The chemical-free production of nanocelluloses from microcrystalline cellulose and their use as Pickering emulsion stabilizer

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Highlight

- Production of cellulose nanoparticles using microfluidization or nearcritical water
- Two environmentally-friendly, entirely water-based pathways
- GPC, WAXS and TEM characterization of the particles revealed key differences
- Both types of particles are able to stabilize oil-in-water emulsions

Abstract

This paper takes a comparative approach in characterizing two types of nano-scale cellulosic particles obtained using chemical-free pathways, either by nearcritical water treatment or by high-shear homogenization from the same microcrystalline cellulose (MCC). The nearcritical water treatment efficiently depolymerized cellulose, producing a solid precipitated fraction of low-molecular-weight material containing cellulose II, while homogenization mechanically
deconstructed MCC without altering its molecular structure. Both pathways yielded nanocellulose-like materials yet with different morphologies. The mechanically produced, rod-like particles were obtained with high yield. In contrast, the hydrothermal precipitate exhibited more hydrophobic ribbon-like particles that provided a greater level of particle-particle interaction. Both materials successfully acted as stabilizers for oil-in-water Pickering emulsions; however, the hydrothermally-produced material exhibited superior performance, with stable emulsions obtained upon addition of as low as 1.0 wt.% cellulose. These two pathways are highly relevant for altering the structure and properties of MCC and for formulating new, sustainably produced nanocellulose-based materials.

Keywords
Microcrystalline cellulose; nearcritical water; microfluidization; nanocellulose; Pickering emulsions

1. Introduction
Cellulose is the most abundant biopolymer on Earth and a versatile raw-material for producing materials, specialty chemicals, and advanced biofuels (Klemm, Heublein, Fink, & Bohn, 2005). Cellulose-based materials can be today obtained using a variety of industrially mature mechanical, chemical, biochemical or thermochemical pathways (Bozell et al., 2000; Hallac & Ragauskas, 2012; Röder et al., 2013). Within the past two decades nano-scale cellulose particles, or nanocellulose, have opened up a new range of potential applications mainly through two categories of products: cellulose nanofibrils (CNF) and cellulose nanocrystals (CNC). These materials, whose properties are strongly influenced by the shape and size of the particles, the raw material, as well as the preparation method, usually exhibit great mechanical strength and are suitable in numerous applications, from reinforcing phases in bio-nanocomposites to hybrid and functional materials (Abitbol et al., 2016).

Among the potential applications for nano-scale cellulose particles is their use as additives in various contexts, for example to take advantage of the rheological properties of cellulose suspensions (Salas, Nypelö, Rodriguez-Abreu, Carrillo, & Rojas, 2014). Nanocellulosic materials have especially attracted significant interest in their ability to stabilize oil-in-water Pickering emulsions. Conventional emulsions are stabilized using surfactants, an alternative to which is the use of nanoparticles that can stabilize two-phase systems at the oil-water interface to
create stable Pickering emulsions (Chevalier & Bolzinger, 2013). The amphiphilic properties of cellulose is one important factor that can explain the stabilization phenomenon (Kalashnikova, Bizot, Cathala, & Capron, 2012). The high aspect ratio of the nanowhiskers or nanofibers also favors the formation of a homogeneous coverage at the hydrophilic-lipophilic interface, which can become as strong as to make these emulsions redispersible after drying (Hu, Marway, Kasem, Pelton, & Cranston, 2016). Thus, numerous types of nanocellulose have recently been shown to successfully stabilize emulsions (Gestranius, Stenius, Kontturi, Sjöblom, & Tammelin, 2016; Jia et al., 2013; Kalashnikova, Bizot, Cathala, & Capron, 2011). However, their production usually involves enzymatic or chemical treatment steps that might limit the extent of their possible use in highly sensitive applications, such as pharmaceuticals, foods, and cosmetics.

CNC and CNF are two distinct materials with very different production pathways that in turn affect the final properties, which can be tailored to specific uses as hierarchical materials (Tingaut, Zimmermann, & Sèbe, 2012). CNF are web-like fibrous mass that have a nano-dimensional cross section. Current CNF production methods involve a preliminary chemical fractionation, or pulping, that extracts cellulose from the lignocellulosic feedstock, such as wood. The obtained pulp fibers are then mechanically deconstructed, or fibrillated, by either grinding or high-shear homogenization. The fibrillation is usually achieved with the help of a pretreatment that decreases the raw energy input and modify the morphological, mechanical, and surface properties of the final material (Dufresne, 2013). Such pretreatments include the TEMPO-mediated oxidation, the mineral acid hydrolysis, or the enzymatic pre-hydrolysis of the fibers. Therefore, conventional CNF production methods are generally multi-step, energy-intensive processes that require various amounts of potentially harmful products. Moreover, CNF typically exhibit high viscosity at low consistency, which limits their maximum consistency to a few percent only. This combined with their inherent hydrophilicity created the need for additional, complex functionalization routes (Missoum, Belgacem, & Bras, 2013).

In contrast, CNC are charged nanoparticles presenting a rod-shaped morphology. They usually possess unique optical properties as they can self-assemble to form chiral, nematic structures (Dufresne, 2013). Producing CNC mainly involves the acid-catalyzed hydrolysis of the cellulosic feedstock using concentrated, strong mineral acids (Habibi, Lucia, & Rojas, 2010). High-purity cellulosic pulp or cotton linters are the usual raw materials for preparing nanocellulose, and the properties of the final CNC varies depending on which acid is used for the hydrolysis (George &
S N, 2015). However, microcrystalline cellulose (MCC), the product of mild acid-catalyzed hydrolysis of cellulosic fibers and a common additive in food, cosmetics, and pharmaceuticals, has also recently gained interest as a valid intermediate material for producing nanocellulosic materials. CNC-like rods can be directly obtained from MCC through a simple mechanical pathway using high-shear homogenization, without the need for any additional treatment step or chemicals. The properties of the resulting material proved sufficient for some applications, such as acting as reinforcing matrix in hydroxyl propyl cellulose-CNC bionanocomposites (Bandera et al., 2014).

