 nm; Emission wavelength: 421 nm).

BC:CMC powders observation was performed using a desktop Scanning Electron Microscope (SEM) coupled with energy-dispersive X-ray spectroscopy analysis (Phenom ProX, Phenom-World BV). Samples were added to aluminium pin stubs with electrically conductive adhesive carbon tape and coated with 2 nm Au prior to analysis.

### *Size measurements*

The measurement of the BC bundle size was carried out in a Malvern Mastersizer 3000 laser diffraction instrument equipped with a Hydro EV sample dispersion unit (Malvern Panalytical, Malvern, UK). Aqueous suspensions of wet-ground BC samples were added to the sample dispersion unit prefilled with tap water, until an obscuration level between 10 and 20% (as recommended by the equipment manufacturer) was reached. The stirring rate in the dispersion unit was set at 1500 rpm with initial ultrasonication (35 W) for 10 s. The detector array measured the scattering pattern during 30 s. Five measurements were performed per sample. The refractive index of cellulose and water were assumed as 1.468 and 1.330 respectively (as provided by the software's database) and Mie scattering model was used for non-spherical particles. The particles size was then expressed as the volume distribution percentiles, D<sub>v</sub>(50) (Mass Median Diameter, the size at which 50% of the sample is smaller and 50% is larger), D<sub>v</sub>(10) and D<sub>v</sub>(90) (the size of particle below which 10% and 90% of the sample lies, respectively); also the Volume Moment Mean, D[4,3], and the Surface Area Mean, D[3,2].

### *Viscosity measurements*

The viscosity profiles of the redispersed BC:CMC samples were measured in a controlled stress rheometer (DHR-1, TA instruments) with TRIOS Software (TA Instruments) at 25 °C using a cone and plate geometry (60 mm, 2.006° cone angle, 64 µm gap). Prior to analysis, dried and ground samples were redispersed either by magnetic stirring or Ultra-turrax homogenizer. Flow tests were performed in duplicate at increasing shear rates (0.01 to 100 s<sup>-1</sup>). Shear rate versus viscosity graphs were drawn in semi-log scale

to better visualise the different rheological profiles at low shear rates.

### Interfacial tension

Interfacial tension between isohexadecane and aqueous suspensions of the non-dried and dry BC:CMC materials (prepared by magnetic stirring) was measured with the pendant drop method using Optical Contact Angle (OCA 20, DataPhysics), and SCA 20 Software module 22 (DataPhysics). A volume of 2 mL of isohexadecane was placed on a disposable optical polystyrene macro standard fluorescence cuvette (1 cm × 1 cm × 4.5 cm) (Labbox). A disposable 1 mL syringe with a needle of 0.71 mm outer diameter, containing the aqueous sample suspensions (heavier phase) was introduced in the cuvette, with the needle always at the same depth. First, maximum drop volume was determined for each sample. For each drop, profile extraction and IFT calculation were made automatically, 10 times per minute until 25 readings, in triplicate for each sample and at  $20 \pm 1$  °C.

Statistical analysis of these results was performed using GraphPad Prism 5 (GraphPad Software). Results were expressed as mean of three separate measurements and respective Standard Deviation. A one-way analysis of variance (ANOVA) was performed, followed by Tukey's Multiple Comparison Test to establish the significance of differences with the H<sub>2</sub>O control and between all datasets.

### Effect of BC:CMC formulations on the suspension stability of cocoa beverage

#### *Effect of drying and grinding*

The effect of particle size of BC:CMC formulations on the suspension stability of cocoa particles in a chocolate milk beverage, was evaluated. For this, BC:CMC samples (i) never-dried; (ii) dried in Hot Plate, ground and sieved to different particle size ranges), were used at a final concentration of 0.15% (m/v dry basis). Pure cocoa (1.2% m/v) and BC:CMC were added to 15 mL of medium-skimmed milk. The mixtures were stirred in a vortex (2,800 rpm) for 3 min at room temperature and then pasteurized at 75 °C for 15 s. A control, where no BC:CMC was added, was also prepared in the same manner. Samples were stored at room temperature and the cocoa

particles sedimentation was evaluated over time. The stabilization of the chocolate drinks was quantified by calculating the percentage of cocoa sedimentation, according Eq. 1:

$$\text{Stability (\%)} = \left( \frac{\text{Volume of milk with suspended cocoa}}{\text{Total milk volume}} \right) \times 100 \quad (1)$$

where 100% stability corresponds to a fully stabilized cocoa suspension.

#### *Benchmark assay*

Several plant-derived commercial celluloses were used in a benchmarking study: hydrocolloidal microcrystalline Avicel RT1133 and CM2159 with CMC, and non-colloidal Avicel LM310 (FMC Biopolymer); microcrystalline Novagel RCN-15 and RCN-10 (FMC Biopolymer), with Guar gum; sulphated Celluforce NCC nanocrystals (from Celluforce Inc.). These materials-differing in size, morphology, presence of additives and chemical modification-are all marketed as stabilizers and rheology/texture modifiers for food products and have been previously characterized (Martins et al. 2019). Furthermore, Bioplus-L Fibrils (lignin-coated cellulose nanofibrils from wood, American Process, Inc.), although not being commercialized for food applications, were also used for their fibrillar nature and nanoscale thickness, similar to BC. According to the manufacturer, these fibres are 5–200 nm wide and 500 nm to several micron in length.

An assay was done using the same conditions as in “[Effect of drying and grinding](#)” Section., with above referred celluloses and CMC. All Avicel and Novagel celluloses were previously activated, for 30 min, at 23 800 rpm, according to the specification's sheets.

