Cellulose

Comparison of cellulose nanocrystals obtained by sulfuric acid hydrolysis and ammonium persulfate treatment, intended as coating onto flexible food packaging materials

--Manuscript Draft--

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Abstract:	Cellulose nanocrystals (CNCs), extracted from trees, plants, or other cellulose-containing species, can be used in combination with other materials to improve their performance or introduce new potentialities; their preparation usually involves a chemical acid hydrolysis process intended to dissolve amorphous chains from the cellulose fibers to release crystal domains. In this work the morphological and chemical characterization of CNCs produced from cotton linters through two different processes, the common acidic hydrolysis (H_2SO_4) and a less investigated ammonium persulfate treatment (APS), was carried out; the main purpose was to compare and understand the potentialities of using these two types of CNCs as coatings for Poly(ethylene terephthalate) (PET) film. CNCs produced by APS treatment showed higher charge density due to the carboxylic groups formed during the process, more clarity of the solution and, as a consequence, higher transparency of the coating, but similar crystallinity pattern; these peculiarities confer higher oxygen barrier respect to the CNCs produced by H_2SO_4 treatment, and the availability of active sites for potential surface modification or chemical grafting. Anyway, both CNCs coatings showed oxygen permeability coefficient lower than synthetic resins commonly used in flexible packaging, they did not affect significantly the optical properties of the substrate and revealed good coefficients of friction. The use of such nanocellulose as water based coating can be considered a possible alternative to conventional food packaging materials: due to the moisture sensitivity of the coating and due to the no sealable property, as happens for EVOH or PVOH oxygen barrier synthetic resins, this type of

	material need to be laminated with another plastic layer such as a polyolefin. In this case it might enhance the final properties of packaging solutions for perishable food products, while reducing their environmental impact with a thin layer of a bio-based polymer.
Response to Reviewers:	Responses to Reviewers Ms. Ref. No.: CELS-D-15-00434 Title: Comparison of cellulose nanocrystals obtained by sulfuric acid hydrolysis and ammonium persulfate treatment, intended as coating onto flexible food packaging materials Thank you for the editor-in-chief and reviewer's constructive comments that contribute to improve the overall quality of our manuscript. We have taken into consideration all the remarks assigned and we tried to incorporate major revisions as suggested. We hope that the revised manuscript will be favourably reconsidered for publication. The answers to the comments are reported in italic in the following paragraph. COMMENTS FOR THE AUTHOR: Editor-in-Chief: We are asking all authors to use the conventions for the Miller indices described in French, Cellulose 21:885-896 (2014). It would be interesting to test your peak deconvolution method on a calculated diffraction pattern, as described in that paper. The authors wish to thank the Editor for the important remark that, together with the specific comment of the reviewer 2 (Line 85) concerning the classical model of crystallinity of the CNCs, led us to study into deep the question concerning the evaluation of the crystallinity of the CNCs. As suggested by the Editor, we decided to use the conventions for the Miller indices. We reported in Figure 3 the powder diffraction patterns calculated with Mercury software in comparison with the experimental data for CNCs from APS and CNCs from H2SO4. The powder diffraction patterns were calculated on the basis of the published atomic coordinates and unit cell dimensions contained in the modified "Crystal information files" (.cif). It is possible to observe in Figure 3 that CNCs have a diffraction pattern more similar to the cellulose Iβ crystal structure with the three main peaks for the Iβ one-chain triclinic unit cell with the Miller Index of (1-10) (110) and (200). The differences in the peak widths correspond to different crystallite sizes. The size can be assessed with the Scherrer equation. We decided to delete the data concerning the calculation of the degree of crystallinity obtained using the XRD analysis. As reported by French, Cellulose 21:885-896 (2014), on these simulated patterns, there is no modelling of amorphous scattering, so it is not appropriate to position a background intensity curve as high as minimum intensity at 18° 2θ. For similar reason the calculation of the degree of crystallinity with the deconvolution method of the same XRD spectra is not appropriate. CI in this case is indeed calculated from the ratio between the area of all crystalline peaks to the total area underneath the diffraction pattern. By this approach, several assumptions about correlation between peak broadening and amorphous contribution are necessary and still under vivid debate. The authors prefer to use the XRD results to stress the self-evident quality of the CNCs obtained. A more common notion of "Degree of crystallinity" is given by NMR analysis (with the Lorenzian functions used to perform the deconvolution of the C4 peaks), providing the relative masses of crystalline and amorphous material in a given sample. Such a method was used in the paper to fulfill the requests of the reviewers. Reviewer #1: This manuscript reported cellulose nanocrystals prepared by H2SO4 hydrolysis and by APS treatment. The morphology, crystallinity, chemical and thermal

properties, charge density as well as surface energy of the two types of CNCs were studied and compared. The application of CNCs as barrier films for food packaging were also investigated. Overall, the manuscript is well conceived and written. The authors have done thorough characterizations to compare CNCs produced by two processes. The science is sound and the topic is interesting to the field of nanocellulose. Several minor revisions are suggested that the authors may consider addressing.

Page 19. Line 441-442. FTIR of CNCs by APS treatment has two peaks around 1700 cm-1. It will be helpful to assign both peaks.
The two peak around 1700 cm-1 were identified. FTIR spectra of the different CNCs show distinct v(C=O)stretching peaks from 1735 cm-1 assigned to the COOH, 1613 cm-1 assigned to the COO- Na+ and around 1630 cm-1assigned to the COO-NH4+ respectively as reported in literature in Lam et al., 2013. In this specific case we tested, with the FTIR technique, the CNCs in acidic form (pH=2), so we are able to identify the COOH peak (1735 cm-1) and the peak of COO-NH4+(1630 cm-1).

Page 19. Table 4. It is suggested to include the clarity of CNCs in water dispersion produced by two processes and correlate with charge density and transparency of the films (table 6).
In agreement with the reviewer's remark, we added the clarity of the CNCs water solution at 7% w/w after ultrasound treatment for 15 minutes (0.7 cycles, 70% output). The clarity was measured as turbidity of the solution using a spectrophotometer at λ=600nm. The results show that turbidity of the APS solution is lower than the H_2SO_4 one and this could be related with surface charge density. Indeed, in the case of APS solution, the higher surface charge density induces a more efficient electrostatic interactions with water (i.e. a tighter hydrogen bonds network), leading to more stable CNCs dispersion. After solution deposition on the substrate, the strong capillary and surface forces due to drying and adsorption trigger a rearrangement of the crystal structure. As indicated also by the second reviewer, cellulose is known to have a very high transparency in solid non-porous form. In this case, we are in presence of a decrease of transparency of the PET film (the support) and this decrease is lower in the case of CNCs from APS treatment. Moreover, SEM micrographs recorded with 180KX Magnification showed homogenous surface of the coating with the presence of some holes, causing a more pronounced roughness in CNCs H_2SO_4 coating than in APS.
In our opinion this set of data fulfills the reviewer’s requests shedding a deeper light with respect to the pristine manuscript, strongly supporting our hypothesis as well.

Figure 2. TEM images with same magnification are suggested for better comparison of dimensions of CNCs produced by two treatments.
In agreement with the reviewer remark, the figures were selected with the same magnification for a better comparison of the CNCs

Reviewer #2: Referee comments on Mascheroni et al.'s "Comparison of cellulose nanocrystals obtained by sulfuric acid hydrolysis and ammonium persulfate treatment, intended as coating onto flexible food packaging materials"

The authors have produced cellulose nanocrystals (CNCs) by two different protocols; the "traditional" sulphuric acid approach and a newer approach based on oxidation with ammonium persulfate (APS). The two CNCs were then characterised and used as coatings on PET. Studies on newly developed chemical protocols are always needed and are of interest. However, I think the authors need to better emphasise what missing piece of information they want to add with this manuscript. If this had been the first time APS had been used for making CNCs, I would have voted for major revision. However, since there already are publications on this protocol, as the authors also mention, I judge this manuscript as borderline between major revision and reject (and resubmit). Since I am absolutely positive that the authors will try to have the manuscript published, in Cellulose or elsewhere, I have listed a few things to that I believe need to be addressed to have this manuscript published.

General 1. Improve the "packaging". In the introduction the authors need to better emphasise on what knowledge gap this manuscript is intended to fill, i.e. why this is interesting for our society and not just a report of experimental results. Both style and language should also be improved. If a thorough proof reading is not enough, the authors are encouraged to subject the manuscript to professional linguistic review.

We added two sentences at the end of Introduction to better emphasize the most important goal of this work, in relation to the packaging sector needs. The chance of producing CNCs from largely available biomass, as well as from industrial byproducts, by a procedure able to remove lignin and that does not need special pre-treatments, can open new frontiers in coating technology of flexible packaging materials. Preliminarily, however, we wanted to check possible differences between CNCs produced by conventional acidic hydrolysis and the APS process, and this comparison is the main target of this paper.

General 2. Numbers are in general given with several decimals. I doubt that they always are significant? When values are given with errors, it is also preferable to inform the reader if they are standard deviations or confidence limits, and on what data they are based. I cannot always find how many measurements that were performed. Write this in the caption of the figure or table, or in Methods.

According to the reviewer remark, we reconsidered the data and we let only the number of decimal essential to compare the different CNCs. All values are reported with standard deviation indicated in the text ad SD

Line 23: (CNC) should read (CNCs).
The word CNC has been changed in CNCs

Line 34: avoid formulations as "a little higher".
The term has been delated

Line 52: "cellulose nanofibrils (NFCs)" should read "cellulose nanofibrils (CNFs)".
The word NFCs has been changed with CNFs

Lines 53-57: There are studies on CNFs that show that it is advantageous to keep the hemicelluloses (e.g. Galland et al. Biomacromolecules 2015, DOI: 10.1021/acs.biomac.5b00678).
Unfortunately, when we wrote the paper, we were not aware of the article of Galland et al. It is definitely pertinent to the subject and, therefore, we added the new citation and a comment

Line 67: There are others that have used periodate, for example Yang et al. Langmuir 2012, who I believe also filed a patent on this. There are also other chemistries that can follow the periodate oxidation.
According to the reviewer's suggestions about lines 67 and 69, we have restructured the initial part of Introduction and inserted two pertinent citations (Yang et al. 2013 - Chinga-Carrasco and Syverud 2014)
Line 69: Carboxymethylation might also deserve being mentioned.
See above

Line 77: The abbreviation NFCs (which should be CNFs) has already been defined on line 52.
Ok correct

Line 85: The classical model is that cellulose is arranged in crystalline and amorphous regions. However, this model is highly debated! As far as I know there is no experimental evidence that support this model and I suggest that you do not use it.
In agreement with this remark, the authors analyzed in a better way the literature, in particular the articles published on "Cellulose". The authors referred to the model presented in literature, for example in the article on Carbohydrate Polymers 83 (2011) 1740–1748 Water sorption behavior and gas barrier properties of cellulose whiskers and microfibrils films by the authors Belbekhouche, S. Bras J, Siqueira G, Chappey C Lebrun L, Khelifi B, Marais S and , Dufresne A.