In addition to mechanical deconstruction, a second chemical-free pathway is being developed that can produce CNC from MCC, only requiring pure water heated at 120 °C and pressurized at 20.3 MPa instead of concentrated mineral acids (Lísias P. Novo, Bras, García, Belgacem, & Curvelo, 2015; Lísias Pereira Novo, Bras, García, Belgacem, & Curvelo, 2016). Such hydrothermal pathway is a promising route for the processing of MCC in general. When working at even higher temperatures and pressures, nearcritical water (water under pressure above 22.1 MPa and temperature between 300 °C and 374°C) triggers the nearly simultaneous hydrolysis and dissolution of a cellulose suspension. The nearcritical water treatment of MCC yields a mixture of soluble glucose and oligomers as well as a fraction of insoluble, low-molecular-weight cellulose precipitate (Sasaki, Fang, Fukushima, Adschiri, & Arai, 2000). The precipitated material, generated through cooling from a dissolved state, is composed of highly crystalline cellulose II allomorph with an unusually low degree of polymerization (DP) (Ehara & Saka, 2002; Sasaki, Adschiri, & Arai, 2004; Yu & Wu, 2009). In addition, it can account for up to 60% of the product mass balance depending on the experimental conditions and on the type of MCC (Tolonen, Penttilä, Serimaa, & Sixta, 2015).

Intense scientific activity over the past 20 years brought substantial knowledge and awareness about the potential of nearcritical water for partially or totally depolymerizing cellulose (Buffiere, Ahvenainen, Borrega, Svedström, & Sixta, 2016; Cantero, Vaquerizo, Mato, Bermejo, & Cocero, 2015; Tolonen, Juvonen, et al., 2015). However, to date no paper has put its focus on characterizing this precipitated fraction nor considered it as a potentially valuable and novel product from MCC. Therefore, the aim of this study was to investigate the potential of both mechanical and hydrothermal processes as two separate chemical-free pathways to produce nanocellulosic materials from MCC. This work covers the structure and morphology of particles
produced from MCC using high-shear homogenization and nearcritical water in a comparative study. In addition, the paper explores the suitability of these particles as stabilizers in oil-in-water Pickering emulsions.

2. Experimental

2.1. Preparation of low-molecular-weight cellulose precipitate

The starting MCC was purchased from Merck (Darmstadt, Germany; Ref. 1.02330) and used as received. A 1.0 wt.% MCC suspension was prepared with deionized water and thoroughly stirred for at least one hour prior to the treatment to ensure sufficient hydration, after which it was fed to a continuous reactor whose description and operating principle were already presented in an earlier paper (Tolonen, Penttilä, Serimaa, Kruse, & Sixta, 2013). In short, the cellulose suspension was mixed with supercritical water at the one end of a tubular reactor (length 55 mm, diameter 3 mm). The mixture was then cooled down to subcritical temperatures at the other end by addition of a cold water flow and depressurized within a few seconds by means of a back-pressure regulator. The operating conditions inside the reactor were chosen at a pressure of 25 MPa, a temperature of 370 °C, and a residence time of 0.4 seconds, conditions which were found to maximize the yield of precipitate for this reactor while only leaving a limited amount of undissolved MCC. Under these conditions the final precipitate concentration was around 350 mg.l⁻¹ based on gravimetry, of which around 100 mg.l⁻¹ accounted for undissolved residue (Buffiere et al., 2016). After the treatment, the sample was let to settle for a period of one week, after which the solid fraction was washed upon three successive centrifugations at 4000 rpm for 20 min where the supernatant was replaced by deionized water. After final removal of the supernatant the resulting material had a white, gel-like texture, and a dry matter content of around 15 wt.%. It is subsequently referred to as hydrothermal cellulose (HTC).

2.2. Preparation of homogenized microcrystalline cellulose

The same MCC was processed via a mechanical pathway according to a method previously described in the literature (Bandera et al., 2014). The operation was achieved using a high-shear homogenizer, where a 15 wt.% suspension of MCC was pumped through two high-shear chambers at 400 kPa. The operation was repeated 15 times until no change in viscosity could be observed when checked with a rotating cylinder rheometer. The first three passes were performed using 400 µm and 200 µm chambers, while the remaining passes were done using 200
µm and 75 µm chambers. Small amounts of water were added to the suspension in between passes to limit the viscosity increase of the suspension and ensure a proper flow through the system. The resulting material had a consistency of around 9 wt.%, increased up to 15 wt.% upon centrifugation and removal of the supernatant. It is subsequently referred to as microfluidized cellulose (MFC).

2.3. Structural characterization

Size-exclusion chromatography (SEC) was performed using a Dionex Ultimate 3000 system equipped with a guard column and four identical Agilent PLgel Mixed-A columns (Agilent, USA) and a Shodex RI-101 refractive index detector (Showa Denko K.K., Japan). The samples were activated and dissolved using an already described method and eluted in 0.75 ml/min DMAc/LiCl 9.0 g.L⁻¹ (Buffiere et al., 2016). The calibration was achieved using a set of eleven narrow pullulan standards with molecular weights comprised between 342 Da and 2,560 kDa (Standard Polymer Service Gmbh, Germany, and Fluka Gmbh, Germany).

Small-angle x-ray scattering (SAXS) measurements were performed using a Bruker Nanostar U diffractometer (Bruker AXS, Germany) with Cu Kα radiation (k=1.54059 Å), and equipped with a Väntec-2000 MikroGap area detection system. MCC was packed as received, while 15 wt.% HTC and MFC suspensions were first frozen using liquid nitrogen, freeze-dried for 24 hours, then ground using a mortar and pestle. Powders were packed into Mark glass tubes of 1 mm outside diameter and 0.01 mm wall thickness. The irradiation time was 3600 s and the sample-to-detector distance was 107 cm.

