Accepted Manuscript

Title: Starch Nanocrystals and Starch Nanoparticles from Waxy Maize as Nanoreinforcement: A Comparative Study

Author: Sihem Bel Haaj Wim Thielemans Albert Magnin
Sami Boufi

PII: S0144-8617(16)30025-X
DOI: <http://dx.doi.org/doi:10.1016/j.carbpol.2016.01.061>
Reference: CARP 10740

To appear in:

Received date: 24-10-2015
Revised date: 13-1-2016
Accepted date: 27-1-2016

Please cite this article as: Haaj, S. B., Thielemans, W., Magnin, A., and Boufi, S., Starch Nanocrystals and Starch Nanoparticles from Waxy Maize as Nanoreinforcement: A Comparative Study, *Carbohydrate Polymers* (2016), <http://dx.doi.org/10.1016/j.carbpol.2016.01.061>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Starch Nanocrystals and Starch Nanoparticles from Waxy Maize as Nanoreinforcement : A Comparative Study

Sihem Bel Haaj^a, Wim Thielemans^{*b}, Albert Magnin^c, Sami Boufi^{*a}

^a, University of Sfax- Sfax Faculty of Science-LMSE BP 802-3018 Sfax, Tunisia

^b Renewable Materials and Nanotechnology Research Group, KU Leuven, Campus Kortrijk, Etienne Sabbelaan 53, 8500 Kortrijk, Belgium

^c CNRS, LRP, F-38000 Grenoble, France

* Corresponding author:

Sami Boufi

University of Sfax-Faculty of science

BP 1171-3000 Sfax-Tunisia-Fax: 216 74 274 437

E-mail Sami.Boufi@fss.rnu.tn

Abstract

The morphological, structural and thermal behaviour of starch nanocrystals (SNCs) extracted from waxy maize starch through an acid hydrolysis were compared with those of starch nanoparticles (SNPs) obtained through an ultrasound treatment starting from the same waxy maize starch. The SNPs were found to be completely amorphous, slightly smaller and had no surface charge, whereas the SNCs had the expected platelet-like morphology with a negative surface charge introduced as a result of the use of sulphuric acid in the acid hydrolysis step. SNCs also showed better thermal stability than SNPs in the presence of water. As a result of their platelet-like morphology, the SNCs performed better in reinforcing a polymer film. On the other hand, SNPs reduced the transparency of the nanocomposite films to a lesser extent than the SNCs due to their smaller size.

Key words; starch nanocrystals, starch nanoparticles, nanocomposite, nanoreinforcement.

Highlights

Starch nanocrystals (SNCs) and starch nanoparticles (SNPs) from waxy maize were prepared. The morphology, crystallinity, colloidal properties and reinforcing potential were investigated and compared.

The inclusion of SNCs in a ductile polymer matrix was shown to bring about stronger reinforcing effect than that of SNPs.

1. Introduction

In recent years, nanoparticles (NPs) derived from polysaccharides have become a topic of intensive research efforts with potential applications in the field of nanocomposite materials, biomedical applications packaging, and cosmetics (Alonso, Janes, & Calvo, 2001; Couvreur, Lemarchand & Gref, 2004; Liu *et al.*, 2008). In addition to their availability in various natural resources, polysaccharides have a number of attractive characteristics such as biocompatibility, biodegradability, and an abundance of functional groups opening the way to a wide spectrum of surface modifications and functionalization pathways (Thielemans, Belgacem, & Dufresne, 2006; Eyley & Thielemans, Nanoscale, 2014; Dufresne, 2010).

The hierarchical organization of polysaccharides such as cellulose, starch and chitin in natural materials along with their semi-crystalline structure has been a key factor in the preparation of nanocrystals through a controlled acid hydrolysis. The amorphous regions are more susceptible to acid hydrolysis than the crystalline regions due to free volume effects and, under controlled conditions, may be removed leaving the integral rigid crystalline regions intact (LeCorre, Bras & Dufresne, 2010). This approach leads to polysaccharide nanocrystals, the morphology of which depends on the polysaccharide origin; rod-like nanocrystals for cellulose (Eichhorn *et al.* 2010), and platelet-like nanocrystals in the case of starch (Putaux, Molina-Boisseau, Momaour & Dufresne 2003).

