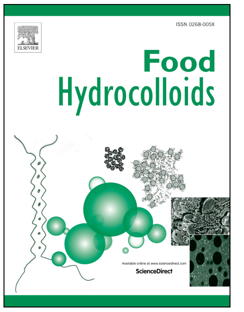


Anti-oxidative and UV-absorbing biohybrid film of cellulose nanofibrils and tannin extract

Panpan Li, Juho Antti Sirviö, Antti Haapala, Alexey Khakalo, Henrikki Liimatainen

PII: S0268-005X(18)32384-1

DOI: https://doi.org/10.1016/j.foodhyd.2019.02.002

Reference: FOOHYD 4928

To appear in: *Food Hydrocolloids*

Received Date: 4 December 2018

Revised Date: 31 January 2019

Accepted Date: 1 February 2019

Please cite this article as: Li, P., Sirviö, J.A., Haapala, A., Khakalo, A., Liimatainen, H., Anti-oxidative and UV-absorbing biohybrid film of cellulose nanofibrils and tannin extract, *Food Hydrocolloids* (2019), doi: https://doi.org/10.1016/j.foodhyd.2019.02.002.

Anti-oxidative and UV-absorbing Biohybrid Film of Cellulose Nanofibrils and Tannin Extract

Panpan Li,[§] *Juho Antti Sirviö,*[§] *Antti Haapala,*[‡] *Alexey Khakalo,*[†] *and Henrikki Liimatainen*[§*]

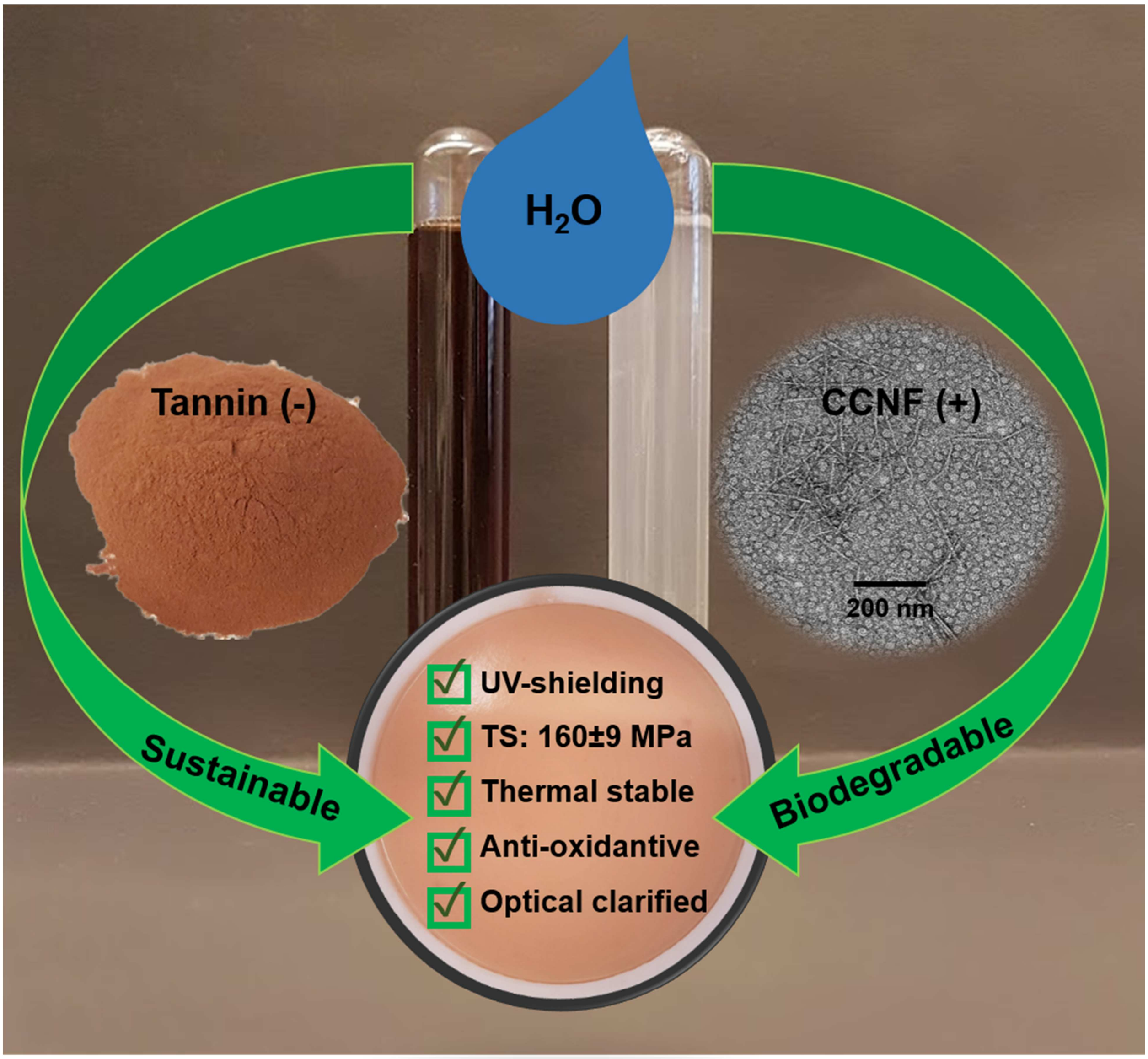
[§]Fibre and Particle Engineering Research unit, University of Oulu, P. O. Box 4300, FI-90014 Oulu, Finland

[‡]Wood Materials Science, University of Eastern Finland, P. O. Box 111, FI-80101 Joensuu, Finland

[†] VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044 VTT, Finland

*Corresponding Author

Graphical abstract



Anti-oxidative and UV-absorbing Biohybrid Film of Cellulose Nanofibrils and Tannin Extract

Panpan Li,[§] Juho Antti Sirviö,[§] Antti Haapala,[‡] Alexey Khakalo,[†] and Henrikki Liimatainen[§]*

[§]Fibre and Particle Engineering Research unit, University of Oulu, P. O. Box 4300, FI-90014 Oulu, Finland

[‡]Wood Materials Science, University of Eastern Finland, P. O. Box 111, FI-80101 Joensuu, Finland

[†] VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044 VTT, Finland

*Corresponding Author (E-mail: Henrikki.Liimatainen@oulu.fi)

ABSTRACT: Cellulose and tannin are both abundant and biodegradable natural polymers. This study proposes a strategy to construct biohybrid films combining the characteristics of cationic cellulose nanofibrils (CCNFs) and tannin extract for film applications. Multi-functional biohybrid films with anti-oxidative and UV-adsorbing characteristics were successfully fabricated from CCNF and tannin mixtures with different mass ratios. The results indicated that pure CCNF could be endowed with multi-functionality by a small amount of introduced flavonoid-rich tannin extract. By adding 5% (w/w) of extract, CCNF-tannin film achieved good anti-oxidant and UV-shielding ability, and simultaneously obtained ca. 15% improved thermal stability and tensile strength of up to 160 ± 9 MPa. In addition, tannin extract was able to enhance the optical clarity of CCNF film with tailorable appearances. The biohybrid films essentially consisted of renewable materials, and they can potentially be exploited in sustainable applications such as biocomposites and packaging materials.

KEYWORDS: cellulose nanofibrils; tannin extract; sustainability; multi-functional; anti-oxidative

1. Introduction

Petroleum-derived plastics, such as polyethylene and polystyrene, have been favoured in everyday applications because they are inexpensive, lightweight and tailorable with mechanical stretching.(Stell, Paul, & Barlow, 2004) However, environmental challenges associated with used plastic products as wastes,(Rochman et al., 2013) as well as the exponentially increased demand for plastics (estimated use is approx. 33 billion tons by the year 2050(Goh et al., 2016)), drive researchers to search for natural renewable resources (*e.g.* collagen, chitosan, tannin and cellulose) as sustainable alternatives. These natural polymers are not only biodegradable and abundant,(Gross & Kalra, 2002) but many of them are also promising to form multi-functional materials. For example, a bio-based matrix made from chitosan and cellulose can provide longer shelf life for food products,(Noshirvani, Ghanbarzadeh, Rezaei Mokarram, & Hashemi, 2017) and hybrid films fabricated from nanocellulose - ZnO(Feng et al., 2017; Y. Jiang et al., 2015) / - lignin(Sadeghifar, Venditti, Jur, Gorga, & Pawlak, 2017) / - aramid nanofibers(J. Luo et al., 2019) were reported to have great performance on UV-shielding while remained good transparencies. In addition to the introduction of UV protection, natural additives such as phenolic substances from plants are also known for the production of materials with anti-microbial(Krepker et al., 2017) and metal chelating abilities(Flora & Pachauri, 2010; Xu, Wang, Jin, Wang, & Qin, 2017).

Complex proanthocyanidins are amongst the most abundant polymeric phenolic compounds generated during the secondary metabolism of plants, and the polymerized components of catechin units are commonly referred to as tannins. They are well known as natural preservatives (for example, to protect a tree from deterioration by insects, fungi and light)(Anttila et al., 2013; Laks, McKaig, & Hemingway, 1988; Thevenon, Tondi, & Pizzi, 2009) with high antioxidant activity(Okuda & Ito, 2011) and binding ability to proteins, alkaloids and certain polysaccharides.(Carn et al., 2012; Haslam, 1998) These prerequisites have made tannins suitable for a variety of applications in the food, medical and leather industries.(Pizzi, 2008; Pranantyo et al., 2015; Gianluca Tondi et al., 2012) However, natural water-soluble tannin has a tendency to be washed away and therefore has a high leachability, which limits the applicability of tannin in many applications.(G. Tondi, Schnabel, Wieland, & Petutschnigg, 2013; G. Tondi, Thevenon, et al., 2013) Nevertheless, slight acidity and negative charged tannin (due to their phenolic and carboxylic structures) can be utilized to form complexes with binding compounds(Weckman, Olsson, & Tufenkji, 2014) and nanoparticles.(Zou et al., 2017)

Cellulose nanofibrils (CNFs) exist commonly as a structural constituent in the cell wall of higher plants. Depending on the raw materials and isolation methods, CNFs are usually 3–100 nm in width and several micrometres in length.(Klemm et al., 2011) The high aspect ratio and inherent chemical structure of cellulose (*e.g.* three reactive hydroxyl groups in each repeating unit) and its chemically modified counterparts also make CNFs (*i.e.* cationic,(T. T. T. Ho, Zimmermann, Hauert, & Caseri, 2011; Thao T. T. Ho, Zimmermann, Ohr, & Caseri, 2012; P. Li, Sirviö, Asante, & Liimatainen, 2018; Sirviö et al., 2014; Aulin, Johansson, Wågberg, & Lindström, 2010; Olszewska et al., 2011) anionic(Isogai, Saito, & Fukuzumi, 2011a; Saito, Nishiyama, Putaux, Vignon, & Isogai, 2006; Selkälä, Sirviö, Lorite, & Liimatainen, 2016; Sirviö, Visanko, & Liimatainen, 2016; Wågberg et al., 2008) and nonderivatizing(Sirviö, Visanko, & Liimatainen, 2015; P. Li, Sirviö, Haapala, & Liimatainen, 2017)) potential complexation agents with polyphenols. Although complex polyphenols such as tannins can provide favourable properties (*e.g.* transparency,(Fukuzumi, Saito, Iwata, Kumamoto, & Isogai, 2009) thermal stability,(P. Li et al., 2017) light weight(Mohieldin, Zainudin, Paridah, & Ainun, 2011) and high mechanical strength(Oksman, Mathew, Bondeson, & Kvien, 2006)) for the CNF materials, homogeneous dispersion of tannin and its strong interaction with CNFs are a challenge to achieve. For example, anionic CNFs have a repulsive interaction with the negatively charged tannin particles, which weakens the formation of complexes,(Thao T. T. Ho et al., 2012) whereas nonderivatized CNFs either have a poor binding ability to tannin or external mechanical forces are required to improve the interaction between CNF and tannin.(Missio et al., 2018)

Recently, an effective and green method was applied to produce cationic CNFs (CCNFs). The reaction medium and reagent, deep eutectic solvent (DES),(Smith, Abbott, & Ryder, 2014) could be recycled five times with no observable effect on reaction efficiency, and CCNF was produced after mild mechanical disintegration.(P. Li et al., 2018) Here, a strategy based on electrostatic attractions between CCNFs and anionic tannin was addressed to form highly compatible, multi-functional biohybrid films.(Thao T. T. Ho et al., 2012) The kinetics of the adsorption of tannins onto the CCNF was previously verified to follow a pseudo-second-order model and indicated a chemisorption process.(Y. S. Ho, McKay, Wase, & Forster, 2000) Moreover, the adsorption process was concluded to be highly pH dependent.

