Universitat de Girona

ENDO-β-1,4-GLUCANASA PARA LA FABRICACIÓN DE MICRO/NANOCELULOSA: PROPIEDADES Y APLICACIONES

Joaquim Agustí Tarrés Farrés

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Universitat de Girona

Endo- β -1,4-glucanasa para la fabricación de micro/nanocelulosa: propiedades y aplicaciones

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Tesis Doctoral

Joaquim Agustí Tarrés Farrés
2017

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Tesis Doctoral

Joaquim Agustí Tarrés Farrés
2017

PROGRAMA DE DOCTORADO EN TECNOLOGÍA

Dirigida por: Dr. Pere Mutjé Pujol y Dr. Marc Delgado Aguilar

Tutor: Dr. Pere Mutjé Pujol

Memoria presentada para optar al título de DOCTOR POR LA UNIVERSITAT DE GIRONA

El Dr. Pere Mutjé Pujol, catedrático del Departamento de Ingeniería Química, Agraria y Tecnología Alimentaria de la Universitat de Girona, y el Dr. Marc Delgado Aguilar, profesor asociado del Departamento de Ingeniería Química, Agraria y Tecnología Alimentaria de la Universitat de Girona,

DECLARAN:

Que el trabajo titulado “Endo- β -1,4-glucanasa para la fabricación de micro/nanocelulosa: propiedades y aplicaciones”, que presenta el Sr. Joaquim Agustí Tarrés Farrés para la obtención del título de doctor ha sido realizado bajo nuestra dirección.

Y para que así conste y a los efectos oportunos, firmamos el presente documento

Dr. Pere Mutjé Pujol

P. Mutjé

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Girona 15 de mayo de 2017

"Restlessness is discontent and discontent is the first necessity of progress.
Show me a thoroughly satisfied man and I will show you a failure."

Thomas A. Edison

Agraïments

L'elaboració d'una Tesi Doctoral, no es només el fruit del treball personal sinó que és necessari l'esforç col· lectiu. Des de l'any 2014, encara sent estudiant d'Enginyeria Química de la Universitat de Girona, he tingut la oportunitat de treballar amb el grup d'investigació LEPAMAP. El treball en equip i el sentit col· lectiu d'aquest han fet possible el desenvolupament de la present Tesi Doctoral.

En primer lloc, voldria agrair al meu director de tesi el Dr. Marc Delgado-Aguilar la seva implicació en el desenvolupament de la mateixa. Durant tot aquest temps el seu suport i dedicació m'han ajudat a créixer acadèmica i professionalment. Només vull expressar la meva eterna gratitud per el teu esforç, comprensió, consell i amistat. Ha estat i espero que segueixi sent un plaer i un honor treballar colze a colze amb tu.

En segon lloc, també voldria expressar el meu agraïment al meu tutor i director de tesi el Dr. Pere Mutjé. Mitjançant la seva professionalitat, dedicació, entusiasme i obstinació per aconseguir els objectius, ha estat i és un exemple per mi. Només puc expressar paraules de gratitud per ser el meu mentor, brindar-me la oportunitat de treballar amb ell i recolzar-me en tots els moments difícils, tant professionals com personals.

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ARTÍCULOS QUE COMPONEN LA PRESENTE TESIS

La presente tesis está compuesta por los siguientes artículos:

1. Tarrés, Q., Saguer, E., Pèlach, M.A., Alcalà, M., Delgado-Aguilar, M. and Mutjé, P. (2016). "The feasibility of incorporating cellulose micro/nanofibers in papermaking processes: the relevance of enzymatic hydrolysis" *Cellulose*, 23, 1433-1445. **Artículo publicado**.

   Factor de impacto 2015: 3.195, posición 1 de 21 en MATERIAL SCIENCE, PAPER & WOOD, 1er Cuartil.

2. Tarrés, Q., Oliver-Ortega, H., Llop, M., Pèlach, M.A., Delgado-Aguilar, M. and Mutjé, P. (2016). "Effective and simple methodology to produce nanocellulose-based aerogels for selective oil removal" *Cellulose*, 23, 3077-3088. **Artículo publicado**.

   Factor de impacto 2015: 3.195, posición 1 de 21 en MATERIAL SCIENCE, PAPER & WOOD, 1er Cuartil.

3. Tarrés, Q., Delgado-Aguilar, M., Pèlach, M.A., González, I., Boufi, S. and Mutjé, P. (2016). "Remarkable increase of paper strength by combining enzymatic cellulose nanofibers in bulk and TEMPO-oxidized nanofibers as coating" *Cellulose*, 23, 3939-3950. **Artículo publicado**.

   Factor de impacto 2015: 3.195, posición 1 de 21 en MATERIAL SCIENCE, PAPER & WOOD, 1er Cuartil.

4. Tarrés, Q., Boufi, S. and Delgado-Aguilar, M. "Enzymatically hydrolyzed and TEMPO-1 oxidized cellulose nanofibers for the production of nanopapers: morphological, optical, thermal and mechanical properties" *Cellulose*. **Artículo en revisión.**

   Factor de impacto 2015: 3.195, posición 1 de 21 en MATERIAL SCIENCE, PAPER & WOOD, 1er Cuartil.

5. Tarrés, Q., Deltell, A., Espinach, F.X., Pèlach, M.A., Delgado-Aguilar, M. and Mutjé, P. (2017). "Magnetic bionanocomposites from cellulose nanofibers: Fast, simple and effective production method" *International Journal of Biological Macromolecules*, 99, 29-36. **Artículo publicado**.

   Factor de impacto 2015: 3.138, posición 26 de 152 en CHEMISTRY, APPLIED, 1er Cuartil.

6. Boufi, S., González, I., Delgado-Aguilar, M., Tarrés, Q., Pèlach, M.A. and Mutjé, P. (2016). “Nanofibrillated cellulose as an additive in papermaking process: A review” *Carbohydrate Polymers*, 154, 151-166. **Artículo publicado.**

   Factor de impacto 2015: 4.219, posición 5 de 72 en CHEMISTRY, APPLIED, 1er Cuartil.

7. Tarrés, Q., Pèlach, M.A., Alcalà, M. and Delgado-Aguilar, M. “Carboard boxes as raw material for high-performance papers through the implementation of alternative technologies: more than closing the loop” *Industrial and Engineering Chemistry*. **Artículo en revisión.**

   Factor de impacto 2015: 4.179, posición 14 de 135 en ENGINEERING, CHEMICAL, 1er Cuartil.

El autor de esta tesis contribuyó a los artículos anexos de la siguiente manera:

Artículo 1

Desarrolló el método para la producción de las nanofibras de celulosa vía hidrólisis enzimática. Diseñó los experimentos y evaluó los resultados. Escribió el trabajo junto con los otros autores

Artículo 2

Realizó los experimentos de producción de aerogeles capaces de eliminar aceite presente en una suspensión acuosa y evaluó los resultados. Ayudo a la redacción del artículo.

Artículo 3

Realizó los experimentos de aplicación de nanofibras de celulosa tanto en masa como en superficie y evaluó los resultados. Participó en la redacción del trabajo.

Artículo 4

Realizó los experimentos de producción de nanopapeles y evaluó los resultados. Participó en la redacción del trabajo.

Artículo 5

Diseñó los experimentos juntamente con los otros autores y evaluó los resultados. Escribió el trabajo junto con los otros autores.

Artículo 6

Realizó el estudio bibliográfico y la redacción del artículo correspondiente a la producción de nanofibras de celulosa mediante pretratamientos de hidrólisis enzimática.

Artículo 7

Realizó los experimentos de biorefinado de la suspensión papelera y la posterior addición de nanofibras de celulosa obtenidas mediante pretratamientos de hidrólisis enzimática y evaluó los resultados. Escribió el artículo.

Otros artículos relevantes no incluidos en esta tesis:

Tarrés, Q., Espinosa, E., Domínguez-Robles, J., Rodríguez, A., Mutjé, P. and Delgado-Aguilar, M. (2017). “The suitability of banana leaf residue as raw material for the production of high lignin content micro/nano fibers: From residue to value-added products” *Industrial Crops and Products*, 99, 27-33.

Tarrés, Q., Ehman, N.V., Vallejos, M.E., Area, M.C., Delgado-Aguilar, M. and Mutjé, P. (2017). “Lignocellulosic nanofibers from triticale Straw: The influence of hemicelluloses and lignin in their production and properties” *Carbohydrate Polymers*, 163, 20-27.

Delgado-Aguilar, M., González, I., Tarrés, Q., Pèlach, M.A., Alcalà, M. and Mutjé, P. (2016). “The key role of lignin in the production of low-cost lignocellulosic nanofibres for papermaking applications” *Industrial Crops and Products*, 86, 295-300.

ABREVIATURAS

AKD:	Dímero de alquilcetena
BNC:	Nanocelulosa bacteriana
C:	Celulosa
CI:	Cohesión interna del papel (J/m^2)
CNC:	Nanocristales de celulosa
CNF:	Nanofibras de celulosa
DP:	Grado de polimerización
E_{CD}:	Módulo de Young en la dirección perpendicular a la de la máquina de papel (GPa)
E_{MD}:	Módulo de Young en la dirección de la máquina de papel (GPa)
G:	Gramaje del papel (g/m^2)
H:	Hemicelulosa
L:	Lignina
LEPAMAP:	Laboratori d’Enginyeria Paperera i Materials Polímers
LR_{CD}:	Longitud de ruptura en la dirección perpendicular a la de la máquina de papel (m)
LR_{MD}:	Longitud de ruptura en la dirección de la máquina de papel (m)
l_w^f:	Longitud de fibra ponderada en longitud (µm)
M:	Lámina media de las fibras celulósicas
P:	Pared primaria de las fibras celulósicas
PA:	Porosidad al aire Gurley (s)
°SR:	Grado de drenaje Schopper Riegler
TEMPO:	2,2,6,6-Tetramethylpiperidine 1-oxyl
W:	Lumen de las fibras celulósicas
σ_t^{NP}:	Resistencia a tracción del nanopapel (MPa)

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RESUM

En els darrers anys, entre la comunitat científica i tecnològica s'ha despertat un gran interès en la producció, caracterització i utilització de nanofibres de cel·lulosa. Aquesta especial atenció es deu, principalment, al seu caràcter biodegradable, el seu origen renovable i la versatilitat que presenten, fent-les aptes per ser utilitzades en multitud d'aplicacions. L'esclat d'aquesta temàtica de recerca va tenir lloc aproximadament deu anys enrere, doncs només cal observar el creixement exponencial de publicacions científiques i patents des d'aleshores.