Starch, like cellulose, is a complex carbohydrate made up of chains of glucose. However, the nature of the links holding the glucose units together is different in starch causing a huge difference in the ultrastructure of these two biopolymers (Mua, & Jackson, 1997). In starch, the glucose units are linked together in an α -conformation, giving rise to a helical twist of the polymer chain while in cellulose the β -conformation leads to a linear stretching of the polysaccharide chains. Starch is composed of two macromolecular α -1,4-D-glucopyranose polymers, namely linear amylose and branched

amylopectin. In additions, four levels of organization make up the starch granule (Gallant, Bouchet & Baldwin, 1997): (i) clusters of amylopectin molecules (0.1–1 nm), (ii) lamellae (~10 nm), (iii) blocklets (20–250 nm) and finally (iv) the whole granule (> 1µm). Within this structure, amylopectin is organized in crystalline clusters of double helices forming stacks of alternating crystalline and amorphous lamellae with a regular repeat distance of 9–10 nm. These are embedded in rounded asymmetric structures named blocklets that have a diameter on the 50 to 100 nm scale. These blocklets, having different sizes, aggregate in alternating amorphous and semi-crystalline radial growth rings of each 100–400 nm thick. The amorphous rings consist of amylose and amylopectin in a disordered conformation, whereas the semi-crystalline rings are formed by the lamellar structure of alternating crystalline and amorphous regions.

The interest in starch nanocrystals is quite recent. The first report of starch nanocrystals was published by Dufresne *et al.* in 1996, in which the authors prepared, analogous to the preparation of cellulose nanocrystals, nanosized crystalline starch particles through a controlled acid hydrolysis of starch. Confirmation of the platelet-like morphology of these starch nanocrystals (SNCs) was provided by Putaux *et al.* in 2003 using TEM observation. It was shown that the nanoparticles resulting from the disruption of waxy maize starch granules by acid hydrolysis were composed of stacks of elongated elements with a width of 5-7 nm, a length of 20-40 nm and a width of 15-30 nm. Since these reports, the interest in the preparation and use of SNCs and other starch nanoparticles (SNPs) has been growing steadily giving rise to simpler and more optimized methods of SNP production and investigations of new applications for these biobased nanoparticles.

Starch nanocrystals and starch nanoparticles can be prepared in different ways, including through acid or enzymatic hydrolysis, regeneration via self-assembly after dissolution or complexation, and physical disintegration (LeCorre, Bras & Dufresne, 2010; Kim, Park & Lim, 2015). The acid hydrolysis procedure involves the hydrolysis of the amorphous fraction of the starch granules and leads to starch nanocrystals with a high crystallinity and a platelet-like

shape. The acid hydrolysis procedure is difficult for practical applications due to its low yield, long treatment period, and use of concentrated acid. New methodologies relying on physical disintegration on the other hand are promising and attractive owing to their relative rapidity, higher yield, and the absence of tedious purification steps. Among these methods, we can cite high-pressure homogenization (HPH), ultrasonication, nanoprecipitation, gamma irradiation and reactive extrusion as approaches that have been investigated (Song, Thio & Deng, 2011; Lamanna, Morales, García & Goyanes, 2013; Bel Haaj, Magnin, Pétrier & Boufi, 2013). HPH is a disintegration process that has been widely adopted for the top-down production of nanoparticles via size reduction of macro- and micro-sized source materials and has been extended to starch to yield SNPs with a large particle size distribution ranging between 5 and 200 nm (Song, Thio & Deng, 2011). Gamma irradiation has been used to generate active free radicals that are then responsible for the hydrolysis of starch, leading to SNPs with sizes of 20 to 30 nm (Lamanna, Morales, García & Goyanes, 2013). A high-intensity ultrasonication method has also been recently reported as an environmentally-friendly method to produce nanosized SNPs in a single step giving nanoparticles with sizes around 40 nm (Bel Haaj, Magnin, Pétrier & Boufi, 2013) after ultrasonication for 75 min at a temperature between 5-10°C. However, the morphology of the recovered SNPs was not well defined and depended strongly on the extraction parameters. Furthermore, a huge decrease, and at times, even a complete loss of the crystallinity occurred during the physical disintegration process.

In the present work, a comparative investigation of the morphological and, structural properties and the reinforcing potential of SNPs produced via high intensity ultrasonication and SNCss produced through conventional hydrolysis route is reported, allowing users to choose the most appropriate nanosized starch for their target application. was reported.

2-Experimental

2-1-Materials

Waxy maize starch (WaxyliTM, >99% amylopectin) was provided by Roquette S.A., Lesterm, France. Sulphuric acid (98%) was obtained from Sigma Aldrich and used as received.

2-2-Starch Nanocrystal Preparation

Sulphuric acid-hydrolysed starch nanoplatelets were prepared from waxy maize starch as described by Angellier *et al.*, 2004). In short, waxy maize starch was hydrolysed under constant stirring for 5 days at 40°C in a 3.16 M aqueous H₂SO₄ solution. Complete hydrolysis was confirmed by transmission electron microscopy and by agreement of the final product yield with earlier results. After the hydrolysis time, the nanocrystals were separated from the acid by centrifugation at 10 000 rpm at 10°C. Subsequent washing and centrifugation with distilled water was performed until neutrality of the eluent (about 8 subsequent wash-centrifugation cycles). A homogeneous dispersion of starch nanocrystals was obtained using an Ultra Turrax T25 homogenizer for 5 min at 13 500 rpm.