In this study, we investigated the fabrication of anti-oxidative and UV-absorbing biohybrid films of CCNF and tannin using a simple vacuum-filtration method. No additional fibrillation force was applied, still homogeneous CCNF-tannin film can be fabricated by electrostatic attraction. The weight ratios between CCNF and tannin were set as 99:1, 95:5 and 90:10. Structural features, thermal stability, mechanical strength, UV-shielding and anti-oxidant properties of biohybrid films were characterized using field emission scanning electron microscopy (FESEM), thermogravimetric analysis (TGA), universal material testing machine, ultraviolet−visible light (UV−vis) spectroscopy and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, respectively.

2. Material and methods

2.1. Materials

Bleached kraft birch (*Betula pendula*) pulp sheets were used as cellulose raw material after disintegration in deionized water. The pulp contents of cellulose (74.8 wt.%), xylan (23.6 wt.%) and glukomannan (1.1 wt.%).(Liimatainen, Sirviö, Haapala, Hormi, & Niinimäki, 2011) Commercial quebracho tannin extract (refined from quebracho colorado, *Schinopsis lorenzii*) in the form of dry powder was obtained from Haarla Limited (Tampere, Finland). Tannin extract contains 117 mg/g of tannin and 10 mg/g residual carbohydrates. Lithium chloride (LiCl) (99%), sodium periodate ($NaIO_4$) (> 99%) and 2,2-diphenyl-1-picrylhydrazyl were obtained from Sigma Aldrich (Germany) to produce dialdehyde cellulose (DAC). Ethanol (CH_3CH_2OH) (96%) and glycerol ($C_3H_8O_3$) (97%) were from VWR International (Fontenay-sous-Bois, France), and aminoguanidine hydrochloride ($CH_6N_4 \cdot HCl$) (> 98%) was from Tokyo Chemicals Industry Co., Ltd. (Tokyo, Japan) to produce cationized dialdehyde cellulose (CDAC).

2.2. Cationization of Cellulose

A two-step method based on consequent periodate oxidation and cationization in DES (composed of aminoguanidine hydrochloride and glycerol) was employed to produce CCNFs from birch pulp.(Sirvio, Hyvakko, Liimatainen, Niinimaki, & Hormi, 2011) In brief, 10 g (abs.) of birch pulp was diluted to 1000 g with deionized water, and the suspension was heated to a final temperature of 55 °C in an oil-bath system. A total of 18 g of LiCl with 8.2 g of $NaIO_4$ was added to react with the cellulose for 3 h at 55 °C to obtain DAC (aldehyde content at 2.2 mmol g^{-1}). The reaction beaker was fully covered with aluminium foil to avoid the light-induced decomposition of the periodate. The DAC was filtered and washed with 1000 ml of 50:50 ethanol water solution, mixed in 500 ml ethanol twice for 15 min and filtrated. Cationization of DAC was done in DES to obtain cationic cellulose with a charge density of 1.1 mmol g^{-1} as determined previously.(P. Li et al., 2018) The DES was formed by mixing aminoguanidine hydrochloride and glycerol in a Scott bottle with a molar ratio of 1:2. A clear DES solution was obtained by melting the compounds at 90 °C after which the reaction temperature was adjusted to 70 °C. The DAC was added to a DES with a mass ratio of 1:20. The reaction was kept at 70 °C for 10 min and stirred continuously with a magnetic bar. After cationization, the reaction bottle was removed from the oil-bath system and 250 ml of ethanol were added. CDAC suspension was filtrated and washed twice with 500 ml of ethanol.

2.3. Fabrication of Cationized Cellulose Nanofibrils

Mechanical disintegration was used to liberate the CCNFs from the CDAC. First, a 1% CDAC solution was mixed with the Ultra-Turrax mixer (IKA T25; Germany) at 12,000 rpm for 10 min and then further disintegrated using a microfluidizer (M-110EH-30; Microfluidics Inc., Westwood, MA, USA) with a pressure of 1000 bars. The suspension was passed twice through the microfluidizer chambers (400 and 200 μm), yielding a relatively transparent suspension.

2.4. Preparation of CCNF-tannin Hybrid Film

A vacuum-filtration method was used to prepare CCNF−tannin biohybrid films (80 g m^{-2}).(Liu, Walther, Ikkala, Belova, & Berglund, 2011; Sehaqui, Liu, Zhou, & Berglund, 2010) The CCNF suspension and tannin dispersion (total solids of 0.353 g in 100 g of deionized water, pH 5.5) were mixed together using a magnetic stirrer for 10 min. The suspensions with CCNF/tannin weight ratios of 100:0, 99:1, 95:5 and 90:10 (coded as CCNF100, CCNF99-Tannin1, CCNF95-Tannin5 and CCNF90-Tannin10, respectively) were vacuum-filtered on a filter membrane with a pore size of 0.65 μm (Millipore, USA), after which the wet film was vacuum-dried at 93 °C for 10 min.

2.5. Transmission Electron Microscopy (TEM)

The morphological features of the CCNFs were analysed with TEM using a JEOL JEM-2200FS (JEOL Ltd., Tokyo, Japan). CCNF samples were diluted with deionized water into 0.1% (w/w), and a tiny droplet (7 μL) of polylysine(Marsich et al., 2012) was at first dosed on the top of a carbon-coated copper grid and allowed to stay for 1 min. The excess polylysine was wiped away with filter paper. Similarly, 7 μL of the CCNF sample solution were then dropped and removed from the grid. Finally, a drop of negative stain agent, 2% (w/v) uranyl acetate, was applied using the same procedure. The stained samples were dried at room temperature and analysed at 100 kV under standard conditions. Images were taken by a Quemesa CCD camera. The widths of the individual nanofibrils were measured by iTEM image analysis software (Olympus Soft Imaging Solutions GMBH, Munster, Germany). The final results were averaged from 50 fibrils, and the standard errors were calculated.

2.6. Field Emission Scanning Electron Microscopy (FE-SEM)

An FESEM (Zeiss Sigma HD VP, Germany) was employed to study the structural properties of CCNF−tannin hybrid films in planar and cross-sectional directions using an accelerating voltage of 5 kV. The cross-sections were captured by snapping the frozen (liquid nitrogen) film strips. The film sample was fixed to a carbon-coated carrier. Prior to imaging, the specimens were sputter-coated with platinum (with thickness of 5 nm).

2.7. UV and Optical Properties

Integrating sphere (DRA 2500) based Cary 5000 spectrophotometers (Agilent Technologies, CA, USA) and Shimadzu UV-2600 spectrophotometers (Kyoto, Japan) were specialized to measure the transmittances of the hybrid films and tannin solutions (at concentrations of 0.01, 0.1 and 1%), respectively. According to the ASTM D1003 Standard,(ASTM International, 2006) the optical haze, *i.e.* the scattering of light as it passes through the biohybrid films and results in poor visibility and/or glare, was measured and calculated by the equation:

$$\text{Haze} = \left[\frac{T_4}{T_2} - \frac{T_3}{T_1}\right] \times 100\%, \qquad (1)$$

where

T_1 = background checking value,

T_2 = total transmitted illumination,

T_3 = beam checking value and

T_4 = pure diffusive transmittance.

The total transmitted illumination, T_2, includes both specular transmittance and pure diffusive transmittance. A wavelength of 550 nm was set for comparison of film transparency.(Zhu, Xiao, et al., 2013)

2.8. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity of the hybrid films was determined according to the DPPH radical scavenging assay(Byun, Kim, & Whiteside, 2010) method with a slight modification. Briefly, a 100 mg sample film was mixed with 2 mL of methanol with a magnetic stirrer for 3 h at room temperature. The supernatant obtained was applied for DPPH radical scavenging activity. Then, 2 mL of methanolic solution of DPPH (0.06 mM) were mixed with 500 μL of the supernatant. The control sample was obtained using 500 μL methanol without the sample film. The mixture was vortexed at room temperature in the dark for 30 min. The remaining DPPH was measured by absorbance at 517 nm with the Shimadzu UV-2600 spectrophotometer. The final DPPH radical scavenging activity of the films was calculated by the equation:(Singh & Rajini, 2004)

$$\text{Radical scavenging activity (\%)} = \left(1 - \frac{A_{sample}}{A_{control}}\right) \times 100\,, \quad (2)$$

where

A_{sample} = the absorbance of the sample solution and

$A_{control}$ = the absorbance of DPPH solution without the addition of the film.

The results were taken as the average of three measurements.

2.9. Thermogravimetric Analysis

The TGA of the CCN-tannin hybrid films was carried out in a thermal analyser (STA 449 F3; Netzsch, Germany) under two separate atmospheres: the nitrogen flow and the air flow (dynamic air), at a constant rate of 60 mL min^{-1}. Approximately 5 mg of the room-temperature dried sample were carried by aluminium pan and heated from 20 °C to 600 °C with a heating rate at 10 °C min^{-1}. The decomposition temperature (T_d) was taken when the temperature at the onset point of the weight loss in the TGA curve was obtained.

2.10. Tensile Test

The mechanical properties of the CCNF−tannin hybrid films were measured with a universal material testing machine (D0724587; Zwick, Switzerland), equipped with a 100 N load cell. Films were kept at constant temperature (23 °C ± 1 °C) and humidity (50% ± 2%) conditions during the sample preparation (films were cut into strips with a uniform width of 5 mm) and were kept in the same controlled environment for over one week prior to testing. The thickness of each specimen was measured by a precision thickness gauge (FT3; Hanatek Instruments, East Sussex, UK). Three different locations within the gauge length were measured to calculate the average thickness of each sample strip. For the tensile tests, a 40 mm gauge length was set at a strain rate of 4 mm min^{-1}.