## Results

BC membranes obtained by static culture were processed by wet grinding, mixed with CMC, dried, ground (or dry comminuted) and sieved to different size ranges. The effect of these different and sequential processing methods on the size of BC, dispersibility, rheological behaviour (viscosity) of BC:CMC and its stabilizing effect on a solid/liquid suspension were studied.

## Effect of Wet grinding method on BC bundle size

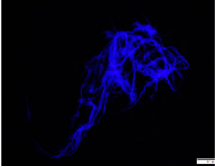
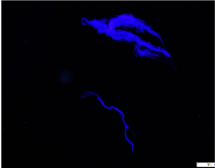
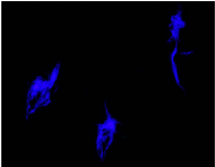
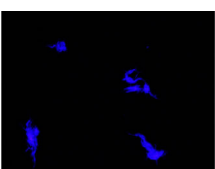
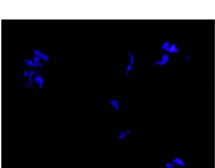
Three BC homogenization methods were used, providing different levels of shear force, leading to different degrees of fibre comminution or disaggregation of the fibre bundles. Table 1 shows the results of the size of the fibre aggregates or flocs, as measured by laser diffraction, as well as illustrative micrographs obtained by staining with Calcofluor White. Images of the same samples observed with a stereo microscope can be found in Supplementary Information (Fig. S1).

When produced under static conditions, BC fibres are organized as a 3D nanofibrillar network of entangled fibres. When submitted to mechanical shear,

the fibre network is broken down into fleeces of flocs, composed mainly by fibre bundles of variable size and shape. These bundles have a star-resembling structure, with loose fibre branches emerging from a deeply entangled core. With increasing grinding shear ( $HB < MHI < HPH$ ), the size of the bundles decreases, the branches become less evident and the core becomes denser. This is especially the case with the use of HPH, where the BC bundles are submitted to cycles of compression and decompression, contrarily to HB and MHI, where the BC bundles are obtained through the mechanical action of the high shear spinning blades.

Regarding the particle size characterization, the  $D[4,3]$  reflects the size of those particles which

**Table 1** Percentiles and Mean Diameters of the particle size distribution (by volume) of BC samples wet-ground by different methods, and respective micrographs (scale bars correspond to 100  $\mu\text{m}$ )

	Percentiles ( $\mu\text{m}$ )			Span	Mean diameters ( $\mu\text{m}$ )		
	Dv(10)	Dv(50)	Dv(90)		D[4,3]	D[3,2]	
HB	254	1228	2299	2	1205	514	
MHI	108	575	1700	3	757	254	
HPH-1	24	79	229	3	107	52	
HPH-2	24	71	187	2	91	50	
HPH-6	22	55	139	2	70	43	

All measurements satisfy the quality criteria since the Residuals (%) and Weighted Residuals (%) are  $\leq 1\%$

*HB* Hand Blender; *MHI* Microcut Head Impeller; *HPH* High-Pressure Homogenizer

constitute the bulk of the sample volume, while D[3,2] is most sensitive to the presence of fine particulates in the size distribution. The differences between these two mean values for the same sample, plus the large variation of the percentiles, indicate its wide size distribution (more evident in the case of HB). HB ground BC showed the highest mean size of fibre bundles. With the increase in the shearing stress (to MHI and HPH) and compression/decompression cycles (HPH 1 to 6 cycles), an increase in the BC defibrillation/fragmentation occurred, as observed in all the values of the percentiles and mean diameters. In all HPH samples only micrometric fragments were observed, most of them being below 100  $\mu\text{m}$ , the micrographs also suggesting that these bundles bear higher density, as mentioned, likely due to the compression forces associated with the homogenization process.

#### Properties of the never-dried BC formulations after CMC addition

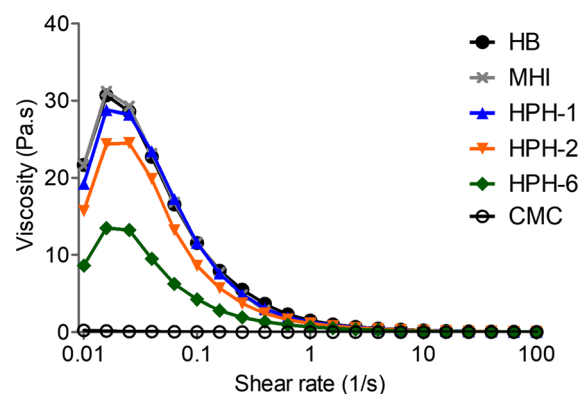
Well-dispersed suspensions are a prerequisite in many industrial applications. Suspensions of cellulosic materials, such as BC, exhibit pronounced aggregation in aqueous media due to strong interfibrillar hydrogen bonds and Van der Waals attractions (Agarwal et al. 2018). To overcome this issue, CMC was added to BC, at mass ratio of 1:1 as described in the “Materials and methods” section. The water-soluble anionic polyelectrolyte plays a determinant role in ensuring the dispersibility of the BC fibres and allowing their stabilization in aqueous media. The negative charge of CMC contributes to the improved dispersion of the BC fibres due to steric hindrance. Non-adsorbed CMC may also prevent the agglomeration of BC fibres due to the creation of a hydration shell around BC, thus also contributing to the improved dispersibility and stability in aqueous media. The presence of CMC improves the final performance of the formulation, even though BC fibres are the responsible for the product’s viscosity and stabilizing ability. The properties of a BC:CMC mixture have been shown to be superior to BC or CMC alone (Martins et al. 2019).