2-3-Starch nanoparticle preparation

The SNPs were prepared by adopting the procedure described in our previous work (Bel Haaj, Magnin, Pétrier & Boufi, 2013). The ultrasound treatment of the starch suspension was carried out as follows: 100 ml of the waxy maize starch suspension with a solids content of 1.5% and kept in a glass cell was immersed in a water bath kept at a temperature of $8 \pm 1^\circ\text{C}$. It was then sonicated at 80% power output for more than 75 min, using a 20 kHz Branson digital Sonifier S-450D (Germany) coupled with a horn with a 13 mm tip diameter. At the end of the ultrasound treatment, the starch suspension turned transparent from the original turbid white appearance.

2-4-Particle size determination

The particle diameters, or more correct their hydrodynamic diameters, were measured at 25°C using a Malvern Nano-Zetasizer ZS (Malvern, UK) with a fixed scattering angle of 173°. The dispersions were not diluted before starting the measurements. Dynamic light scattering (DLS) measurements gave a Z-average size that was used for comparison of the different particles.

2-5-Zeta-potential measurement

The zeta-potentials were measured at 25°C using a laser Doppler electrophoresis apparatus (Malvern Nano-Zetasizer ZS, Malvern, UK). The sample concentration in water was set at 0.01% (w/v). The measurements were performed in triplicate for each sample and average values are reported.

2-6-Wide-angle X-ray diffraction (WAXD)

Wide-angle X-ray diffraction was carried out on powders obtained from freeze-dried SNP and SNC suspensions. The measurements were performed on a PANalytical, X'Pert PRO MPD diffractometer equipped with an X'celerator detector. We used a CuK α radiation source, with 2 θ diffraction angles measured between 4° and 44° with a step size of 0.084° and an acquisition time of 250s per step.

The crystalline index (CrI) was estimated as the ratio of the amplitude of the crystalline peak at about 17° (2 θ) (H_c), to the sum of the amplitude of the amorphous halo plus that of the crystalline peaks (H_t) measured from the horizontal baseline corrected by drawing a straight line from a diffraction angle of 13° to 27° (2 θ). The crystallinity index $X_c = H_c / H_t \times 100$ is proportional to the relative crystallinity of the sample.

2-7- DSC characterization

DSC analyses were carried out on a Perkin-Elmer DSC 7 instrument. The starch samples (approximately 10 mg) were hydrated to about 70% moisture content and then transferred to a sealed aluminum crucible and heated from 0°C to 120 °C at a rate of 10 °C/min. An empty sealed aluminum crucible was used as a reference.

2-8-Field Emission Scanning Electron Microscopy (FE-SEM)

Field Emission Scanning Electron Microscope (FE-SEM) images were obtained with a Zeiss Supra40. Images were created using SMARTSEM software. The electron source, based on a hot cathode producing electrons through the Schottky effect, was a tungsten filament coated with a ZrO layer. A drop of a diluted suspension of starch (with a solid content of about 0.02

wt%) was deposited on a silicon wafer, evaporated at room temperature and then coated with a thin carbon layer limited to 3 nm applied by sputtering.

2.9 AFM observation

The morphology of the nanofibers was studied by atomic force microscopy (AFM) (FlexAFM from Nanosurf) using a tapping mode. The samples were prepared by depositing a drop of diluted suspension of nanoparticles (with a solid content about 0.001 wt%) on the surface of a silicon wafer and leaving it to dry for 1h.

2-10 Nanocomposite film preparation

Nanocomposite films were prepared via casting of a mixture of a latex dispersions of poly(butylmethacrylate) with the starch nanofiller suspension in a Teflon mold and stored at 50°C until water evaporation was complete. A transparent to translucent film, depending on the nanofiller content, was obtained with a thickness of 200–300µm.

2-11-Dynamic mechanical analysis (DMA)

Dynamic mechanical analysis (DMA) experiments were conducted in tension mode using a PYRIS™ Diamond DMA (Perkin-Elmer, Waltham, MA, USA). Temperature scans were run from -50 °C to 100 °C at a heating rate of 1°C min⁻¹, while deforming the sample at a frequency of 1 Hz and an amplitude of 10 µm. The storage E' was measured as a function of temperature. Sample dimensions were about 20 mm (length), 10 mm (width) and 0.1–0.3 mm (thickness).

2.12 Viscosity measurements

Dynamic measurements were performed using a DHR3 rheometer supplied by TA Instruments working under constraint strain, and conducted in flow mode using Couette geometry. After loading, the test samples were placed in the Couette cylinder at 25°C. The viscosity of starch suspensions was measured from a shear rate 0.1 to 10 s⁻¹ for different solid contents.

3. Results and discussion

3.1 Colloidal properties of SNC and SNP

The colloidal properties of SNCs and SNPs are given in Table 1.