Una gran quantitat de documents fan referència a metodologies de producció i possibles aplicacions. No obstant això, la majoria de tècniques de pretractament desenvolupades per a la producció de nanofibres de cel·lulosa s'han centrat en processos químics, tals com oxidació catalitzada per TEMPO, la carboximetil·lació o la carboximetil·lació amb clorit-periodat, carbonat sòdic, sulfonació o hidròlisi àcida suau. Tanmateix, aquests pretractaments químics representen un elevat consum de productes químics no respectuosos amb el medi ambient i, addicionalment, un cost de producció inviable quan les nanofibres de cel·lulosa van destinades a sectors tecnològics madurs com pot ser la industria paperera.

D'altra banda, altres metodologies de producció de nanofibres de cel·lulosa com el pretractament per hidròlisi enzimàtica o pretractaments mecànics es presenten com a alternatives amb un menor cost econòmic e impacte ambiental. Tot i això, a la bibliografia també es demostra que les nanofibres de cel·lulosa obtingudes mitjançant aquesta tipologia de pretractaments presenten característiques molt inferiors. En aquest sentit, la present tesi explora la optimització del pretractament d'hidròlisi enzimàtica per a la obtenció de nanofibres de cel·lulosa amb elevades prestacions. La optimització d'aquest procés s'ha realitzat mitjançant l'estudi de l'efecte de la temperatura de treball, concentració de la suspensió, pH, concentració d'enzim i temps d'hidròlisi.

L'aplicació d'aquestes nanofibres de cel·lulosa enzimàtiques, un cop optimitzat el pretractament d'hidròlisi, sobre suspensions papereres ha estat demostrat com a altament efectiu, arribant a obtenir increments en les propietats mecàniques del mateix ordre que produeixen les nanofibres de cel·lulosa obtingudes per oxidació TEMPO. D'altra banda, també s'ha estudiat l'aplicació d'aquestes en combinació amb altres mètodes per a l'increment de les propietats mecàniques del paper com són el refí mecànic i el biorefí, per tal d'obtenir papers amb elevades prestacions. També en aquest sentit, s'ha estudiat la seva compatibilitat quan són utilitzades en massa amb una posterior aplicació superficial de nanofibres de cel·lulosa TEMPO. Una de les principals avantatges que presenten aquests resultats són la conservació en certa mesura de les propietats originals de les fibres, la possibilitat de reduir el gramatge dels papers i/o l'addició d'una major quantitat de cargues minerals. Totes aquestes mesures, permetrien allargar la vida útil de les fibres i l'augment del nombre de cicles de reciclat al que aquestes poden ser sotmeses.

Tanmateix, en la present tesi també s'ha avaluat l'ús d'aquestes nanofibres de cel·lulosa en altres camps diferents al paperer. Els experiments duts a terme han demostrat que es possible la seva utilització en la fabricació d'aerogels amb una superfície especifica suficient com per generar una gran capacitat d'absorció i per altre banda nanopapers amb unes elevades propietats mecàniques i òptiques. Tanmateix, aquests nanopapers es van modificar amb nanopartícules de magnetita, donant lloc a membranes amb potencial ús en altaveus.

De manera general, la present tesi pretén demostrar que les nanofibres de cel·lulosa obtingudes mitjançant un pretractament d'hidròlisi enzimàtica poden ser produïdes de forma efectiva, de manera que pugin ser aplicades en una gran varietat de camps científics i tecnològics. La seva gran versatilitat, disponibilitat, baix cost i baix impacte mediambiental, justifiquen que en el futur es continuï amb la seva investigació sobre noves aplicacions.

RESUMEN

En los últimos años, entre la comunidad científica y tecnológica se ha despertado un gran interés en la producción, caracterización y utilización de las nanofibras de celulosa. Esta especial atención se debe, principalmente, a su carácter biodegradable, su origen renovable y la versatilidad que presentan, haciéndolas aptas para ser utilizadas en multitud de aplicaciones. El estallido de esta temática de recerca tuvo lugar aproximadamente diez años atrás, tal y como se puede observar mediante el crecimiento exponencial de publicaciones científicas y patentes des de ese momento.

Una gran cantidad de documentos hacen referencia a metodologías de producción y posibles aplicaciones. Sin embargo, la mayoría de técnicas de pretratamiento desarrolladas para la producción de nanofibras de celulosa se han centrado en procesos químicos, tales como la oxidación catalizada por TEMPO, la carboximetilación o la carboximetilación con clorito-periodato, carbonato sódico, sulfonación o hidrólisis ácida suave. Así mismo, estos pretratamientos químicos representan un elevado consumo de productos químicos no respetuosos con el medio ambiente y, adicionalmente, un coste de producción inviable cuando las nanofibras de celulosa van destinadas a sectores tecnológicos maduros como puede ser la industria papelera.

Por otra parte, otras metodologías de producción de nanofibras de celulosa como el pretratamiento por hidrólisis enzimática o pretratamientos mecánicos se presentan como a alternativas con un menor coste económico e impacto ambiental. Aun así, en la bibliografía también se demuestra que las nanofibras de celulosa obtenidas mediante esta tipología de pretratamientos presentan características muy inferiores. En este sentido, la presente tesis explora la optimización del pretratamiento de hidrólisis enzimática para la obtención de nanofibras de celulosa con elevadas prestaciones. La optimización de este proceso se ha realizado mediante el estudio del efecto de la temperatura de trabajo, concentración de la suspensión, pH, concentración de enzima y tiempo de hidrolisis.

La aplicación de estas nanofibras de celulosa enzimáticas, una vez optimizado el pretratamiento de hidrólisis, sobre suspensiones papeleras ha sido demostrado como a altamente efectivo, alanzando incrementos en las propiedades mecánicas del mismo orden que el que producen las nanofibras de celulosa obtenidas mediante oxidación TEMPO. Por otra parte, también se ha estudiado la aplicación de estas en combinación con otros métodos para al incremento de las propiedades mecánicas del papel como son el refino mecánico y el biorefino, con tal de obtener papeles con elevadas prestaciones. También en este sentido, se ha estudiado su compatibilidad cuando son utilizadas en masa con una posterior aplicación superficial de nanofibras de celulosa TEMPO. Una de las principales ventajas que presentan estos resultados son la conservación en cierta medida de las propiedades originales de las fibras, la posibilidad de reducir el gramaje de los papeles y/o la adición de una mayor cantidad de cargas minerales. Todas estas medidas, permitirían alargar la vida útil de las fibras y el aumento del número de ciclos de reciclado al que estas pueden ser sometidas.

Así mismo, en la presente tesis también se ha evaluado el uso de estas nanofibras de celulosa en otros campos diferentes al papelero. Los experimentos realizados han demostrado que es posible su utilización en la fabricación de aerogeles con una superficie específica suficiente como para generar una gran capacidad de absorción y por otra parte nanopapeles con unas elevadas propiedades mecánicas y ópticas. Estos nanopapeles fueron modificados con nanopartículas de magnetita, dando lugar a membranas con un potencial uso en altavoces.

De forma general, la presente tesis pretende demostrar que las nanofibras de celulosa obtenidas mediante un pretratamiento de hidrólisis enzimática pueden ser producidas de forma efectiva, de modo que puedan ser aplicadas en una gran variedad de campos científicos y tecnológicos. Su gran versatilidad, disponibilidad, bajo coste y bajo impacto medioambiental, justifican que en el futuro se continúe con su investigación sobre nuevas aplicaciones.

ABSTRACT

In the recent years, significant interest on the production, characterization and application of cellulose nanofibers has brought out among scientific and technic authorities. This special attention mainly comes from their biodegradability, renewability and versatility, making them able to be used for several applications and fields. This topic outbreak took place about ten years ago, fact that is confirmed by the exponential increase on scientific publications and patents since then.

Most of this scientific documents deal with production methods and potential applications. However, most of those methods are mainly focused in chemical processes, such as TEMPO-mediated oxidation, carboxymethylation, sodium carbonate, sulfonation or soft acid hydrolysis. In addition, such treatments imply high non-environmentally friendly chemicals consumption and, moreover, the production cost becomes unaffordable when cellulose nanofibers are intended to be used in mature technological sectors such is the case of papermaking industry.