	Other articles published on Cellulose and more focused on the crystallinity structure of cellulose investigated the issue more deeply and surely it is possible to assess that the question of cellulose structure is highly debated. In particular in Nishiyama Y, Structure and properties of the cellulose microfibril Journal of Wood Science (2009), 55: 241-249 the structure of microfibil is presented and discussed. The article reports that cellulose microfibril can be considered as a single thin and long crystalline entity with highly anisotropic physical properties and the two models (series model and microfibril model) are compared and discussed. Also in two other articles (Nishiyama Y, Johnson GP and French AD Diffraction from nonperiodic models of cellulose crystals, Cellulose 2012 19:319-336 and Nishiyama Y, Kim UJ, Kim DY, Katsumata KS, May RP, Lagan P. Periodic disorder along ramie cellulose microfibrils. Biomacromolecules, 2003: 4, 1013-1017) it is reported that observation by microscopy hypothesis on the morphogenesis, and molecular modeling often lead to structural models that do not necessarily fit the simplistic two-phase model. Cellulose is thought to crystallize in proximity to the polymerization site where many chains are simultaneously produced and deposited, leading to a continuous, fine but crystalline filament. In this filaments, no amorphous regions have been observed, and disordered, or strained regions seems to be very small with the presence of periodic defects. The measurement of chemical reactivity shows the rupture of hydrogen bonding O3H-O5(of the ring) only in this region of defect. The text was so modified according to these knowledges. Line 131: I assume that the expression "elongated voids" refers to the lumen of the fibre. Yes, the technical word for cellulose matrix is “Lumen of the fiber”. So the text was corrected Line 161: CNC should read CNCs. Ok it has been corrected. Line 188: What do the authors mean with "to avoid decomposition or burning"? Cellulose will not burn at this temperature. The standard way to determine dry content of cellulosic fibres is to dry them overnight at 105 °C. The observation of the reviewer is right, the official method to determine the dry content is to put the sample at 105°C overnight. The text was corrected Line 192: Why was the dispersion freeze dried? Would this not only generate a problem to re-disperse the CNCs? The authors would like to explain the reason of the freeze drying treatment. The production was done on laboratory scale and the variables that are inserted time by time, for a research approach, are not able to guarantee always the same dry content for each production. For the coating application, we needed solutions with the same concentration of CNCs so we prefer to dry the CNCs production. Even more, the authors have seen that the ultrasound treatment conditions of the CNCs solution are able to determine the final properties of the CNCs solution and, as a consequence, also of the coating. For these reasons, freeze dried product led us to modulate the final solution properties Line 256: Why is 1.6 g/cm3 used there and 1.58 g/cm3 on line 319? The text was corrected Line 274: Hold the Alt key and type 0197 to produce an Å. The text was corrected Line 301: Here and on many other places in the text the authors cannot differentiate between the proper use of CNC and CNCs. Here it should be CNC. See my general comment about linguistic review. The auhors decided to use uniform style , so they will always use CNCs (plural form) Line 307: Very short. How many samples and how many spots in each sample were evaluated? The transmittance of the two different CNC coating was measured at 550 nm, according to the ASTM D 1746-70, by means of a Perkin-Elmer L650 UV-VIS spectro photometer. Each measure was replicated three times, by analyzing four spots, each

	replica. Line 312: I gravimetric determination the best way to determine the thickness? This can only give an average and no indication on the structure and homogeneity of the coating. Cross-sections under SEM would presumably confirm the thickness and give additional information about the coating. In agreement with the reviewer remark, cross section under SEM observation were done. The author agree with the comment of the reviewer that the method previously used by the author is not able to provide indication about the structure and the homogeneity of the coating. The authors decided to delete the data obtained with the weight method because this method require a precise estimation of CNCs density that we considered 1.6 g/cm3, a value currently found in literatureattributed to the powder form of the CNC and probably it is not the density of the CNCs in form of coating. SEM observation permitted to verify, in fact, that the surface of the coating is homogeneous (the thickness was quite the same in different point of the surface)but the value was lower than the value obtained with the previous method, the reason could be an underestimation of the density. The values of the thickness for both the coating was around 450±50 nm. The text was corrected according to these results and a figure of SEM observation was inserted Line 328: Odd choice of reference. A more obvious choice would be any edition of Crank's "Mathematics of diffusion". The authors should also be aware that this equation assumes two (or several) layers that in no way interact with each other, i.e. it neglects the interface between the PET and coating. According to the reviewer's remark, the authors changed the citation and added a sentence to underline the assumption behind the equation Line 330: "Strength of (wet) adhesion" is an odd title for this evaluation. Water resistance or something would be more suitable. Since the sample is wiped (wet) wear or abrasion resistance might be a fair description. The authors agree completely with the reviewer remark, in this paper no measure of strength adhesion are presented but only a test of the water resistance of the CNCs. The aim was to test the reaction of the coating to the high humidity because it is well known the problem of cellulose water sensitivity Line 377: "… to lead the quantitative obtainment of cellulose nanocrystals." Please rephrase. The sentence was changed: "The two different processes used on the same batch of cotton linters were able to give cellulose nanocrystals that are characterized to see their peculiarities." Line 399: avoid "perfectly matches". The word perfectly was delated Table 2: With an error of 47 nm in length, there is no need what so ever to use two decimals on the average (110 nm)!! Furthermore, if simple error propagation is performed, the uncertainty of the L/d for APS is 8, i.e. from Table 2 it is impossible to with any certainty say that APS results in CNCs with higher aspect ratio! The criticism is completely justified and the authors changed accordingly the table and the comments Line 424: "little higher"… Neither evaluation technique shows any significant difference in crystallinity. Unless measurements were performed under inert gas, the indication of a difference could simply be due to different moisture content. The author agree with the reviewer and also according to the Editor's suggestions, this part of the text was revised and the original Table 3 was delated, leaving only the crystallinity value obtained by NMR analysis. The measurement were done under an inert gas, anyway the difference are not statistically significant as reported in the text.. Line 500: Omit + in CNC-COONa+, or write CNC-COO-Na+ Ok, it has been corrected with the second version Line 518: Why is the coating reducing the transparency? Cellulose is known to have a

very high transparency in solid non-porous form. An increase in transparency or haze might come from particles that are large enough to scatter visible light, or from an increased surface roughness.

According to the reviewer's suggestion the authors explained the small increases of haze and the small reduction of transparency with a comment related to surface roughness (displayed by SEM observation) and the clarity of the CNCs solutions that was added in Table 5.

Line 524: avoid "huge".
The word huge was delated

Line 557: If this is a comparison between two types of CNCs, please describe why the APS process is better than sulphuric acid.
The authors have underlined many times in the text that CNCs obtained by the APS process seem different and better than CNCs produced by sulfuric acid hydrolysis, according to the final use to which they are intended. The nanocrystals from APS lead to a more transparent film, a lower COF, a more stable coating in a moist condition and, mostly, a higher barrier to oxygen permeation. Moreover, concerning the APS process, the authors underlined, with reference to the literature cited, the utility of a process that can be applied to lignin containing cellulose sources

Table 7: To really test the coatings from the two CNCs, I suggest that measurements are performed also at 80% RH, or even 90% RH. Table 7 shows that the permeability increases 6 times for sulphuric acid CNCs and 22 times for APS CNCs when the RH is increased from 0 to 50 %. This indicates that the sulphuric acid CNCs might be better than APS CNCs at higher (more relevant) humidities. Also, what is the reason behind the higher sensitivity? Is it only due to higher charge or is it something else?
These reviewer's comments are justified by the results presented. Therefore, the authors added the following sentence: The apparent higher moisture sensitivity of CNCs from the APS process, which shows a higher increase of permeability when measured at 50% RH in comparison with H_2SO_4 CNCs coated film, is consistent with the higher wettability shown by optical contact angle evaluation (Table 5) and can be attributed to the higher charge density. In any case, the moisture sensitivity of the coating, as happens for the most common oxygen barrier synthetic resins (EVOH or PVOH), is overcome by the need of providing a sealable layer, thus poliolefinic and moisture barrier, to the possible final laminate in a real application.

Line 561: Shorten the discussion by moving some parts to Discussion.
The authors removed about 80 words from the Conclusion

Line 581: Define in what way the APS coating is better, or at least under what conditions.
As the author stated in the conclusion: "…transparency, friction coefficient and wettability of the coating were always better for the CNCs coating obtained by the APS process, and especially the oxygen barrier property revealed as a very interesting and promising feature. Therefore, these advantages must be correlated to the efficacy of cellulose fragmentation and to the higher charge density due to the presence of carboxylic groups, which in turn gave rise to a more performing coating." Also, with reference to the APS process, the authors wrote in the conclusion that: "The use of ammonium persulfate to obtain cellulose nanocrystals has been initially proposed in order to perform in one single step a complete fragmentation of various cellulosic biomasses, in contrast to acid hydrolysis which requires pretreatment steps for cellulose isolation. This is a very important target that deserves to be fully explored to valorize largely available, renewable resources" The same arguments were reported along the discussion of results

Figure 1: No real need for this figure. I the authors want to show the structure, use this structure in a chemical reaction to simultaneously explain what happen when APS reacts with cellulose.
The figure was deleted

Figures 2 and 3: Use the images of same magnification.
The figures were corrected and renamed Fig1a and Fig1b

	Figure 4: What is meant by arbitrary units? Please read up on DLS. The y-axis should be intensity, number or volume, and all three mean different things. I suggest that signal intensity is used since it does not "filter away" the effect of larger particles. The figure was corrected using the signal intensity as suggested and renamed as Fig 3 Figure 5: Is this one single measurement or how many measurements were performed? The authors performed three measurement and the curve can be stackable so just one curve was reported

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Dr. Alfred D. French, Editor-in-Chief, Cellulose,

Milan, 3rd November 2015

Dear Dr. French,

I am pleased to submit the revised copy of the original manuscript entitled "*Comparison of Cellulose nanocrystals obtained by Sulfuric acid hydrolysis and Ammonium persulfate treatment, intended as coating onto flexible food packaging materials*", for possible publication as a research paper in *Cellulose*.

We very much appreciated the comments from the two referees that we found properly addressed. We tried to fulfil as much as possible their requests. According to the comments, we revised our manuscript writing in red all the changes operated in the manuscript. In the pages "Responses to Reviewers" we answered to all the questions raised by the referees and tried to explain our views.

We confirm that this manuscript has not been published elsewhere in whole or in part and is not under consideration by another journal. Approvals of all of the Authors' institutions have been granted to publish this work. All authors have approved the manuscript in this revised version and agree with submission to Cellulose.

The study was supported by our own funding. The authors have no conflicts of interest to. We have read and understood the "Ethical Responsibilities of Authors" in the journal's "Instructions for Authors", including the passage on screening for plagiarism with computer software.

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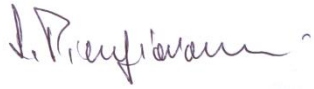
We look forward to hearing from you at your earliest convenience.

Yours sincerely,



Comparison of cellulose nanocrystals obtained by sulfuric acid hydrolysis and ammonium persulfate treatment, intended as coating onto flexible food packaging materials

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ABSTRACT

Cellulose nanocrystals (CNCs), extracted from trees, plants, or other cellulose-containing species, can be used in combination with other materials to improve their performance or introduce new potentialities; their preparation usually involves a chemical acid hydrolysis process intended to dissolve amorphous chains from the cellulose fibers to release crystal domains. In this work the morphological and chemical characterization of CNCs produced from cotton linters through two different processes, the common acidic hydrolysis (H_2SO_4) and a less investigated ammonium persulfate treatment (APS), was carried out; the main purpose was to compare and understand the potentialities of using these two types of CNCs as coatings for Poly(ethylene terephthalate) (PET) film. CNCs produced by APS treatment showed higher charge density due to the carboxylic groups formed during the process, more clarity of the solution and, as a consequence, higher transparency

of the coating, but similar crystallinity pattern; these peculiarities confer higher oxygen barrier respect to the CNCs produced by H_2SO_4 treatment, and the availability of active sites for potential surface modification or chemical grafting. Anyway, both CNCs coatings showed oxygen permeability coefficient lower than synthetic resins commonly used in flexible packaging, they did not affect significantly the optical properties of the substrate and revealed good coefficients of friction. The use of such nanocellulose as water based coating can be considered a possible alternative to conventional food packaging materials: due to the moisture sensitivity of the coating and due to the no sealable property, as happens for EVOH or PVOH oxygen barrier synthetic resins, this type of material need to be laminated with another plastic layer such as a polyolefin.