Table 1: Physical characteristics of SNCs and SNPs produced from waxy-maize

	SNC	SNP
Particle size (nm)	58	37
PDI*	0,218	0,192
Zeta-potential (mV)	-32	-3
Morphology	Platelet	Ellipsoidal
CrI	69	0
Viscosity at 1 s ⁻¹	8	3
(mPa.s)2%	14	7
3%	28	15
5%		

* PDI: Polydispersity index

Both types of starch nanoparticles were clearly in the nanoscale range with respective sizes of 58 and 38 nm for SNCs and SNPs. They displayed a monomodal size distribution (Figure 1A) which was relatively narrow (PDI) around 0.2. The zeta-potential (ζ -potential) of SNCs and SNPs (Figure 1B) were about -32 mV and -3 mV, respectively, indicating that SNCs were negatively charged and virtually no charge was present on the SNPs. The negative charges on SNCs are due to sulphate groups ($-\text{OSO}_3^-$) arising from the reaction of the hydroxyl groups on the SNC surface in an esterification reaction with sulfuric acid during the acid hydrolysis process (Angellier, Hchoisnard, Molina-Boisseau, Ozil, & Dufresne, 2004). These negative charges are expected to enhance the colloidal stability by electrostatic repulsion between the double layers and to reduce the tendency of SNCs to self-aggregate. The absence of surface charges on SNPs is explained by the absence of any chemical additive during the sonication treatment that could introduce negatively charged chemical functionalities.

Fig. 1: (A) Particle size and (B) ζ -potential distribution of SNC and SNP suspension from waxy maize

The morphology of the starch nanoparticles was analyzed by FE-SEM observation after deposition of a drop of a diluted starch suspension (with a solid content of about 0.02%) on a silicon wafer, followed by drying at room temperature (Figure 2). The SNCs exhibited a platelet like shape with a well defined parallelipedic form and a size range between 35 and 130 nm. The SNPs have a less distinct shape and sizes ranging from about 30 to 60 nm. The presence of starch nanoparticles in FE-SEM observation with size larger than 100 nm, namely for SNC might be the result of the strong tendency of SNC to self-aggregate during water evaporation, even though a dilute suspension (about 0.02%) was used. The self-aggregation of SNCs is favoured by their

platelet morphology and the presence of surface hydroxyl groups favouring interaction via hydrogen bonding. The thickness of the nanoplatelets could unfortunately not be determined from FE-SEM observations since all nanoparticles laid flat on the surface. Further confirmation of a 2D dimension of both SNC and SNP was provided from AFM observation (see Figure 1 supplementary data) with thickness around 8 and 6 nm for SNCs and SNPs, respectively. These values were in agreement with literature data (Putaux, Molina-Boisseau, Momaur, & Dufresne 2003).

Figure 2: FE-SEM images of (A) SNCs derived from H_2SO_4 hydrolysis and (B) SNPs derived from ultrasound treatment from waxy maize.

Figure 3: (A) UV–vis transmittance spectra of the SNC and SNP suspension at 2 and 5% (wt. %), and (B) the visual aspect of the suspension. (Samples were sonicated for 2 min and left at room temperature for 24 h before measurement)

The nanoscale dimension of both SNC and SNP is further highlighted by the Tyndall (Quinten 2001) seen through light scattering caused by the dispersed starch nanoparticles when their suspension is illuminated by a laser beam (see inset Figure 3). In pure water, light passes without scattering and no Tyndall effect is observed. However, at 2% and 5% a bright “pathway” can be found caused by Tyndall scattering, the intensity of which is dependent on the solid content and the particle size. At 5%, the SNP suspension appeared more translucent than that of SNC, as a result of the lower particle size of the former. This evolution in the transparency of the nanosized starch suspension is further confirmed by transmittance measurements in the visible range. As shown in Figure 3, at 2% by mass concentration, both the SNC and SNP suspension exhibited roughly the same transmittance spectra with a maximum transmittance exceeding 80%. At a concentration of 5% however, a huge decrease in the transmittance is observed with the stronger effect seen for SNP suspensions. This evolution is indicative of the greater sensitivity of SNPs to aggregation when the concentration is increased, presumably due to their low ζ -potential and the absence of surface charges on the SNPs. The strong dependence of transmittance on wavelength (lower transmittance at shorter wavelengths) is explained by the strong dependence of scattering by nanoparticles on wavelength (I is inversely proportional to the fourth power of wavelength $I \propto 1/\lambda^4$).