Powder X-ray diffraction (XRD) was carried out with a Bruker D8 Advance diffractometer. The measurements were achieved using the same freeze-dried powders as for SAXS. Cu Kα source was used with a Göbel mirror in a parallel beam setup with a 1.2 mm aperture slit. The applied accelerator voltage and current were 40 kV and 40 mA, respectively, which allowed collecting information from a large area. The detector was a Vantec-1 position sensitive detector. The different powders were filled in flat plastic holders with 20 mm diameter and 1.5 mm depth. Standard theta-two theta scans between the two theta values of 5° and 40° were used, while the specimen holder was also rotated for the best averaging. Apart from the cellulose powders, the signal from an empty specimen holder (for background determination) and a corundum standard (to measure the instrument function for peak width determination) were also measured. The
crystallite size was determined by the Scherrer equation using the 200 lattice plane for MCC and MFC, and the 020, 110 and 1-10 lattice planes for HTC. The peak width was measured using the standard DIFFRAC EVA software. The crystallinity was determined by two methods, the XRD peak deconvolution and the XRD peak height method, when the latter was possible (Park, Baker, Himmel, Parilla, & Johnson, 2010). For the deconvolution method, the background from the specimen holder was subtracted and the remaining signal was fitted into six peaks, of which five were assumed crystalline and one amorphous.

2.4. Morphological characterization

Transmission electron microscopy (TEM) was performed using a Jeol FS 2200 microscope operated at an acceleration voltage of 200 kV in scanning mode. Lacey carbon film on copper grid were treated for ten seconds with a Fischline 1020 plasma cleaner, after which a drop of dilute 0.01 wt.% cellulose suspension was deposited. The samples were negatively stained using uranyl acetate in order to achieve better contrast. The excess of water was removed after both applying the suspension and the staining solution after 20 s by means of capillary forces by placing a filter paper in contact with the bottom side of the grid. The micrographs were recorded using a High-Angle Annular Dark Field detector.

Atomic force microscopy (AFM) was performed using a Bruker Nanoscope V MultiMode apparatus. Imaging was done in tapping mode in air using a MicroMash silicon cantilever with a resonance frequency range of 300-360 kHz. The resulting images were flattened using Nanoscope Analysis software, which also provided direct quantification of the thickness of the particles through a height profile.

Scanning electron microscopy (SEM) images were obtained by placing one drop of 0.1 wt.% cellulose suspension on a high-purity mica surface that was preliminarily cleaned by removing its upmost layer with double-sided tape. The drop was dried in vacuum and sputtered directly with a platinum layer of 8 nm (BAL-TEC AG, Lichtenstein). Images of films were obtained by drying 5 g of 15 wt.% suspension placed into a glass petri dish overnight in an oven at 60 °C. The images were recorded on a FEI Nova Nano SEM 230 instrument (FEI, USA) with an accelerating voltage of 5 kV and a working distance of 5 mm.

2.5. Material properties
Rheological investigations were performed at 23 °C by means of an Anton Paar 300 oscillatory rheometer (Anton Paar, Germany) with parallel-plate geometry. The upper plate had a diameter of 25 mm and the gap between the plates was set at 2 mm. The linear visco-elastic range of the sample was preliminarily obtained from an amplitude sweep using a constant angular frequency of 1.0 s⁻¹ with strain amplitude varying from 0.01% to 500%. Complex viscosity, storage modulus (G'), and loss modulus (G'‘) were measured as a function of the angular frequency using oscillatory measurements in the range 0.01 s⁻¹ - 100 s⁻¹ with a fixed strain amplitude of 0.7%.

The surface free energy of the materials was assessed using contact angle (CA). A thin film of materials was deposited by spin coating one drop of 0.1 wt.% cellulose suspension onto a silicon wafer. The wafer was preliminarily cleaned using an UV-ozone treatment, washed with deionized water, and pre-adsorbed with a 1.0 wt.% poly(ethylene imine) solution prior to the spin coating (Wang, Hauser, Laine, & Rojas, 2011). Four liquids were used in static CA mode using a CAM 200 (KSV Instruments, Finland) goniometer, by depositing four liquids: deionized water, diiodomethane, formamide, and ethylene glycol. The CA value was taken one second after the deposition of a 5-10 µl sessile drop onto the substrate and extrapolated from the pictures using the Laplace-Young equation. The measurements were repeated three times per liquid and per cellulose sample. The London-van der Waals and acid-base contributions of the surface free energy were extracted from the contact angle values according to a well-established theory and equations already developed elsewhere (Oh & Luner, 1999).

### 2.6. Preparation of Pickering emulsions

Oil-in-water Pickering emulsions were prepared based on a procedure and emulsion ratios already reported in the literature (Kalashnikova et al., 2011). 70 vol.% aqueous suspensions containing various amounts of cellulose were mixed with 30 vol.% commercial refined almond oil into glass tubes, for a total volume of 5.0 ml. After, the samples were shaken by hand and sonicated for three seconds in order to ensure sufficient dispersion of the cellulose within the two-phase system. The stability and homogeneity of the emulsions was assessed using a light scattering Turbiscan MA 2000 (Formulaction, France) apparatus. The samples were inserted into transparent glass tubes and both transmitted and back-scattered light profiles were recorded 24 hours after formulation.
Emulsion polymerizations of styrene were performed using azobisisobutyronitrile (AIBN) as oil-soluble initiator and cellulose particles as stabilizing phase. The emulsion polymerization was inspired by recent work on nanocellulose-stabilized Pickering emulsions (Kalashnikova et al., 2011). 1.5 g of styrene and 1.0 wt.% AIBN were simultaneously added to the cellulose suspension. The emulsion was sonicated for three seconds before being left for 20 hours at 50 °C. The product was diluted 1:1 with water and the remaining styrene was evaporated for 48 hours in a fume hood. A drop of this suspension was sampled and diluted to 0.1wt.% for SEM observations.