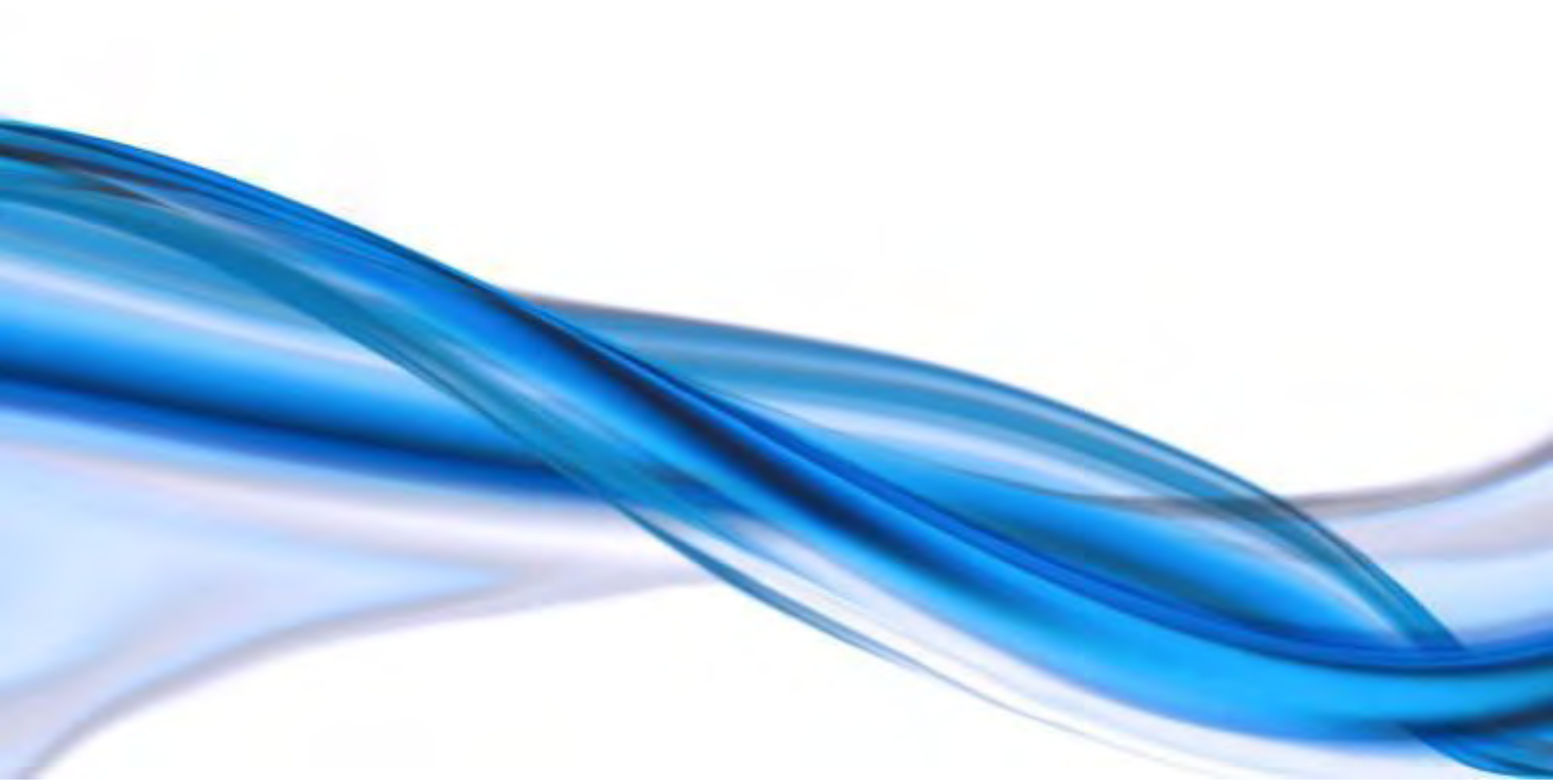
On the other hand, other methodologies for the production of cellulose nanofibers such enzymatic hydrolysis or mechanical treatment have appeared as strong alternative in terms of production costs and environmental impact. Nonetheless, these nanofibers have been also reported to have lower properties. In this sense, this thesis explores the enzymatic hydrolysis process optimization for the obtaining of cellulose nanofibers with higher properties and characteristics. This optimization has been carried out by means of operating temperature, pulp consistency, pH, enzyme dosage and hydrolysis time.

Once the process was optimized, the incorporation of the obtained cellulose nanofibers to pulp slurries demonstrated their high efficiency, thus the obtained properties of paper where of the same magnitude than when TEMPO-oxidized cellulose nanofibers were used, even in combination with mechanical and/or enzymatic refining. Besides being used in bulk, their combination with coated TEMPO-oxidized cellulose nanofibers was also studied, leading to papers even stronger. In fact, these strategies could allow decreasing basis weight of paper products or adding higher mineral filler content, with the subsequent resources savings in paper production. In addition, since such treatments do not affect the fiber structure, the lifespan of paper could be significantly increased.

A part from using cellulose nanofibers as paper additive, this thesis also explores their utilization in other sectors and fields. Exemplarily, they were used for the production of high-absorbent aerogels for oil and water separation, high-performance nanocellulose-based membranes (nanopapers) and functional magnetic membranes for their use for loudspeakers production.

In a nutshell, the present thesis aims to demonstrate that enzymatically hydrolyzed cellulose nanofibers can be successfully produced and used in several sectors. Their versatility, availability, low cost and low environmental impact justify the ongoing research in this field.

Introducción y Estado del Arte



1. Introducción y Estado del Arte

El estudio de los comúnmente llamados biomateriales y nanomateriales con altas prestaciones ha adquirido durante los últimos años un gran interés por parte de la comunidad científica y tecnológica. Durante los últimos treinta años, los avances en biotecnología, nanotecnología, tecnología de los materiales y la tecnología de la información han entrado en un proceso de aceleración que nos ha brindado la oportunidad de alcanzar cambios radicales en todas las dimensiones de nuestra vida diaria (Silbertglitt et al., 2010). En este sentido, se pueden clasificar los biomateriales y nanomateriales con altas prestaciones según el tipo de aplicación al que estos van dirigidos: aplicaciones comunes o aplicaciones novedosas.

Como aplicaciones comunes, se entiende aquellas aplicaciones derivadas de sectores tecnológicos maduros como pueden ser la fabricación de papel o la fabricación de materiales plásticos. La aplicación de biomateriales y/o nanomateriales en este tipo de aplicaciones se sustenta en la obtención de materiales más sostenibles con el medio ambiente (Theng et al., 2015), la menor utilización de materias primeras (Vallejos et al., 2016), la substitución de materiales de origen mineral (Jimenez and Jana, 2009; Sanchez-Garcia, Lagaron and Hoa, 2010) o la producción de materiales más ligeros (Krueger, 2010). Se puede decir por lo tanto, que en el caso del uso de bio/nano materiales en aplicaciones comunes, el objetivo principal es la obtención de materiales con las mismas o mejores prestaciones físico-mecánicas que los materiales presentes actuales en el mercado pero con una mayor sostenibilidad medioambiental.

Por otro lado, la utilización de estos bio/nano materiales en aplicaciones novedosas, permite el incremento de la cuota de mercado disponible para su uso, además de generar nuevos materiales capaces de realizar funciones diferentes o de forma más efectiva que los actuales. Dando lugar, a infinidad de aplicaciones distintas algunas de las cuales serán comentadas más adelante.

1.1. Nanocelulosa

La celulosa es el polímero más importante en cuanto a abundancia en la naturaleza. Ésta compone la estructura básica de las plantas y se encuentra formada por carbono, hidrogeno y oxígeno en forma de cadena de homopolisacárido lineal con repeticiones de celobiosa. Cada bloque de la cadena se encuentra compuesto por dos anillos adyacentes de anhidro-glucosa enlazados mediante enlaces β-1,4-glucosidicos, tal y como se muestra en la figura 1.

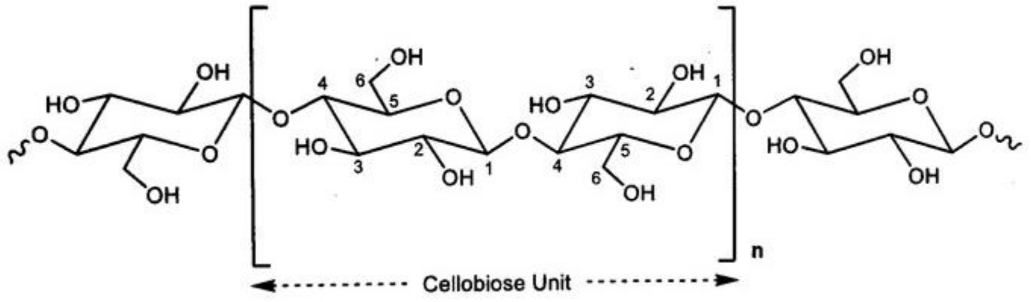


Figura 1: Esquema parcial de la estructura de la cadena de cel·lulosa (Fuente: Future Markets, 2017*)*

Por otra parte, bajo el término nanocelulosa podemos encontrar al menos tres tipos distintos de materiales según sus distintas características. Estos son, nanofibras de celulosa (CNF), nanocristales de celulosa (CNC) i nanocelulosa bacteriana (BNC). Las nanofibras de celulosa se caracterizan por tener un diámetro entre 3 y 300 nm, una longitud superior a los 2000 nm y una cristanilidad inferior al 70 % (Saito et al., 2007). Por lo que a los nanocristales de celulosa se refiere, estos se caracterizan por presentar un diámetro entre 3 y 5 nm, una longitud entre 50 y 500 nm y una cristalinidad superior al 90 % (Habibi, Lucia and Rojas, 2010; Thielemans, Warbey and Walsh, 2009). Finalmente, la nanocelulosa bacteriana se caracteriza por tener un diámetro entre los 10 y 100 nm, una longitud entre los 100 y 1000 nm y una cristalinidad cercana al 70 % (Dufresne, 2013).

1.1.1. Nanofibras de celulosa

Las nanofibras de celulosa (CNF) son un material que en los últimos años ha captado la atención de la comunidad científica debido a sus interesantes propiedades tales como gran resistencia a tracción, transparencia, posibilidad de modificación química, baja densidad, biodegradabilidad y alta disponibilidad. Las nanofibras de celulosa son fibras de celulosa con un diámetro de partícula comprendido entre 3 y 300 nm. La producción de nanofibras de celulosa se basa en la desestructuración de las fibras, que pueden ser tanto madereras como provenientes de plantas anuales, de residuos agrícolas o forestales e incluso papel reciclado tal como se refleja en la figura 2, donde se muestran diferentes tipos de materia prima. Como fuente de nanocelulosa se observa un árbol de Pino, pacas de papel reciclado, tallos de cáñamo y tallos de maíz. Una vez extraídas la fibras (madera de pino, tallos de cáñamo o de maíz) o individualización de las fibras (papel reciclado), se procede a pretratamientos que faciliten la desestructuración de las fibras.