The viscosity of the SNC and SNP suspensions (Table 1) did not increase in an appreciable manner and is independent of shear rate up to a solids content of 5%. Increasing the solids content from 2 to 5% increased the viscosity from 8 to 28 mPa.s for SNC and from 3 to 15 mPa.s for SNP. These viscosities were quite low indicating that both the SNC and SNP suspensions flowed easily and behaved like Newtonian fluids in the shear rate range studied. Such a low viscosity is beneficial for nanocomposite processing where the solids content of nanofillers in a suspension should be as high as possible in order to avoid excessive dilution upon addition of the nanofillers to the polymer emulsion. The higher viscosity for SNC suspension is likely due to the larger square section of SNCs compared to that of SNPs, which gives a higher effective volume concentration for the same mass concentration

3.2 WAXD analysis

WAXD diffraction patterns of freeze-dried starch samples are shown in Fig. 4. Native waxy-maize exhibited the A-type structure typical of cereal starch with 2θ diffraction peaks at 15, 17, 18, and 23° (Zobel 1984) and a crystalline index (CrI) around 47° . The SNCs exhibited an A-type crystallinity similar to native starch with diffraction peaks at (2θ) 15, 17, 18 and 23° and a CrI of 69. The substantial increase in the crystalline index (CrI) for SNCs relative to the native starch grain is explained by the degradation of the amorphous and paracrystalline regions of the starch granules during the acid hydrolysis treatment.

Fig. 4: WAXD diffraction patterns of freeze-dried native starch, SNCs and SNPs from waxy maize. The SNP diffractogram on the other hand only showed a broad halo between 5 and 30°, associated with amorphous material. The SNPs were thus completely amorphous and the starch had totally lost its crystallinity during the ultrasound fragmentation process.

3.3 Thermal behavior of starches and their corresponding SNC and SNP:

The thermal behavior of native starch and their corresponding SNCs and SNPs was studied by differential scanning calorimetry (DSC), ramping the temperature at a rate of 10 °C/min in the presence of an excess of water (70% by weight) (Figure 3 supplementary data). Native waxy maize showed a large endothermic peak extending from 65 to 80°C assigned to the gelatinization of starch and the melting of crystallites, in agreement with literature data (Atichokudomchai, Varavinit, & Chinachoti, 2012). SNC and SNP exhibited a single endothermic peak around 90 and 78°C, respectively. However, the heat effect is rather low and presumably did not correspond to the melting of crystallites. These data were different than those reported in the literature where two thermal transitions were observed around 90-95°C and 105-110°C (LeCorre, Bras & Dufresne 2012a) with the first peak being attributed to the first stage of crystallites melting (unpacking of the double helices) and the second transition to the second stage of crystallites melting (unwinding of the helices). However, it should be noted that no consensus on the thermal transition of SNCs is established, presumably due to the limited number of publications and the difference on the origin of starch used in the preparation of SNCs. The absence of a clear melting endotherm for SNP is likely due to their amorphous character and the absence of crystallites.

3.3 Reinforcing effect of SNC and SNP

Starch nanoparticles have been investigated in a broad range of applications including as nanoreinforcement, emulsion stabilizer (Bel Haaj, Ben Mabrouk, Thielemans & Boufi, 2013; Bel Haaj, Thielemans, Magnin & Boufi, 2014), drug carrier, (Santander-Ortega *et al.*, 2010) fat replacement (García *et al.*, 2009), and adsorbent for wastewater treatment (Alila, Aloulou, Thielemans & Boufi, 2011). However, so far, the main application of starch-based nanoparticles is

in the field of nanocomposites where their inclusion in a polymer matrix is expected to enhance the stiffness and the strength, but also the barrier properties to reduce water vapor transmission and oxygen permeability (Angellier, Molina-Boisseau & Dufresne, 2005). However, it was shown that the reinforcing capability was dependent on the botanic source of starch. More specifically, the content of amylose of native starch appeared to affect the nanofiller properties (LeCorre, Bras & Dufresne 2012b).

In order to investigate the SNP reinforcing effect, nanocomposite films with different nanofiller loadings were prepared by solvent-casting a mixture of SNC or SNP suspensions and a latex dispersions of poly(butylmethacrylate). The resulting films were analysed by DMA. The temperature dependence of the storage tensile modulus, E' , as a function of temperature at 1 Hz, for the nanocomposite films with different content in nanofiller is shown in Fig. 5. At low temperature, i.e. below the T_g , the storage moduli underwent only a slight enhancement with respect to the unfilled matrix upon inclusion of the nanoparticles. This behavior is expected and common to nanocomposite materials based on nanosized polysaccharide particles. It can be explained by the relatively low difference between the modulus of the glassy matrix (close to 1 GPa) and that of the nanofiller network (in the range of 10–15 GPa) while taking into account the low filler content (Boufi, Kaddami & Dufresne, 2014).