In this case it might enhance the final properties of packaging solutions for perishable food products, while reducing their environmental impact with a thin layer of a bio-based polymer.

Keywords: Cellulose Nanocrystals, Ammonium Persulfate, Oxygen permeability, Film coating

1. INTRODUCTION

Cellulose, the most abundant natural polymer on the earth, can potentially become a widely used renewable nanomaterial for various applications in both its forms: cellulose nanocrystals (CNCs) and cellulose nanofibrils (CNFs) (Li et al. 2015; Dufresne 2012). Two main approaches are commonly used for obtaining cellulose nano-particles: mechanical treatments and acid hydrolysis, being the last the classical method for CNCs production.

For nanocellulose production by mechanical treatment, several variants and technological supports were proposed in the last 30 years (Moon et al. 2011; Rebouillat and Pla 2013; Nakagaito and Yano 2004; Berglund 2005). Besides the mechanical process of high-pressure homogenization, pre-treatments like further refining, oxidation by 2,2,6,6-tetramethylpiperidinyl-1-oxy, known as TEMPO, (Saito et al. 2007) or periodate-chlorite mixture (Liimatainen et al. 2012, Yang et al. 2013), enzymatic (Paakko et al. 2007), ultrasound treatment (Peng et al. 2010), combination of carboxymethylation and periodate oxidation (Chinga-Carrasco and Syverud 2014) have been used. The large number of hydrogen bonds among cellulose fibrils hinders the transverse cleavage required to obtain the nano-sized dimensions, rendering the processes to obtain nanocellulose difficult and variable according to the cellulose source used (Iwamoto et al. 2008). In addition, the plant-based raw materials (i.e. the most common cellulose sources) contain also hemicellulose, lignin, extractives and minerals, which have been considered hurdles to polymer fragmentation, to be thoroughly removed in order to obtain nanocellulose (Spence et al. 2011; Spence et al. 2010a; Spence et al. 2010b). More recently, however, Galland et al. (2015) demonstrated, in the preparation of CNFs by a mild peracetic acid delignification process, that highly preserved cellular molar

mass with up to 24% of hemicellulose content, contributes to CNF dispersion. This multistep process led to unique final properties and provided a possible physical model structure of the CNFs obtained. The cellulose oxidation, based on TEMPO, as well as on other chemicals, has been widely proposed in combination with acid hydrolysis or mechanical treatment, leading to effective transformation of cellulose hydroxyls groups into carboxyl. TEMPO oxidation, in particular, has been also proposed as an intermediate step in the grafting of various functional groups on cellulose chains (Araki et al. 2000), and in the enhancement of nanofibrils properties (Saito and Isogai 2007; Habibi et al. 2006). The different procedures in the mechanical treatment can produce cellulose nanofibrils with a broad range of morphologies and different performances. However, considering the different steps and the large number of variants, it is generally considered a high-energy demanding and time consuming process (Siro and Plackett 2010).

The production of nanocellulose by acid hydrolysis is the most classical procedure for cellulose fragmentation (Nickerson and Habrle 1947; Favier et al. 1995; Håkansson and Ahlgren 2005; Bondeson et al. 2006; Elazzouzi-Hafraoui et al. 2008). The structure of cellulose is highly debated in the scientific field (Belbekhouche et al. 2011; Nishiyama et al. 2009), but the measure of chemical reactivity showed the rupture of hydrogen bonding between O3H and the ring oxygen O5 only in the region of defect that are periodically present along the microfibril and due to the biological synthesis of the cellulose (Nishiyama et al. 2009). The dimensions and the morphologies of these particles mainly depend on the cellulose source and the process used. Different acids have been tested and used in nanocellulose production (sulfuric, hydrochloric, phosphoric, hydrobromic and maleic acid (Filpponen 2009; Filson and Dawson-Andoh 2009; Okano et al. 1999) leading to distinct properties and morphologies of CNCs. Concentrated sulfuric acid

is the most common medium for the hydrolysis process because it determines the formation of surface charged sulfate ester groups on the cellulose chains which promotes water dispersion of the nano-particles, avoiding the aggregation phenomena observed using hydrochloric acid (Araki et al. 2000). This process is easy to apply and low energy consuming but it can be time consuming, not ecofriendly and pretreatments are necessary starting from lignocellulosic matrices (Espino et al. 2014). This method indeed is not able to eliminate lignin, hemicelluloses and other impurities within plant raw materials.

An alternative to these processes is ammonium persulfate (APS) treatment (Leung et al. 2011). APS is a chemical widely used as strong oxidizer in polymer chemistry (Jayakrishnan and Shah 1984) as etchant, cleaning and bleaching agent in various industries (Turrentine 1906) and it has low long-term toxicity, high water solubility and low cost. Ammonium persulfate is generally preferred to potassium and sodium persulfates because of its higher solubility, lower pH, lower density and viscosity.

Persulfates decompose thermally in aqueous solutions by two independent reactions, which occur simultaneously (Kolthoff and Miller 1951):

(A) an un-catalyzed reaction leading to the symmetrical rupture of the O-O bond and the formation of two sulfate free-radicals which disappear reacting with water and producing anion bisulfate and atomic oxygen;

$$A\colon\ S_2O_8^{=} \xrightarrow{T} 2SO_4^{-\circ}$$

$$2SO_4^{-\circ} + 2H_2O \xrightarrow{T} 2HSO_4^{-} + 2HO^{\circ}$$

$$2HO^{\circ} \xrightarrow{T} H_2O + {}^{1}/_{2}\,O_2$$

(B) an acid catalyzed reaction leading to the unsymmetrical rupture of the O-O bond to form sulfur tetroxide and bisulfate.

$$B\colon S_2O_8^{=} + H^{+} \xrightarrow{T} HS_2O_8^{-}$$

$$HS_2O_8^- \xrightarrow{T} SO_4 + HSO_4^-$$

$$SO_4 \xrightarrow{T} SO_3 + {}^1/_2\, O_2$$

In diluted acidic solutions sulfur tetroxide decomposes to form atomic oxygen and sulfuric acid but if the acidic concentration increases (> 0.5M), the sulfur tetroxide reacts with water (Gall et al. 1943; Beer and Muller 1962) leading to mono peroxysulfuric acid (Caro's acid, H_2SO_5) which is patented as an effective delignification agent (Springer,Minor 1991).

$$SO_4 + H_2O \xrightarrow{T} H_2SO_5$$

$$H_2SO_5 + H_2O \xrightarrow{T} H_2O_2 + H_2SO_4$$

Therefore, the uniqueness of this treatment is that a simultaneous hydrolysis and oxidation process of cellulose fibers occurs on the surface and within the inner amorphous regions due to the fast penetration of free radical ions ($SO_4^{\cdot-}$) and H_2O_2 through the lumen of the fibers. In addition, the effectiveness of the phenomena is related to the progressive increase in acid concentration and peroxysulfuric acid generation.

Recent literature reports that APS is able to produce CNCs from different type of lignocellulosic materials containing up to 20% of lignin and the process has been patented as effective method to produce CNCs from renewable biomass (Leung et al. 2012; Leung et al. 2011). The reaction conditions (i.e. reaction time, temperature and APS concentration) can be tuned to have satisfactory yields and crystals with different aspect ratio. Moreover this treatment gives the opportunity to obtain carboxylated CNCs and to increase the crystallinity (Cheng et al. 2014). It is well known that carboxyl groups on the surface of the materials can provide active sites for template synthesis of nanoparticles, surface modification and chemical grafting (Ifuku et al. 2009; Qi et al. 2011; Arola et al. 2012). The oxidized cellulose obtained

by APS treatment shows a higher charge density that can improve interface interactions. Furthermore, the polymer crystallinity generally drives to better mechanical properties and higher gas barrier properties (Lasoski and Cobbs 1959; Salame 1989; Miller and Krochta 1997).

These peculiar characteristics of CNCs obtained by APS treatment and the huge availability of various lignin containing cellulose sources in agricultural biomasses as well as in industrial byproducts, addressed our research towards the potential uses of these carboxylated cellulose nanocrystals as coatings of novel, high performing and more sustainable flexible packaging materials (Li et al. 2013b; Li et al. 2013a). A process able to make feasible the obtainment of large amount of CNCs from cheap and largely available sources, would be welcome by a sector, such as packaging materials production, requiring novel materials better performing and more sustainable, as coming from renewable sources. So far, however, to our best knowledge, does not exist an accurate comparison between CNCs obtained by the two processes and addressed to their potential use as coating for flexible packaging materials. Therefore, in this paper we propose a comparison between the cellulose nanocrystals obtained by sulfuric acid hydrolysis and APS oxidation-hydrolysis starting from the same cotton linters, and a characterization of PET films coated with the two differently obtained CNCs, intended for food packaging applications.

2. **MATERIALS AND METHODS**

2.1 *Materials*

Cotton linters used as raw material to produce CNCs were kindly supplied by Innovhub (Milano, Italy). Sulfuric acid 96%, ammonium persulfate ≥98%,

hydrochloric acid 37%, sodium hydroxide ≥97%, ion exchange resin Dowex Marathon MR-3 were purchased from Sigma-Aldrich (Milan, Italy).

Poly(ethylene terephthalate) (PET) film, having a thickness of 12±0.5 μm, was provided by Sapici s.p.a (Cernusco sul Naviglio, Italy)

2.2 CNC extraction by Sulfuric Acid hydrolysis

Cellulose nanocrystals (CNCs) were obtained from cotton linters by a common procedure used by many Authors (Dong et al. 1996; Li et al. 2013b). Milled cotton linters were hydrolyzed by 64% w/w sulfuric acid under vigorous stirring at 45°C for 45 minutes (fibers/acid ratio 1:17.5 g/mL). To quench the reaction, the mixture was diluted ten times with deionized water (18 MΩ cm, Millipore Milli-Q Purification System). The solution was centrifuged 5 times at 10000 rpm for 15 minutes with addition of deionized water for each time in order to remove the excess of acid until the supernatant became turbid. For further purification, the centrifuged solution was posed inside dialysis tubes (Molecular Weight Cut off 12000 Da) immersed in deionized water for 72 hours to remove the acid still present and the low molecular weight contaminants. The suspension was then sonicated (UP 400S 400 W, Hielscher ultrasonics GMBH, Teltow, Germany) repeatedly (cycles 0.7, time 5 minutes at 70% output) for bringing cellulose crystals to colloidal dimensions. During the ultrasonic treatment, the suspension was cooled with an ice water bath to avoid overheating. Ion exchange resin was added to the sonicated suspension (resin/solution ratio 10:1 g/L) to complex any residual ions. After that, the suspension was filtered under vacuum, using Munktell filter discs GF/C 1.2 μm in order to remove the largest fibers agglomerates and the microfibers possibly present. The cellulose content of the resulting aqueous suspension was determined by drying several samples (1 mL) at 105 °C overnight. The pH of purified

suspensions was adjusted to 8 by 1M NaOH in order to gain fully charged CNCs. Secondly, the dispersion was frozen at -18 °C overnight and then moved to freeze dryer (LIO-10P, Cinquepascal, Trezzano s/N (MI), Italy). Finally, the freeze-dried powder obtained was stored in tightly close bottles under dry conditions.