2.11. Statistical Analysis

Mechanical properties were completed using values from more than five strips in each sample for tensile strength, maximum strain and Young’s modulus. One-way analysis of variance (ANOVA) was applied with (★) $p < 0.05$ suggesting a significant difference and with (★★) $p < 0.01$ providing a critical assessment.(Majdzadeh-Ardakani & Sadeghi-Ardakani, 2010)

3. Results and discussion

3.1. Characteristics of CCNF-tannin Suspensions

Biohybrid films of CCNF and tannin were fabricated using a simple vacuum filtration and drying technique. The CCNF was isolated from birch pulp which was pre-treated into CDAC in the DES, followed by a mechanical nanofibrillation procedure (Fig. 1A). The average lateral dimension of individual nanofibrils of CCNF based on TEM imaging was 4.6 ± 1.1 nm, while the length of nanofibrils was several hundreds of nanometres, indicating a high aspect ratio of CCNF (Fig. 1B; Fig. S1 in supplementary material). The chemical characteristics of CCNF were analysed in our previous study.(P. Li et al., 2018) Water-soluble tannin at different ratios to CCNF (up to 10:90, w/w) were all effectively dispersed into the CCNF solution without formation of any visible large particles or fibrous aggregates (Fig. S2 in supplementary material).

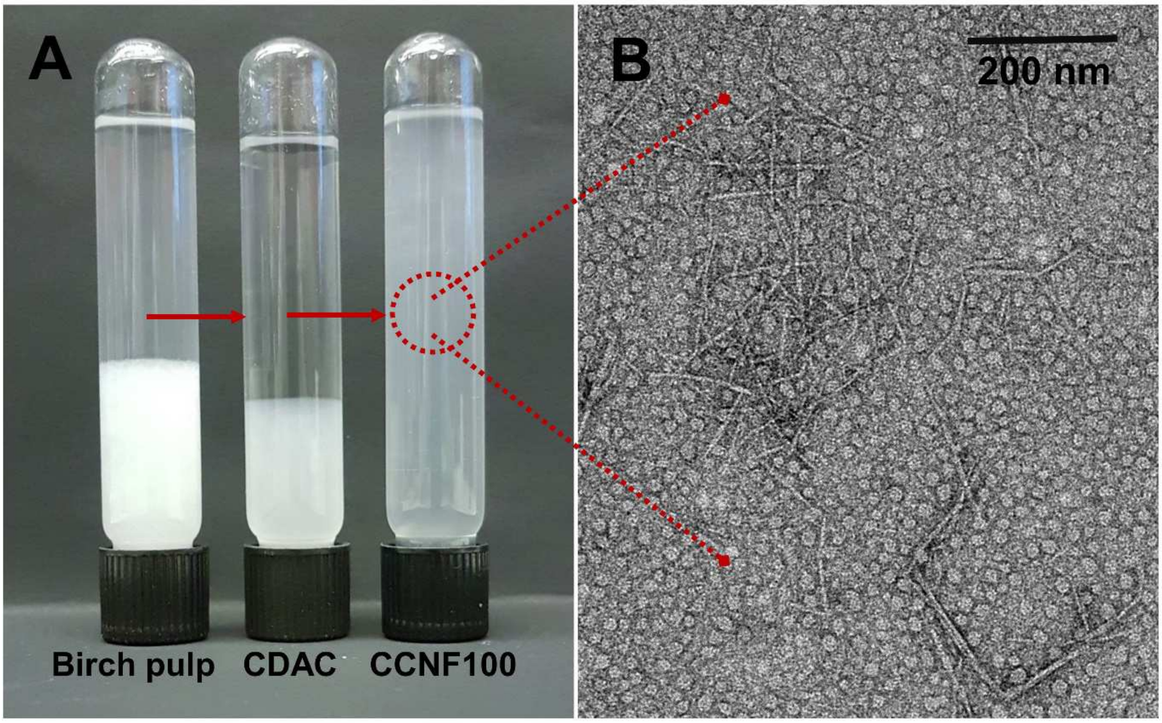


Fig. 1. Suspension (1%) of birch pulp, cationized cellulose (CDAC) and cationic cellulose nanofibrils without tannin extract (CCNF100) at room temperature (A), and the TEM image of CCNF100 (B).

Depending on the percentage of tannin, the CCNF-tannin suspension showed a brownish to reddish colour and formed a cake-like structure after vacuum filtration (Fig. 2A). Nevertheless, due to high tannin adsorption capacity of CCNF, all the filtered water from CCNF-tannin suspensions were transparent and colourless. Tannin was distributed in the CCNFs, and all the dried biohybrid films (with thickness of c.a. 60 µm) were translucent, as they appear when they cling to an object (Fig. 2B). The optical properties, including specular transmittance, diffusive transmittance and haze of the hybrid films, were further analysed and are shown in the section about UV-shielding and optical performance.

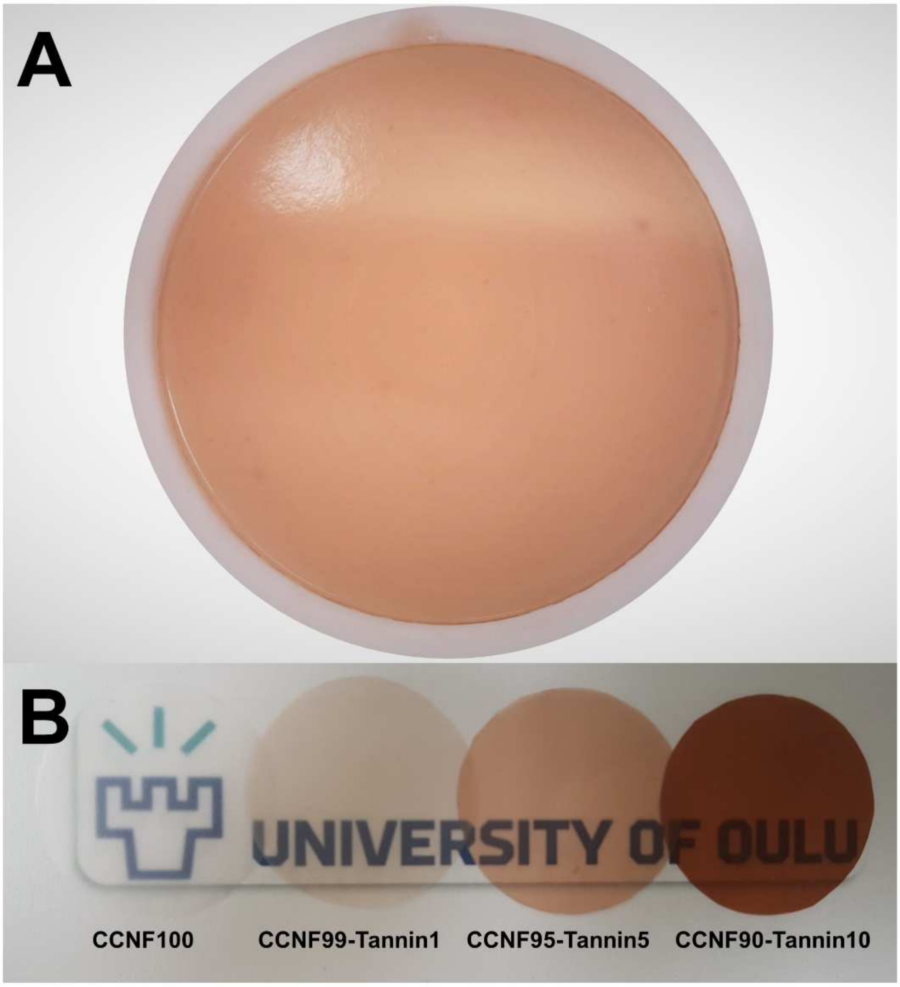


Fig. 2. Vacuum-filtrated CCNF95-Tannin5 hybrid cake (wet) after filtration (A) and the translucent CCNF biohybrid films (dry) with different amounts of tannin (B).

3.2. Morphological Properties of Films

Although the visual appearance of all the films were smooth to naked eye and the addition of tannin extract did not make significant visible differences, FESEM imaging (Fig. 3) still indicated variances on both the surfaces and the cross-section of the hybrid films. Compared with pure CCNF film (Fig. 3A), hybrid films contain higher amount of tannin (Fig. 3C-D) usually showed more flat surfaces yet with less nanofibrils under FESEM random checking. This was most likely because of tannin extract that was applied as fillers in hybrid film fibrous networks. In addition, different from previous films that were made from anionic(Selkälä et al.,

2016) and non-derivative nanofibrils,(P. Li et al., 2017) lesser amount of big fiber bundles (i.e. several micrometres in width) were detected on the CCNF and their hybrid films. Absence of fiber aggregates confirmed present CCNFs are fine and homogeneous. It should be noted that films that made previously from anionic nanofibrils(Selkälä et al., 2016) and non-derivative nanofibrils(P. Li et al., 2017) were easily removed from the filter membrane (0.65 μm PVDF) after press-drying (93 °C), whereas CCNF and its hybrid films tended to stick to the surface of filter membrane and thus difficult to be removed completely. This phenomenon was presumably associated with adhesion caused by slightly negatively charged filter membrane, which adhered on the positively charged surface of CCNF film through electrostatic interaction.

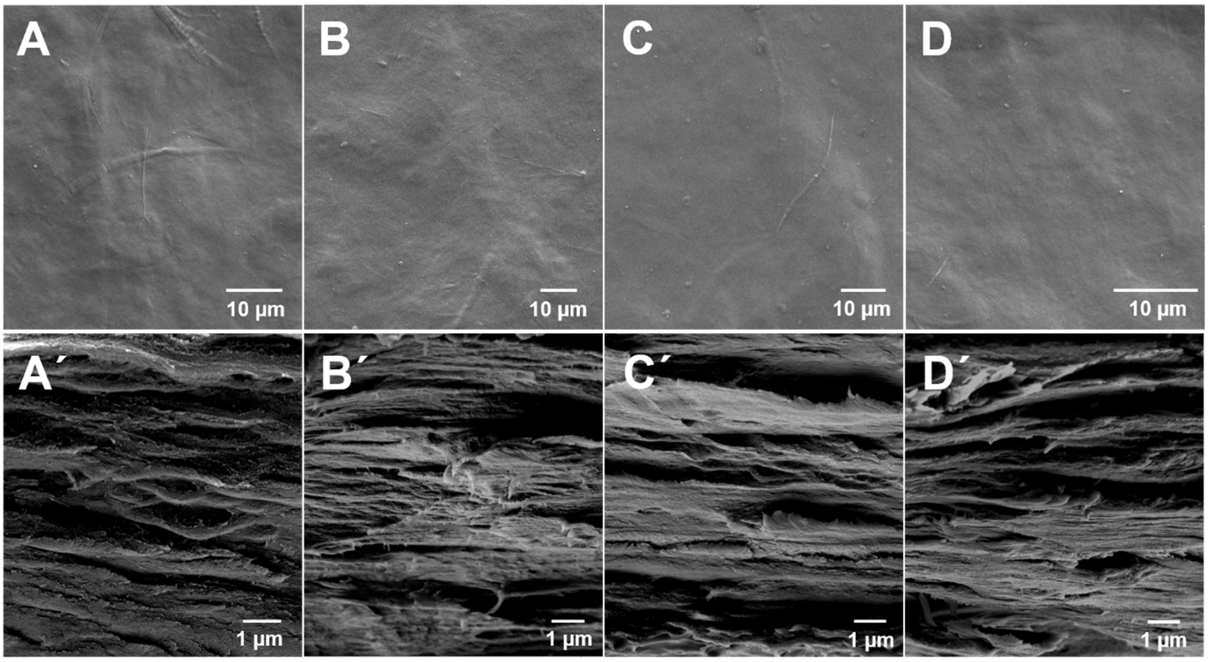


Fig. 3. FESEM images on the surface of CCNF100 (A), CCNF99-Tannin1 (B), CCNF95-Tannin5 (C) and CCNF90-Tannin10 (D). Cross-sections of CCNF100 (A´), CCNF99-Tannin1 (B´), CCNF95-Tannin5 (C´) and CCNF90-Tannin10 (D´).