3. Results and Discussion

The two processing pathways were distinct from each other in terms of their effect on MCC. The mechanical pathway consisted in creating high-shear within a chamber to create friction and trigger the physical deconstruction of the particles. The process took multiple passes but the final yield achieved was practically 100%. In contrast, mixing the MCC suspension and nearcritical water under elevated pressure and temperature in a quick one-step process, followed by the physical separation of the solid fraction that precipitated after the treatment, resulted in a yield close to 25%. However, both pathways yielded similar white, stable gel-like materials. This indicated a change in the structure and/or morphology of the particles since a MCC suspension of similar dry matter content quickly sedimented. The following three sections focus on characterizing the structure, the morphology, and some of the material properties of the two MCC-derived products, MFC and HTC.

3.1. Degree of polymerization and supramolecular arrangement

This section presents structural data for MCC, MFC and HTC from SEC, XRD and SAXS measurements. The starting MCC had a weight-average DP of 283 and a polydispersity index (PDI) of 2.6, as obtained by SEC. These values represent both low DP and low PDI for a cellulosic material, but are compatible with the fact that MCC production involves the partial acid-catalyzed hydrolysis of cellulosic pulp. The microfluidized material, MFC, exhibited a very similar molecular weight distribution, as shown in Figure 1. The mechanical treatment resulted in no or negligible chain cleavage. This indicates that microfluidization did not chemically alter the composition of the MFC material, which remained very similar to that of the initial MCC. In contrast, HTC was composed of chains of significantly lower molecular weight, with a weight-
average DP of 50 and a PDI of only 1.5. These values are exceptionally low for cellulose, and confirm the observations of previous studies as well as the singular depolymerization triggered by nearcritical water conditions on MCC. The precipitated fraction is composed of cellulose chains which are soluble in nearcritical water but insoluble in water at ambient temperature and pressure (Ehara & Saka, 2002).

Figure 1. Molecular-weight distributions of microcrystalline cellulose (MCC), microfluidized cellulose (MFC), and hydrothermal cellulose (HTC).

In addition to the degree of polymerization of cellulose, its supramolecular structure, or the arrangement of the polymer chains at a mesoscopic level, significantly influences the intrinsic properties of the corresponding materials. As expected, MCC had a cellulose I diffraction pattern typical for native cellulose, shown in Figure 2.a. As for the molecular weight distribution, the mechanical treatment had no visible effect on the structure of cellulose, and the XRD intensities of the MFC sample were highly similar to that of MCC. However, XRD confirmed that the arrangement of cellulose chains in the HTC precipitate is not the same as in the initial cellulose, an observation already noticed in earlier studies (Buffiere et al., 2016; Tolonen et al., 2013). The precipitate exhibited high cellulose II lattice content, a characteristic of regenerated cellulose. An estimation based on peak fitting using pure cellulose I and pure cellulose II patterns gave a value of around 85% allomorph II in HTC. This indicates that the MCC was not fully dissolved during hydrothermal treatment. However, the residual cellulose I peak in the 15° scattering region was different from that of MCC and similar to the arrangement known as cellulose IVs, suggesting that this fraction was not unaffected by the treatment, an observation that was previously made on a similar sample (Buffiere et al., 2016). In addition, XRD spectra confirmed the high
crystallinity of MCC, as shown in Figure 2.a. by the high crystalline peak area relative to the amorphous base, with a ratio of 80% (±5%) based both peak deconvolution and peak height methods. This ratio was approximately the same for all products: the crystallinity was not visibly affected by microfluidization and HTC samples were highly crystalline as well. As for the crystallite sizes, the Scherrer equation gave sizes of around 7 nm (±1 nm) for MCC and MFC, whereas it was closer to 12 nm (±2 nm) for HTC. The presence of crystallites of higher dimensions in HTC than in the initial MCC indicates a reorganization of the cellulose chains. These values are compatible with earlier observations and confirm that unlike microfluidization, nearcritical water treatment had a radical impact on the way in which individual cellulose chains are organized within the HTC material (Buffiere et al., 2016).

While XRD measurements revealed the effect of the MCC treatments on the supramolecular arrangement of the materials, SAXS involved scattering caused by inhomogeneity within the material at the nanometer range. The spectra revealed similar interfacial structures between MCC, MFC and HTC, as shown in Figure 2.b. The smooth nature of the decreasing curve in the 0.1-1 nm⁻¹ range indicated densely ordered materials, with no significant pore distribution. In addition, the good quality of the measurements enabled modelling at both ends of the SAXS spectra. At lower angles, a Guinier plot extrapolated the scattering intensity at zero scattering angles, giving the radius of gyration for the molecules composing the materials. The radiiuses of gyration obtained from the Guinier plot were 24.2 nm for MCC and MFC, and 20.7 nm for HTC. This supports the previous observations made with SEC and XRD that the mechanical treatment left the supramolecular structure of cellulose unaffected. Conversely, at the higher end of the SAXS region, a Porod plot allowed for characterization of the interface between the molecules. The plot gave straight lines with slopes slightly above -4, indicating rather smooth interfaces and a monodisperse system in all three materials, as expected for highly crystalline cellulose. The exponents of the Porod trend lines were -3.7 for MCC and -3.8 for MFC and HTC, respectively.
3.2. Morphological properties of individual particles

The present section focuses on investigating the size and shape of the particles composing the MFC and HTC materials, revealed by TEM. Imaging MCC was not possible due to the high dimensions of the particles. In both cases, the micrographs revealed sub-micrometer particles with significant aspect ratio. The MFC micrographs exhibited rather homogeneously elongated, rod-like cellulose particles with a relatively high aspect ratio, as shown in Figure 3.a. Their dimensions were about 100 nm to 200 nm in length and about 10 nm to 20 nm in width, with some longer particles due to apparent interconnection. The rod-like morphology reminded that of conventional CNC. In addition, a significant amount of particle bundles was visible, indicating an incomplete defibrillation during high-shear homogenization, even after 15 passes. The particle...
dimensions and presence of bundles are compatible with earlier observations for a similarly produced material (Bandera et al., 2014).