The never-dried BC:CMC samples were magnetically stirred (low energy mixing), or homogenized with Ultra-turrax (high shear mixing). Samples dispersed using only magnetic stirring still showed small fibre bundles and agglomerates, but after treatment

with UT a homogeneous suspension was obtained (minimum of fibre bundles were visually observed). These results also show that, in the conditions used, the wet-grinding of BC is insufficient to yield a fully dispersed fibre suspension after adding CMC, an additional high energy mixing with the UT being necessary to achieve that goal. The viscosity profiles of the BC:CMC samples dispersed with UT are shown in Fig. 2. Although not shown, the analysis of the never-dried BC:CMC samples without UT treatment was performed and the general trend is similar to the one obtained with those processed with the UT, suggesting that UT does not compromise the fibres viscosity—it mainly further disaggregates the larger bundles producing a more homogeneous mixture.

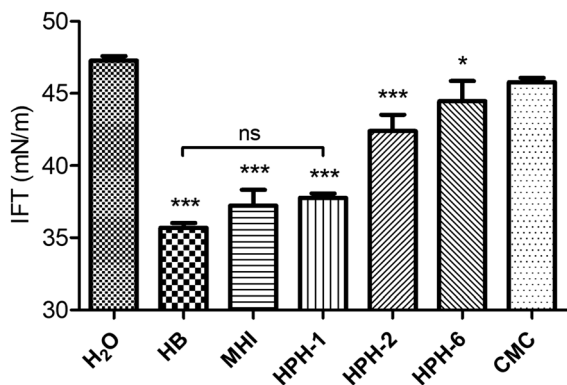
From Fig. 2 it can be seen that after a first shearing, where the molecules in the sample rearrange and orient, all BC:CMC samples show a shear-thinning behaviour. It is also clear that the wet grinding method affected the viscosity profile of the BC:CMC mixtures: in general, the smaller the initial BC fibre bundle size, the lower the viscosity of the BC:CMC dispersions. CMC alone has much lower viscosity than the BC:CMC formulations at the same concentration, so the increase in viscosity can be imparted on the BC fibres.

It was not possible to analyse samples of wet-ground BC without CMC. BC suspensions exhibit pronounced aggregation in aqueous media, making it impossible for the sample to be continuously sheared in the rheometer without entanglements (for the



**Fig. 2** Viscosity profiles of 0.5% (m/v) of CMC and never-dried BC:CMC mixtures with BC from the different grinding methods, diluted and dispersed using Ultra-turrax. HB: Hand Blender; MHI: Microcut Head Impeller; HPH: High-Pressure Homogenizer





**Fig. 3** Effect of wet grinding of BC on the Interfacial Tension of Isohexadecane/Water containing 0.5% (m/v) never-dried BC:CMC. Results are expressed as average of triplicate measurements and bars are representative of the Standard Deviation. All datasets were compared with each other using one-way ANOVA followed by Tukey's Multiple Comparison Test (significant at  $*p < 0.05$ ,  $***p < 0.001$ , when compared with the H<sub>2</sub>O control; ns – not significant comparing HB with HPH-1). HB: Hand Blender; MHI: Microcut Head Impeller; HPH: High-Pressure Homogenizer

viscosity measurements), or to flow through a syringe (for the IFT determination, further below).

Figure 3 shows the results of the interfacial tension between water dispersions of never-dried BC:CMC and isohexadecane, using the Pendant Drop method. Isohexadecane is a lightweight hydrocarbon (0.79 g/mL at 20 °C) used in cosmetic applications and the energy of its interface with distilled water was  $47.3 \pm 0.3$  mN/m. The IFT value significantly decreased in the presence of 0.5% BC:CMC, in BC samples wet ground by HB, MHI and HPH-1, indicating that, despite differences in the mean particle size (Table 1), the fibres possess similar interface activity and can promote the stabilization of this heterogeneous liquid–liquid system. However, the IFT reduction was less and less evident as the BC was more fragmented, becoming very close to that of pure water for the HPH-6 sample. As the effect of non-dried BC:CMC on the isohexadecane/water interface becomes smaller, we can say there's a decrease in the formulation's functionality with the reduction in fibres' size. A similar effect and functionality loss was observed for the viscosity with increasing fragmentation of the fibres (Fig. 2).

### Effect of drying and redispersion methods

Different drying methods have been tested with nanocelluloses (Peng et al. 2013), including BC (Amin et al. 2014; Clasen et al. 2006; Pa'E et al. 2014). Freeze-drying yields best results in preventing hornification in whole BC membranes, since the water is sublimated and the 3D structure is maintained, avoiding collapsing and aggregation of the fibres (Clasen et al. 2006; Klemm et al. 2001; Pa'E et al. 2014). However, freeze-drying is an expensive technology for a scaled-up production process. On the other hand, Amin et al. (2014) also prepared dry powders with purified BC by spray-drying. They employed particle size analysis to determine the ease of redispersion of the powders, only concluding that no further agglomeration occurred between the particles after drying. Besides, the reported swelling index and water retention capacity values show that the powders were only limitedly redispersible, as compared to the water retention capacity of the never-dried BC (over 100 fold the dry weight) (Klemm et al. 2001). In addition, no further tests were performed to assess the preservation of the fibres functionality. In this work we produced a fully redispersible powdered BC:CMC formulation, using simple and more scalable methods: hot plate and oven drying.

Table 2 summarizes the aqueous dispersibility scores for the dried and ground BC:CMC mixtures (size < 300 µm). Dispersibility of the formulations was classified as follows: (1)–sample is homogeneous and no visible particles or aggregates are observable; (2)–sample contains some very small particles or aggregates; (3)–the sample contains some larger particles or aggregates; (4)–non-dispersible, the water remains transparent and the well separated particles or aggregates are observed. Corresponding images of samples bearing the different dispersibility scores can be found in Supplementary Information (Fig. S2).