However, above the glass transition temperature (around 35°C), the storage modulus is more sensitive to the presence of the nanofiller and it can be seen to increase significantly with SNP and SNC addition, in line with the well-known reinforcing effect of nanobased polysaccharide nanoparticles (Boufi, Kaddami & Dufresne, 2014). The reinforcing effect brought by the starch nanofiller was different for both types of starch nanoparticles. This can directly be seen through comparison of the relative modulus $E_r = E_{com}/E_{matrix}$ (with E_{com} and E_{matrix} the storage modulus of the nanocomposite and unfilled matrix respectively, measured in the rubbery region taken here at 70°C) versus the nanofiller content (Figure 6). Above a nanofiller loading of 2%, the increase in

the modulus was much higher when reinforcing the polymer matrix with SNCs than with SNPs. For instance, at 10 wt% of nanofiller, the modulus is 50 times higher than the modulus of the neat matrix when SNCs were used, whereas it is only about 20-fold that of the neat matrix modulus when SNPs were used as reinforcement. As reported by Angellier *et al.* (2005) the reinforcing effect of SNCs is attributed to the formation of a percolating starch nanocrystal network through hydrogen linkages between starch nanoparticle clusters. The lower aspect ratio of the SNPs relative to the SNCs, and the higher tendency to aggregate will thus reduce the establishment of this percolated network. This will result in a lower reinforcement for the same content of nanofillers. In addition, the SNPs are also almost completely amorphous, which inevitably leads to a lower intrinsic modulus of SNPs relative to the modulus of crystalline SNCs.

Figure 5: Evolution of the storage tensile modulus, E' , versus temperature at 1 Hz for nanocomposites films obtained from (A) SNCs, and (B) SNPs.

It is also worth to note the lower reinforcing effect brought by the inclusion of SNCs or SNPs when compared to nanocellulose such as nanofibrillar cellulose. For the purpose of comparison, we have included the improvement in the modulus produced by the inclusion of nanofibrillated cellulose (NFC) from wood. Two reasons are likely to account for this difference. Firstly, the difference in the morphological features of the nanofiller; NFC is a high aspect-ratio nanoparticle while SNC is a 2D nanofiller. This difference led to a much higher aspect ratio (L/d , L being the length and d the diameter of the fibrils) for NFC. Secondly, individual cellulose nanofibrils display a much higher stiffness of nanofibril around 65-70 GPa according to Chang and Wang (Chang & Wang 2008) than that of SNCs or SNPs, albeit no published data was reported in the literature regarding the direct measurement of the stiffness of SNCs. The higher modulus of NFCs results from the packing of stretched cellulose chains in the form of elementary crystallites along the cellulose nanofibril axis. On the other hand, starch nanocrystals with a platelet-like structure are composed of a hundred parallel stranded double helices densely packed, each consisting of about 10 to 20 glucose units. The axis of the chains is lying almost perpendicular to the nanocrystal plane and the cohesion is mainly held through hydrogen bonding.

Figure 6: Evolution of the relative storage modulus nanofiller content for SNC and SNP at 70 °C. For sake of comparison, data for nanocomposite based on nanofibrillar cellulose was included from Besbes *et al.* 2011.2011)

Tan δ curves (Figures 3a and 3b in supplementary data) exhibited α relaxation of the matrix, associated with the glass transition, around 40°C. Increasing the increase in the nanoparticulate starch/SNP content leads to a drop in the magnitude of the relaxation but to a different extent of which depended on according to the type of nanofiller. The drop is much more pronounced in the presence of SNCs than in the presence of SNPs, which is in agreement with the lower reinforcing effect of SNPs. Furthermore, one can note that the maximum position of tan δ undergoes an apparent shift towards lower temperatures as the content of starch nanofiller is increasing. This behavior is in agreement with published data (Angellier, Molina-Boisseau & Dufresne 2005) commonly observed in nanocomposites based on polysaccharide nanoparticles and is ascribed to a mechanical coupling effect (Boufi, Kaddami & Dufresne 2014).

3.4 Optical Properties of nanocomposite films

In addition to the mechanical reinforcing effect expected from the inclusion of a nanofiller, the optical transparency that can be achieved with nanoinclusions is another sought-after property.

The optical transparency of the nanocomposite film was evaluated by transmittance measurement from 400 to 800 nm (Figure 7). The neat PBMA matrix was an optically transparent sheet with light transmittance above 90% in the wavelength range of visible light (400–800 nm). The inclusion of starch nanofillers is accompanied by a decrease in the transparency. However, the drop in the transmittance was more pronounced for SNCs than for SNPs. In the presence of SNPs, the transmittance was little affected up to a content of 8% and then strongly decreased for filler contents over 10%. In contrast, for SNCs a huge decrease in the transmittance was noted even in the presence of 2% nanofiller. The drop in the transparency with the inclusion of nanoparticles within a polymer matrix is expected and is due to light scattering by the particles, the magnitude of which strongly depends on the particle size. In general, 40 to 50 nm is the upper limit of the nanoparticle diameter to avoid intensity loss of the transmitted light via Rayleigh scattering (Althues, Henle & Kaskel, 2007). Thus, the degree of transparency of a nanocomposite film will strongly depend on the dispersion of the nanofiller within the host matrix. Any aggregation of nanoparticles during film processing will increase the scattering effect and inherently reduce film transparency. Therefore, the higher drop in the transmittance in presence of SNCs is likely due to

the stronger tendency of SNC to aggregate into a percolated network during the film-formation process as a result of the higher aspect ratio, presumably through self-interaction via hydrogen bonding. The difference in the particle size distribution between SNC and SNP is another reason likely to account for the lower transparency of SNCs based nanocomposite. The presence in SNC of nanoparticles with size exceeding 60 nm (see figure 1) brought about excessive light scattering that reduced the fraction of transmitted light.