As for HTC, the particles also exhibited nano-scale particles with high aspect ratio. The particles were larger than that of MFC, typically up to 500 nm in length and 100 nm, as shown in Figure 3.b. However, the overall HTC morphology was less homogeneous, with significant variability in both length and width. The particles had sharp edges that may result from particle breakage upon sonication. Overall, the HTC particles looked somewhat similar to low-molecular-weight cellulose II precipitated from hydrolysis in a concentrated phosphoric acid solution (Helbert & Sugiyama, 1998). Indeed, phosphoric acid is known to simultaneously hydrolyze and dissolve cellulose, and is thus able to produce either conventionally-looking CNC or a low-molecular-weight precipitate depending on the treatment conditions (Camarero Espinosa, Kuhnt, Foster, & Weder, 2013; Jia et al., 2013). In this sense, hydrothermal treatment seemed to have a similar effect on MCC than concentrated phosphoric acid, producing particles with similar overall dimensions yet with different cross-sectional morphology than conventional CNF.

![Figure 3. Left: TEM images of a. MFC and b. HTC particles. Right: AFM images of c. MFC and d. HTC. Inserts show the height profile of particle crosscut. Scale bar 200 nm.](image-url)
In addition to TEM, AFM confirmed the overall 2D morphology and allowed quantifying the particle thickness, as shown in Figure 3.c for MFC and Figure 3.d for HTC. The images revealed a thickness for MFC particles around 10 to 20 nm. This makes the MFC particles rod-like, with similar dimensions in thickness and in width. In contrast, the thickness of HTC particles was around 5 to 10 nm only, giving the particles a ribbon-like morphology. An analogy can be drawn between the precipitation from nearcritical water and from phosphoric acid. In the latter, the chains were shown to assemble in single crystals upon stacking perpendicular to the growth direction of the crystal, giving a particle thickness roughly equivalent to the length of the cellulose chains (Helbert & Sugiyama, 1998). Thus, both nearcritical water treatment and high-shear homogenization yielded nano-scale cellulose particles, yet with radically different morphologies.

3.3. Material properties and particle interactions.

This section discusses the influence of structure and particle morphology on some of the material properties of MFC and HTC. A summary of morphological, rheological and surface energy data for MFC and HTC is presented in Table 1. The behavior of cellulosic materials as fluids in aqueous suspensions is of critical importance for most applications. The complex viscosity of the MFC and HTC materials as a function of the angular frequency gave steadily decreasing slopes up to relatively high frequencies at nearly all tested conditions, as shown in Figure 4. This thixotropic behavior, or time-dependent shear-thinning, was observed for both materials and at all consistencies except for MFC at 5.0 wt.%. Shear-thinning is a sought-after property when using cellulose as an thickening additive, and is commonly observed in nano-scale cellulosic materials (Lasseuguette, Roux, & Nishiyama, 2008).

Table 1. Summary of morphology, rheology and surface free energy data for MFC and HTC.

|     | L (nm) | W (nm) | T (nm) | μ (Pa.s) | Cgc (wt.%) | γ_LW (mJ.m²) | γ_AB (mJ.m²) | γ_TOT (mJ.m²) |
|-----|--------|--------|--------|----------|------------|--------------|--------------|--------------|
| MFC | 100-200| 10-20  | 10-20  | 52,900   | 5-10       | 35.9         | 1.5          | 37.4         |
| HTC | 200-500| 40-100 | 5-10   | 280,000  | < 5        | 43.0         | 4.7          | 47.7         |

L: approximate particle length. W: approximate particle width. T: approximate particle thickness. μ: complex viscosity for a 15 wt.% suspension at 0.01 s⁻¹. Cgc: critical gelation concentration. γ_LW: London-van der Waals forces. γ_AB: acid-base forces. γ_TOT: total surface free energy.
Figure 4. Complex viscosity of microfluidized cellulose (MFC) and hydrothermal cellulose (HTC) suspensions for consistencies of 5 wt.%, 10 wt.%, and 15 wt.%. In addition, the storage modulus, or elastic contribution, was higher than the loss modulus, or viscous contribution. This confirms that both materials behave like gels at sufficiently high consistencies. A visualization of the storage and loss moduli of the materials is shown in the supplementary information file through Figures S1 and S2. The critical gelation concentration, or the cellulose consistency above which the material behaves like a gel, was below 5.0 wt.% for HTC, while it was above 5.0 wt.% for MFC. In addition, the former was significantly more viscous; the complex viscosity values were several times higher for HTC for a given consistency and angular frequency. This indicates a significantly higher level of interactions between the particles in the hydrothermal precipitate. The higher viscosity of HTC is likely the result of particles of larger dimensions while retaining high specific surface area due to the limited particle thickness, which favors contact between the particles, and results in a greater density of particle-particle interactions.

The difference in particle-particle interaction between MFC and HTC could be directly observed on the SEM micrographs of dried suspensions and films, shown in Figures S3 through S7 in the electronic supplementary information file. The images of dried suspensions revealed that the MFC samples also contained numerous large particles, indicating an incomplete disintegration during the mechanical processing of MCC. This observation was made before, with SEM images showing large residual aggregates after high-shear homogenization (Bandera et al., 2014). The presence of large bundles reduced the overall specific surface area of the material, also leading to fewer particle-particle interactions. However, the smaller particles interacted with each other and
formed thin, three-dimensional fibrillar networks. In case of HTC, some larger particles were also visible, which is in accordance with the hypothesis that the HTC contained some residual, undissolved cellulose as indicated by XRD results. The particles also formed extensive three-dimensional networks, significantly denser and less fibrillar in nature than for MFC. In addition, air-dried, self-standing films made of pure HTC exhibited significantly denser structures, while MFC particles organized themselves in loosely organized sheets. This confirms the hypothesis of larger particle-particle interactions between HTC particles, most likely thanks to larger dimensions and a morphology favoring interparticular contact points. The nature of the treatment changed the morphology of the particles, which in turn had an influence on the interaction and network-forming ability of the nanocellulose, without any chemical modification of the particles.