For dried BC:CMC samples redispersed in water under low shear (magnetic stirring) some fibre bundles or particle fragments were still visible at naked eye, whereas with a high shear treatment (UT), the BC fibres were completely dispersed. This behaviour was consistent in all samples regardless of the wet grinding method; moreover, the material dispersibility was also independent of the presence of residual moisture in the sample, for both 80% and 100% TS materials showed

**Table 2** Dispersibility of BC:CMC mixtures after drying, grinding and sieving to a particle size of < 300  $\mu\text{m}$ 

Wet grinding	Drying	Dispersion	Dispersibility	
HB	Hot Plate, Oven	Magnetic stirrer	2	
		UT Homogenizer	1	
MHI	Hot plate	Magnetic stirrer	2	
		UT Homogenizer	1	
	Oven	80% TS, 100% TS	Magnetic stirrer	2
			UT Homogenizer	1
HPH-1, 2 and 6	Hot plate	Magnetic stirrer	2	
		UT Homogenizer	1	

*HB* Hand Blender; *MHI* Microcut Head Impeller; *HPH* High-Pressure Homogenizer

good dispersion in water (also further improved with UT homogenization).

It is worth to mention that UT homogenization is efficient in redispersing all BC:CMC formulations in aqueous media, independently of the previous processing used; furthermore, with UT homogenization it was also possible to disperse larger size fractions than < 300  $\mu\text{m}$ , and even the whole BC:CMC dry powders before sieving. The need for a formulation with smaller sized particles is only applicable when pursuing a material that can be redispersed using low energy mixing.

In addition to Table 2, redispersion of dry BC (HB treatment, hot plate and oven dried, sieved to < 300  $\mu\text{m}$ ) without CMC was unsuccessful—classified as 4, non-dispersible, even after UT homogenization.

BC:CMC samples dried by either of the tested methods and then ground and sieved to different granulometries were redispersed using low shear (magnetic stirring) and high shear (UT homogenizer). The obtained samples were analysed by rheological tests (Fig. 4).

As observed previously for the never-dried samples, the harsher the wet-grinding treatments (yielding smaller wet BC fibre bundles), the lower the viscosity of the corresponding samples obtained after undergoing drying and redispersion. This tendency can be observed in all graphics from Fig. 4, for both drying methods and for the different dry particle sizes. Thus, the influence of wet-grinding of the BC membranes on viscosity still remains following drying and redispersion.

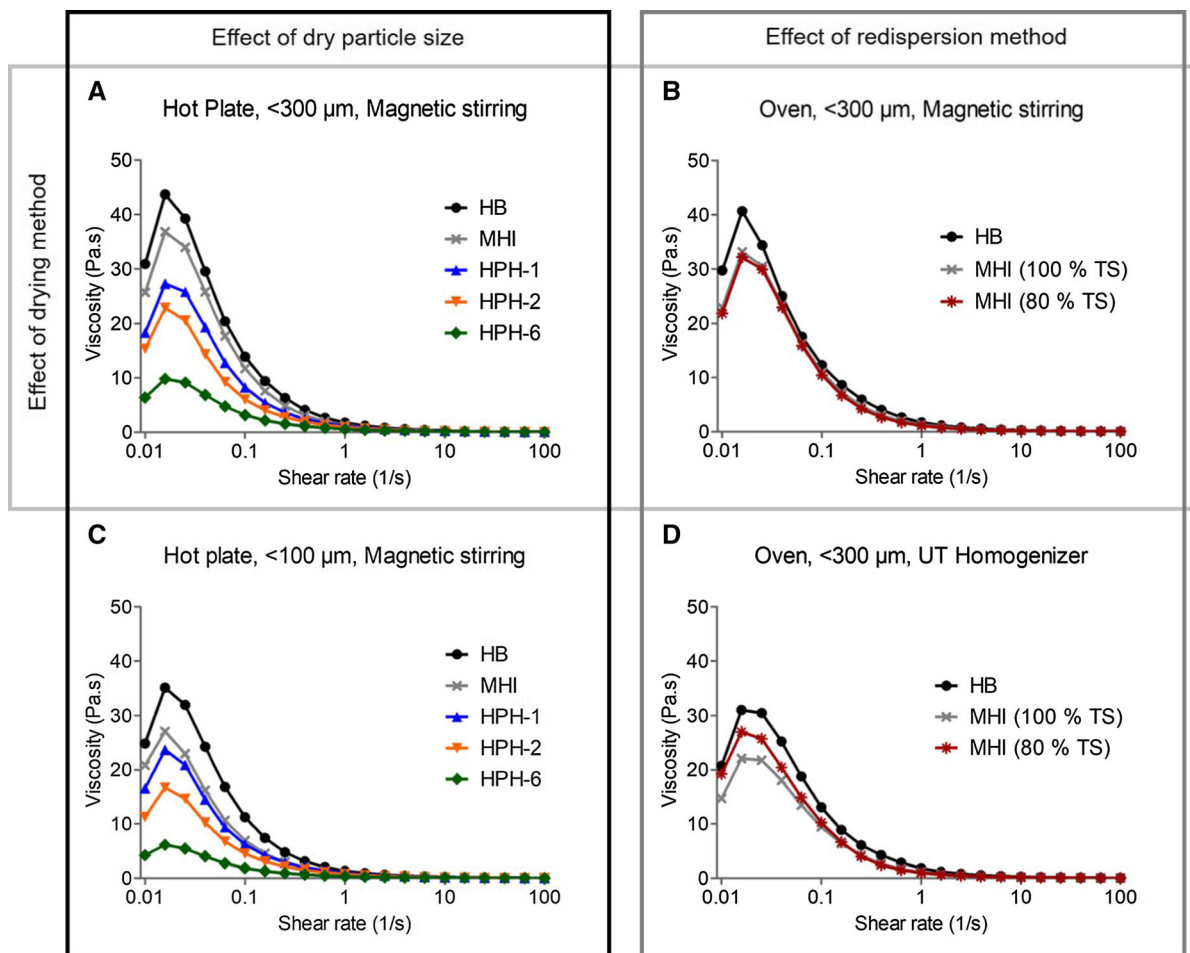
Furthermore, a comparison of Fig. 4A, C shows that samples processed similarly (wet grinding, drying, redispersion), but dry ground to different granulometry present significantly different viscosity

profiles. Samples with particle size < 100  $\mu\text{m}$  present much lower viscosity than the < 300  $\mu\text{m}$  counterpart, confirming the relevance of the grinding process (both on the wet and dry state) on the viscosity of the BC:CMC fibrous suspensions.