2.3 CNC extraction by Ammonium Persulfate treatment

CNCs were produced from cotton linters by the hydrolyzing-oxidative method proposed by Leung and coworkers in 2011(Leung et al. 2011). Milled cotton linters and 1M ammonium persulfate (APS) (ratio between fibers and APS 10:1 g/L) were introduced into a large beaker, onto a magnetic stirrer hotplate, equipped with a Vertex Digital thermoregulator (VELP Scientifica, Usmate (MB) Italy). The mixture was heated and continuously stirred at 75°C for 16 hour, limiting the evaporation by means of an aluminum foil cover. The suspension of CNCs obtained was centrifuged at 10000 rpm for 15 minutes with addition of deionized water in order to rinse the suspension. The centrifugation/washing procedure was repeated 4 times until the suspension pH was around 4. In order to have the sodium form of CNCs, NaOH 1M was added until the suspension reached pH 8 and then it was sonicated for 15 minutes (0.7 cycles, 70% output). The purified suspension was frozen at -18 °C overnight and freeze-dried. Finally, the freeze-dried powder obtained was stored in tightly close bottles under dry conditions.

2.4 Morphological characterization of CNCs

Drops of aqueous dispersion of CNCs 0.5% w/w were deposited on carbon-coated electron microscope grids, negatively stained with uranyl acetate and allowed to dry. The samples were analyzed with a Hitachi Jeol-10084 transmission electron microscope (TEM) operated at an accelerating voltage of 80kV. Representative

micrographs have been selected for measuring the diameter and length of nanocrystals by digital image analysis (Image-Pro Plus software). The aspect ratio was also calculated. Lengths average and diameters average of the crystals were determined by analyzing 70 crystals.

In addition, the hydrodynamic size distributions of diluted aqueous dispersions of CNCs were determined by dynamic light scattering (DLS) (Malvern Instruments Nano Series Zetasizer optical units). Measurements were performed at (23.0±0.1)°C with a class 14 mW continuous wave He–Ne laser light (λ = 632.8 nm). The scatterers in solution are undergoing Brownian motions, constantly changing their instantaneous position and causing thus temporal fluctuations in the scattered light intensity. By applying correlation analysis and Stokes-Einstein relation, the hydrodynamic dimension and the size distribution of the scatterers can be calculated (Berne,Pecora 2000). Prior to DLS measurement the samples were diluted to 1:500 (w/w) with distilled water previously adjusted to pH 8 and maintained at 25°C under stirring until measurement. 1 ml of the diluted solution was injected in the measurement cell after 30 s homogenization with ultrasonic bath.

2.5 Determination of the degree of oxidation (DO) of CNCs, charge density and clarity of the CNCs solutions.

Conductometric titrations were performed to determine the carboxylic acid content of the CNCs. 50 mg of dry powders of CNCs were suspended into 15 mL 0.01M HCl for complete protonation of the COOH groups and sonicated for 10 min to disperse the nanocrystals. The CNCs suspensions were then titrated against 0.01M NaOH and the carboxylic acid contents were determined from the resulting

conductivity curves. The DO of the CNCs was calculated using the following equation:

$$DO = \frac{162(V_2 - V_1)C}{w - 36(V_2 - V_1)C}$$

Where (V_2-V_1) is the volume of NaOH (L) required to deprotonate the carboxylic acids groups, C is the concentration (M) of NaOH, w is the weight of the CNCs samples, the values 162 and 36 correspond to the molecular weight of an anhydroglucose unit (AGU) and the molecular weight difference between an AGU and sodium gluconate, respectively. The degree of oxidation (DO) was also quantified used FTIR spectra. FTIR spectroscopy was performed on Perkin Elmer instrument (Spectrum 100), equipped with ATR, at room temperature, on CNCs in their acidic form (pH=2). The data were collected over 64 scans with a resolutions of 4 cm^{-1} and the DO was calculated by the ratio of the intensity of the carbonyl peak (absorbance bands at 1735 cm^{-1} (ν (C=O) in the acid form)) to that of the band near 1060 cm^{-1}, relating to the backbone structure of cellulose. The equation used in this case is the following:

$$D0 = 0.01 + 0.7(I1735 - I1060)$$

Surface charge density was estimated using the dimensions of CNC determined by TEM, assuming a cylindrical shape and a density of 1.6 g/cm^3 for cellulose nanocrystals (Beck-Candanedo et al. 2005). The clarity of the CNCs water solution at 7% w/w was also tested after ultrasound treatment for 15 minutes (0.7 cycles, 70% output). It was measured as turbidity of the solution using a spectrophotometer at λ=600nm.

2.6 Cristallinity evaluation by Solid-state nuclear magnetic resonance (NMR) spectroscopy and by X-ray diffraction (XRD).

All NMR spectra (three replication for each type of CNCs) were acquired at room temperature on a Bruker AVANCE-600 spectrometer (Bruker Spectrospin GmbH, Rheinstetten, Germany), equipped with a 4 mm broad-band CP-MAS probe for solid state measurements. About 100 mg of CNCs sample were directly pressed into a 4 mm ZrO_2 rotor without further treatment. 13C spectra were acquired at 150.9 MHz using Cross Polarization (CP) and Magic Angle Spinning (MAS) at 6-10 kHz (Pines et al. 1973). Proton decoupling was achieved with GARP-based composite pulse. Standard acquisition parameters were as follows: Spectral width: 75.7 kHz; acquisition time: 3.4 ms; relaxation delay: 2s (fast acquisition conditions); contact time for Cross Polarization: 1 ms; number of scans: 7000-24000. The contact time was optimized by systematic variation of the corresponding pulse within the 0.3-3.0 ms range. Adamantane was used as external chemical shift reference.

X-ray diffraction measures were conducted using an X-ray diffractometer (D8-Advance Bruker AXS GmbH) at room temperature with a monochromatic Cu-Ka radiation source (Wavelength 1.5418 Å) in the step-scan mode with a 2θ angle ranging from 5° to 59.98° with a step size of 0.02 and 2750 number of points. The freeze-dried CNC powders were placed on the sample holder and leveled to obtain uniform X-ray exposure.

The NMR analysis was used to provide the relative masses of crystalline and amorphous material. Gaussian function was used to perform the deconvolution of the C4 peaks.CI is calculated by dividing the area of the crystalline peak (integrating the peak from 87 to 93 ppm) by the total area assigned to the C4 peaks (integrating the region from 80 to 93 ppm).

XRD diffraction were done to determine the crystal type (polymorph). Diffraction patterns from cellulose Iα and Iβ were calculated based on the published atomic

coordinates and unit cell dimension contained in modified "crystal information files"(.cif). Diffraction intensities, output by Mercury program from the Cambridge Crystallographic Data Centre, has been compared with the experimental data (French, 2014, Nishiyama et al., 2012).

2.7 Thermogravimetric analysis (TGA)

TGA was carried out to determine the thermal stability of the different kinds of CNC by employing a thermogravimetric analyser Perkin Elmer, TGA 4000. Samples were heated from 30°C to 800°C under air or nitrogen atmosphere at a heating rate of 10°Cmin^{-1}; three replication were done for each CNCs type.

2.8 Coating process

A 7% wt CNCs water dispersion (pH = 8) was coated onto PET 12 μm film, according to ASTM D823-07, practice C. After activation of the external side of the substrate by using a corona treater, (Arcotech GmbH, Monsheim, Germany), the CNCs solution was coated by an automatic film applicator (model 1137, Sheen Instruments, Kingston, UK) at a constant speed of 2.5 mm s^{-1}. Water was evaporated using a constant mild air flow (25±0.3 °C for 5 minutes).

2.9 Transparency, Haze and Water Resistance of the coatings

The transmittance of the sample was measured at 550 nm, according to the ASTM D 1746-70, by means of a Perkin-Elmer L650 UV-VIS spectro-photometer. Haze (%) was measured in accordance with ASTM D 1003-61 with the same instrument equipped with a 150 mm integrating sphere. Each sample was replicated three times, by analyzing four spots for each replica. The moisture sensitivity of the hydrophilic coatings was done, by using an empirical test, measuring the weight losses after 4 days of immersion in a water bath at 37 ± 0.5 °C, of ten samples

(diameter 12 mm) cut from the two CNCs coated PET films. Samples of the two kinds of CNCs were submerged in distilled water, avoiding any floating then wiped up, dried and weighed to measure the weight loss (%).

2.10 Thickness

The thickness of the two different CNCs coatings were measured analyzing the cross section by Scanning Electron Microscopy (SEM). Scanning electron microscopy images were obtained from a Sigma Field Emission microscope (Carl Zeiss Microscopy, LLC) at accelerating 5 kV voltage and 6 mm working distance, with a 30 m width slit. The samples were first gold sputtered (Sputtering Polaron E 5100) for 30 s (rate 1 nm s−1) with argon and 18 mA current intensity.

2.11 Oxygen Permeability measurements

The oxygen permeability (PO_2) of CNCs coated plastic films were assessed (mL m^{-2} d^{-1} bar^{-1}) by isostatic method (Multiperm, Extra_Solution S.r.l. Capannori (LU), Italy) at 23°C and two different relative humidity values (0% and 50% RH), complying with ASTM D-3985. The oxygen permeability coefficients of the CNCs coating alone (i.e., KPO_2 of the coating) was calculated using the following equation (Crank 1979) and assuming that the substrate surface (PET film) does not interact with the CNCs coating above or that the interface between them, negligibly affects the final permeation measure.

$$\frac{L}{KPO_2(coating)} = \frac{1}{PO_2(coated\ film)} - \frac{1}{PO_2(bare\ film)}$$

2.12 Contact angles and surface energies

Self-standing films of the two different CNCs were obtained by casting, leaving their suspension evaporate at pH 7 for one night at 30°C in Petri dishes Ø 9 cm. Their surface free energies (SFE) were achieved by measuring static contact angles of polar Milli-Q water (18.3 MΩ cm) and apolar diiodomethane (99%, Sigma Aldrich), using OCA 15 Plus angle goniometer (Data Physics Instruments GmbH, Filderstadt, Germany), then elaborated by the Owens-Wendt-Rabel and Kaelblem (OWRK) method (Owens and Wendt 1969; Kaelble 1970). The measurements of static contact angles were performed at room temperature, on five different positions for each sample; the sessile drop method was used, by gently dropping a droplet of 4.0 ± 0.5 μL of each liquid onto the substrate. The instrument was equipped with a high-resolution CCD camera and a high performance digitizing adapter. SCA20 and SCA21 software (Data Physics Instruments GmbH, Filderstadt, Germany) were used, respectively, for contact angle measurements and surface energy calculation. According to the OWRK theory, the SFE is divided into two distinct parts, i.e. the polar γ_s^P and dispersive γ_s^D. These components are the square values, respectively, of slope and intercept of the following first order equation:

$$\frac{\gamma_l(1+\cos\theta)}{2\sqrt{\gamma_l^D}} = \sqrt{\gamma_s^P}\left(\frac{\sqrt{\gamma_l^P}}{\sqrt{\gamma_l^D}}\right) + \sqrt{\gamma_s^D}$$

Therefore, knowing the polar and dispersive components (γ_l^P, γ_l^D) of at least two liquids (the ones used in this work are reported in Table 1), and the corresponding apparent contact angles of these liquids onto the solid surface of interest, a linear regression permits to estimate the SFE components γ_s^P and γ_s^D.