The difference between the two materials was not only in morphology but also in surface properties, as showed by contact angle measurements and surface free energy values for MFC and HTC. The contact angle values and corresponding standard deviations can be found in Table S1 of the supplementary information file. With deionized water, the average contact angle was 43° for HTC and 69° for MFC. Both the London-van der Waals component and the acid-base component were significantly higher for HTC than for MFC, which made HTC overall more hydrophilic. With 47.7 mJ.m\(^{-2}\) for HTC against 37.4 mJ.m\(^{-2}\) for MFC, the difference was substantial, although the values are in the range of previously measured surface free energy for MCC using capillary intrusion (Steele et al., 2008). The polar and acid-base components of the surface free energy were both higher in case of HTC. This disparity in surface free energy is likely to be attributed to a difference in the surface properties of the materials. The different supramolecular structure of HTC as well as a ribbon-like morphology are likely to have resulted in more hydrophilic hydroxyl groups exposed, which in turn increased the surface free energy of the material. The difference in morphology and in coverage of the silicon wafers might have also influenced the surface free energy values.

### 3.4. Stabilization of oil-in-water Pickering emulsions.

This section aims at exploring an example of applications in which the two types of nanocelluloses could be desirable. Almond oil is widely incorporated into a wide range of cosmetic products and is an increasingly used additive in foods. These products are often water-based, thus creating the need to efficiently stabilize oil-in-water emulsions, for example using nano-scale cellulosic particles in a Pickering system. In case of a 30 vol.% almond oil and
70 vol.% aqueous suspension, the oil and water phase separated instantaneously without additives, as shown in Figure 5. Progressively adding MFC or HTC in small amounts to the aqueous phase first mitigated the separation, and eventually prevented it entirely. Samples with stable emulsion ranged from 1.0 wt.% with HTC and between 2.0 and 5.0 wt.% with MFC. The 10 wt.% HTC sample could not be emulsified because of the too high viscosity of the aqueous phase. Therefore, the ideal amount of cellulose to stabilize 30 vol.% of almond oil ranged between 1.0 and 5.0 wt.% for HTC and 5.0 wt.% to 10 wt.% for MFC.

Figure 5. Visual appearance of a. HTC-stabilized and b. MFC-stabilized oil-in-water Pickering emulsions prepared with 0 to 10 wt.% cellulose in the aqueous phase, after shaking.

Above the critical cellulose consistency, the emulsions were very stable over time. Turbiscan measurements taken for samples 24 hours after formulation with cellulose consistencies of 0.5 wt.% and 2.0 wt.% gave information about the particle distribution within the emulsified phase and revealed clear differences between MFC and HTC materials, as shown in Figure 6. In case of HTC, the 0.5 wt.% emulsion was not physically stable as indicated by the variation in back-scattered light across the sample height, suggesting an inhomogeneous particle distribution. In contrast, the particles composing the emulsion prepared with 2.0 wt.% cellulose were highly stable and homogeneously distributed across the sample height. In addition, the cellulose migrated entirely to the upper part of the sample, leaving a clear aqueous phase layer at the bottom and indicating good compatibility between the oil and the cellulose suspension.

The situation was different in case of MFC: at 2.0 wt.% consistency, no clear aqueous phase could be observed and cellulose was distributed across the whole sample height; however, a phase separation was clearly visible, as indicated by the shift in back-scattered light around sample mid-height. In addition, the profile revealed an uneven particle distribution and a sedimentation phenomenon, as more particles were found at the bottom of the two phases. Since
neither of the two treatments is known to create any significant charges at the surface of the particles, the previously identified differences between the two materials can explain the difference in behavior in stabilizing emulsions and the better performance of HTC in stabilizing emulsions. The HTC particles exhibited a higher interfacial contact and particle-particle interaction, as well as higher overall network formation ability. In addition, the higher hydrophilicity as well as Van der Waals contribution seemed to be the drivers for a better affinity to the oil phase. This is likely due to the higher exposure of the amphiphilic regions of cellulose to the oil droplets, thus resulting in more complete and homogeneous mixing between cellulose and oil. Moreover, in case of MFC the presence of large particle bundles is likely to have contributed to the sedimentation observed even at moderate cellulose consistency.

Figure 6. Transmitted and back-scattered light profiles across sample height through a. HTC-stabilized and b. MFC-stabilized emulsions, 24 hours after shaking. The photographs show the visual aspect of the two emulsions stabilized with 2.0 wt.% cellulose.
While turbidity analysis gave a macroscopic description of the emulsions, emulsion polymerization with styrene revealed the arrangement of individual cellulose particle within an oil-in-water emulsion. Emulsion polymerization with HTC yielded highly homogeneous, sub-micron polystyrene beads, with a few larger spheres with up to a few micrometers in diameter, as shown in Figure 7.a. In addition, the cellulose particles were clearly visible at the surface of the larger spheres, homogeneously covering the beads and giving them a rough-looking surface, as depicted in Figure 7.b. However, only few individual HTC particles were visible at the surface of the smaller beads. The high viscosity of HTC and its ribbon-like morphology may have prevented the migration of styrene during emulsion polymerization, leading to a large share of beads of dimensions too small to allow the adsorption of cellulose.

In contrast, MFC-stabilized emulsions yielded a significantly larger share of larger beads, as shown in Figure 7.c. In this case, the individual cellulose rods were clearly visible, and homogeneously distributed at the surface of most polystyrene spheres, including the smaller ones, as depicted in Figure 7.d. The difference in morphology and in particle size is likely one reason for this difference in behavior, in which the MFC particles did not prevent the migration of styrene during emulsion polymerization. In addition, the higher surface free energy of HTC likely led to lower adhesion on hydrophobic surfaces. If the absence of cellulose on the smaller beads is confirmed, these characteristics would also make HTC an especially good candidate for substituting surfactants in emulsion polymerization.
4. Conclusions

This work reported the formation of two types of nano-scale cellulose particles, their characterization, and their behavior as emulsifier. The results emphasize the importance of structure-property relationship in nanocelluloses and show that MCC is a relevant starting material for producing nanocelluloses. MCC as starting material was easy to process with both high-shear homogenization and nearcritical water treatment; no plugging was observed, and the limited viscosity of the final MFC and HTC materials compared to conventional CNF makes these conversion routes easy to upscale and the final products easy to transport.