Comparing Fig. 4A, B, the drying method (slow oven drying or the fast hot plate drying) did not affect significantly the viscosity profile of redispersed BC:CMC, with the HB and MHI samples having close viscosity profiles for the two drying methods. Also, regarding residual moisture, the MHI sample dried to 80% TS had equal profile to the one dried to 100% TS (Fig. 4B), such that the extent of drying also does not significantly affect the viscosity.

All the redispersed samples in Fig. 4A showed a similar or higher viscosity than the never-dried ones (Fig. 2). Thus, redispersion using low shear stress mixing, at room temperature and under 5 min, was enough to allow for the recovery (even slight improvement) of the rheological profile of the non-dried BC:CMC formulations. However, the occurrence of small aggregates of BC in the redispersed BC:CMC suspension (Table 2) may contribute to the increase in the apparent viscosity profile. Indeed, dispersion of the dry materials with UT slightly reduced the rheological profile (as compared to the samples dispersed in the magnetic stirrer), as can be seen from the HB and MHI samples viscosity in Fig. 4A, D, possibly due a better hydration of the powders and the reduction of some fibre agglomerates.

Overall, these results demonstrate that it is the BC wet-grinding, not the drying process, that has a relevant impact on the properties of its powdered formulations. In the presence of CMC, BC fibres are somewhat protected and less exposed to the negative effects that some drying methods might have on the



**Fig. 4** Dynamic viscosity profiles of 0.5% (m/v) BC:CMC powders redispersed in water, following different drying (hot plate or oven) and comminution (to sizes  $< 300 \mu\text{m}$  or  $< 100$

$\mu\text{m}$ ), and redispersed using UT homogenizer or Magnetic stirring. HB: Hand Blender; MHI: Microcut Head Impeller; HPH: High-Pressure Homogenizer

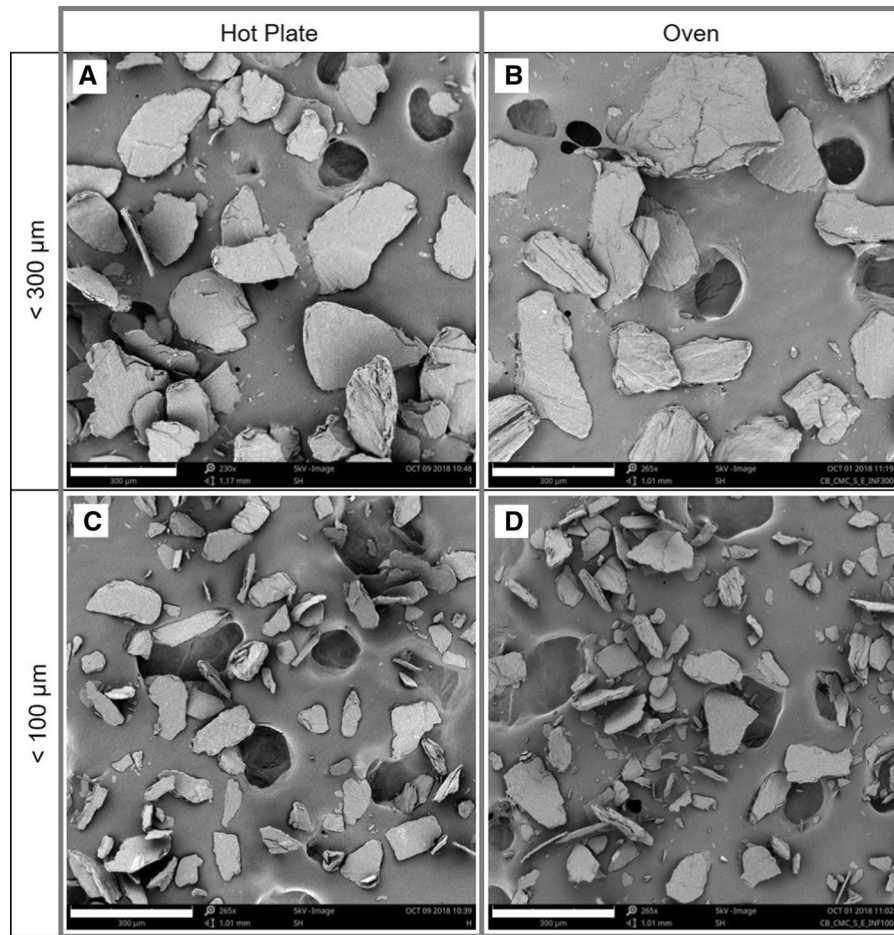
fibres. This way, higher temperatures and less time and energy consuming processes can be employed, without jeopardizing the functionality of the material after redispersion and rehydration. In sum, the drying method (either slower or faster) and temperature do not affect the redispersion and the rheological profile of BC:CMC formulations and, contrarily to what has been proposed in other works (Watanabe et al. 2000), drying can be done to the full extent.

#### Effect of dry comminution

As an example, Fig. 5 shows SEM micrographs of BC:CMC powders produced from HB processed BC and comminuted to different particle sizes.