The cross-section images demonstrated that all the films achieved clearly layered structures (Fig. 3A´–D´), which is similar to previously cellulose nanofibril films(Henriksson, Berglund, Isaksson, Lindström, & Nishino, 2008; Y. Luo, Zhang, Li, Liao, & Li, 2014; Mautner, Lucenius, Österberg, & Bismarck, 2017) and talc-nanofibril hybrid film(Liimatainen et al., 2013) fabricated by other researchers. The introduction of tannin extract to CCNFs (Fig. 3B´– D´) showed higher amount of thinner individual inner-layers than what pure CCNFs film had (Fig. 3A´), even though all the film samples had similar thickness as a whole. Besides, very little notable particle aggregation was detected in their either the planar or cross-sectional views, whereas clear trace of breaking fibres were observed in all the cross sections with larger magnification (Fig. S3 in supplementary material). In general, anionic tannin was evenly incorporated into the positive-charged CCNF, and these hybrid films achieved flat and multi-layered structures.

3.3. UV-shielding and Optical Performance

Conventional opaque cellulose paper has a strong incident light scattering, because of its porous structures.(Zhu, Fang, Preston, Li, & Hu, 2014) Nanofibril-based films are, in turn, optically tailorable because they are built from fine fibril strands, which have small and tuneable light scattering based on their fibril size, film density, surface smoothness etc.(F. Jiang et al., 2018; Zhu, Parvinian, et al., 2013) Therefore, hybrid nanofibril films made with different approaches possess unique optical characteristics, which offer variable options for different applications. Due to the high content of phenolic units, tannin extract has great potential to be applied as a UV-absorber.(Northey, Glasser, & Schultz, 2000) To study the UV-shielding ability and the optical properties, the hybrid films were tested and compared with the pure CCNF film (thickness of films was ca. 50 µm) in terms of specular transmittance (Fig. 4A), diffusive transmittance (Fig. 4B) and total transmittance (Fig. 4C). The specular transmittance detects the light that transmits along the same axis with the incident light, diffusive transmittance describes the light that diffuses in other directions than the axis and total transmittance collects all the light that penetrates the sample.

Fig. 4C indicates the clear trend of a UV-shielding property while adding tannin compounds into pure CCNF film, that is, a higher percentage of introduced polyphenolics leads to a better UV absorption of CCNF film. Similar results are also shown in Fig. 4E for when tannin extract was diluted by deionized water into a different consistency. Specifically, CCNF90-Tannin10 had 88% protection of UV-A (320–400 nm) and was able to block almost 100% of UV light below 320 nm (covered UV-B and UV-C ranges), whereas pure CCNF100 had a 40% and 12% of transmittance at 320 and 280 nm, respectively. Yet, after introducing 1% tannin extract, the film CCNF99-Tannin1 reduced the transmittance to 19% and 1% at 320 and 280 nm, respectively. Meanwhile, CCNF95-Tannin5 was able to absorb 92% of UV light when the wavelength was below 320 nm (Table S1 in supplementary material). In general, CCNF-tannin hybrid films achieved good UV-shielding ability, which was also comparable with previous lignin-derivate film.(Sadeghifar et al., 2017)

Besides the UV-shielding property, it was notable that the visual appearance of CCNF films was also greatly affected by the introduction of tannin. For example, pure CCNF had a total transmittance of 58% (at 550 nm), whereas the transmittance of CCNF99-Tannin1, CCNF95-Tannin5 and CCNF90-Tannin10 was reduced to 53%, 44% and 29%, respectively. Interestingly, although the optical transmittance decreased when adding tannin extract, the hybrid films seemed to be clearer to the naked eye (Fig. 2B). Therefore, we tested both the specular and diffusion transmittances, and the optical property was reflected as haze, which indicated visual transmittance for the eye.(Zhu, Parvinian, et al., 2013) It was observed that introduction of tannin decreased the haziness of the CCNF film. Among the hybrid films, CCNF95-Tannin5 offered the best optical clarity, whereas CCNF99-Tannin1 and CCNF90-Tannin10 had similar haziness, although CCNF99-Tannin1 had a higher total transmittance. Hence, the optical performance of CCNF film was tailorable by the tannin extract dose. Additionally, both CCNF90-Tannin10 and pure CCNF100 water suspensions (1% w/w) showed a clear Tyndall scattering after keeping them in a stationary state at 5 °C for one month (Fig. 4F) without any flocculation and sedimentation observed, indicating a good stability of homogenous CCNF or CCNF-Tannin mixtures (Fig. S4 in supplementary material).(Carn et al., 2012)

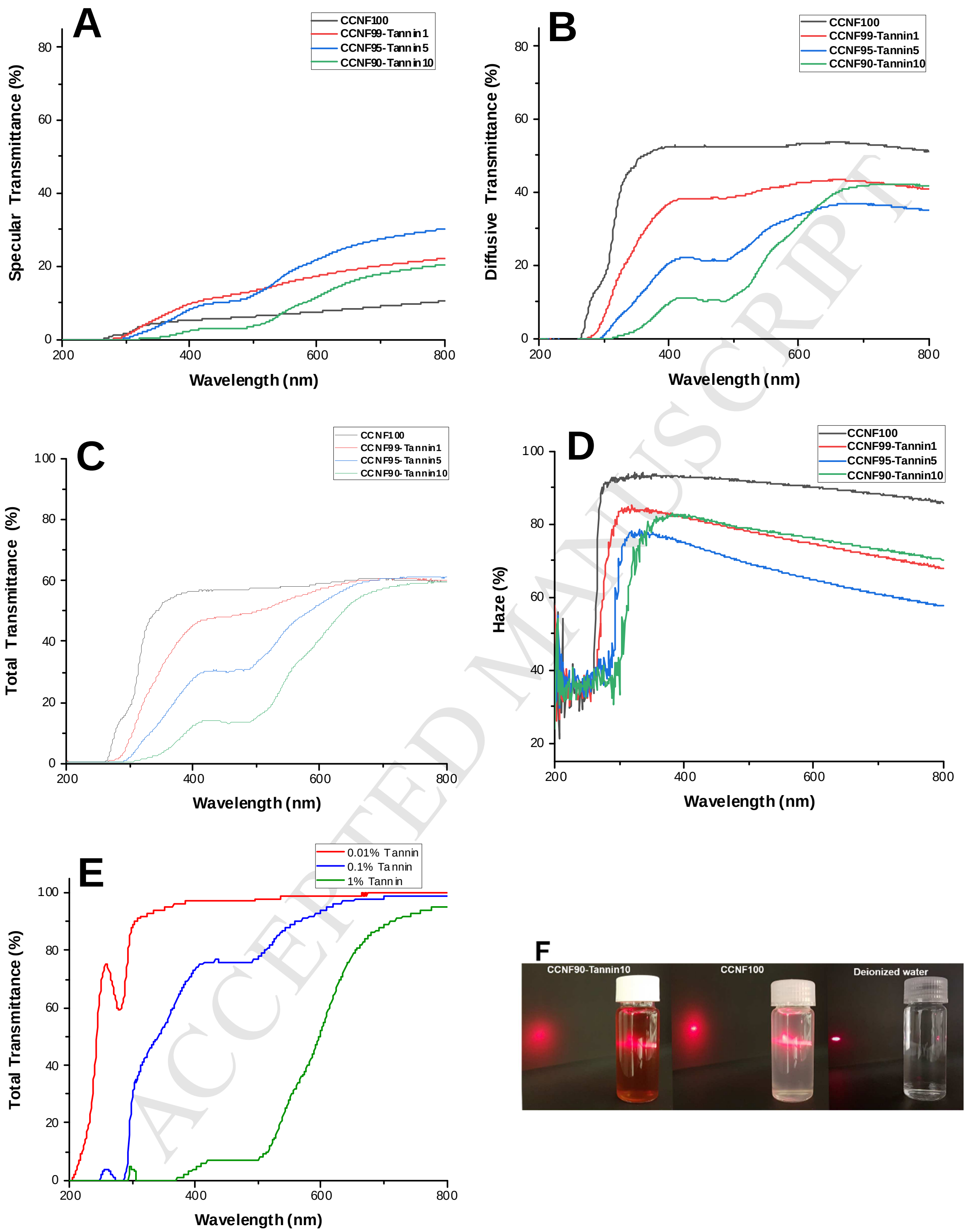


Fig. 4. Specular transmittance (A), diffusive transmittance (B), total transmittance (C) and calculated haze (D) of CCNF and CCNF-tannin hybrid films. The UV-absorbing property of tannin was tested in aqueous suspensions at different concentrations (E). The suspension of

CCNF90-Tannin10, CCNF100 and water (stored at 5 °C for one month) were incident through a horizontal laser (F).

3.4. Antioxidant Activity

Free radicals, including reactive oxygen species, are capable of damaging biomolecules and causing a wide range of degenerative diseases.(Halliwell & Gutteridge, 1990) Therefore, antioxidant and radical scavenging properties are desired in food packaging applications,(Byun et al., 2010; Gemili, Yemenicioğlu, & Altınkaya, 2010; López de Dicastillo et al., 2011) biomedical products such as drug deliveries,(Digge, Moon, & Gattani, 2012) anti-aging cosmetics(Jadoon et al., 2015) and dietetics.(Bhat, Liong, Abdorreza, & Karim, 2013) Natural antioxidants containing phenolic groups can prevent the impact on oxidants, *e.g.* by donating hydrogen atoms (reduction) and suppressing the formation of hydroxyl radicals.(Andrade et al., 2005; I. Gülçin, Mshvildadze, Gepdiremen, & Elias, 2006)

The antiradical activity of tannin hybrid CCNF films was investigated by the reduction of DPPH. In the DPPH assay, the dark-coloured (violet(İ. Gülçin, Huyut, Elmastaş, & Aboul-Enein, 2010)) DPPH radical becomes less coloured DPPH-H by reacting with antioxidants, which is detected as a decrease in the absorbance at 517 nm.(I. Gülçin, 2007) Fig. 5 shows a gradually improved DPPH radical scavenging activity with the increase in the amount of tannin. Compared with the control sample, films of pure CCNF100, CCNF99-Tannin1, CCNF95-Tannin5 and CCNF90-Tannin10 showed 7 ± 5, 8 ± 3, 28 ± 2 and 31 ± 1% enhanced antiradical activity, respectively. Similar results were reported previously when tannic acid was tested solely,(İ. Gülçin et al., 2010) whereas tannic acid was tested with a much higher concentration (15–45 μg/mL). Although not many noticeable changes occurred with 1% tannin addition, films containing 5% and 10% of tannin showed a significant decrease ($p < 0.01$) in the concentration of the DPPH radical due to the effective scavenging ability of polyphenols.(Lopes, Schulman, & Hermes-Lima, 1999)