Table 1 Surface tension parameters of the liquids used in contact angle determination (in mJ/m^2) at 20°C (van Oss 2003)

Liquid	γ_l	γ_l^D	γ_l^P
Water	72.8	21.8	51
Diiodomethane	50.8	50.8	0

2.13 Coefficient of Friction

The static (μ_S) and dynamic (μ_D) friction coefficients (COF) were measured by a dynamometer (model Z005, Zwick Roell, Ulm, Germany), according to the standard method ASTM D 1894-87. Firstly, the uncoated side of CNCs coated film was attached on a specific sled (6.2×6.2 cm^2, 197.99 g), while the un-coated film was covered on the sliding plane (exposing the un-treated side). Then the sled was connected to the force sensor of dynamometer and was horizontally pulled by the instrument on the covered sliding plane. The raw data (pulling force) were recorded and analyzed by software TestXpert V10.11 (Zwick Roell, Ulm, Germany).

3. RESULTS

3.1 CNCs characterization

The two different processes used on the same batch of cotton linters were able to give cellulose nanocrystals that were characterized to determine the differences of CNCs obtained.

The CNCs were characterized in terms of morphology with TEM (Fig. 1a; Fig 1b) and hydrodynamic dimension distribution (DLS), (Fig. 2). Electron microscopies show quite similar rod-like shapes with comparable rod diameters (Table 2) for CNCs obtained by sulfuric acid and APS treatment. On the other hand a 10% difference in rod length, and around 20% aspect ratio and PDI are detected. The higher values were measured for the CNCs achieved through the APS process.

Fig. 1a Trasmission Electron Migrograph (TEM) of cotton linters nanocrystals obtained by APS

Fig. 1b Trasmission Electron Migrograph (TEM) of cotton linters nanocrystals obtained by H_2SO_4

Fig. 2 CNCs hydrodynamic diameter distributions obtained with DLS

The fact that CNCs are not spherical poses some issues on the CNCs diameter calculated by DLS. DLS analyzes the data in spherical approximation, mathematically treating the CNCs as spheres moving with Brownian motion independently from their real physical morphology. Particularly, when a rod-like particle is subjected to Brownian motion, it will be dragged along and rotate, as well as for a spherical particle. Differently from spheres, the hydrodynamic stresses will depend on the relative orientation of the rods, causing an anisotropic motion of the scatterer (Mewis and Wegner 2012). Prolated rod-like structures of length L are detected by DLS as equivalent Brownian spheres with a smaller average diameter (Kroeger et al. 2007). The average TEM dimension matches the DLS measured size distribution (Table 2 and Fig. 2), stressing the good overlap between size distribution in CNCs solution and electron micrographies (EM) of drop-casted CNCs. Such an average size persistence in presence of strong capillary forces associated to drying and absorption before EM measurements underlines the stability of the morphology of both the CNCs obtained (Kralchevsky and Nagayama 1994).

Table 2 TEM/Image and hydrodynamic size distribution of dispersed CNC.

CNC	**TEM-Image ProPlus**			**DLS**	
	Length (nm)	**Diameter (nm)**	**L/d**	**Nanocrystal diameter in uniform**	*PDI**

				spherical approximation (nm)	
CNC $_{H_2SO_4}$	110 ±47	6.7 ± 2.3	16.4 ± 8.9	80.8	0.19
CNC $_{APS}$	121 ± 46	6.2 ± 0.9	19.7 ± 8.0	101.2	0.23

* polydispersity index, defined as $PDI = (\sigma/D)^2$, is a measure of the width of particle size distribution

The length of the nanocrystals, in particular, can be relevant in the networking consolidation during the coating process. The similar values and the uncertainty of the measures of the aspect ratio of crystals from the two preparations leads to assume similar dimensions of the crystals. Other important analyses for the goal of this research are the chemical indexes assessed by XRD, NMR, FTIR and conductometric titrations. NMR analysis with deconvolution based on Gaussian Equation is still often used to interpret the diffraction profile to provide the relative masses of crystalline and amorphous material in a given sample (Nishiyama et al. 2013). In this specific case the values are 62.6±1.1 % for $CNC_{H_2SO_4}$ and 63.8±1.2% for CNC_{APS} therefore no significant difference in crystallinity content is present.

More interesting is the analyses of the XRD diffraction pattern of our experimental data compared with the powder diffraction patterns form cellulose Iα and Iβ based on the published atomic coordinates and unit cell dimension contained in modified "crystal information files"(.cif) calculated with Mercury Software. The powder diffraction patterns are reported in Figure 3.

Fig.3. Powder diffraction patterns calculated with Mercury software based on the cellulose Iβ crystal structure and experimental data obtained by CNCs from APS and from H_2SO_4.

Because of the strong oxidative potential of the APS treatment, an essential characterization of the two different CNCs produced is related to the oxidation degree and the assessment of the charge density. Both FTIR spectra and conductometric titrations were carried out for this purpose and the overall results obtained are reported in Table 3. FTIR spectra of APS CNCs (Fig. 4) clearly show the typical absorption bands of carboxylic groups. The two peaks recognizable in the inset of Figure 4 are referable, according to Lam et al. (2013), to C=O stretching peaks of the carboxylic group (1733 cm^{-1}) and to $COO^{-}NH_4^{+}$ (1630 cm^{-1}), respectively.

Fig. 4 FTIR spectra and enlargement recorded for raw cotton linters (blue line), from CNC obtained by sulphuric acid hydrolysis (black line) and CNC-COOH produced by APS treatment (red line)

Concentration of COOH groups resulted to be 0.98 mmol/g of cellulose nanocrystals, leading to an oxidation degree around 0.15. According to Elazzouzi-Hafraoui et al. (2009) and assuming that all the glucose units on the surface of each nanocrystalline cellulose particle are completely carboxylated, the charge content measured is an estimate of nanocrystals diameter between 6 and 10 nm. The charge density that was accounted for APS CNCs is 4.5 times the value obtained for sulfuric acid hydrolysis and this achievement seems consistent with the goal of producing a suspension that can be casted, as a thin and functional coating, on the activated surface of flexible packaging materials.

Table 3 Extraction yield (%), degree of oxidation (DO) and charge density of CNC

CNC	Yield (%)	DO	COOH content (mmol/g)	Sulfur Content (mmol/g)	Charge density (e/nm^2)

		Conductimetric	FT-IR			
CNC $_{H_2SO_4}$	52.7	-	-	-	0.21±0.9	0.32
CNC $_{APS}$	34.4	0.15	0.16	0.98±0.10	-	1.46

The results concerning the oxidation degree and the charge densities of the two different CNCs appear well correlated to the values of contact angles and surface energies, measured on specimens obtained by casting the cellulose nanocrystals from their 7% suspension in water (pH = 7), and reported in Table 3. Both the samples presented water contact angles consistent with high hydrophilicity largely expected for their cellulosic origin; however the value of sample from APS process is lower, showing a much higher wettability. Surface free energies (SFE) were measured using also the static contact angles of apolar diiodomethane, by means of the Owens-Wendt-Rabel and Kaelblem (OWRK) method, and they also showed a significant difference between the two samples. CNCs from APS process has a surface free energy which is 13.6% higher than that of CNCs coming from sulfuric acid hydrolysis of cotton linters: this is mainly due to the polar part of this energy (32.54 versus 21.71 mN/m), in accordance with the large presence of carboxyl groups in the cellulose nanocrystals obtained using ammonium persulfate. It is reasonable to assume that such a high surface energy can be useful in anchoring the CNCs layer onto a common activated substrate.

Table 4: Static contact angles (θ) and Surface Energy (SFE) of CNC in form of thin casted films.

CNC	θ Water	θ DIM	Polar part (mN/m)	Dispersive part (mN/m)	SFE (mN/m)
CNC $_{H_2SO_4}$	45.72±1.69	44.39±1.87	21.72	37.34	59.09

CNC_{APS}	29.29±1.73	49.67±3.23	32.54	34.46	67.01

The yields recorded for the two processes are rather different, showing the APS treatment leading just to a 34,4% in comparison with almost 53% of the sulfuric acid hydrolysis. It is worth noting that these values are only indicative and related to a specific lab scale production process. In particular, they strongly depend on the preparation condition and post treatment filtration. The extraction yield should correspond to the proportion of crystalline domains of the cellulosic raw materials but usually lower yields are obtained. It was supposed that continued action of the reactive agents like acids for long time can cause dissolution of cellulose in crystallites (Dufresne 2012), or other reaction byproducts.

Thermal stability of CNCs was also tested given its importance for thermoplastic applications where the processing temperature is often above 200°C. In literature different degradation temperature for nanocrystals are reported because the degradation temperature depends from different experimental variables (Roman and Winter, 2004).

In this case (Fig. 5) TGA curves report a small loss (about 6%) from 30°C until 250°C, where cellulose undergoes to the most important degradation. Even if the TGA analysis was performed in the temperature range between 30°C and 800°C, only the range from 100°C to 500°C is reported in Figure 5 because this is the zone where the differences between the samples are more visible and the behavior is more influenced by the CNCs production process and by the initial humidity of the samples. During the hydrolysis reaction, for example, the sulfate or hydroxyl group are introduced on the surface of the nanoparticles giving at the same time improved stability of the aqueous suspension and lower thermal stability.

Fig 5: TGA plots, from 100°C to 500°C, of CNC from H_2SO_4 hydrolysis, CNC-COO-Na^+ in Nitrogen atmosphere and CNC-COO-Na^+ in air

Increasing quantity of sulfate and hydroxyl groups led to lower degradation temperature (Fig.5) and a broader degradation temperature range was observed in comparison to unhydrolyzed sample as shown in literature (Roman and Winter 2004). In literature is also reported that the -COOH form of CNCs is more stable than the $COONa^+$ (Lam et al. 2012); also in our case the $COONa^+$ form is more stable in water solution than the COOH form but it is less thermostable. Complete decomposition of CNCs to volatile products including CO_2 was observed at T > 400°C; this phenomenon could be attributed to the depolymerization and decomposition of the cellulose chain. The presence of sulfate groups on nanocrystals brings to charred residue at 350°C. Heating in air causes oxidation of the hydroxyl groups resulting, as the temperature increases, in the increase of carbonyl, carboxyl and hydroperoxide groups; also free radicals appear. The thermal degradation temperature in this case is higher as shown in Fig. 5.

3.2 *Coated PET film characterization*

The turbidity of the APS solution used for coating preparation is lower than the H_2SO_4 one and this could be related with the surface charge density reported in Table 3. Indeed, in the case of APS solution, the higher surface charge density induces a more efficient electrostatic interactions with water (i.e. a tighter hydrogen bonds network), leading to more stable CNCs dispersion.

The nanocrystals were used, in the form of 7% water dispersion at pH 8, as lacquers for coating the properly corona treated side of thin PET films. The thickness of the coatings obtained were well below 1 µm but enough to affect the optical properties, reducing the transparency and increasing the haze (Table 6) in comparison with the

uncoated PET film (T% 84.2, Haze 2.8) After solution deposition on the substrate, the strong capillary and surface forces due to drying and adsorption trigger a rearrangement of the crystal structure.

Cellulose is known to have very high transparency in solid non-porous form. In this case, we are in presence of a decrease of transparency of the PET film and this decrease is higher in the case of CNCs from H_2SO_4 treatment (Table 5). Moreover, SEM micrographs of the coatings surface, recorded at 1 KK and 100KX Magnification, showed homogenous surface of the coatings with the presence of some holes (Figure 6), with a more pronounced roughness in CNCs H_2SO_4 coating than in APS that can be correlated with the higher decrease in transparency of the coating produced with CNCs H_2SO_4 .

Fig.6: SEM micrographs at 1KX and 100KX Magnification of the of the coatings surface produced by CNCs from H_2SO_4 (up) and CNS form APS (down)

SEM observation of the same sample but in cross section permitted to verify, that the surface of the coating is homogeneous in thickness and the values of the thickness for both the coating is around 450±50 nm (Figure 7).