The HTC material was similar to that obtained from dissolution and precipitation in phosphoric acid, but prepared from MCC using water and heat only, without any additional chemicals. Moreover, the low molecular weight of this material differentiates it from typical CNC, which could be of advantage for situations in which the properties of nanocellulose particles are needed, but where there is also a concern for biodegradability or rapid degradation. While the process requires significant heating as well as a specific high-pressure reactor system, the
reaction transforming MCC into HTC is extremely fast, thus allowing for low environmental footprint, high upscaling potential, and industrial relevance as soon as the process heat is recovered.

In contrast, the simplicity of MFC production using a mechanical treatment only as well as a product yield near 100% ensures low production costs and easy process implementation. The final material is obtained without any chemical or biochemical pretreatments directly from commercial MCC, which makes it potentially suitable in many of the sensitive applications where MCC is used today.

The materials were especially suitable as additives in oil-in-water Pickering emulsions. When stabilized with more than 1.0 wt.% HTC or more than 5.0 wt.% MFC, the emulsions were highly homogeneous and stable over time. In addition, the properties of HTC could make it relevant in surfactant-free emulsion polymerization.

Therefore, both HTC and MFC are believed to be of high relevance in formulating and stabilizing creams and other biphasic mixtures, especially for use in cosmetic and pharmaceutical areas where the purity and chemical neutrality of the stabilizer are important. Further characterization of the emulsions and of their stability is needed to explain and understand the role of the materials in stabilizing them. Nevertheless, the inherent properties of HTC are attractive and unusual for nanocellulose, whereas MFC provides a cheap and more sustainable alternative to conventional CNC while still being able to act as Pickering stabilizer.

Supporting information

Attached to this article is a supplementary information file that contains the previously cited figures depicting the standard deviation for contact angle values (Table S1), rheology curves (Figures S1 and S2), as well as SEM images (Figures S3 through S7).

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References

Abitbol, T., Rivkin, A., Cao, Y., Nevo, Y., Abraham, E., Ben-shalom, T., … Shoseyov, O. (2016). Nanocellulose, a tiny fiber with huge applications. Current Opinion in Biotechnology, 39(1), 76–88. https://doi.org/10.1016/j.copbio.2016.01.002

Bandera, D., Sapkota, J., Josset, S., Weder, C., Tingaut, P., Gao, X., … Zimmermann, T. (2014). Influence of mechanical treatments on the properties of cellulose nanofibers isolated from microcrystalline cellulose. Reactive and Functional Polymers, 85, 134–141. https://doi.org/10.1016/j.reactfunctpolym.2014.09.009

Bozell, J. J., Moens, L., Elliott, D. C., Wang, Y., Neuenschwander, G. G., Fitzpatrick, S. W., … Msin, K. (2000). Production of levulinic acid and use as a platform chemical for derived products. Resources, Conservation and Recycling, 28, 227–239. https://doi.org/10.1016/S0921-3449(99)00047-6

Buffiere, J., Ahvenainen, P., Borrega, M., Svedström, K., & Sixta, H. (2016). Supercritical water hydrolysis: a green pathway for producing low-molecular-weight cellulose. Green Chem., 18, 6516–6525. https://doi.org/10.1039/C6GC02544G

Camarero Espinosa, S., Kuhnt, T., Foster, E. J., & Weder, C. (2013). Isolation of thermally stable cellulose nanocrystals by phosphoric acid hydrolysis. Biomacromolecules, 14(4), 1223–1230. https://doi.org/10.1021/bm400219u

Cantero, D. A., Vaquerizo, L., Mato, F., Bermejo, M. D., & Cocero, M. J. (2015). Energetic approach of biomass hydrolysis in supercritical water. Bioresource Technology, 179, 136–143. https://doi.org/10.1016/j.biortech.2014.12.006

Chevalier, Y., & Bolzinger, M.-A. (2013). Emulsions stabilized with solid nanoparticles: Pickering emulsions. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 439, 23–34. https://doi.org/10.1016/j.colsurfa.2013.02.054

Dufresne, A. (2013). Nanocellulose : a new ageless bionanomaterial. Materials Today, 16(6), 220–227. https://doi.org/10.1016/j.mattod.2013.06.004
Ehara, K., & Saka, S. (2002). A comparative study on chemical conversion of cellulose between the batch-type and flow-type systems in supercritical water. *Cellulose*, 9(3–4), 301–311. https://doi.org/10.1023/A:1021192711007

George, J., & S N, S. (2015). Cellulose nanocrystals: synthesis, functional properties, and applications. *Nanotechnology, Science and Applications*, 8, 45–54. https://doi.org/10.2147/NSA.S64386

Gestranius, M., Stenius, P., Kontturi, E., Sjöblom, J., & Tammelin, T. (2016). Phase behaviour and droplet size of oil-in-water Pickering emulsions stabilised with plant-derived nanocellulosic materials. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 519, 60–70. https://doi.org/10.1016/j.colsurfa.2016.04.025

Habibi, Y., Lucia, L. A., & Rojas, O. J. (2010). Cellulose nanocrystals: Chemistry, self-assembly, and applications. *Chemical Reviews*, 110(6), 3479–3500. https://doi.org/10.1021/cr900339w

Hallac, B. B., & Ragauskas, A. J. (2012). Analyzing cellulose degree of polymerization and its relevancy to cellulosic ethanol. *Biofuels, Bioproducts and Biorefining*, (5), 215–225. https://doi.org/10.1002/bbb

Helbert, W., & Sugiyama, J. (1998). High-resolution electron microscopy on cellulose II and α-chitin single crystals. *Cellulose*, 5, 113–122. https://doi.org/10.1023/A:1009272814665

Hu, Z., Marway, H. S., Kasem, H., Pelton, R., & Cranston, E. D. (2016). Dried and Redispersible Cellulose Nanocrystal Pickering Emulsions. *ACS Macro Letters*, 5(2), 185–189. https://doi.org/10.1021/acsmacrolett.5b00919