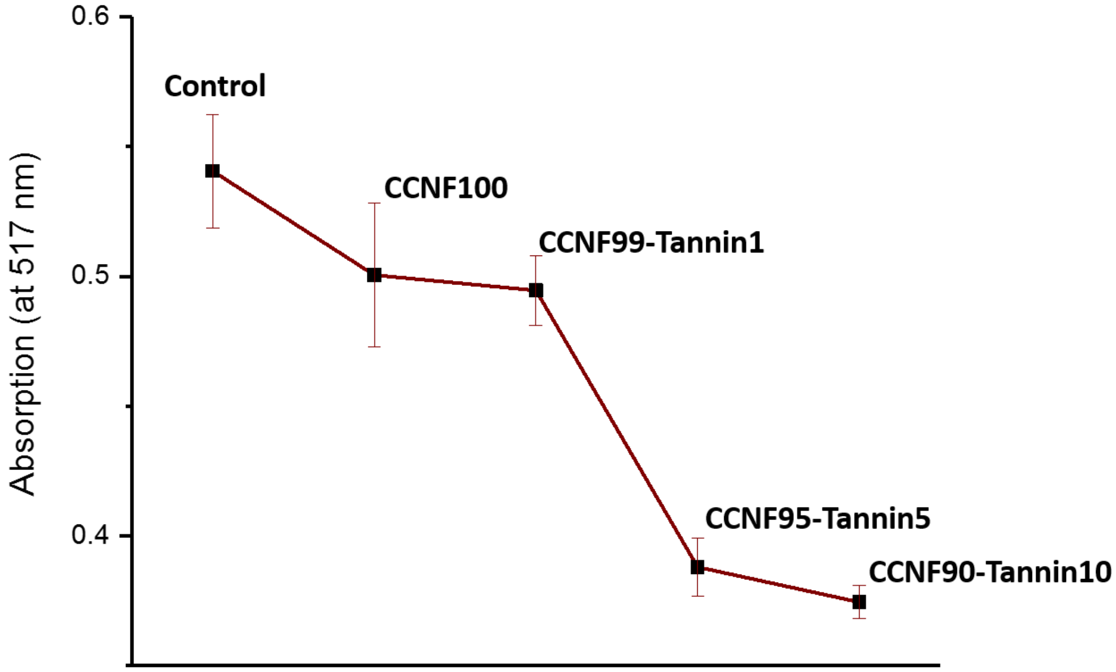


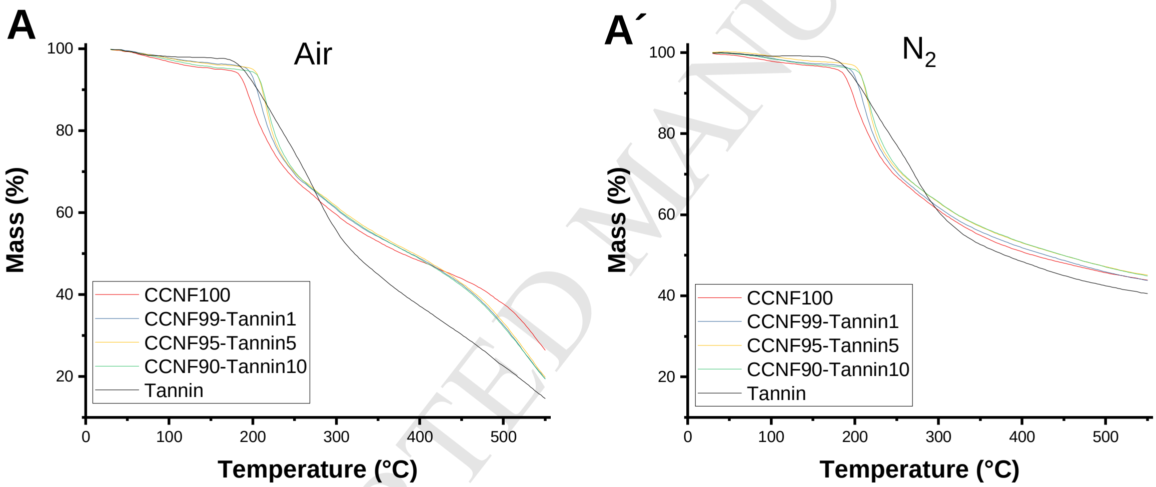
Fig. 5. UV-absorbance (at 517 nm) of DPPH residual solution. (DPPH solution was reacted with CCNF100, CCNF99-Tannin1, CCNF95-Tannin5 and CCNF90-Tannin10, respectively. DPPH solution without the addition of the film was set as the control.)

3.5. Thermal Stability of Tannin Hybrid Films

Thermal stability tests of the mixtures/hybrids in an emerging temperature profile indicate the degree of interaction (elevated energy is required for decomposition of interactions, *e.g.* hydrogen bonding in a mixure(Abbott, Capper, & Gray, 2006; Chen et al., 2018)), as well as compositional and structural differences among the samples.(Visanko et al., 2017) Tannin hybrid films were tested and compared with pure CCNF film under both air and N_2 atmospheres. Generally, the introduction of tannin can make CCNF films bear a relative higher decomposition temperature under both air and N_2 atmospheres (Fig. 6). Unlike the original birch fibres (containing 24.7% hemicelluloses)(Liimatainen et al., 2011) and lignin reserved nanopaper(Visanko et al., 2017) that usually have a steep weight loss curve, both pure CCNF100 and tannin hybrids showed a gentle mass loss step (Fig. 6 A and A´). In addition to the evaporation of water, the major weight loss of pure CCNF and tannin started at similar temperatures of 167 °C and 170 °C, respectively, which were lower than the onset decomposition temperature of original birch fibre (T_{Onset} 250 °C)(P. Li et al., 2017) under air atmosphere. However, it is well known that chemically modified cellulose nanofibres usually have a decreased thermal stability when compared to their starting materials.(Eyholzer et al., 2010; Fukuzumi et al., 2009; Visanko et al., 2017) The decreased thermal stability of CCNF100 was mostly due to the chemical hydrolysis of pristine cellulose fibres and the sequentially mechanical disintegration procedure.(Dhar, Bhardwaj, Kumar, & Katiyar, 2014; J. Li et al., 2012) However, the tannin hybrid films usually obtained improved T_{Onset} from 167 °C (of

CCNF100) to 185, 192 and 195 °C, respectively (Fig. 6A) as a function of increased tannin content. In addition to the T_{Onset} comparisons among films, aligned trends were also observed from their maximum mass loss rates (Fig. 6 B), which verifies the formation of hybrid materials by electrostatic attraction between cationic cellulose and anionic tannin, therefore resulting in an enhanced thermal stability.

Under N_2 atmosphere, films exhibited similar thermal stability compared to air, yet a higher amount of residuals were left because of the inhibited process of oxidation (Fig. 6 A´ and B´). It was notable that all the CCNF films were able to maintain over 50% of total mass at 400 °C; however, conventional non-derivative cellulose fibres (*e.g.* birch pulp(P. Li et al., 2017) and softwood dissolving cellulose(Selkälä et al., 2016)), anionic modified cellulose nanofibrils (*e.g.* via succinylation(Selkälä et al., 2016) and TEMPO-oxidation(Fukuzumi et al., 2009)) and lignin-reserved fibres (*e.g.* ground wood pulp and wood nanofibre(Visanko et al., 2017)) had less than 40% residuals. Thus, this thermal behaviour in an inert atmosphere might make CCNF-tannin hybrid material to be utilized as advanced carbonized materials in several applications.(Cao et al., 2016; Ruan, Wang, Lindh, & Strømme, 2018)



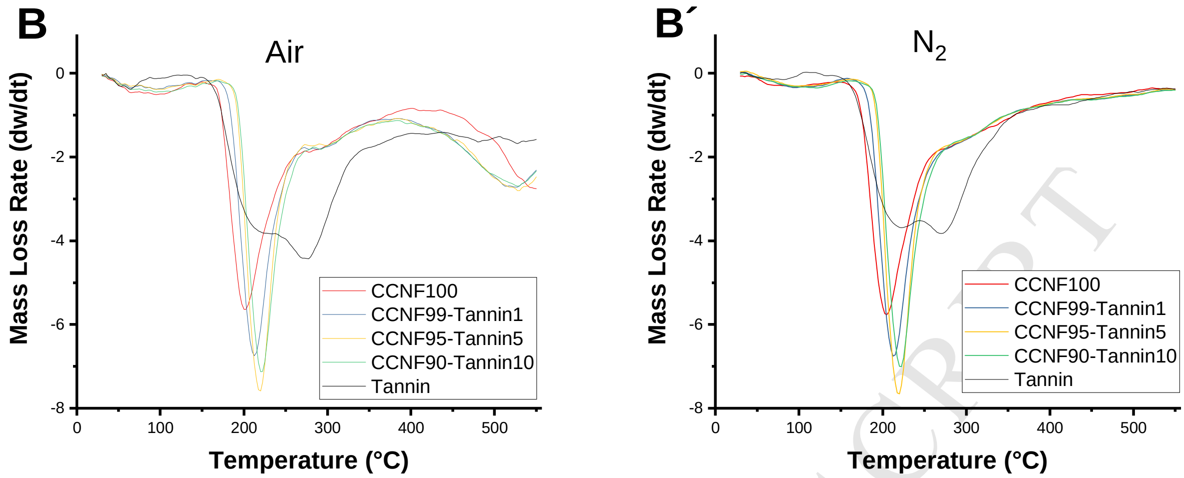
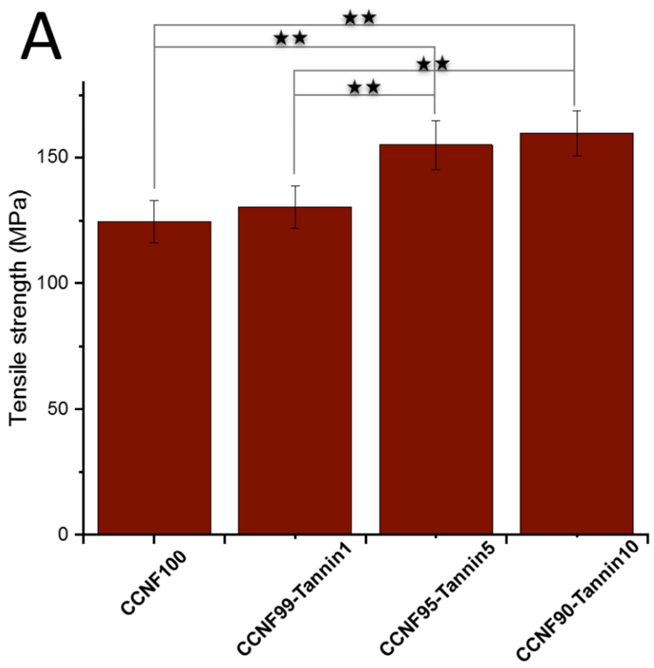


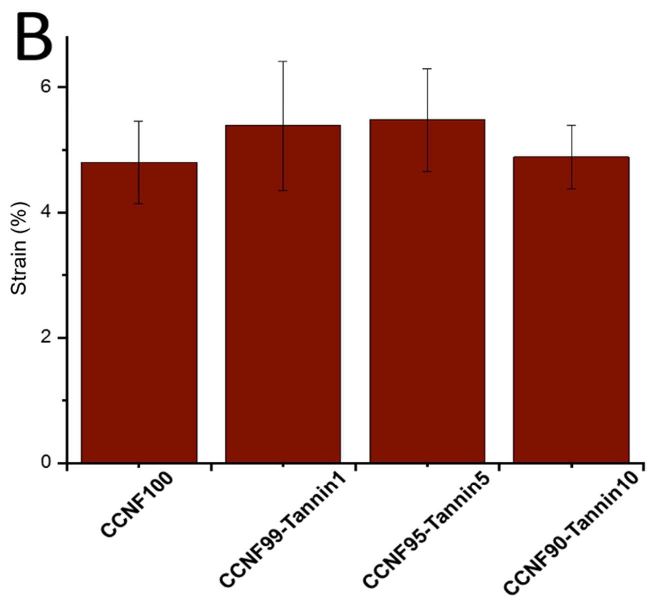
Fig. 6. TGA curve of the hybrid film samples under air (A) and N_2 (A´) atmospheres, respectively, with their first derivate curves of the TGA (DTG) under air (B) and under N_2 (B´).