The separation of the BC:CMC particles after grinding was done by mechanical means (sieves with different mesh apertures). The BC:CMC powders resemble thin flakes of varying shape, such that some particles with larger length than the mesh aperture may have passed perpendicularly through the sieve. Thus, although the size assigned to the samples ( $< 100$  or  $< 300 \mu\text{m}$ ) is in fact indicative, two populations of particles (larger and smaller) were obtained through sieving.

Table 3 summarizes the main observations pertaining to the dispersibility of BC:CMC dry formulations ground and sieved to a size  $< 100 \mu\text{m}$ . To simplify the demonstration of the results, only hot plate dried samples' results are shown. The same profile was observed for samples dried in an oven.



**Fig. 5** SEM images of BC:CMC powders obtained from HB processed BC, **A** dried in a Hot plate, ground and sieved to < 300  $\mu\text{m}$ ; **B** dried in an Oven, ground and sieved

to < 300  $\mu\text{m}$ ; **C** dried in a Hot plate, ground and sieved to < 100  $\mu\text{m}$ ; **D** dried in an Oven, ground and sieved to < 100  $\mu\text{m}$ . All white scale bars correspond to 300  $\mu\text{m}$

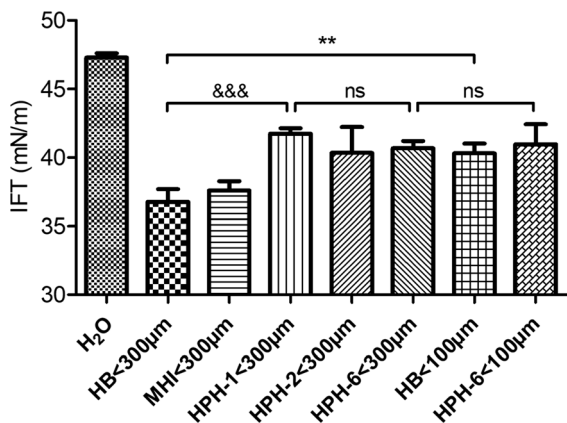
**Table 3** Dispersibility of BC:CMC mixtures after drying, grinding and sieving to a particle size < 100  $\mu\text{m}$

Wet Grinding	Drying	Dispersion	Dispersibility
HB, MHI, HPH-1, 2 and 6	Hot plate	Magnetic stirrer	1
		UT Homogenizer	1

*HB* Hand Blender; *MHI* Microcut Head Impeller; *HPH* High-Pressure Homogenizer

Decreasing the dry particle size of the BC:CMC samples to < 100  $\mu\text{m}$  allows for a faster dispersion (complete under 1 min) of the powders at low shear magnetic stirring (Table 3), as compared to particles < 300  $\mu\text{m}$ , where a complete dispersion was only achieved after UT homogenization of all samples (Table 2).

However, with the decrease in particle size there is also a decrease in the viscosity profile, as noted previously (Fig. 4A, C). Increasing the particle size (from < 100  $\mu\text{m}$  to < 300  $\mu\text{m}$ ), decreases the ease of dispersion under low shear mixing, but maintains more of the fibre's functionality, as perceived by the higher viscosity profiles.



**Fig. 6** Interfacial tension of Isohexadecane/Water containing dried BC:CMC at 0.5%, ground and sieved to different size ranges. Results are expressed as average of triplicate measurements and bars are representative of the Standard Deviation. All datasets were compared with each other using one-way ANOVA followed by Tukey's Multiple Comparison Test (all samples were significantly different at  $p < 0.001$  when comparing with the H<sub>2</sub>O control; &&&  $p < 0.001$  comparing HB < 300 µm with HPH-1 < 300 µm; \*\*  $p < 0.01$ , comparing HB < 300 µm and HB < 100 µm; ns – not significant, comparing HPH-1 < 300 µm with HPH-6 < 300 µm, and HPH-6 < 300 µm with HPH-6 < 100 µm). HB: Hand Blender; MHI: Microcut Head Impeller; HPH: High-Pressure Homogenizer

The redispersed samples were also analysed for their ability to stabilize isohexadecane/water interfaces (Fig. 6).

Overall, the effect of the technology used for BC wet-grinding on the ability of BC:CMC to reduce the interfacial tension, observed in the non-dried samples (Fig. 3), is still patent in the corresponding samples obtained after drying and redispersion. Actually, the results obtained with samples HB and MHI after drying and redispersion are similar to those of the never dried counterpart (Fig. 6 vs Fig. 3), while a deterioration of the activity of the sample HPH-1 was observed as a consequence of further processing. Further decreasing the size of wet BC (from HPH-1 Pass to HPH-6), has no further influence on the IFT values. Importantly, these results also show that too extensive dry comminution (e. g. HB, ground from < 300 to < 100 µm) affects the functionality of the BC:CMC mixture, as shown also concerning the viscosity of the samples (Fig. 4A, C).

#### Functionality assessment: suspension stability of a cocoa beverage

The dried and non-dried BC:CMC were used in a stability test using a solid-in-liquid suspension, a cocoa and milk system. The stabilizing effect of the different samples was determined using Eq. 1 and the results are summarized in Table 4. An example of the aspect of samples with different levels of stabilization percentage can be found in Supplementary Information (Fig. S3). Percentages of stability under 10% were considered as complete sedimentation (c.s.) of the cocoa particles. Only one of each Avicel and Novagel celluloses are shown because all the results were similar.

Hydrocolloids are used in heterogeneous systems such as chocolate milk and other beverages (solid-in-liquid suspensions) to increase their viscosity, having a double function of improving the mouthfeel and preventing the sedimentation of suspended particles-improved rheology (Saha and Bhattacharya 2010).