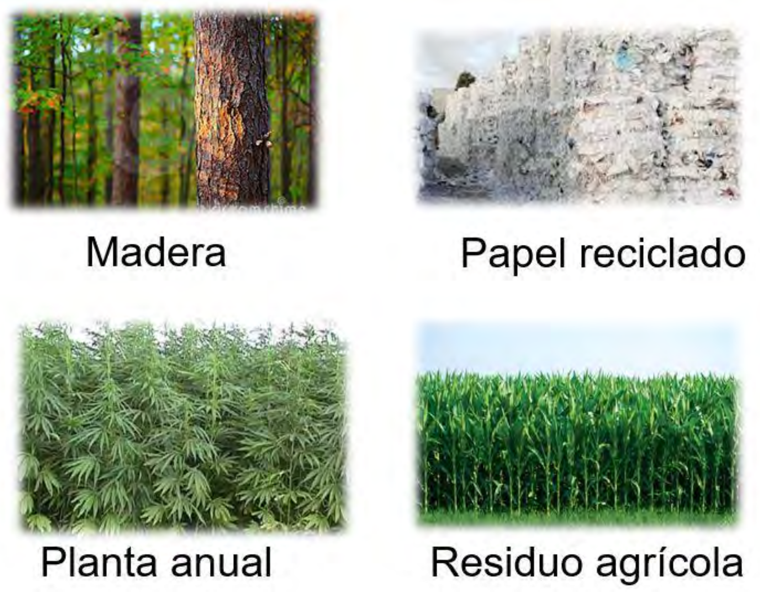


Figura 2. Posibles fuentes de fibra para la producción de nanofibras de celulosa

Como ejemplo en la figura 3 se muestra una secuencia del proceso de obtención de nanofibras de celulosa a partir de madera. Se puede observar que de la madera (1) se obtienen paquetes de fibras y fibras (2). A continuación se muestra la estructura de las fibras (3) con lámina media (ML), pared primaria (P), pared secundaria a su vez formada por tres subcapas (S1, S2 y S3) y el lumen (W). La composición química de la pared secundaria (4) está formada por hemmicelulosas (H), lignina (L) y celulosa (C). Aun así en la producción de nanofibras de celulosa casi la totalidad de la lignina presente en las fibras puede ser eliminada con tal de facilitar su desestructuración.

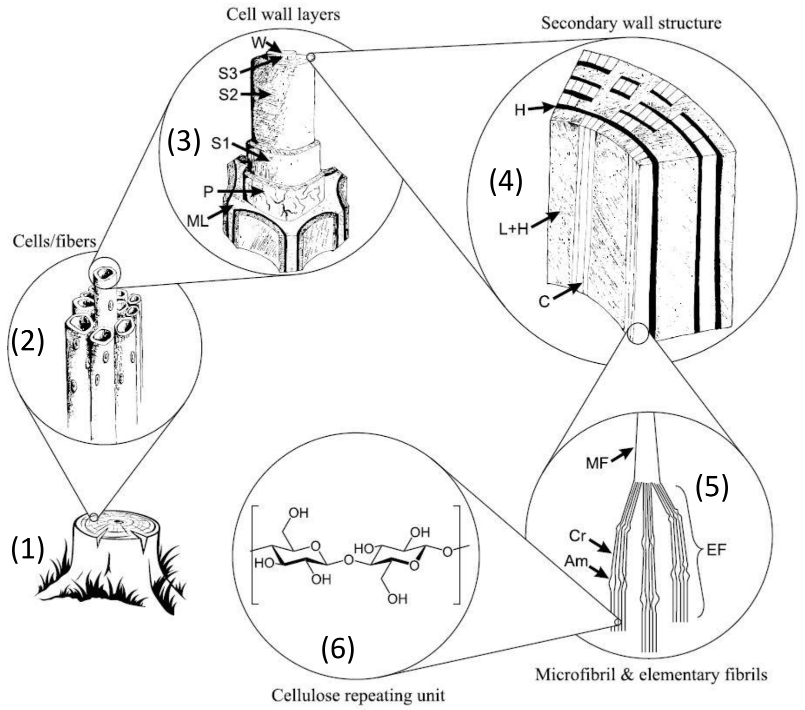


Figura 3. Evolución de las fibra de celulosa hasta las nanofibras de celulosa (Fuente: Nechyporchuk, Belgacem and Bras, 2015*)*

La desestructuración (5) de estas fibras da lugar a microfibras (MF) que a su vez están compuestas por fibras elementales las cuales contienen parte cristalina (Cr) y parte amorfa (Am). Finalmente, estas fibras elementales están compuestas por cadenas de celulosa (6).

Si bien este material, la nanocelulosa, se conoce desde principios de los años 80 (Herrick et al., 1983; Turbak, Snyder and Sandberg, 1983a, 1983b), no ha sido hasta hace unos 10 años que el interés en su producción y utilización se ha incrementado considerablemente. Esto se debe al desarrollo de métodos de pretratamiento de las fibras que han facilitado la fabricación de las CNF a costos más moderados. La producción de nanofibras de celulosa normalmente comprende una serie de etapas que generalmente pueden estructurarse en pretratamientos, desestructuración y caracterización, tal y como se muestra en la siguiente figura.

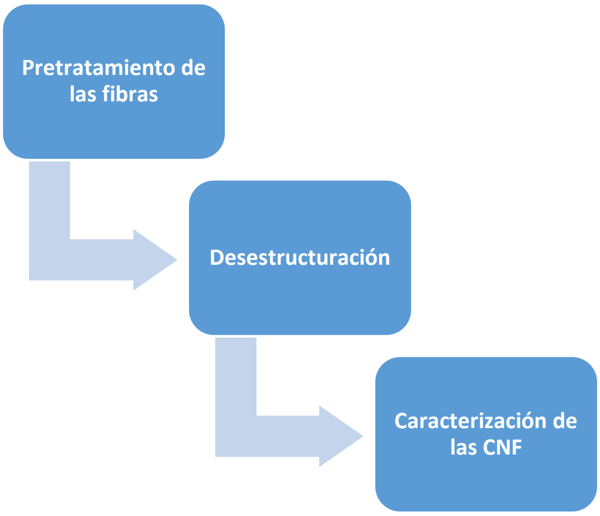


Figura 4: Etapas en el proceso de obtención de CNF.

El pretratamiento de las fibras para la producción de nanofibras de celulosa ha sido ampliamente estudiado con tal de disminuir la energía necesaria durante el proceso de desestructuración. Entre los métodos desarrollados para el pretratamiento de las fibras estos se pueden clasificar como pretratamientos químicos, enzimáticos (Henriksson et al., 2007; Pääkko et al., 2007) y mecánicos (Delgado-aguilar et al., 2015; Tarrés et al., 2017). Sin embargo, dentro de esta clasificación son los pretratamientos químicos los que presentan una mayor diversidad. Entre ellos destacan, la oxidación catalizada por TEMPO a pH básico (Saito et al., 2007), la oxidación catalizada por TEMPO a pH neutro (Besbes, Alila and Boufi, 2011), el pretratamiento por hidrólisis ácida suave (Zimmermann, Pöhler and Geiger, 2004), la carboximetilación de las fibras (Aulin et al., 2010), carboximetilación con ácido cloroacetico (Ankerfors, 2012), carboximetilación con clorito-periodato (Tejado et al., 2012), pretratamiento con carbonato sódico (Zhang, Tsuzuki and Wang, 2015) o sulfonación (Liimatainen et al., 2013). Se ha demostrado en diversos artículos que el pretratamiento químico produce CNF altamente individualizadas y con una gran superficie específica. No obstante, los pretratramientos químicos, en especial la oxidación TEMPO, son aún costosos para su aplicación a escala industrial en sectores maduros. Por otro lado, la utilización de reactivos químicos durante la etapa de pretratamiento puede originar contratiempos ambientales. Si tenemos, por ejemplo, en cuenta la producción de nanofibras de celulosa mediante oxidación catalizada por TEMPO a pH básico para un nivel de oxidación de 5 mmoles, resulta en que para la producción de un kilogramo de nanofibras son necesarios 16 gramos de catalizador TEMPO, 100 gramos de bromuro de sodio, 2.5 litros de hipoclorito sódico al 15 %, es decir 375 gramos de hipoclorito sódico, y 20 gramos de hidróxido de sodio. Resultando un consumo de productos químicos de 511 gramos por cada kilogramo de CNF TEMPO 5 mmoles producidas. En el mismo sentido, el consumo de productos químicos para un grado de oxidación de 10 mmoles haciende a 886 gramos por cada kilogramo de CNF. Por tanto, no es de extrañar que este tipo de nanofibras se encuentren actualmente en el mercado a un precio mínimo de 10 €/kg. Otros tipos de pretratamiento químico enumerados anteriormente también generan consumos importantes de disolventes y reactivos químicos.

El pretratamiento mediante la hidrólisis enzimática con la enzima endo-β-1,4-glucanasa, es un método que facilita la obtención de CNF a un costo mucho más razonable y con posibilidad de ser escalado a nivel industrial. Por otra parte, el proceso de desestructuración es un proceso mecánico que puede realizar-se mediante distintos equipos tales como homogeneizadores de elevada presión (Turbak et al., 1983b), microfluidizadores (Zimmermann et al., 2004) o grinders de tipo Mazuko (Taniguchi and Okamura, 1998). El nivel de desestructuración de las fibras es sensiblemente inferior debido a la falta de pretratamiento, en este último caso.