3.6. Mechanical Characteristics of Nanofibril Films

The mechanical properties of the films are crucial to different applications.(Cordero, Yoon, & Suo, 2007) Hydrogen bonding based cellulose nanopapers have typically adjustable stress−strain curves when extra components are added, for example, as crosslinkers,(Özkan, Borghei, Karakoç, Rojas, & Paltakari, 2018) plasticizers(Azeredo et al., 2010) and grafting agents.(Soeta, Fujisawa, Saito, Berglund, & Isogai, 2015) Unlike many previous works in which the tensile strength and modulus of a nanopaper are reduced linearly with the increase of the additives, such as minerals, Fig. 7 indicates a positive effect on mechanical properties when tannin was introduced to CCNF films.(Liimatainen et al., 2013) Compared to the pure CCNF100 with a tensile strength of 124 ± 8 MPa, CCNF99-Tannin1, CCNF95-Tannin5 and CCNF90-Tannin10 achieved tensile strength of 130 ± 8, 155 ± 9 and 160 ± 9 MPa, respectively. The results from ANOVA analysis confirmed significant a difference when 5% or 10% (w/w) of tannin was added to the CCNF, whereas only a small improvement was obtained with CCNF99-Tannin1. However, all the tannin hybrid films obtained similar strains with that of pristine CCNF100 (4.8% ± 0.6%), which was in line with previously reported sepiolite hybrid nanopaper(Campo et al., n.d.), yet was higher than lignin-containing nanopaper (3.5% at maximum).(Visanko et al., 2017) In addition, Young's modulus of the tannin hybrid nanopapers (average improvement of ca. 20%) was comparable with previously reported chemically modified (*e.g.* TEMPO-oxidized nanopaper with 6−7 GPa)(Isogai, Saito, & Fukuzumi, 2011b) and non-derivatizing nanopaper (*e.g.* DES pre-treated nanopaper with ca. 7 GPa).(P. Li et al., 2017)

Previously, hybrid films made from positively charged CNF and negatively charged muscovite mica (up to 25% by weight) also had improvement in tensile strength and Young's modulus (without noticeable changes in strain) compared to the pure reference film.(Thao T. T. Ho et al., 2012) However, non-charged CNF film was reported to have a ca. 20% decrease in tensile strength after the addition of tannin, because tannin can partially prohibit the hydrogen bonds between the nanofibrils.(Missio et al., 2018) Herein, the mechanical features of CCNF matrices were significantly improved by incorporation of tannin through good electrostatic attraction.


A
★★
★★
★★
★★
Tensile strength (MPa)
0
50
100
150
CCNF100
CCNF99-Tannin1
CCNF95-Tannin5
CCNF90-Tannin10


B
Strain (%)
0
2
4
6
CCNF100
CCNF99-Tannin1
CCNF95-Tannin5
CCNF90-Tannin10

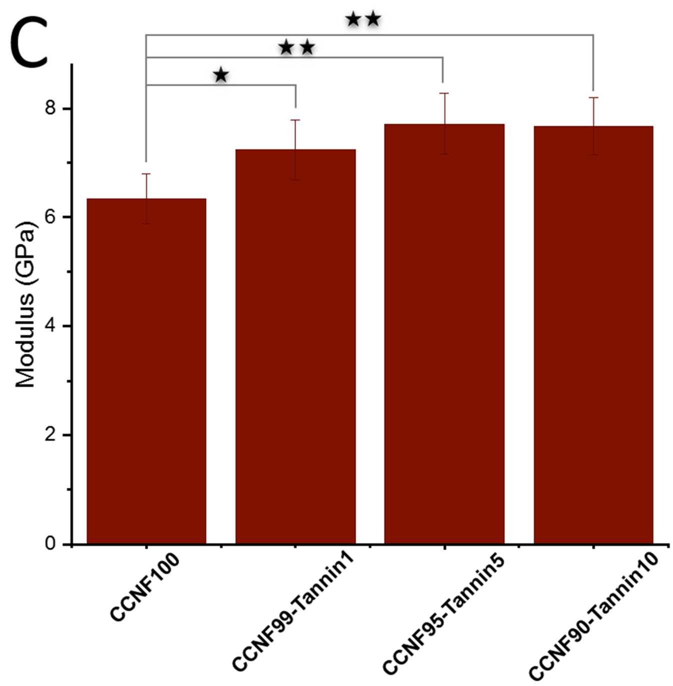


Fig. 7. Tensile strength (A), strain (B) and Young's modulus (C) of hybrid films. Data presented as mean ± standard error and analysed using a one-way ANOVA; (*) $p < 0.05$; (**) $p < 0.01$; otherwise, with no statistical significance.

4. Conclusion

Functional biohybrid nanofilm was fabricated from cationic cellulose nanofibrils and natural anionic Quebracho tannin extract through good electrostatic attraction, no additional force was applied to bind tannin into CCNF. In a water solution, the mixtures of CCNFs and tannin were stable, without the forming of flocculation; in a dry form, the hybrid films were homogeneous, with a gradually shaded colour. All the tested properties of hybrid films were tailorable with the ratio between CCNFs and tannin, and the addition of tannin could enhance the functionalities of CCNF films comprehensively. The results suggest that introduction of 5% (w/w) tannin extract would be sufficient for CCNF films to achieve improved properties in UV-shielding, anti-oxidant characteristics, thermal decomposition and tensile strength. In addition, tannin extract was also able to reduce optical haziness. Hence, inexpensive and nature-based tannin compounds may offer a greener route to the production of bio-based multi-functional materials.

Acknowledgments

We thank Tommi Kokkonen for the thermogravimetric analysis. Sami Saukkos is gratefully acknowledged for his help with TEM images.

AUTHOR INFORMATION

Corresponding Author

*E-mail: Henrikki.Liimatainen@oulu.fi

ORCID: Henrikki Liimatainen 0000-0002-7911-2632

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

References

Abbott, A. P., Capper, G., & Gray, S. (2006). Design of Improved Deep Eutectic Solvents Using Hole Theory. *ChemPhysChem*, *7*(4), 803–806. https://doi.org/10.1002/cphc.200500489

Andrade, R. G., Dalvi, L. T., Silva, J. M. C., Lopes, G. K. B., Alonso, A., & Hermes-Lima, M. (2005). The antioxidant effect of tannic acid on the in vitro copper-mediated formation of free radicals. *Archives of Biochemistry and Biophysics*, *437*(1), 1–9. https://doi.org/10.1016/j.abb.2005.02.016

Anttila, A.-K., Pirttilä, A. M., Häggman, H., Harju, A., Venäläinen, M., Haapala, A., … Julkunen-Tiitto, R. (2013). Condensed conifer tannins as antifungal agents in liquid culture. *Holzforschung*, *67*(7), 825–832. https://doi.org/10.1515/hf-2012-0154

ASTM International. (2006). *Standard Test Method for Haze and Luminous Transmittance of Transparent Plastics* (Vol. D1003-11e1). West Conshohocken, PA. Retrieved from http://file.yizimg.com/175706/2011120519401287.pdf

Aulin, C., Johansson, E., Wågberg, L., & Lindström, T. (2010). Self-Organized Films from Cellulose I Nanofibrils Using the Layer-by-Layer Technique. *Biomacromolecules*, *11*(4), 872–882. https://doi.org/10.1021/bm100075e

Azeredo, H. M. C., Mattoso, L. H. C., Avena-Bustillos, R. J., Filho, G. C., Munford, M. L., Wood, D., & McHugh, T. H. (2010). Nanocellulose Reinforced Chitosan Composite Films as Affected by Nanofiller Loading and Plasticizer Content. *Journal of Food Science*, *75*(1), N1–N7. https://doi.org/10.1111/j.1750-3841.2009.01386.x

Bhat, R., Liong, M.-T., Abdorreza, M. N., & Karim, A. A. (2013). Evaluation of Free Radical Scavenging Activity and Antioxidant Potential of a Few Popular Green Leafy Vegetables of Malaysia. *International Journal of Food Properties*, *16*(6), 1371–1379. https://doi.org/10.1080/10942912.2011.584202

Byun, Y., Kim, Y. T., & Whiteside, S. (2010). Characterization of an antioxidant polylactic acid (PLA) film prepared with α-tocopherol, BHT and polyethylene glycol using film cast extruder. *Journal of Food Engineering*, *100*(2), 239–244. https://doi.org/10.1016/j.jfoodeng.2010.04.005

Campo, M. M. G. del, Darder, M., Aranda, P., Akkari, M., Huttel, Y., Mayoral, A., … Ruiz□Hitzky, E. (n.d.). Functional Hybrid Nanopaper by Assembling Nanofibers of Cellulose and Sepiolite. *Advanced Functional Materials*, *0*(0), 1703048. https://doi.org/10.1002/adfm.201703048

Cao, S., Feng, X., Song, Y., Liu, H., Miao, M., Fang, J., & Shi, L. (2016). In Situ Carbonized Cellulose-Based Hybrid Film as Flexible Paper Anode for Lithium-Ion Batteries. *ACS Applied Materials & Interfaces*, *8*(2), 1073–1079. https://doi.org/10.1021/acsami.5b10648

Carn, F., Guyot, S., Baron, A., Pérez, J., Buhler, E., & Zanchi, D. (2012). Structural Properties of Colloidal Complexes between Condensed Tannins and Polysaccharide Hyaluronan. *Biomacromolecules*, *13*(3), 751–759. https://doi.org/10.1021/bm201674n

Chen, W., Xue, Z., Wang, J., Jiang, J., Zhao, X., & Mu, T. (2018). Investigation on the Thermal Stability of Deep Eutectic Solvents. *Acta Physico-Chimica Sinica*, *34*(8), 904–911. https://doi.org/10.3866/PKU.WHXB201712281

Cordero, N., Yoon, J., & Suo, Z. (2007). Channel cracks in a hermetic coating consisting of organic and inorganic layers. *Applied Physics Letters*, *90*(11), 111910. https://doi.org/10.1063/1.2713134

Dhar, P., Bhardwaj, U., Kumar, A., & Katiyar, V. (2014). Cellulose Nanocrystals: A Potential Nanofiller for Food Packaging Applications. In *Food Additives and Packaging* (Vol. 1162, pp. 197–239). American Chemical Society. Retrieved from http://dx.doi.org/10.1021/bk-2014-1162.ch017

Digge, M. S., Moon, R. S., & Gattani, S. G. (2012). Applications of carbon nanotubes in drug delivery: a review. *International Journal of PharmTech Research*, *4*(2), 839–847.