Microcrystalline cellulose (MCC) and cellulose derivatives, namely CMC, are employed for this purpose. Concentrations over 0.75% of CMC are needed for the change in rheological behaviour of milk to be noticeable (Bayarri et al. 2009). For MCCs such as Avicel products, concentrations of 0.5% are necessary to completely avoid cocoa particles sedimentation. MCC acts beyond the simple thickening of the media: it forms aggregate structures with the cocoa particles, also further interacting with the milk components, creating network structures responsible for the stable suspensions (Yaginuma and Kijima 2006). BC:CMC formulations (1:1) are also able of creating structured networks and having a positive effect on rheological properties of heterogeneous systems, at concentrations as low as 0.1% (Martins et al. 2019).

Results in Table 4 were obtained with only 0.15% of the different powders or non-dried formulations, and simple vortex agitation was employed, followed by a short heat treatment. HB and MHI samples preserved their stabilizing capabilities after drying and comminution, even with the smaller sizes (< 100 µm), maintaining fully stable suspensions for up to 4 days. A more extensive homogenization with HPH-1 only showed stability for the never-dried BC:CMC and the dry formulation with a size of < 300 µm; for 2 and 6 cycles of HPH, not even the never dried material was capable of complete stabilization for 4 days, and the

**Table 4** Stabilization of cocoa particles in chocolate milk with BC:CMC, added at 0.15% (m/v), at room temperature

Wet grinding	Drying	Particle size ( $\mu\text{m}$ )	Stability (%)		
			1 h	24 h	4 days
HB	Hot plate	< 100	100	100	83
		< 300	100	100	100
	Never-dried	-	100	100	100
MHI	Hot plate	< 100	100	100	100
		< 300	100	100	100
	Never-dried	-	100	100	100
HPH-1	Hot plate	< 100	100	67	50
		< 300	100	100	100
	Never-dried	-	100	100	100
HPH-2	Hot plate	< 100	100	67	50
		< 300	100	100	83
	Never-dried	-	100	100	83
HPH-6	Hot plate	< 100	100	27	33
		< 300	100	100	50
	Never-dried	-	87	83	67
Avicel CM 2159	UT, 30 min		95	c.s	c.s
Novagel RCN-15	UT, 30 min		96	c.s	c.s
Bioplus-L Fibrils	UT, 30 min		70	40	35
CelluForce NCC	UT, 30 min		100	100	100
CMC	-	-	40	c.s	c.s
Control	-	-	c.s	c.s	c.s

HB Hand Blender; MHI Microcut Head Impeller; HPH High-Pressure Homogenizer; c.s. complete sedimentation

difference in the dry particle size becomes more evident, as the materials < 100  $\mu\text{m}$  are much less efficient than the ones < 300  $\mu\text{m}$ . As seen before, extensive grinding of the wet fibres, and then of the dried material to a particle size < 100  $\mu\text{m}$ , reduces the stabilizing effect of BC:CMC. To the exception of Celluforce, all colloidal plant celluloses and nanocelluloses used as benchmark showed a very low stabilizing effect on cocoa particles. It is also worth to notice that CMC alone was unable to fully stabilize the system, so the stabilizing effect can be imparted to the BC fibres in the BC:CMC formulations.

## Conclusions

Dry formulations of BC:CMC, able to redisperse in a few minutes under magnetic stirring, were developed. The redispersed material preserves the properties of

the non-dried samples, namely concerning viscosity, the ability reduction of the interfacial activity of oil/water interface and the stabilization of multiphase systems (liquid–liquid and solid–liquid).

It is demonstrated for the first time that the size of the BC fibre bundles is of paramount relevance concerning BC:CMC functionality and potential applications in the food and cosmetic fields. Thus, the control of the grinding process, both on the wet and dry materials, is of utmost importance, while the drying process (time and temperature) seems not to be very relevant in this regard after adding CMC as co-drying agent.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