Jia, X., Chen, Y., Shi, C., Ye, Y., Wang, P., Zeng, X., & Wu, T. (2013). Preparation and characterization of cellulose regenerated from phosphoric acid. *Journal of Agricultural and Food Chemistry*, 61(50), 12405–12414. https://doi.org/10.1021/jf4042358

Kalashnikova, I., Bizot, H., Cathala, B., & Capron, I. (2011). New Pickering Emulsions Stabilized by Bacterial Cellulose Nanocrystals. *Langmuir*, (27), 7471–7479. https://doi.org/10.1021/la200971f

Kalashnikova, I., Bizot, H., Cathala, B., & Capron, I. (2012). Modulation of cellulose nanocrystals amphiphilic properties to stabilize oil/water interface. *Biomacromolecules*, 13, 267–275.
Klemm, D., Heublein, B., Fink, H. P., & Bohn, A. (2005). Cellulose: Fascinating biopolymer and sustainable raw material. *Angewandte Chemie - International Edition*, 44(22), 3358–3393. https://doi.org/10.1002/anie.200460587

Lasseuguette, E., Roux, D., & Nishiyama, Y. (2008). Rheological properties of microfibrillar suspension of TEMPO-oxidized pulp. *Cellulose*, 15, 425–433. https://doi.org/10.1007/s10570-007-9184-2

Misoum, K., Belgacem, M. N., & Bras, J. (2013). Nanofibrillated Cellulose Surface Modification: A Review, 1745–1766. https://doi.org/10.3390/ma6051745

Novo, L. P., Bras, J., García, A., Belgacem, N., & Curvelo, A. A. da S. (2016). A study of the production of cellulose nanocrystals through subcritical water hydrolysis. *Industrial Crops and Products*, 93, 88–95. https://doi.org/10.1016/j.indcrop.2016.01.012

Novo, L. P., Bras, J., García, A., Belgacem, N., & Curvelo, A. A. S. (2015). Subcritical Water: A Method for Green Production of Cellulose Nanocrystals. *ACS Sustainable Chemistry and Engineering*, 3(11), 2839–2846. https://doi.org/10.1021/acssuschemeng.5b00762

Oh, E., & Luner, P. E. (1999). Surface free energy of ethylcellulose films and the influence of plasticizers. *International Journal of Pharmaceutics*, 188(2), 203–219. https://doi.org/10.1016/S0378-5173(99)00224-0

Park, S., Baker, J. O., Himmel, M. E., Parilla, P. A., & Johnson, D. K. (2010). Cellulose crystallinity index: measurement techniques and their impact on interpreting cellulase performance. *Biotechnology for Biofuels*, 3(10). https://doi.org/10.1186/1754-6834-3-10

Röder, T., Moosbauer, J., Wöss, K., Schlader, S., Kraft, G., & Ag, L. (2013). Man-Made Cellulose Fibres – a Comparison Based on Morphology and Mechanical Properties. *Lenzinger Berichte*, 91, 7–12.

Salas, C., Nypelö, T., Rodriguez-Abreu, C., Carrillo, C., & Rojas, O. J. (2014). Nanocellulose properties and applications in colloids and interfaces. *Current Opinion in Colloid and Interface Science*, 19(5), 383–396. https://doi.org/10.1016/j.cocis.2014.10.003

Sasaki, M., Adschiri, T., & Arai, K. (2004). Kinetics of Cellulose Conversion at 25 MPa in Sub-
and Supercritical Water. *AIChe Journal*, 50(1), 192–202. https://doi.org/10.1002/aic.10018

Sasaki, M., Fang, Z., Fukushima, Y., Adschiri, T., & Arai, K. (2000). Dissolution and Hydrolysis of Cellulose in Subcritical and Supercritical Water. *Industrial & Engineering Chemistry Research*, 39(8), 2883–2890. https://doi.org/10.1021/ie990690j

Steele, D. F., Moreton, R. C., Staniforth, J. N., Young, P. M., Tobyn, M. J., & Edge, S. (2008). Surface energy of microcrystalline cellulose determined by capillary intrusion and inverse gas chromatography. *The AAPS Journal*, 10(3), 494–503. https://doi.org/10.1208/s12248-008-9057-0

Tingaut, P., Zimmermann, T., & Sèbe, G. (2012). Cellulose nanocrystals and microfibrillated cellulose as building blocks for the design of hierarchical functional materials. *Journal of Materials Chemistry*, 22(38), 20105–20111. https://doi.org/10.1039/c2jm32956e

Tolonen, L. K., Juvonen, M., Niemelä, K., Mikkelsen, A., Tenkanen, M., & Sixta, H. (2015). Supercritical water treatment for cello-oligosaccharide production from microcrystalline cellulose. *Carbohydrate Research*, 401, 16–23. https://doi.org/10.1016/j.carres.2014.10.012

Tolonen, L. K., Penttilä, P. A., Serimaa, R., Kruse, A., & Sixta, H. (2013). The swelling and dissolution of cellulose crystallites in subcritical and supercritical water. *Cellulose*, 20(6), 2731–2744. https://doi.org/10.1007/s10570-013-0072-7

Tolonen, L. K., Penttilä, P. A., Serimaa, R., & Sixta, H. (2015). The yield of cellulose precipitate from sub- and supercritical water treatment of various microcrystalline celluloses. *Cellulose*, 22(3), 1715–1728. https://doi.org/10.1007/s10570-015-0628-9

Wang, Z., Hauser, P. J., Laine, J., & Rojas, O. J. (2011). Multilayers of Low Charge Density Polyelectrolytes on Thin Films of Carboxymethylated and Cationic Cellulose. *Journal of Adhesion Science and Technology*, 25, 643–660. https://doi.org/10.1163/016942410X525876

Yu, Y., & Wu, H. (2009). Characteristics and Precipitation of Glucose Oligomers in the Fresh Liquid Products Obtained from the Hydrolysis of Cellulose in Hot-Compressed Water. *Industrial & Engineering Chemistry Research*, 48(23), 10682–10690. https://doi.org/10.1021/ie900768m