Fig.7 SEM micrographs at 180KX Magnification of the of the cross section of the films containing the coating of CNCs from H_2SO_4 (up) and CNS form APS (down)

At the same time, the test of the water resistance showed that APS coating was removed only for 49.2%, while the sulfuric acid CNCs layer was almost completely removed (87.4%). Despite its empiricism, the test perceptibly reveals a higher stability of the APS coating compared to the H_2SO_4 one. In Table 5, also the coefficients of friction (COF, both dynamic and static) measured for the two coated films are reported. The values, pertinent to the sulfuric acid CNCs coating, are quite similar to the ones obtained in a previous work (Li et al. 2013a), for various films (OPET, OPP, Cellophane and OPA) coated with the same CNCs. The achievement

of similar COF values for different coated films were assumed as an evidence of a complete and uniform covering of the substrates. The values obtained for APS CNCs coated PET are significantly lower, both as dynamic and static coefficients. This achievement can be interpreted as a possible better performance on the automatic machineries for converting the coated material or for a potential packaging operation, but it also confirms a very good adhesion to the plastic film and a compact and uniform coating offered by the cellulose nanocrystals.

Table 5: Optical properties, clarity of the CNCs in water dispersion (7%), water resistance and coefficients of friction (COF).

Coating	Clarity (Abs 600 nm)	Haze (%)	Transparency (%)	Water Resistance (% losses)	COF (μ_D)	COF (μ_S)
CNCs H_2SO_4	3.1±0.1	5.99±0.11	77.48±0.02	87.4±3.0	0.28±0.01	0.31±0.01
CNCs APS	1.8±0.05	6.19±0.10	79.55±0.01	49.2±3.3	0.21±0.01	0.24±0.01

Owing to the highly hydrophilic nature of these coatings and the results obtained in previous researches (Li et al. 2013a), the most promising property of such CNC coatings is gas barrier. Therefore, the oxygen permeability was measured at two different relative humidity values at room temperature for both the coated PET films and the uncoated substrate. The results obtained are presented in Table 6 and show a very sharp reduction of gas diffusion trough the PET film, once coated by the CNCs. To get the same permeability, just increasing the plastic thickness, a 1.5 mm thick PET film would be necessary to have the same performance offered by the sulfuric acid nano-cellulose coating. In the case of APS CNCs coated film, the thickness of the "standard" PET sheet having the same permeability should be around 8.2 mm. The very high oxygen barrier demonstrated by APS CNCs coating,

584 which is much higher than that of the majority of synthetic barrier resins commonly
585 used in food packaging applications, realistically derives from the inherent
586 morphological, chemical and physical characteristics of these nanocrystals, which
587 lead to low diffusion. The apparent higher moisture sensitivity of CNCs from the
588 APS process, which shows a higher increase of permeability when measured at 50%
589 RH in comparison with H_2SO_4 CNCs coated film, is consistent with the higher
590 wettability shown by optical contact angle evaluation (Table 4) and can be
591 attributed to the higher charge density. In any case, the moisture sensitivity of the
592 coating, as happens for EVOH or PVOH oxygen barrier synthetic resins, doesn't
593 permit the use of such materials at high relative humidity (80% or higher) and it
594 could be overcome by the need of providing a sealable layer, thus poliolefinic and
595 moisture barrier, to the possible final laminate in a real application.

596
597 *Table 6: Oxygen permeability (PO_2) and permeability coefficients (KPO_2) of*
598 *CNC coated films and coatings alone.*

Sample	**PO_2 (mL $m^{-2}d^{-1}$ bar^{-1}) 0%RH, 23°C**	**PO_2 (mL $m^{-2}d^{-1}$ bar^{-1}) 50%RH, 23°C**
PET uncoated film	74.95±1.83	87.96±1.13
CNC H_2SO_4 coated PET	1.06±0.07	5.99±2.21
CNC APS coated PET	0.17±0.01	3.53±1.92
Sample	**KPO_2 (mL µm $m^{-2}d^{-1}$ bar^{-1}) 0%RH, 23°C**	**KPO_2 (mL µm $m^{-2}d^{-1}$ bar^{-1}) 50%RH, 23°C**
PET uncoated film	899.4±1.83	1,055±1.13
CNC H_2SO_4 coating alone	0.48±0.01	2.89±0.01
CNC APS coating alone	0.075±0.01	1.65±0.01

4. CONCLUSIONS

The use of ammonium persulfate to obtain cellulose nanocrystals has been initially proposed in order to perform in one single step a complete fragmentation of various cellulosic biomasses, in contrast to acid hydrolysis which requires pretreatment steps for cellulose isolation. This is a very important target that deserves to be fully explored to valorize largely available, renewable resources. In this paper the advantage of using this kind of oxidized CNCs for improving the performance of common food packaging materials, while increasing their possible sustainability, was proved. Transparency, friction coefficient, wettability of the coating were always better for the CNCs coating obtained by the APS process, and especially the oxygen barrier property revealed as a very interesting and promising feature. Therefore, these advantages must be correlated to the higher charge density (due to the presence of carboxylic groups), which in turn gave rise to a more performing coating and availability of functional groups that can be used for grafting the CNCs to other molecules that could be involved in a lamination process. In fact, as the tests performed demonstrated, for its intrinsic nature the hydrophilic coating is very sensitive to moisture and it is not thermoplastic, thus neither thermo-sealable as shown in the Differential Scanning Calorimetry analysis (Online Resource 1); therefore such a high performing coating has to be protected by means of a hydrophobic and sealable polymeric layer in case of a practical application as food packaging material. Nevertheless this is the same problem that must be faced when synthetic barrier polymer, such as polyvinyl alcohol (PVOH) or ethylene vinyl alcohol copolymer (EVOH) are used. Because CNCs coating showed oxygen permeability coefficient, which are lower than the synthetic resins, it can be

623 considered as their possible alternative to increase gas barrier properties, while
624 reducing the oil resources dependency.
625
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631

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Figure 1a

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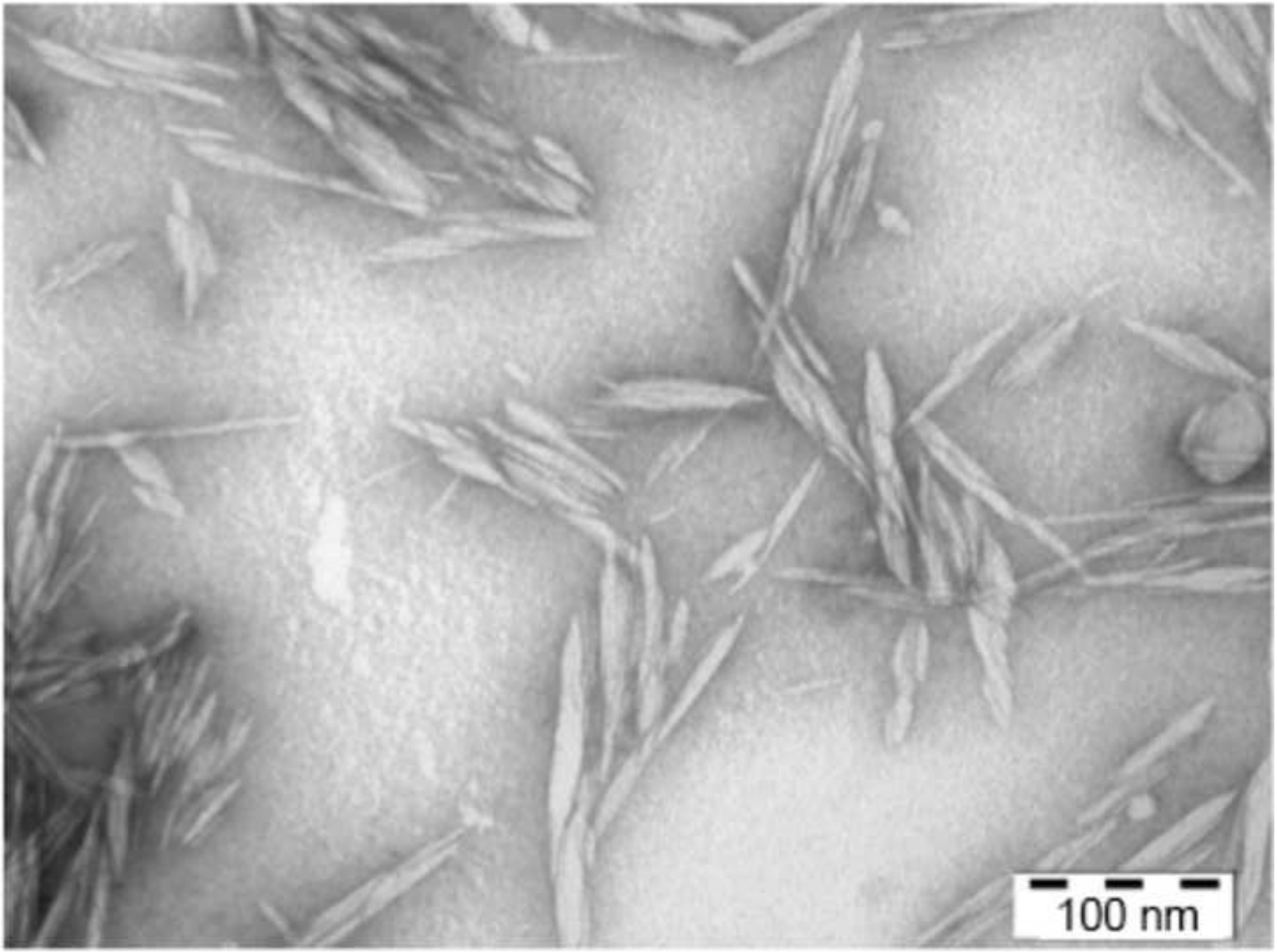

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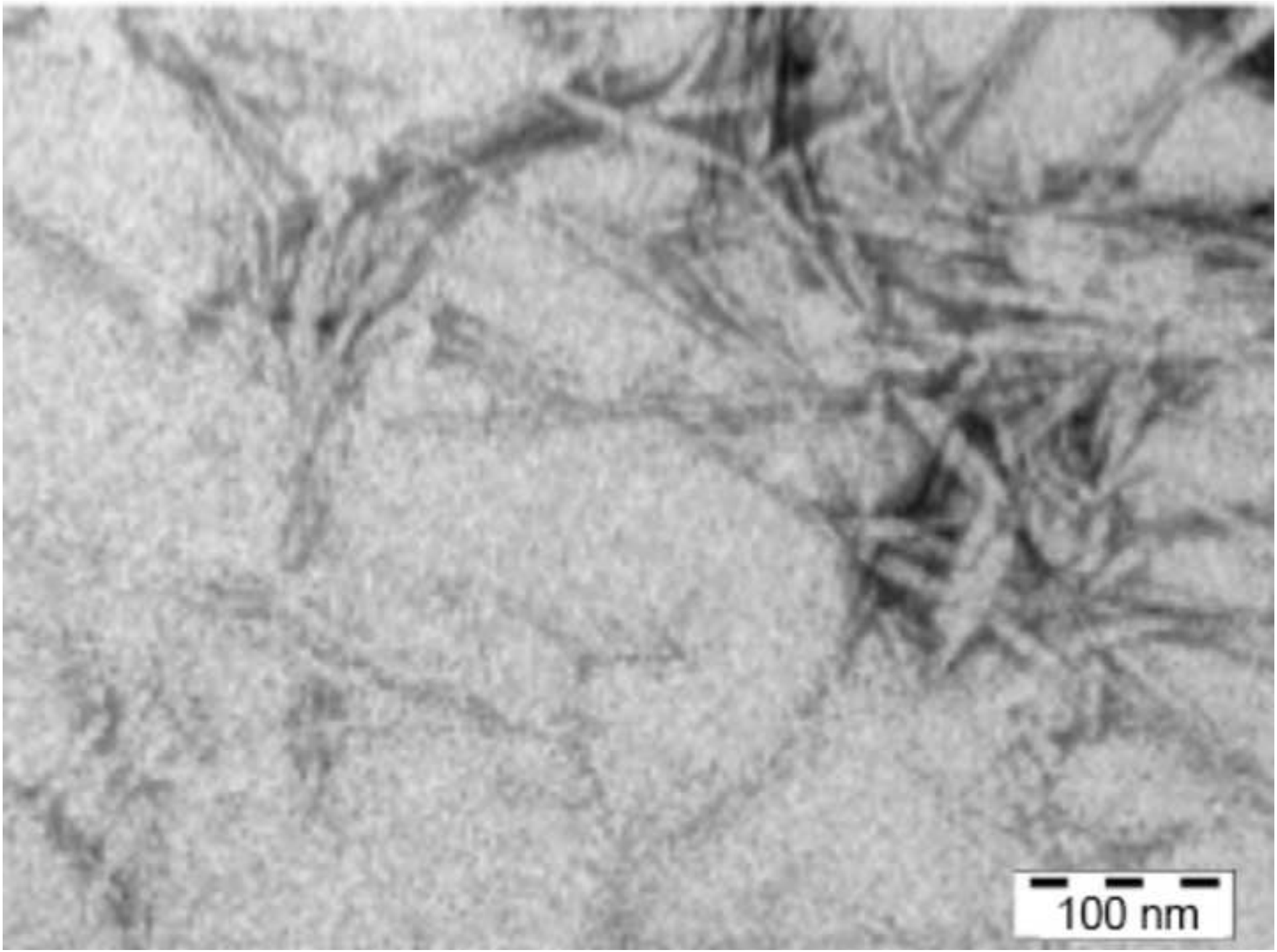