Eyholzer, C., Bordeanu, N., Lopez-Suevos, F., Rentsch, D., Zimmermann, T., & Oksman, K. (2010). Preparation and characterization of water-redispersible nanofibrillated cellulose in powder form. *Cellulose*, *17*(1), 19–30. https://doi.org/10.1007/s10570-009-9372-3

Feng, X., Zhao, Y., Jiang, Y., Miao, M., Cao, S., & Fang, J. (2017). Use of carbon dots to enhance UV-blocking of transparent nanocellulose films. *Carbohydrate Polymers*, *161*, 253–260. https://doi.org/10.1016/j.carbpol.2017.01.030

Flora, S. J. S., & Pachauri, V. (2010). Chelation in Metal Intoxication. *International Journal of Environmental Research and Public Health*, *7*(7), 2745–2788. https://doi.org/10.3390/ijerph7072745

Fukuzumi, H., Saito, T., Iwata, T., Kumamoto, Y., & Isogai, A. (2009). Transparent and High Gas Barrier Films of Cellulose Nanofibers Prepared by TEMPO-Mediated Oxidation. *Biomacromolecules*, *10*(1), 162–165. https://doi.org/10.1021/bm801065u

Gemili, S., Yemenicioğlu, A., & Altınkaya, S. A. (2010). Development of antioxidant food packaging materials with controlled release properties. *Journal of Food Engineering*, *96*(3), 325–332. https://doi.org/10.1016/j.jfoodeng.2009.08.020

Goh, K., Heising, J. K., Yuan, Y., Karahan, H. E., Wei, L., Zhai, S., … Chen, Y. (2016). Sandwich-Architectured Poly(lactic acid)–Graphene Composite Food Packaging Films. *ACS Applied Materials & Interfaces*, *8*(15), 9994–10004. https://doi.org/10.1021/acsami.6b02498

Gross, R. A., & Kalra, B. (2002). Biodegradable Polymers for the Environment. *Science*, *297*(5582), 803–807. https://doi.org/10.1126/science.297.5582.803

Gülçin, I. (2007). Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa. *Amino Acids*, *32*(3), 431–438. https://doi.org/10.1007/s00726-006-0379-x

Gülçin, İ., Huyut, Z., Elmastaş, M., & Aboul-Enein, H. Y. (2010). Radical scavenging and antioxidant activity of tannic acid. *Arabian Journal of Chemistry*, *3*(1), 43–53. https://doi.org/10.1016/j.arabjc.2009.12.008

Gülçin, I., Mshvildadze, V., Gepdiremen, A., & Elias, R. (2006). Screening of antiradical and antioxidant activity of monodesmosides and crude extract from Leontice smirnowii tuber. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, *13*(5), 343–351. https://doi.org/10.1016/j.phymed.2005.03.009

Halliwell, B., & Gutteridge, J. M. (1990). Role of free radicals and catalytic metal ions in human disease: an overview. *Methods in Enzymology*, *186*, 1–85.

Haslam, E. (1998). *Practical Polyphenolics: From Structure to Molecular Recognition and Physiological Action*. Cambridge University Press.

Henriksson, M., Berglund, L. A., Isaksson, P., Lindström, T., & Nishino, T. (2008). Cellulose Nanopaper Structures of High Toughness. *Biomacromolecules*, *9*(6), 1579–1585. https://doi.org/10.1021/bm800038n

Ho, T. T. T., Zimmermann, T., Hauert, R., & Caseri, W. (2011). Preparation and characterization of cationic nanofibrillated cellulose from etherification and high-shear disintegration processes. *Cellulose*, *18*(6), 1391–1406. https://doi.org/10.1007/s10570-011-9591-2

Ho, Thao T. T., Zimmermann, T., Ohr, S., & Caseri, W. R. (2012). Composites of Cationic Nanofibrillated Cellulose and Layered Silicates: Water Vapor Barrier and Mechanical Properties. *ACS Applied Materials & Interfaces*, *4*(9), 4832–4840. https://doi.org/10.1021/am3011737

Ho, Y. S., McKay, G., Wase, D. A. J., & Forster, C. F. (2000). Study of the Sorption of Divalent Metal Ions on to Peat , Study of the Sorption of Divalent Metal Ions on to Peat. *Adsorption Science & Technology*, *18*(7), 639–650. https://doi.org/10.1260/0263617001493693

Isogai, A., Saito, T., & Fukuzumi, H. (2011a). TEMPO-oxidized cellulose nanofibers. *Nanoscale*, *3*(1), 71–85. https://doi.org/10.1039/C0NR00583E

Isogai, A., Saito, T., & Fukuzumi, H. (2011b). TEMPO-oxidized cellulose nanofibers. *Nanoscale*, *3*(1), 71–85. https://doi.org/10.1039/C0NR00583E

Jadoon, S., Karim, S., Bin Asad, M. H. H., Akram, M. R., Khan, A. K., Malik, A., … Murtaza, G. (2015). Anti-Aging Potential of Phytoextract Loaded-Pharmaceutical Creams for Human Skin Cell Longetivity. *Oxidative Medicine and Cellular Longevity*, *2015*, 709628. https://doi.org/10.1155/2015/709628

Jiang, F., Li, T., Li, Y., Zhang, Y., Gong, A., Dai, J., … Hu, L. (2018). Wood-Based Nanotechnologies toward Sustainability. *Advanced Materials*, *30*(1), 1703453. https://doi.org/10.1002/adma.201703453

Jiang, Y., Song, Y., Miao, M., Cao, S., Feng, X., Fang, J., & Shi, L. (2015). Transparent nanocellulose hybrid films functionalized with ZnO nanostructures for UV-blocking. *Journal of Materials Chemistry C*, *3*(26), 6717–6724. https://doi.org/10.1039/C5TC00812C

Klemm, D., Kramer, F., Moritz, S., Lindström, T., Ankerfors, M., Gray, D., & Dorris, A. (2011). Nanocelluloses: A New Family of Nature-Based Materials. *Angewandte Chemie International Edition*, *50*(24), 5438–5466. https://doi.org/10.1002/anie.201001273

Krepker, M., Shemesh, R., Danin Poleg, Y., Kashi, Y., Vaxman, A., & Segal, E. (2017). Active food packaging films with synergistic antimicrobial activity. *Food Control*, *76*, 117–126. https://doi.org/10.1016/j.foodcont.2017.01.014

Laks, P. E., McKaig, P. A., & Hemingway, R. W. (1988). Flavonoid Biocides: Wood Preservatives Based on Condensed Tannins. *Holzforschung*, *42*(5), 299–306. https://doi.org/10.1515/hfsg.1988.42.5.299

Li, J., Wei, X., Wang, Q., Chen, J., Chang, G., Kong, L., … Liu, Y. (2012). Homogeneous isolation of nanocellulose from sugarcane bagasse by high pressure homogenization. *Carbohydrate Polymers*, *90*(4), 1609–1613. https://doi.org/10.1016/j.carbpol.2012.07.038

Li, P., Sirviö, J. A., Asante, B., & Liimatainen, H. (2018). Recyclable deep eutectic solvent for the production of cationic nanocellulose s. *Carbohydrate Polymers*, *199*, 219–227. https://doi.org/10.1016/j.carbpol.2018.07.024

Li, P., Sirviö, J. A., Haapala, A., & Liimatainen, H. (2017). Cellulose Nanofibrils from Nonderivatizing Urea-Based Deep Eutectic Solvent Pretreatments. *ACS Applied Materials & Interfaces*, *9*(3), 2846–2855. https://doi.org/10.1021/acsami.6b13625

Liimatainen, H., Ezekiel, N., Sliz, R., Ohenoja, K., Sirviö, J. A., Berglund, L., … Niinimäki, J. (2013). High-Strength Nanocellulose–Talc Hybrid Barrier Films. *ACS Applied Materials & Interfaces*, *5*(24), 13412–13418. https://doi.org/10.1021/am4043273

Liimatainen, H., Sirviö, J., Haapala, A., Hormi, O., & Niinimäki, J. (2011). Characterization of highly accessible cellulose microfibers generated by wet stirred media milling. *Carbohydrate Polymers*, *83*(4), 2005–2010. https://doi.org/10.1016/j.carbpol.2010.11.007

Liu, A., Walther, A., Ikkala, O., Belova, L., & Berglund, L. A. (2011). Clay Nanopaper with Tough Cellulose Nanofiber Matrix for Fire Retardancy and Gas Barrier Functions. *Biomacromolecules*, *12*(3), 633–641. https://doi.org/10.1021/bm101296z

Lopes, G. K. B., Schulman, H. M., & Hermes-Lima, M. (1999). Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions1This study is dedicated to the memory of Botany Professor Luiz F.G. Labouriau (1921–1996).1. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1472*(1), 142–152. https://doi.org/10.1016/S0304-4165(99)00117-8

López de Dicastillo, C., Nerín, C., Alfaro, P., Catalá, R., Gavara, R., & Hernández-Muñoz, P. (2011). Development of New Antioxidant Active Packaging Films Based on Ethylene Vinyl Alcohol Copolymer (EVOH) and Green Tea Extract. *Journal of Agricultural and Food Chemistry*, *59*(14), 7832–7840. https://doi.org/10.1021/jf201246g

Luo, J., Zhang, M., Yang, B., Liu, G., Tan, J., Nie, J., & Song, S. (2019). A promising transparent and UV-shielding composite film prepared by aramid nanofibers and nanofibrillated cellulose. *Carbohydrate Polymers*, *203*, 110–118. https://doi.org/10.1016/j.carbpol.2018.09.040

Luo, Y., Zhang, J., Li, X., Liao, C., & Li, X. (2014). The Cellulose Nanofibers for Optoelectronic Conversion and Energy Storage. *Journal of Nanomaterials*, *2014*, 1–13. https://doi.org/10.1155/2014/654512

Majdzadeh-Ardakani, K., & Sadeghi-Ardakani, S. (2010). EXPERIMENTAL INVESTIGATION OF MECHANICAL PROPERTIES OF STARCH/NATURAL RUBBER/CLAY NANOCOMPOSITES. *Digest Journal of Nanomaterials & Biostructures (DJNB)*, *5*(2), 307–316.

Marsich, L., Bonifacio, A., Mandal, S., Krol, S., Beleites, C., & Sergo, V. (2012). Poly-l-lysine-Coated Silver Nanoparticles as Positively Charged Substrates for Surface-Enhanced Raman Scattering. *Langmuir*, *28*(37), 13166–13171. https://doi.org/10.1021/la302383r

Mautner, A., Lucenius, J., Österberg, M., & Bismarck, A. (2017). Multi-layer nanopaper based composites. *Cellulose*, *24*(4), 1759–1773. https://doi.org/10.1007/s10570-017-1220-2

Missio, A. L., Mattos, B. D., Ferreira, D. de F., Magalhães, W. L. E., Bertuol, D. A., Gatto, D. A., … Tondi, G. (2018). Nanocellulose-tannin films: From trees to sustainable active packaging. *Journal of Cleaner Production*, *184*, 143–151. https://doi.org/10.1016/j.jclepro.2018.02.205

Mohieldin, S. D., Zainudin, E. S., Paridah, M. T., & Ainun, Z. M. (2011). Nanotechnology in Pulp and Paper Industries: A Review. *Key Engineering Materials*, *471–472*, 251–256. https://doi.org/10.4028/www.scientific.net/KEM.471-472.251

Northey, R. A., Glasser, W. G., & Schultz, T. P. (Eds.). (2000). *Lignin: historical, biological, and materials perspectives*. Washington, DC : [Cary, NC]: American Chemical Society ; Distributed by Oxford University Press.