Conclusions

We compared the properties of starch nanocrystals (SNCs) extracted from waxy maize starch through an acid hydrolysis with those of amorphous starch nanoparticles (SNPs) obtained through a purely mechanical ultrasound treatment also starting from waxy maize starch. The amorphous SNPs were slightly smaller and had no surface charge, whereas the SNCs had the expected platelet-like morphology with a negative surface charge introduced as a result of the use of sulphuric acid in the acid hydrolysis step. As a result of their platelet-like morphology, the SNCs performed better (2.5x modulus improvement at 10wt% nanoparticle loading when compared to SNPs) in reinforcing a polymer film. On the other hand, SNPs reduced the transparency of the composite films to a lesser extent than the SNCs due to their smaller size.

Although SNPs exhibited a lower reinforcing potential than compared to that brought by SNCs, the easier method to produce SNPs through a green approach based on ultrasonication without the necessity to add any chemical additive nor implement further purification steps should contribute to arouse interest for the usefulness of this class of starch nanoparticles. One possible application is their use as a Pickering emulsifier to prepare emulsions free from any added surfactant. This approach was shown to be efficient for the Pickering emulsion polymerization of acrylic monomers (Bel Haaj, Magnin & Boufi 2014).

Acknowledgments

The authors thank the CRNS (France), DGRS (Tunisia), and Research Foundation - Flanders (Grant G.0C60.13 N) for their financial support. The Laboratoire Rhéologie et Procédés is part of the Labex Tec 21 (Investissement d'Avenir – grant agreement n° ANR-11-LABX-0033) and of the Polynat Carnot Institut (Investissement d'Avenir – grant agreement n° ANR-11-CARN-030-01).

References

- Alila, S., Aloulou, F., Thielemans & W., Boufi, S. (2011). Sorption potential of modified nanocrystals for the removal of aromatic organic pollutant from aqueous solution. *Ind. Crop. Prod.*, 33, 350-357.
- Alonso, M. J., Janes, K. A., & Calvo, P. (2001). Polysaccharide colloidal particles as delivery systems for macromolecules. *Adv. Drug Delivery Rev.*, 47, 83-97.
- Althues, H., Henle, J. & Kaskel, S. (2007). Functional Inorganic Nanofillers for Transparent Polymers. *Chem. Soc. Rev.*, 36, 1454–1465.
- Angellier, H., Choisnard, L., Molina-Boisseau, S., Ozil, P. & Dufresne, A. (2004). Optimization of the Preparation of Aqueous Suspensions of Waxy Maize Starch Nanocrystals Using a Response Surface Methodology. *Biomacromolecules*, 5, 1545–1551.
- Angellier, H., Molina-Boisseau, S. & Dufresne, A. (2005). Mechanical properties of waxy maize starch nanocrystals reinforced natural rubber. *Macromolecules*, 38, 9161-9170.
- Atichokudomchai, N., Varavinit, S. & Chinachoti, P. (2002). Gelatinization transitions of acid-modified tapioca starches by differential scanning calorimetry (DSC). *Stärke*, 54, 296–302.
- Bel Haaj, S., Ben Mabrouk, A., Thielemans, W. & Boufi, S. (2013). A one-step miniemulsion polymerization route towards the synthesis of nanocrystal reinforced acrylic nanocomposites. *Soft Matter*, 9, 1975–1984.
- Bel Haaj, S., Magnin, A., Pétrier, C. & Boufi, S. (2013). Starch nanoparticles formation via high power ultrasonication. *Carbohydrate Polymers*, 92, 1625-1632.
- Bel Haaj S., Magninb A. & Boufi S. (2014) Starch nanoparticles produced via ultrasonication as a sustainable stabilizer in Pickering emulsion polymerization. *RSC Advances* 4, 42638.
- Bel Haaj, S., Thielemans, W., Magnin, A. & Boufi, S. (2014). Starch Nanocrystal Stabilized Pickering Emulsion Polymerization for Nanocomposites with Improved Performance. *ACS Applied Mater*, 6, 8263-8273.