- Agarwal D, MacNaughtan W, Foster TJ (2018) Interactions between microfibrillar cellulose and carboxymethyl cellulose in an aqueous suspension. *Carbohyd Polym* 185:112–119. <https://doi.org/10.1016/j.carbpol.2017.12.086>
- Amin MCIM, Abadi AG, Katas H (2014) Purification, characterization and comparative studies of spray-dried bacterial cellulose microparticles. *Carbohyd Polym* 99:180–189. <https://doi.org/10.1016/j.carbpol.2013.08.041>
- Bayarri S, González-Tomás L, Costell E (2009) Viscoelastic properties of aqueous and milk systems with carboxymethyl cellulose. *Food Hydrocoll* 23:441–450. <https://doi.org/10.1016/j.foodhyd.2008.02.002>
- Butchosa N, Zhou Q (2014) Water redispersible cellulose nanofibrils adsorbed with carboxymethyl cellulose. *Cellulose* 21:4349–4358. <https://doi.org/10.1007/s10570-014-0452-7>
- Clasen C, Sultanova B, Wilhelms T, Heisig P, Kulicke WM (2006) Effects of different drying processes on the material properties of bacterial cellulose membranes. *Macromol Sy* 244:48–58. <https://doi.org/10.1002/masy.200651204>
- Kargl R, Mohan T, Bračč M, Kulterer M, Doliška A, Stana-Kleinschek K, Ribitsch V (2012) Adsorption of carboxymethyl cellulose on polymer surfaces: evidence of a specific interaction with cellulose. *Langmuir* 28:11440–11447. <https://doi.org/10.1021/la302110a>
- Keshk SM (2014) Bacterial cellulose production and its industrial applications. *J Bioprocess Biotech*. <https://doi.org/10.4172/2155-9821.1000150>
- Khoshkava V, Kamal MR (2014) Effect of drying conditions on cellulose nanocrystal (CNC) agglomerate porosity and dispersibility in polymer nanocomposites. *Powder Technol* 261:288–298. <https://doi.org/10.1016/j.powtec.2014.04.016>
- Klemm D, Schumann D, Udhardt U, Marsch S (2001) Bacterial synthesized cellulose—Artificial blood vessels for microsurgery. *Prog Polym Sci* 26:1561–1603. [https://doi.org/10.1016/S0079-6700\(01\)00021-1](https://doi.org/10.1016/S0079-6700(01)00021-1)
- Klemm D, Kramer F, Moritz S, Lindström T, Ankerfors M, Gray D, Dorris A (2011) Nanocelluloses: a new family of nature-based materials. *Angew Chem Int Edit* 50:5438–5466. <https://doi.org/10.1002/anie.2010101273>
- Kondo T, Rytczak P, Bielecki S (2016) Bacterial nanocellulose characterization. In: Gama M, Dourado F, Bielecki S (eds) *Bacterial nanocellulose—From biotechnology to bioeconomy*. Elsevier B.V, Amsterdam, pp 59–71
- Lee KY, Buldum G, Mantalaris A, Bismarck A (2014) More than meets the eye in bacterial cellulose: biosynthesis, bioprocessing, and applications in advanced fiber composites. *Macromol Biosci* 14:10–32. <https://doi.org/10.1002/mabi.201300298>
- Martins D, Estevinho B, Rocha F, Dourado F, Gama M (2019) A dry and fully dispersible bacterial cellulose formulation as a stabilizer for oil-in-water emulsions. *Carbohyd Polym* 230:115657. <https://doi.org/10.1016/j.carbpol.2019.115657>
- Naderi A, Lindström T, Sundström J, Flodberg G (2015) Can redispersible low-charged nanofibrillated cellulose be produced by the addition of carboxymethyl cellulose? *Nord Pulp Paper Res J* 30:568–577. <https://doi.org/10.3183/npprj-2015-30-04-p568-577>
- Nechita P, Panaitescu DM (2013) Improving the dispersibility of cellulose microfibrillated structures in polymer matrix by controlling drying conditions and chemical surface modifications. *Cell Chem Technol* 47:711–719
- Newman RH (2004) Carbon-13 NMR evidence for cocrystallization of cellulose as a mechanism for hornification of bleached kraft pulp. *Cellulose* 11:45–52. <https://doi.org/10.1023/B:CELL.0000014768.28924.0c>
- Ougiya H, Watanabe K, Morinaga Y, Yoshinaga F (1997) Emulsion-stabilizing effect of bacterial cellulose. *Biosci Biotechnol Biochem* 61:1541–1545. <https://doi.org/10.1271/bbb.61.1541>
- Pa'E N, Hamid NIA, Khairuddin N, Zahan KA, Seng KF, Siddique BM, Muhamad II (2014) Effect of different drying methods on the morphology, crystallinity, swelling ability and tensile properties of Nata De Coco. *Sains Malays* 43:767–773. [https://journalarticle.ukm.my/7159/1/16\\_Norhayati\\_PaE.pdf](https://journalarticle.ukm.my/7159/1/16_Norhayati_PaE.pdf)
- Paximada P, Koutinas AA, Scholten E, Mandala I (2016) Effect of bacterial cellulose addition on physical properties of WPI emulsions. Comparison with common thickeners. *Food Hydrocoll* 54:245–254
- Peng Y, Gardner DJ, Han Y, Kiziltas A, Cai Z, Tshabalala MA (2013) Influence of drying method on the material properties of nanocellulose I: thermostability and crystallinity. *Cellulose* 20:2379–2392. <https://doi.org/10.1007/s10570-013-0019-z>
- Rodrigues AC, Fontão AI, Coelho A, Leal M, Soares da Silva FAG, Wan Y, Dourado F, Gama M (2019) Response surface statistical optimization of bacterial nanocellulose fermentation in static culture using a low-cost medium. *New Biotechnol* 49:19–27. <https://doi.org/10.1016/j.nbt.2018.12.002>
- Saha D, Bhattacharya S (2010) Hydrocolloids as thickening and gelling agents in food: a critical review. *J Food Sci Tech* 47:587–597. <https://doi.org/10.1007/s13197-010-0162-6>
- Tamarate P (1999) US Pat. No. 5962676-Processes for the modification and utilization of bacterial cellulose. Thailand
- Watanabe K, Shibata A, Ougiya H, Hioki N, Morinaga Y (2000) US Pat. No. 6153413-Method for processing bacterial cellulose. Japan
- Yaginuma Y, Kijima T (2006) Effects of microcrystalline cellulose on suspension stability of cocoa beverage. *J Disper Sci Technol* 27:941–948. <https://doi.org/10.1080/01932690600766306>
- Yang Z-F, Sharma S, Mohan C, Kobzeff J (2001) WO 01/05838A1-process for drying reticulated bacterial cellulose without co-agents. United States

- Yang Z-F, Morrison NA, Talashek TA, Brinkmann DF, DiMasi D, Chen YL (2011) US Pat. No. 8053216B2-Bacterial cellulose-containing formulations. United States
- Zemljic FL, Stenius P, Laine J, Stana-Kleinschek K, Ribitsch V (2006) Characterization of cotton fibres modified by carboxymethyl cellulose. *Lenzing Berichte* 85:68–76
- Zhai X, Lin D, Liu D, Yang X (2018) Emulsions stabilized by nanofibers from bacterial cellulose: new potential food-grade pickering emulsions. *Food Res Int* 103:12–20. <https://doi.org/10.1016/j.foodres.2017.10.030>
- Zhao GH, Kapur N, Carlin B, Selinger E, Guthrie JT (2011) Characterisation of the interactive properties of microcrystalline cellulose–carboxymethyl cellulose hydrogels. *Int J Pharm* 415:95–101. <https://doi.org/10.1016/j.ijpharm.2011.05.054>

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