Noshirvani, N., Ghanbarzadeh, B., Rezaei Mokarram, R., & Hashemi, M. (2017). Novel active packaging based on carboxymethyl cellulose-chitosan-ZnO NPs nanocomposite for increasing the shelf life of bread. *Food Packaging and Shelf Life*, *11*, 106–114. https://doi.org/10.1016/j.fpsl.2017.01.010

Oksman, K., Mathew, A. P., Bondeson, D., & Kvien, I. (2006). Manufacturing process of cellulose whiskers/polylactic acid nanocomposites. *Composites Science and Technology*, *66*(15), 2776–2784. https://doi.org/10.1016/j.compscitech.2006.03.002

Okuda, T., & Ito, H. (2011). Tannins of Constant Structure in Medicinal and Food Plants—Hydrolyzable Tannins and Polyphenols Related to Tannins. *Molecules*, *16*(3), 2191–2217. https://doi.org/10.3390/molecules16032191

Olszewska, A., Eronen, P., Johansson, L.-S., Malho, J.-M., Ankerfors, M., Lindström, T., … Österberg, M. (2011). The behaviour of cationic NanoFibrillar Cellulose in aqueous media. *Cellulose*, *18*(5), 1213–1226. https://doi.org/10.1007/s10570-011-9577-0

Özkan, M., Borghei, M., Karakoç, A., Rojas, O. J., & Paltakari, J. (2018). Films based on crosslinked TEMPO-oxidized cellulose and predictive analysis via machine learning. *Scientific Reports*, *8*(1), 4748. https://doi.org/10.1038/s41598-018-23114-x

Pizzi, A. (2008). Chapter 8 - Tannins: Major Sources, Properties and Applications. In M. N. Belgacem & A. Gandini (Eds.), *Monomers, Polymers and Composites from Renewable Resources* (pp. 179–199). Amsterdam: Elsevier. https://doi.org/10.1016/B978-0-08-045316-3.00008-9

Pranantyo, D., Xu, L. Q., Neoh, K.-G., Kang, E.-T., Ng, Y. X., & Teo, S. L.-M. (2015). Tea Stains-Inspired Initiator Primer for Surface Grafting of Antifouling and Antimicrobial Polymer Brush Coatings. *Biomacromolecules*, *16*(3), 723–732. https://doi.org/10.1021/bm501623c

Rochman, C. M., Browne, M. A., Halpern, B. S., Hentschel, B. T., Hoh, E., Karapanagioti, H. K., … Thompson, R. C. (2013). Policy: Classify plastic waste as hazardous. *Nature*, *494*(7436), 169–171. https://doi.org/10.1038/494169a

Ruan, C.-Q., Wang, Z., Lindh, J., & Strømme, M. (2018). Carbonized cellulose beads for efficient capacitive energy storage. *Cellulose*, *25*(6), 3545–3556. https://doi.org/10.1007/s10570-018-1811-6

Sadeghifar, H., Venditti, R., Jur, J., Gorga, R. E., & Pawlak, J. J. (2017). Cellulose-Lignin Biodegradable and Flexible UV Protection Film. *ACS Sustainable Chemistry & Engineering*, *5*(1), 625–631. https://doi.org/10.1021/acssuschemeng.6b02003

Saito, T., Nishiyama, Y., Putaux, J.-L., Vignon, M., & Isogai, A. (2006). Homogeneous Suspensions of Individualized Microfibrils from TEMPO-Catalyzed Oxidation of Native Cellulose. *Biomacromolecules*, *7*(6), 1687–1691. https://doi.org/10.1021/bm060154s

Sehaqui, H., Liu, A., Zhou, Q., & Berglund, L. A. (2010). Fast Preparation Procedure for Large, Flat Cellulose and Cellulose/Inorganic Nanopaper Structures. *Biomacromolecules*, *11*(9), 2195–2198. https://doi.org/10.1021/bm100490s

Selkälä, T., Sirviö, J. A., Lorite, G. S., & Liimatainen, H. (2016). Anionically Stabilized Cellulose Nanofibrils through Succinylation Pretreatment in Urea-Lithium Chloride Deep Eutectic Solvent. *ChemSusChem*. https://doi.org/10.1002/cssc.201600903

Singh, N., & Rajini, P. S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry*, *85*(4), 611–616. https://doi.org/10.1016/j.foodchem.2003.07.003

Sirviö, J. A., Anttila, A.-K., Pirttilä, A. M., Liimatainen, H., Kilpeläinen, I., Niinimäki, J., & Hormi, O. (2014). Cationic wood cellulose films with high strength and bacterial anti-adhesive properties. *Cellulose*, *21*(5), 3573–3583. https://doi.org/10.1007/s10570-014-0351-y

Sirviö, J. A., Visanko, M., & Liimatainen, H. (2015). Deep eutectic solvent system based on choline chloride-urea as a pre-treatment for nanofibrillation of wood cellulose. *Green Chem.*, *17*(6), 3401–3406. https://doi.org/10.1039/C5GC00398A

Sirviö, J. A., Visanko, M., & Liimatainen, H. (2016). Acidic Deep Eutectic Solvents As Hydrolytic Media for Cellulose Nanocrystal Production. *Biomacromolecules*, *17*(9), 3025–3032. https://doi.org/10.1021/acs.biomac.6b00910

Sirvio, J., Hyvakko, U., Liimatainen, H., Niinimaki, J., & Hormi, O. (2011). Periodate oxidation of cellulose at elevated temperatures using metal salts as cellulose activators. *Carbohydrate Polymers*, *83*(3), 1293–1297.

Smith, E. L., Abbott, A. P., & Ryder, K. S. (2014). Deep Eutectic Solvents (DESs) and Their Applications. *Chemical Reviews*, *114*(21), 11060–11082. https://doi.org/10.1021/cr300162p

Soeta, H., Fujisawa, S., Saito, T., Berglund, L., & Isogai, A. (2015). Low-Birefringent and Highly Tough Nanocellulose-Reinforced Cellulose Triacetate. *ACS Applied Materials & Interfaces*, *7*(20), 11041–11046. https://doi.org/10.1021/acsami.5b02863

Stell, J. R., Paul, D. R., & Barlow, J. W. (2004). Mechanical properties of oriented polyethylene/polystyrene blends. *Polymer Engineering & Science*, *16*(7), 496–506. https://doi.org/10.1002/pen.760160706

Thevenon, M.-F., Tondi, G., & Pizzi, A. (2009). High performance tannin resin-boron wood preservatives for outdoor end-uses. *European Journal of Wood and Wood Products*, *67*(1), 89–93. https://doi.org/10.1007/s00107-008-0290-0

Tondi, G., Schnabel, T., Wieland, S., & Petutschnigg, A. (2013). Surface properties of tannin treated wood during natural and artificial weathering. *International Wood Products Journal*, *4*(3), 150–157. https://doi.org/10.1179/2042645313Y.0000000047

Tondi, G., Thevenon, M. F., Mies, B., Standfest, G., Petutschnigg, A., & Wieland, S. (2013). Impregnation of Scots pine and beech with tannin solutions: effect of viscosity and wood anatomy in wood infiltration. *Wood Science and Technology*, *47*(3), 615–626. https://doi.org/10.1007/s00226-012-0524-5

Tondi, Gianluca, Palanti, S., Wieland, S., Thevenon, M.-F., Petutschnigg, A., & Schnabel, T. (2012). DURABILITY OF TANNIN-BORON-TREATED TIMBER. *BioResources*, *7*(4), 5138–5151. https://doi.org/10.15376/biores.7.4.5138-5151

Visanko, M., Sirviö, J. A., Piltonen, P., Sliz, R., Liimatainen, H., & Illikainen, M. (2017). Mechanical fabrication of high-strength and redispersible wood nanofibers from unbleached groundwood pulp. *Cellulose*, *24*(10), 4173–4187. https://doi.org/10.1007/s10570-017-1406-7

Wågberg, L., Decher, G., Norgren, M., Lindström, T., Ankerfors, M., & Axnäs, K. (2008). The Build-Up of Polyelectrolyte Multilayers of Microfibrillated Cellulose and Cationic Polyelectrolytes. *Langmuir*, *24*(3), 784–795. https://doi.org/10.1021/la702481v

Weckman, N. E., Olsson, A. L. J., & Tufenkji, N. (2014). Evaluating the Binding of Selected Biomolecules to Cranberry Derived Proanthocyanidins Using the Quartz Crystal Microbalance. *Biomacromolecules*, *15*(4), 1375–1381. https://doi.org/10.1021/bm401909c

Xu, Q., Wang, Y., Jin, L., Wang, Y., & Qin, M. (2017). Adsorption of Cu (II), Pb (II) and Cr (VI) from aqueous solutions using black wattle tannin-immobilized nanocellulose. *Journal of Hazardous Materials*, *339*, 91–99. https://doi.org/10.1016/j.jhazmat.2017.06.005

Zhu, H., Fang, Z., Preston, C., Li, Y., & Hu, L. (2014). Transparent paper: fabrications, properties, and device applications. *Energy Environ. Sci.*, *7*(1), 269–287. https://doi.org/10.1039/C3EE43024C

Zhu, H., Parvinian, S., Preston, C., Vaaland, O., Ruan, Z., & Hu, L. (2013). Transparent nanopaper with tailored optical properties. *Nanoscale*, *5*(9), 3787. https://doi.org/10.1039/c3nr00520h

Zhu, H., Xiao, Z., Liu, D., Li, Y., Weadock, N. J., Fang, Z., … Hu, L. (2013). Biodegradable transparent substrates for flexible organic-light-emitting diodes. *Energy & Environmental Science*, *6*(7), 2105. https://doi.org/10.1039/c3ee40492g

Zou, Y., Zhong, J., Pan, R., Wan, Z., Guo, J., Wang, J., … Yang, X. (2017). Zein/tannic acid complex nanoparticles-stabilised emulsion as a novel delivery system for controlled release of curcumin. *International Journal of Food Science & Technology*, *52*(5), 1221–1228. https://doi.org/10.1111/ijfs.13380

Anti-oxidative and UV-absorbing Biohybrid Film of Cellulose Nanofibrils and Tannin Extract

Panpan Li,[§] *Juho Antti Sirviö,*[§] *Antti Haapala,*[‡] *Alexey Khakalo,*[†] *and Henrikki Liimatainen*[§*]

[§]Fibre and Particle Engineering Research unit, University of Oulu, P. O. Box 4300, FI-90014 Oulu, Finland

[‡]Wood Materials Science, University of Eastern Finland, P. O. Box 111, FI-80101 Joensuu, Finland

[†] VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044 VTT, Finland

*Corresponding Author (E-mail: Henrikki.Liimatainen@oulu.fi)

Highlights:

- Biodegradable films were made from natural tannin extract and cellulose
- Functionalities including anti-oxidative and UV-absorbing were applied to pristine film
- Mechanical strength and thermal stability of biohybrid film were enhanced by tannin extract
- Electrostatic attractions and self-assembly lead to formation of biohybrid film