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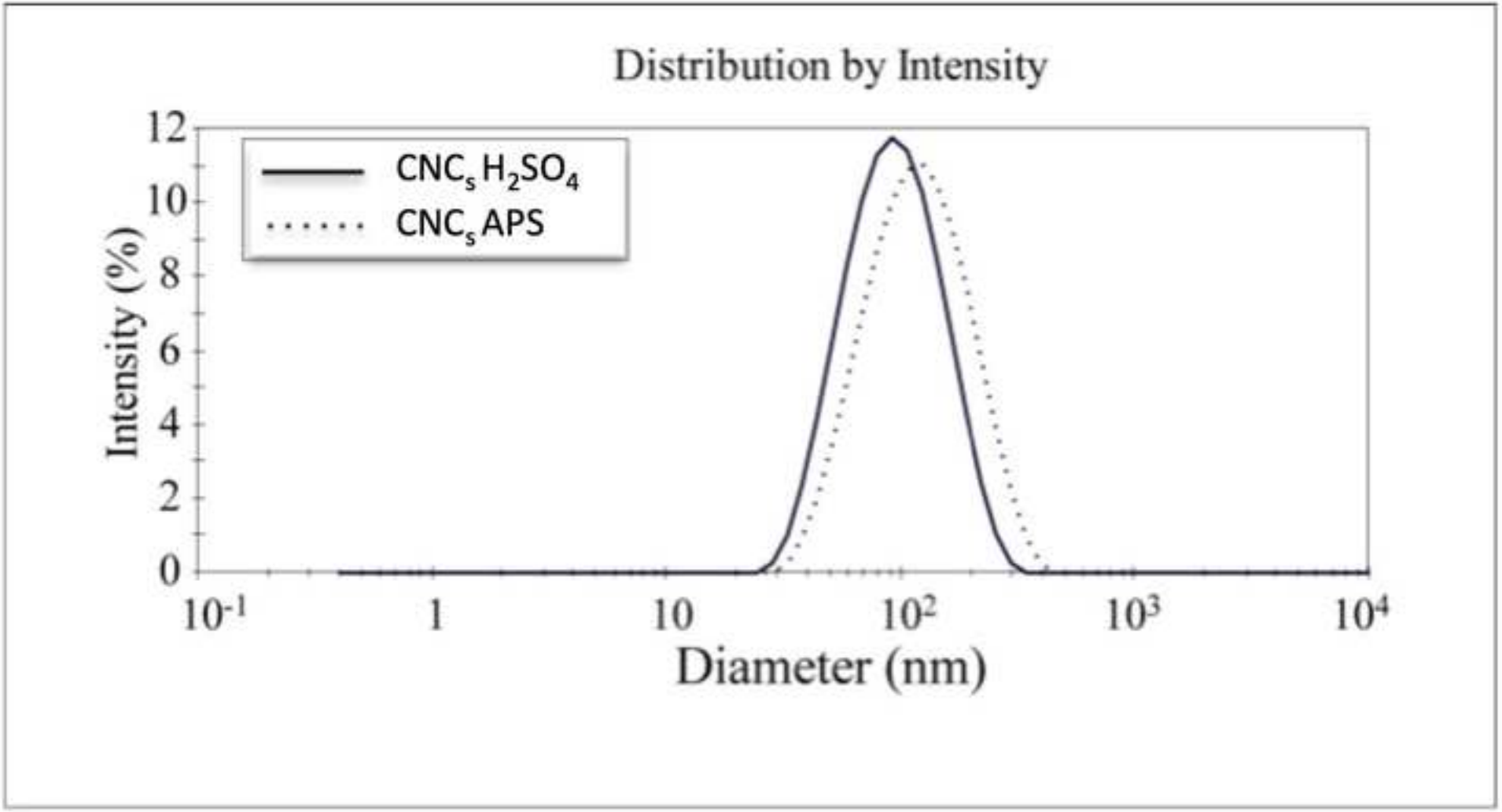

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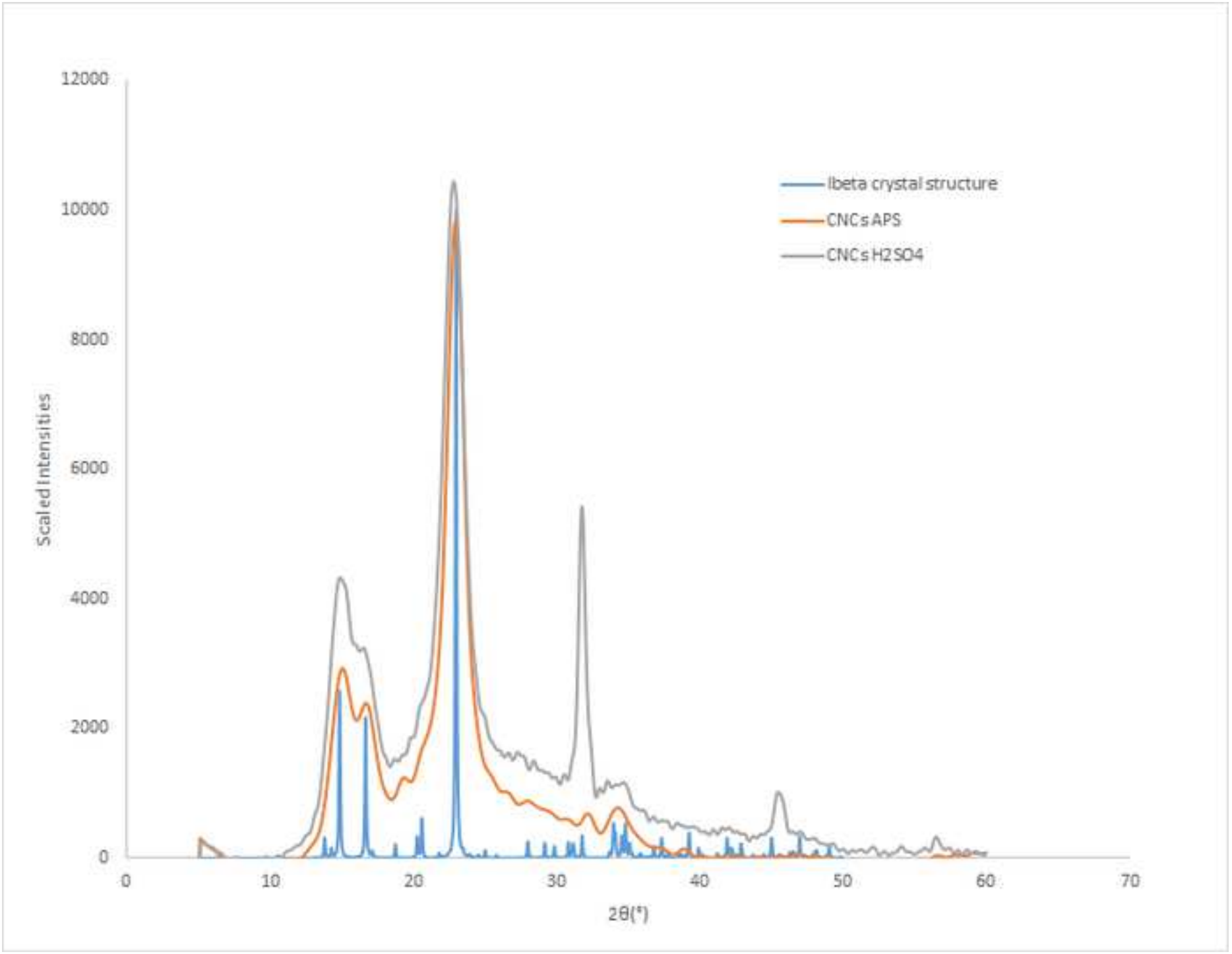

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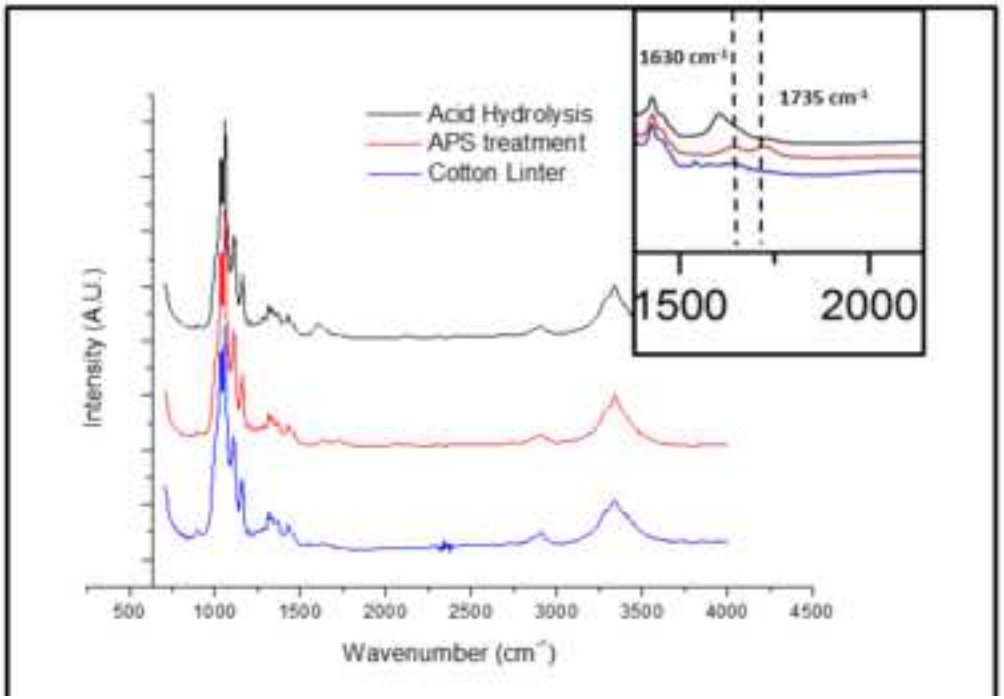