1.2. Aplicaciones de las nanofibras de celulosa

1.2.1. Producción de papel

Uno de los ámbitos donde las CNF han encontrado aplicación inmediata, al menos a nivel de laboratorio, es en la fabricación de papel (Afra et al., 2013; Delgado-Aguilar et al., 2015; González et al., 2012; Hietala et al., 2015; Sehaqui, Zhou and Berglund, 2013). Cuando se adicionan en masa a suspensiones papeleras, tanto de fibras vírgenes como de recicladas, las nanofibras aumentan la resistencia del papel y mejoran algunas propiedades físicas como la densidad y la porosidad. Esto las convierte en una alternativa interesante al refino tradicional mecánico, que es el método industrial más utilizado para mejorar las propiedades del papel. Si bien el refino mecánico está ampliamente establecido, tiene la desventaja de dañar considerablemente la fibra dependiendo de la intensidad del mismo, lo que puede reducir la vida útil de la misma y su capacidad de reciclaje.

El trabajo publicado por González et al., 2013, mostro el efecto de las nanofibras de celulosa como agentes de resistencia en la fabricación de papel a partir de eucalipto blanqueado en combinación con un proceso de biorefinado. Además se comprobó cómo dichos procesos permitían la sustitución de la metodología clásica (refinado mecánico), evitando de este modo el deterioro que este provoca en la morfología de las fibras.

Por otro lado, las nanofibras de celulosa también pueden ser aplicadas superficialmente en la fabricación de papel. En este caso, el incremento en las propiedades mecánicas se encuentra limitado a la porosidad del papel, es decir en la capacidad de penetración de las mismas en la estructura tridimensional del papel. Por otra parte, la modificación química de estas nanofibras puede permitir que mediante su aplicación en la superficie del papel se obtengan papeles con funcionalidades específicas (Lavoine et al., 2012, 2014; Luu, Bousfield and Kettle, 2011).

1.2.2. Aerogeles

En los últimos años la producción de aerogeles de nanofibras de celulosa se ha presentado como una gran oportunidad para la producción de dispositivos/materiales con una estructura tridimensional con una elevada porosidad, extremada baja densidad y elevada superficie específica (Du et al., 2013). A estas propiedades, que hacen de los aerogeles de nanofibras de celulosa un material con múltiples aplicaciones finales, hay que además añadir su carácter biobasado (Ayadi et al., 2016). La metodología de preparación de dichos aerogeles, comúnmente se basa en la dispersión de las nanofibras de celulosa obtenidas en una solución acuosa a la concentración deseada, seguido de un proceso de congelación. Finalmente, mediante liofilización el agua presente en la suspensión en estado sólido es eliminada, dando lugar a la estructura de nanofibras de celulosa tridimensional (Sescousse, Gavillon and Budtova, 2011). La fácil modificación de las nanofibras de celulosa debido a la presencia de grupos hidroxilos superficiales, permite la funcionalización de estos aerogeles para su aplicación en múltiples campos. Algunas de estas modificaciones son la modificación mediante surfactantes o polielectrolitos, silanización, uretanización, amidación o el injerto de polímeros (Habibi, 2014).

Estudios recientes, han demostrado que entre sus diferentes usos los aerogeles de nanofibras de celulosa pueden ser aplicados como agentes retardantes del fuego o aislantes acústicos. A modo de ejemplo, en el estudio de Bendahou, Bendahou, Seantier, Grohens and Kaddami, 2015 se produjeron aergoeles mediante nanofibras obtenidas por oxidación TEMPO, juntamente con la adición de partículas de nanozeolita con el objetivo de obtener materiales de muy baja conductividad térmica. Los materiales obtenidos, en el mejor de los casos, presentaron valores de conductividad térmica de 18 $mW \cdot m^{-1} \cdot K^{-1}$, con una baja perdida de propiedades mecánicas de los aerogeles.

1.2.3. Nanopapeles y dispositivos electrónicos

La producción de nanopapeles a partir de nanofibras de celulosa se presenta como una interesante opción en el campo de los films para embalaje de alimentos (Sorrentino, Gorrasi and Vittoria 2007), materiales con propiedades barrera, etc. Al igual que el proceso de producción de papel, los nanopapeles pueden ser producidos mediante la filtración y posterior secado de una suspensión de nanofibras de celulosa. Estos films de CNF, presentan como ventajas unas elevadas propiedades mecánicas (Henriksson et al., 2008) y son más respetuoso con el medio ambiente que los films plásticos. Estos nanopapeles además, pueden ser utilizados como soportes para la producción de dispositivos electrónicos flexibles, films conductores para la producción de sensores, actuadores o materiales magnéticos (Fang et al., 2014; Olsson et al., 2010; Walcarius et al., 2013).

En este sentido, el estudio realizado por Hsieh, Kim, Nogi and Suganuma, 2013 reportó el potencial uso de los nanopapeles producidos con CNF como sustratos conductores de la electricidad para la producción de dispositivos electrónicos. En este estudio, se comparó la resistencia eléctrica de líneas conductoras de nanoparticulas de plata en papeles producidos con microfibras y los producidos con nanofibras sin ningún recubrimiento. Los resultados mostraron los nanopapeles producidos con nanofibras de celulosa, como uno de los mejores candidatos a ser utilizados como sustrato para la fabricación de dispositivos electrónicos flexibles, debido a su elevada conductividad eléctrica.

1.2.4. Membranas de filtración

La elevada superficie específica de las nanofibras de celulosa, permite una gran interacción con otras sustancias. En los últimos años, una de las aplicaciones de nanofibras de celulosa que han suscitado un elevado interés entre la comunidad científica y tecnológica ha sido las membranas activas. La fácil modificación de la superficie de las nanofibras junto con la ya mencionada elevada superficie específica, hace de ella unas excelentes candidatas a dicha aplicación. Algunos ejemplos de ello son las membranas bioactivas para filtración, las membranas para el tratamiento de aguas, para la filtración de aires o la eliminación de olores (Huang et al., 2011; Zhang, Menkhaus and Fong, 2008).

El trabajo presentado por Ma, Burger, Hsiao and Chu, 2011, mostro la viabilidad de utilizar membranas producidas mediante nanofibras de celulosa para la purificación del agua. Los resultados obtenidos, mostraron que mediante el uso de estas membranas es posible la eliminación de un mayor flujo de bacterias que las membranas comerciales actuales, por exclusión de tamaño debido al diámetro de las nanofibras. A su vez, se reportó la eliminación simultáneamente de virus por absorción a éstas.

1.2.5. Dispositivos médicos

El desarrollo de nuevos materiales biomédicos a partir de recursos naturales ha sido siempre uno de los principales temas de investigación por parte de los investigadores. En ese aspecto, las nanofibras de celulosa pueden ser utilizadas para la producción de dispositivos de entrega de fármacos, como elemento modificador de la reología de fármacos, producción de tejidos, agentes antibacterianos o gasas para heridas (Hu et al., 2014; Lin and Dufresne, 2014; Liu et al., 2012; Yoo, Kim and Park, 2009).

A modo de ejemplo, el articulo reportado por Son, Youk and Park, 2006 mostró la producción de agentes antibacterianos. Los filtros antimicrobianos fueron producidos mediante iones de plata fotoreducidos adicionados a las nanofibras de celulosa. Los resultados obtenidos, mostraron un importante efecto antimicrobiano.

1.2.6. Dispositivos de almacenamiento de energía

Actualmente, existe una fuerte demanda para el desarrollo de dispositivos de almacenamiento de energía que sean económicos, flexibles, ligeros y respetuosos con el medio ambiente. Como respuesta a estas necesidades, la investigación en este campo se centra actualmente en el desarrollo de nuevos materiales para la fabricación de electrodos flexibles como alternativa a los materiales utilizados actualmente. En el caso de baterías y supercapacitadores, los polímeros conductores de la electricidad, tales como el polipirrol, la polianilina o el politiofeno son particularmente interesantes. Por otra parte, estos materiales necesitan un soporte que permita su estabilidad, flexibilidad y resistencia mecánica. En este sentido, el uso de nanofibras de celulosa como soporte se presenta como una opción interesante (Kim et al., 2013; Nyholm et al., 2011; Qiu et al., 2013).

En el trabajo reportado por Chun et al., 2012, se estudió la producción de separadores ecológicos basados en nanofibras de celulosa para su uso en baterías de iones de litio. Mediante el uso de nanofibras de celulosa, se pretendía solventar las limitaciones que los soportes de macro/microfibras presentan. Dichas limitaciones son principalmente el tamaño y distribución de poro y las propiedades mecánicas del soporte producido. Mediante el uso de estos soportes, se logró desarrollar exitosamente separadores para baterías de iones de litio con una mejora sustancial en la conductividad iónica, humectabilidad del electrolito y contracción térmica, en comparación con los separadores presentes actualmente en el mercado fabricados con polipropileno y polietileno.

1.2.7. Aditivos alimentarios

La utilización de nanofibras de celulosa en alimentos, actualmente se limita a estudios preliminares sobre la modificación reologíca y de retención de agua que estas producen. El elevado coste de producción actual de las nanofibras de celulosa y los requerimientos legales a superar para la comercialización de productos con la presencia de estas han limitado su uso en esta campo (Gómez et al., 2016; Ström, Öhgren and Ankerfors, 2013). Sin embargo, es posible encontrar algunas publicaciones relevantes en el potencial de las nanofibras de celulosa como aditivos alimentarios y la seguridad de su uso (Mendes et al., 2015; Cunha et al., 2014; Winuprasith and Suphantharika, 2013, 2015).

1.2.8. Otras aplicaciones

Entre muchas otras aplicaciones, las nanofibras de celulosa también pueden ser utilizadas como agentes para la modificación de la reología de pinturas, recubrimientos, lacas, como agentes de aplicación superficial en papeles de impresión o bien en la fabricación de pigmentos, pinturas, cosméticos, cementos, etc. (Turbak et al., 1983a).