- 468 Besbes I., Rei Vilar M. & Boufi S. (2011) Nanofibrillated cellulose from alfa, eucalyptus and pine
469 fibres: preparation, characteristics and reinforcing potential, Carbohydr. Polym. 86, 1198–
470 1206.
- 471 Boufi, S., Kaddami, H., & Dufresne, A. (2014). Mechanical performance and transparency of
472 nanocellulose reinforced polymer nanocomposites. Macromolecular Materials and Engineering,
473 299, 560–568.
- 474 Cheng, Q. & Wang, S. (2008) A method for testing the elastic modulus of single cellulose fibrils
475 via atomic force microscopy. Composites Part A: Applied Science and Manufacturing,
476 39(12):1838-1843
- 477 Couvreur, P., Lemarchand, C. & Gref, R. (2004). Polysaccharide-decorated nanoparticles.
478 European Journal of Pharmaceutics and Biopharmaceutics, 58, 327-341.
- 479 LeCorre D, Bras J & Dufresne A. a(2012) Influence of native starch's properties on starch
480 nanocrystals thermal properties, Carbohydrate Polymers 87, 658– 666.
- 481 LeCorre D.S., Bras J. & Dufresne A. b (2012) Influence of the Botanic Origin of Starch
482 Nanocrystals on the Morphological and Mechanical Properties of Natural Rubber
483 Nanocomposites. Macromol. Mater. Eng. 297, 969–978
- 484 Dufresne, A., Cavaillé, J.Y. & Helbert, W. (1996). New nanocomposite materials:
485 microcrystalline starch reinforced thermoplastic. Macromolecules, 29, 7624–7626.
- 486 Dufresne, A. (2010). Processing of Polymer Nanocomposites Reinforced with Polysaccharide
487 Nanocrystals. Molecules, 15, 4111-4128.
- 488 Eichhorn S. J., Dufresne A., Aranguren M., Marcovich N. E., Capadona J. R., Rowan S. J., Weder
489 C., Thielemans W., Roman M., Renneckar S., Gindl W., Veigel S., Keckes J., Yano H., Abe K.,
490 Nogi M., Nakagaito A. N., Mangalam A., Simonsen J., Benight A. S., Bismarck A., Berglund L.
491 A. & Peijs T.. (2010) Review: current international research into cellulose nanofibres and
492 nanocomposites, J. Mater. Sci. 45, 1–33.
- 493
- 494 Eyley, S., Thielemans, W. (2014). Surface modification of cellulose nanocrystals. Nanoscale, 6,
495 7764-7779.
- 496 Gallant, D.J., Bouchet, B. & Baldwin, P.M. (1997). Microscopy of starch: evidence of a new level
497 of granule organization. Carbohydrate Polymers, 32, 177-191.
- 498 García, N. L., Ribba, L., Dufresne, A., Aranguren, M. I. & Goyanes, S. (2009). Physico-
499 Mechanical Properties of Biodegradable Starch Nanocomposites. Macromol.Mater. Eng., 294,
500 169–177.
- 501 Kim, H.-Y., Park, S. S. & Lim, S.-T. (2015). Preparation, characterization and utilization of starch
502 nanoparticles. Colloids and Surfaces B: Biointerfaces, 126, 607–620.

Quinten M. in *Optical Properties of Nanoparticle Systems: Mie and Beyond*, Wiley-VCH 2001, pp23.

Lamanna, M., Morales, N. J., García, N. L. & Goyanes, S. (2013). Development and characterization of starch nanoparticles by gamma radiation: potential application as starch matrix filler. *Carbohydr. Polym.*, 97, 90-97.

LeCorre, D., Bras, J. & Dufresne, A. (2010). Starch nanoparticles: a review. *Biomacromolecules*, 11, 1139–1153.

Liu, Z., Jiao, Y., Wang, Y., Zhou, C. & Zhang, Z. (2008). Polysaccharides-based nanoparticles as drug delivery systems. *Adv Drug Deliv Rev*, 60, 1872-8294.

Mua, J. P., Jackson, D. S. (1997). Fine Structure of Corn Amylose and Amylopectin Fractions with Various Molecular Weights. *J. Agric. Food Chem.*, 45, 3840-3847.

Putaux, J. L., Molina-Boisseau, S., Momaaur, T. & Dufresne, A. (2003). Platelet Nanocrystals Resulting from the Disruption of Waxy Maize Starch Granules by Acid Hydrolysis. *Biomacromolecules*, 4, 1198-1202.

Santander-Ortega, M. J., Stauner, T., Loretz, B., Ortega-Vinuesa, J. L., Bastos-Gonzalez, D., Wenz, G., Schaefer, U. F. & Lehr, C. M. (2010). Nanoparticles made from novel starch derivatives for transdermal drug delivery. *J. Control. Release.*, 141, 85-92

Song, D., Thio, Y.S. & Deng, Y. (2011). Starch nanoparticle formation via reactive extrusion and related mechanism study. *Carbohydr. Polym.*, 85, 208-214.

Thielemans, W., Belgacem, M. N. & Dufresne, A. (2006). Starch Nanocrystals with Large Chain Surface Modifications. *Langmuir*, 22, 4804-4810.

Zobel H., in R.L. Whistler, J.N. BeMiller &, and E.F. Paschall (Eds.), *Starch: Chemistry and Technology*, 2nd ed., Academic Press, Orlando, FL, 1984, pp 285-309.