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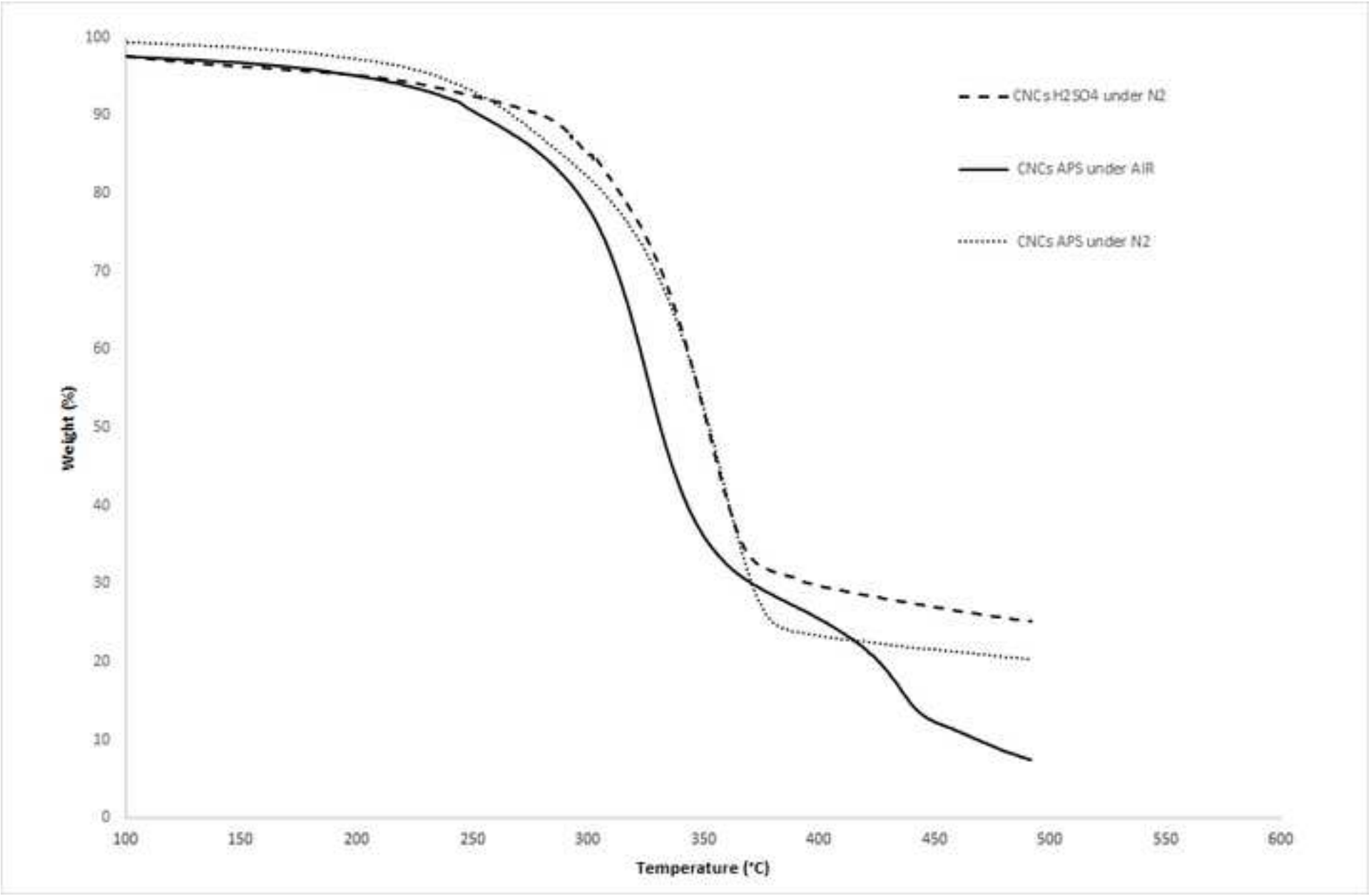

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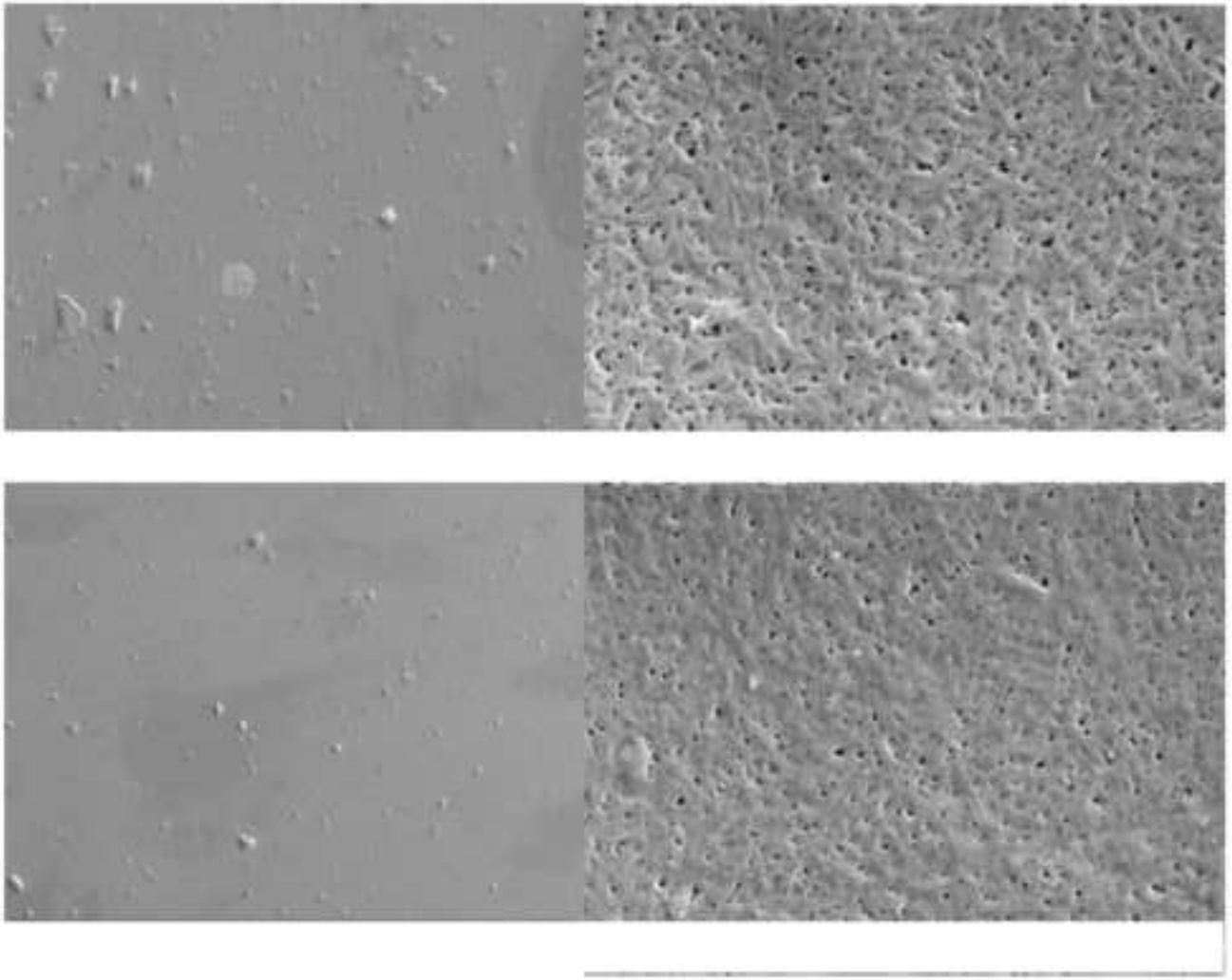

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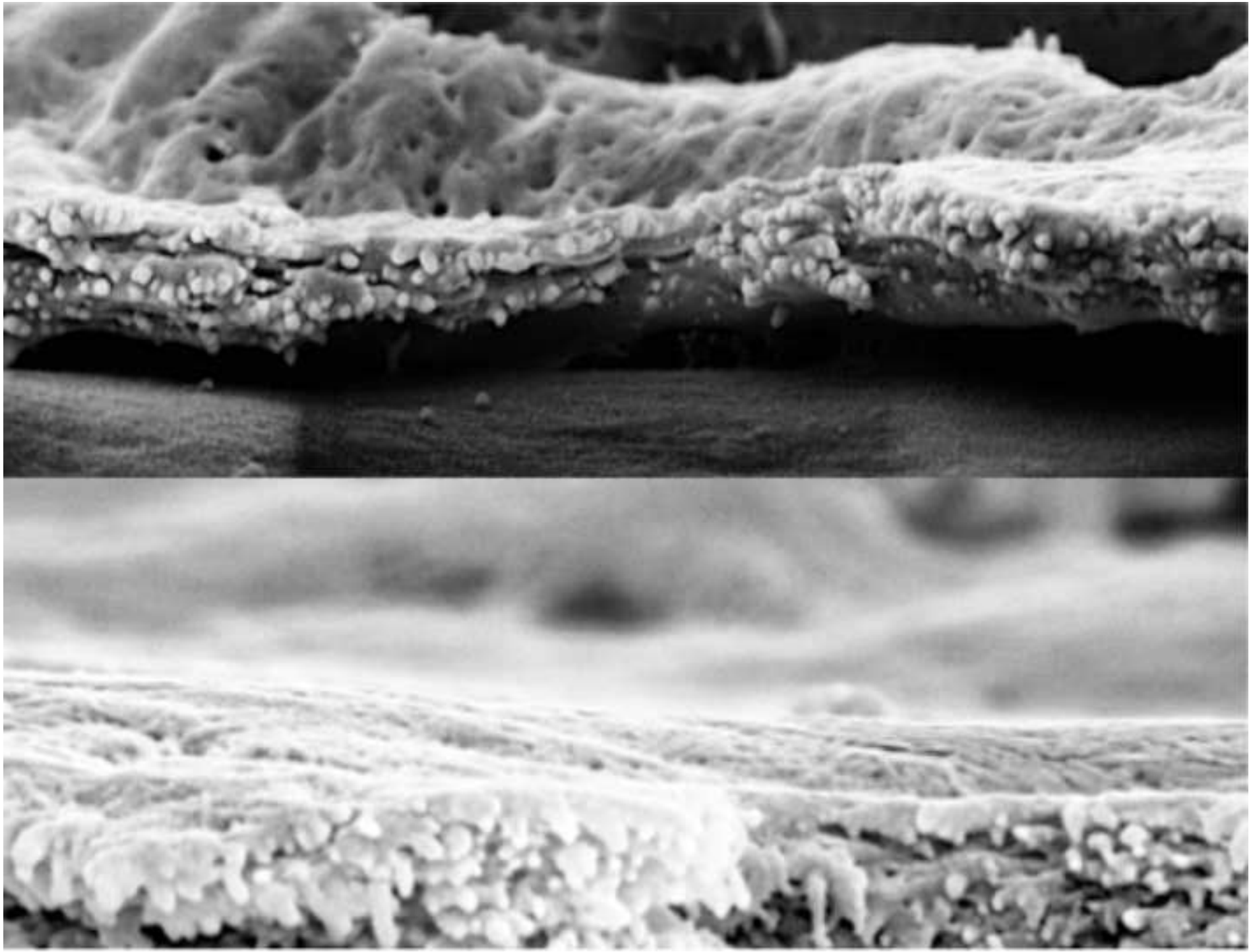

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