1.3. Relevancia de las nanofibras de celulosa de hidrólisis enzimática

Aunque desde principio de los años 70 las enzimas han sido utilizadas en la industria papelera para la reducción de costes energéticos, incremento de la vida útil de las fibras, aumento de la drenabilidad o bien para su uso en las etapas de blanqueo (García et al., 2002; Lecourt, Sigoillot and Petit-Conil, 2010), su uso en la producción de nanofibras de celulosa aunque ha sido investigado (Henriksson et al., 2007; Pääkko et al., 2007; Wang et al., 2014; Yoo and Hsieh, 2010) no ha alcanzado el nivel de repercusión que si han logrado los pretratamientos químicos.

Es conocido que el uso de enzimas cataliza la hidrólisis de la celulosa aumentando de este modo su fibrilación. Esta actividad característica de algunas enzimas ha sido estudiada con el fin de disminuir o eliminar el refinado mecánico de las fibras en la producción de papel (Delgado-Aguilar et al., 2015; González et al., 2013). Por otro lado, mediante dicha actividad catalítica es posible alcanzar, mediante una hidrólisis severa, la obtención de unidades de glucosa para la producción de bioetanol (Tangu, 1982).

Las enzimas comúnmente utilizadas con estos fines son las denominadas celulasas. Estas pueden dividirse en tres grupos principales: endoglucanasas, celobiohidrolasas y beta-glucosidasas (Cadena et al., 2010). Las endoglucanasas son enzimas con la capacidad de catalizar la rotura aleatoria de los enlaces internos de la cadena de celulosa. Por otro lado, las celobiohidrolasas actúan sobre el extremo de la cadena de celulosa liberando celobiosa, mientras que las beta-glucosidasas solo actúan en los celo-oligosacaridos y celobiosa. Entre las diferentes celulasas, las endoglucanasas son las enzimas con una mayor efectividad en la interacción con las partes amorfas presentes en las fibras. Al contrario, presentan una baja efectividad frente la parte cristalina. Este fenómeno resulta de vital importancia en la producción de nanofibras de celulosa mediante hidrólisis enzimática, ya que como han reportado distintos autores, su interacción se sitúa en la parte amorfa de las fibras de celulosa (Hildén, Väljamäe and Johansson, 2005).

Los cuatro estudios mencionados anteriormente (Henriksson et al., 2007; Pääkko et al., 2007; Wang et al., 2014; Yoo and Hsieh, 2010), son los únicos de los que se tiene constancia en cuanto a producción de nanofibras de celulosa mediante hidrólisis enzimática de las fibras. En estos casos se evaluó la posibilidad de sustituir los reactivos químicos utilizados comúnmente en la producción de nanofibras de celulosa por enzimas del tipo enduglocanasa. Pääkko et al., 2007 propuso como método para la obtención de nanofibras de celulosa una primera etapa de refinado mecánico, con el objetivo de aumentar la capacidad de absorción de agua de las fibras, haciéndolas de este modo más accesibles para la enzima. A continuación realizó la etapa de hidrólisis enzimática con el fin de facilitar la desestructuración de las fibras. Dicha hidrólisis enzimática fue realizada a pH neutro, 50 °C, con una consistencia del 3 % durante dos horas. Posteriormente realizó una etapa de lavado, con el propósito de eliminar las enzimas presentes y una etapa de refino mecánico antes de una etapa de desestucturación realizada mediante la recirculación durante ocho veces de una suspensión al 2 % de las fibras pretratadas a través de un microfluidizador. Por su parte Henriksson et al., 2007 propuso un procedimiento similar sin embargo utilizando distintos porcentajes de enzima y realizando la desestructuración mediante el paso de la suspensión de fibras pretratadas al 2 % mediante un homogeneizador de elevada presión 20 veces.

Por otro lado, Wang et al., 2014 realizaron un estudio de obtención de nanofibras de celulosa mediante hidrolisis enzimática mediante dos enzima del tipo endoglucanasa a pH 5.0, a temperaturas de 50 y 70 °C, un 5 % de consistencia durante 48 horas a concentraciones de enzima de entre 0.1 y 10 mg/g de fibra. Finalmente, las fibras pretratadas fueron sometidas a un proceso de desestructuración mediante su paso a una consistencia del 0.3 %, durante 40 veces en un equipo del tipo microfluidizador.

En el estudio presentado por Yoo and Hsieh, 2010, se evaluó la producción de nanofibras de celulosa mediante un pretratamiento de hidrólisis enzimática mediante una enzima del tipo celulasa. Dicho pretratamiento fue realizado a una consistencia del 3 %, a un pH entre 7 y 9, 50 °C de temperatura durante dos horas con una concentración de enzima del 0.02 % respecto al peso de fibra seca. Finalmente, el proceso de desestructuración fue realizado mediante un equipo del tipo grinder recirculando una suspensión de fibras pretratadas al 0.5 % durante 90 minutos.

Por una parte, las propiedades de las nanofibras de celulosa obtenidas mediante hidrólisis enzimática, tales como rendimiento de nanofibrilación, superficie específica o diámetro, reportadas en la bibliografía, son claramente inferiores a las obtenidas mediante pretratamientos químicos como la oxidación TEMPO a pH básico. En general, cada tipo de aplicación requiere un determinado tipo de nanofibras y así mismo unos costes de producción competitivos. La aplicación de nanofibras en la fabricación de papel, hoy en día es una de las que se encuentra más cerca de alcanzar su uso industrial. Por otra parte, como ya se ha mencionado anteriormente éste es un sector maduro que no permite el uso de materiales con un alto coste de producción. Como ejemplo paradigmático, la aplicación de nanofibras para la fabricación de papeles reciclados (embalaje), es posible mediante la adición de nanofibras pretratadas químicamente (oxidación TEMPO), enzimáticamente y nanofibras lignocelulosicas mecánicas obtener los mismos incrementos en las propiedades mecánicas. Además, el volumen de producción de papel de esta industria resulta muy elevado con lo cual la aplicación de nanofibras requeriría una gran cantidad de éstas. A modo de ejemplo, una máquina de producción de papel de un gramaje de 120 g/m^2 con un ancho de cinco metros a una velocidad de 500 m/min, produce 18 toneladas de papel cada hora. Por lo tanto, si se adicionara un 3 % de CNF respecto al peso de fibra seca, sería necesaria la adición de 540 kg de CNF cada hora. Por otro lado, el coste actual de las nanofibras de celulosa obtenidas por oxidación TEMPO a un nivel de oxidación de 5 mmoles se sitúa alrededor de los 10 €/kg, en el caso de la utilización de reactivos con poca pureza, o de 130 €/kg en el caso de reactivos de elevada pureza. Ello conllevaría un coste de 0.3 €/kg de papel en el mejor caso, coste claramente insostenible en este tipo de producto. Sin embargo, la utilización de nanofibras de celulosa obtenidas mediante hidrólisis enzimática con un coste cercano a los 2 €/kg. Estos 2 €/kg tendrían una repercusión sobre el coste del papel de 0.06 €/kg de papel para una aplicación del 3 % en peso, con la misma capacidad de refuerzo que supondría la viabilidad de su uso. Estudios industriales recientes realizados por el grupo LEPAMAP, han demostrado la necesidad de alcanzar un contenido óptimo en lignina presente en las fibras, para la correcta nanofibrilación en el caso de producción de nanofibras de celulosa mediante únicamente desestructuración mecánica. La presencia de dicho contenido en lignina limita su uso en las distintas aplicaciones debido a la modificación en la blancura que éstas generan. En este caso, el precio de estas nanofibras lignocelulosicas mecánicas con un coste de producción inferior al 1 €/kg, comportarían un incremento en el precio de producción de papel de 0.03 €/kg de papel.

Además, hay que añadir que mediante el uso del pretratamiento de hidrólisis enzimática permitiría la obtención de nanofibras de celulosa con una mayor sostenibilidad mediambiental debido a la importante reducción de reactivos químicos utilizados, solamente ácido clorhídrico para la adecuación del pH.

Objetivo y justificación de la tesis

2. Justificación y objetivos de la tesis

2.1. Justificación

El grupo de investigación LEPAMAP ha estado trabajando en la producción, caracterización y aplicación de nanofibras de celulosa desde el año 2011. Principalmente, al igual que ha ocurrido a nivel internacional se ha centrado esta investigación en la producción de nanofibras de celulosa pretratadas químicamente. A raíz de contratos industriales privados que el grupo LEPAMAP tiene en curso, se ha puesto de manifiesto la inviabilidad del uso de dichas nanofibras con un elevado coste de producción en sectores maduros como es la industria papelera. Esta situación dio lugar a la exploración de metodologías alternativas de pretratamiento de las fibras, entre ellas la hidrólisis enzimática, con un menor coste de producción y eficacias similares. Es en este contexto en el que se enmarca esta tesis.

La bibliografía actual sobre el uso del pretratamiento de hidrólisis enzimática para la producción de nanofibras de celulosa es escasa y con resultados que no son satisfactorios. La presente tesis está basada en siete artículos que en su conjunto analizan de manera extensiva la optimización de la hidrólisis enzimática para la obtención de nanofibras de celulosa y su uso como aditivos de refuerzo en suspensiones papeleras, además de explorar nuevas aplicaciones para las CNF enzimáticas. En el primer artículo se optimiza la etapa de hidrólisis enzimática de fibras blanqueadas, pH, tiempo, temperatura, consistencia y concentración de enzima sobre el sustrato. La caracterización conduce a un tipo de nanofibras de celulosa óptimo, que muestra la máxima capacidad de incremento en las propiedades del papel producido con una suspensión de fibras blanqueadas comerciales. En el segundo artículo se estudia la aplicación de las CNF enzimáticas en campos distintos al papelero, en concreto la fabricación de aerogeles hidrofóbicos para su uso en la recolección de hidrocarburos derramados en cuerpos de agua. Las nanofibras obtenidas mediante pretratamiento de hidrolisis enzimática, optimizadas, se modifican químicamente, en diferentes grados con dímero de alquillcetena (AKD). Con las mismas se fabrican aerogeles de distinta hidrofobicidad y se estudia la absorción de hidrocarburos en suspensión acuosa. El tercer artículo analiza los resultados de la aplicación en primer lugar de CNF de hidrólisis enzimática optimizadas en masa y posteriormente de manera superficial sobre el papel ya formado. La aplicación de las nanofibras de celulosa enzimáticas en superficie no fue satisfactoria. Se dedujo, que el nivel de nanofibrilación (diámetro y longitud) no permitía su penetración en la estructura porosa del papel. En consecuencia se procedió a la aplicación en superficie de nanofibras de celulosa TEMPO con un nivel de oxidación de 15 mmoles, que se caracterizan por su elevado grado de despolimerización, longitudes relativamente bajas y alto grado de individualización con diámetros realmente muy bajos (7-9 nm). El cuarto artículo evalúa las propiedades morfológicas, ópticas, térmicas y mecánicas de los nanopapeles obtenidos a partir de las CNF enzimáticas comparativamente con los nanopapeles obtenidos con CNF TEMPO. El quinto artículo estudia el uso de nanopapeles con propiedades magnéticas, producto de la adición de ferrita cuya funcionalidad permite ser utilizados como membranas para la fabricación de altavoces. El sexto artículo es una revisión bibliográfica del uso de nanofibras de celulosa, entre ellas las procedentes de pretratamiento mediante hidrólisis enzimática, como agentes de resistencia en seco en la fabricación de papel, mediante suspensiones de fibra virgen y reciclada, tanto destintadas como sin destintar. En el transcurso de las investigaciones llevadas a término durante este periodo se han desarrollado aplicaciones de las enzimas tipo endo-β-1,4-glucanasa con la finalidad de encontrar una alternativa al refinado mecánico, que podemos denominar como biorefinado. El séptimo artículo trata del uso de fibras biorefinadas previamente a las que se incorporan en masa, CNF pretratadas mediante hidrólisis enzimática. La aplicación se desarrolló sobre papel de embalaje tipo test-liner. El resultado fueron suspensiones con altas prestaciones de resistencia sin el uso de refino mecánico.

2.2. Objetivo general

El objetivo general de la presente tesis es la obtención de nanofibras de celulosa a partir de fibras pretratadas mediante hidrólisis enzimática con características que las hagan viables tanto técnica como económicamente en un amplio rango de aplicaciones. Principalmente, se estudiará la optimización del pretratamiento de hidrólisis enzimática, la aplicación de las nanofibras de celulosa obtenidas en la fabricación de papel mediante distintos soportes y la aplicación de dichas nanofibras en sectores novedosos como son la fabricación de aerogles y nanopapeles.

2.3. Objetivos específicos

En base a los objetivos generales y la problemática planteada, los objetivos específicos de esta tesis doctoral son los que se numeran a continuación:

1. La optimización de las condiciones del pretratamiento de hidrólisis enzimática en la producción de nanofibras de celulosa.
2. Evaluar y comparar el efecto de la adición en masa de estas CNF en una suspensión de fibras vírgenes de eucalipto blanqueadas. Y constatar su eficacia con las CNF pretratadas mediante oxidación TEMPO.
3. Investigar el uso de nanofibras enzimáticas hidrofobizadas, mediante modificación química, en la adsorción de hidrocarburos en suspensión acuosa, utilizando como soporte aerogeles de las mismas.
4. Estudiar los ciclos de regeneración de los aerogeles adsorbentes de hidrocarburos producidos.
5. Estudiar el uso combinado de nanofibras enzimáticas en masa y nanofibras TEMPO aplicadas en superficie para la obtención de papeles con elevadas propiedades mecánicas físico-mecánicas.

6. Constatar las propiedades de los nanopapeles producidos mediante CNF enzimáticas con los nanopapeles obtenidos a partir de nanofibras TEMPO.
7. Investigar el uso de nanofibras hidrolizadas enzimáticamente para la producción de membranas con propiedades magnéticas para ser utilizadas en la producción de altavoces
8. Una vez optimizadas la producción de nanofibras de celulosa mediante un pretratamiento de hidrólisis enzimática, se procederá a la comparación de las mismas con las nanofibras obtenidas con otros procesos de pretratamiento en cuanto a su aplicación en la fabricación de papel.
9. Estudiar la sinergia entre la aplicación de nanofibras enzimáticas y otras tecnologías alternativas en la fabricación de papeles mediante suspensiones de fibras recicladas.

Resultados

Tarrés, Q., Saguer, E., Pèlach, M.A., Alcalà, M., Delgado-Aguilar, M. and Mutjé, P. (2016). “The feasibility of incorporating cellulose micro/nanofibers in papermaking processes: the relevance of enzymatic hydrolysis”. *Cellulose*, 23, 1433-1445

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Abstract

Cellulose nanofiber (CNF) is becoming a topic of great interest among the industrial and academic communities, mainly due to their potential applications in very well-differentiated industrial sectors. Among this wide range of applications, papermaking is one of the most accepted and studied. However, it is widely known that the papermaking sector is forced to compete in markets where products do not have huge added value and production margins are very low. Therefore, papermakers are constantly looking for new technologies that balance efficiency and production costs. In line with this, the present work attempts to assay the enzymatic hydrolysis of cellulose fibers to obtain CNFs. Accordingly, pH, pulp consistency, treatment time, enzyme dosage and temperature were varied to find a combination of parameters that could lead to highly efficient CNF in terms of the mechanical properties of paper enhancement and production costs. For this, CNFs were applied to unrefined and refined bleached kraft pulps and their properties were assessed. The obtained results demonstrated that it is possible to obtain highly efficient CNFs from bleached pulp at affordable costs for papermakers. Moreover, it was found that the treatment time has a key role during the production of this CNF but at low enzyme dosages since the obtained results, in terms of intrinsic properties and reinforcing potential, for high enzyme charges did not vary significantly as time was increased. In sum, the present work offers a cost-efficient solution for the application of CNF in the production of paper from bleached pulp as well as a promising alternative to those conventional processes from a technical point of view.

Keywords

Cellulose nanofibers

Enzymatic hydrolysis

Papermaking

Mechanical properties

Endo-β-1

4-Glucanases

Tarrés, Q., Oliver-Ortega, H., Llop, M., Pèlach, M.A., Delgado-Aguilar, M. and Mutjé, P. (2016). "Effective and simple methodology to produce nanocellulose-based aerogels for selective oil removal". *Cellulose*, 23, 3077-3088.

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Abstract

Spilled oil in seas has a direct impact on the environment and biodiversity. Moreover, there is no clear relationship between the amount of oil in the aquatic environment and its impact, since it mainly depends on the time and season that the oil is spilled. Nowadays, there are several techniques to clean up and recover oil from the sea, including the use of microorganisms, chemicals, controlled burning, dispersants and solidifiers, among others. Sometimes, unfortunately, the best option is to watch and wait for natural attenuation. Cellulose nanofibers have potential environmental applications due to their availability, light weight, mechanical and optical properties, and renewability. Several studies have dealt with modification of their hydrophilic character through silanation and acetylation. Both treatments, despite having a significant impact on the environment, are not plausible on a large scale because of the cost of chemicals and complexity of the modification. In this sense, the present work aims to develop hydrophobic nanocellulose-based aerogels from bleached kraft eucalyptus fibers modified with alkyl ketene dimers. For this, an experimental batch of 24 aerogels was prepared, including three types of CNF (TEMPO-oxidized, enzymatically hydrolyzed and mechanical) and eight modification degrees. The obtained aerogels were characterized in terms of morphology, hydrophilicity and water–oil absorption capacity under static and dynamic conditions, as well as their suitability for recycling and reuse for selective oil removal. The results showed that it is possible to obtain 3D-structured aerogels with a high oil absorption capacity by a simple and presumably low-cost methodology.

Keywords

Cellulose nanofibers

Aerogels

Oil removal

Alkyl ketene dimer

Tarrés, Q., Delgado-Aguilar, M., Pèlach, M.A., González, I., Boufi, S. and Mutjé, P. (2016). "Remarkable increase of paper strength by combining enzymatic cellulose nanofibers in bulk and TEMPO-oxidized nanofibers as coating". *Cellulose*, 23, 3939-3950.

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Abstract

Cellulose nanofibers (CNF) have been proposed by several authors as a reinforcing additive for papermaking pulps; their use allows one to avoid mechanical refining of pulp fibers, which damages the fibers and reduces their lifespan and consequently recycling cycles. In the present work, use of CNF obtained through enzymatic pretreatment as a bulk pulp additive is studied versus traditional (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO)-oxidized CNF. Results indicate that enzymatic CNF improves the overall paper properties in a similar way to TEMPO-oxidized CNF; however, the enzymatic pretreatment is supposed to be significantly cheaper and environmentally friendly compared with the chemical treatment. Paper reinforced with enzymatic CNF was later surface-coated with solution containing TEMPO nanofibers oxidized with 15 mmol NaClO to further promote the mechanical strength. The results indicate that the combination of bulk addition of enzymatic CNF to the papermaking pulp followed by surface application of TEMPO-oxidized CNF induced significant improvement in the mechanical properties of the paper at lower cost than sole use of TEMPO-oxidized CNF as bulk additive. To the best of our knowledge, this is the first work in which enzymatic and TEMPO-oxidized CNF are combined as paper additives for bulk and surface application to improve overall paper properties.

Keywords

Cellulose nanofibers

Papermaking

Mechanical properties

Drainage rate

TEMPO oxidation

Enzymatic hydrolysis

3.4. Artículo IV

Enzymatically hydrolyzed and TEMPO-oxidized cellulose nanofibers for the production of nanopapers: morphological, optical, thermal and mechanical properties

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Abstract

In the present study, CNF prepared by TEMPO-mediated oxidation and enzymatic hydrolysis were used for the production of nanopapers using a papermaking-like route. Nanopapers were characterized in terms of tensile, thermal, optical and morphological properties. Those prepared from enzymatically hydrolyzed CNF were found to be weaker at tensile than those resulting from TEMPO-mediated oxidation, but with similar level of stiffness. Enzymatically obtained CNF presented lower transparency due to their higher diameter and lower fibrillation yield. Moreover, TEMPO-oxidized CNF presented lower onset of the thermal degradation temperature (230°C) due to the presence of carboxylic groups. Overall, the influence of increasing the amount of enzyme during enzymatic hydrolysis and the amount of sodium hypochlorite during TEMPO-mediated oxidation was assessed as function of the ultimate properties of nanopapers.

Keywords: cellulose nanofibers; nanopaper; enzymatic hydrolysis; TEMPO-mediated oxidation

Introduction

In the last century, many efforts have been paid to sustainable, green and environmental friendly materials development for a wide range of applications. Among these materials, cellulose deserves special attention due to its abundance, renewability, lightweight, recyclability, biodegradability and many other interesting properties and characteristics (Saito, Kimura et al. 2007). Cellulose is the most common and abundant organic compound and biopolymer on Earth, and it is present in many living species. Among these species, the main source of cellulose worldwide is trees, annual plants and agricultural residues, which, unfortunately, have a finite supplying capacity. In addition, forests and croplands are continuously competing with the need of using such lands for agricultural purposes. In this sense, bearing in mind that the supplying capacity is such limited, cellulose fibers extraction processes must be improved and optimized to obtain the maximum efficiency in terms of natural resources exploitation (Hubbe 2014).

Cellulose nanofibers (CNF) have become a topic of increasing interest with promising applications in a broad field of innovative nanostructured materials. CNF are the smallest structural unit of plant fiber, consisting of a bundle of stretched cellulose chain molecules with long, flexible and entangled cellulose nanofibers which alternate crystalline and amorphous domains. In the same way than common cellulose fibers, nanocellulose extends the potential application to papermaking (Eriksen, Syverud et al. 2008), nanopapers (Sehaqui, Liu et al. 2010), biomedicine (Jorfi and Foster 2015) and nanocomposites (Spence, Habibi et al. 2011), among others. Furthermore, CNF have greater mechanical properties than common fibers, contributing to strongly enhance the mechanical performance in comparison with cellulosic fibers. In this sense and in the aforementioned frame of increasing the efficiency of natural resources exploitation, the higher performance of CNF confer them the feasibility to be used in common applications, but using lower amounts, contributing thus both to economy and environment (Hubbe 2014).

CNF can be produced by high shear mechanical disintegration process using high-pressure homogenizers, grinders or microfluidizers. To facilitate the disintegration process and increase the yield in cellulose nanofibrils, pretreatment such as TEMPO-mediated oxidation, enzymatic hydrolysis, carboxymethylation and mechanical refining, is widely used (Delgado-Aguilar, González et al. 2015). Depending on the selected pre-treatment, the obtained CNF will present specific properties, as well as environmental impact and production costs. In that way, several authors have reported the need of producing tailor-made CNF for each application, always pursuing a well-balanced triangle between properties, costs and environmental impact (Henriksson, Henriksson et al. 2007). Nonetheless, several efforts must to be already paid to further understand production methods and properties of cellulose nanofibers (Naderi 2017).

One of the most promising CNF-based products is nanopaper, which consists of a membrane of a 3-D structured CNF network. Mimicking papermaking process, nanopapers can be produced by filtering a CNF suspension, leading to a fully cellulose-based membrane with high mechanical properties (Henriksson, Berglund et al. 2008), being more environmentally friendly than plastic films and made of renewable and biodegradable raw materials. Moreover, when TEMPO-oxidized CNF are used, nanopapers present high transparency that able them to be used as substrate for optoelectronic devices, loudspeaker membranes, photovoltaic devices and transistors, among others (Olsson, Samir et al. 2010, Zhu, Fang et al. 2014). However, such kind of CNF have a great capacity to absorb water, increasing the filtration time and, thus, increasing the energy consumption for their production. In this sense, several strategies must be implemented to develop nanopapers from CNF with lower capacity to retain water without decreasing their mechanical properties. Thus, the present work aims to compare the mechanical, morphological, optical and thermal properties of nanopapers made both from TEMPO-oxidized and enzymatically hydrolyzed CNF.

Materials and Methods

Materials

Bleached kraft hardwood (*Eucalyptus globulus*) pulp (BKHP) was kindly provided by Torraspapel S.A. (Spain), originally with 16 µm of diameter and 700 µm of length, according to the supplier. The enzymatic hydrolysis was performed using the commercial enzyme cocktail Novozym 476, kindly provided by Novozymes A/S (Denmark), that contains 2% of endo-β-1,4-glucanases with an activity factor of 4500 CNF-CA/g of cellulose (tested over a CMC substrate). All the reagents required for conducting both enzymatic hydrolysis and TEMPO-catalyzed oxidation, as well as for CNF characterization, were supplied by Sigma Aldrich (Spain).

Cellulose nanofibers production

TEMPO-mediated oxidation was performed maintaining pH at 10, according to the methodology reported by Saito, Kimura et al. (2007). For the production of fifteen grams of CNF, 1.5 g NaBr and 0.24 g TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl radical) were diluted into 1.2 liters distilled water. When both TEMPO and NaBr were completely dissolved in water, 15 g of BKHP fibers, prior disintegration, were added to the solution, adding extra water until reaching a total volume of 1.5 liters. Keeping the suspension under gentle stirring, NaClO was gradually dropped in. The amount of NaClO was ranged between 5 and 15 mmols/g. Once all NaClO was added, NaOH at 0.1 M was dosed until achieving a constant pH of 10. The oxidized suspensions then were washed with distilled water and kept at 4 °C.

Enzymatic hydrolysis was carried out according to the methodology reported by (Henriksson, Henriksson et al. 2007). However, several parameters were changed according to the optimization carried out by Tarrés, Saguer et al. (2016). BKHP was dispersed at 1.5 wt% in water in a laboratory pulper for 30 minutes at 3,000 rpm. Then, the fibers were filtered until 10 wt% consistency and refined in a PFI mill for 4,000 revolutions. This process was carried out to swell the fibers and thus to promote the activity of enzymes. Briefly, refined fibers were suspended in water again (until reaching a pulp consistency of 5 wt%), and 0,1 N HCl was dropped until achieving a pH of 5. Then, the suspension was heated until 50 °C under constant stirring to avoid temperature gradients. At this step, the enzyme cocktail was dropped into the suspension and stirred for 4 hours. The enzymatic process was stopped by heating the suspension to 80 °C for 15 minutes, where the enzyme activity is strongly decreased (Marco and Felix 2007). Enzyme dosage was ranged between 80 and 320 g/Tn. The enzymatically hydrolyzed pulp then was washed with distilled water and kept at 4 °C.

The treated suspensions (1 wt% consistency) were gradually homogenized in a PANDA Plus laboratory homogenizer (Gea Niro Soavi, Italy) following the sequence of three passes at 300 bar, three passes at 600 bar and three passes at 900 bar. All the suspensions followed the abovementioned sequence, making the difference only on the type of pretreatment. Moreover, the pressure was increased gradually to avoid clogging in the pressure chambers.

The produced CNF received the following nomenclature: CNF-Treatment/Dosage. TEMPO-oxidized CNF received the following codes: CNF-T/5, CNF-T/10 and CNF-T/15, being the oxidation performed at 5, 10 and 15 mmols/g of NaClO, respectively. Enzymatically hydrolyzed CNF were labeled as follows: CNF-E/80, CNF-E/160, CNF-E/240 and CNF-E/320.

Nanopapers production

The obtained CNF gels at 1 wt% consistency after fibrillation were dispersed in higher amount of water, until reaching a consistency of 0.2 wt%. These suspensions were then vacuum-dewatered in a Rapid Köthen-like sheet former equipment. The screen in the stock container was provided with a 0.22 µm nitrocellulose filter membrane to retain the CNF. The sheet former operated at -0.7 bars of pressure. The suspension was left to dewater until a gel-like cake of CNF was observed at the top of the membrane. The obtained CNF-membranes were then vacuum dried during 15-20 minutes and finally conditioned in a weather chamber at 23 °C and 50 % of relative humidity for 24 hours before testing.

Cellulose nanofibers characterization

The characterization of CNF was performed according to previous works (Delgado-Aguilar, González et al. 2015). Cationic demand was determined by colloidal titration using a Mütek PCD04 charger analyzer from BTG, S.L. (UK). This titration was performed through surface adsorption of poly-DADMAC and the excess was titrated with PES-Na and anionic standard polymer, both from BTG. The molecular weight (MW) of the poly-DADMAC was 107 kDa.

The carboxyl content of the oxidised cellulose was determined using conductometric titration, as described elsewhere (Besbes et al. 2011).

Specific surface and diameter were estimated from cationic demand and carboxyl content, as described by Espinosa, Tarrés et al. (2016). Briefly, it was considered that the interaction between the CNF surface and the added cationic polymer (poly-DADMAC) occurred through two different mechanisms: on the one hand, part of the polymer got retained by ionic interaction between carboxylic groups from CNF and the polymer thereof. On the other, the rest of the consumed poly-DADMAC during cationic demand determination was assumed to be retained by hydrogen bonding and Van der Waals forces. This can be assumed due to the high molecular weight of the poly-DADMAC that was used. Indeed, low-MW polymers could penetrate into the cellulose fiber cell walls, while high-MW polymers cannot (Lizundia, Delgado-Aguilar et al. 2016).

Moreover, the estimation of the specific surface was accomplished taking into account two assuptions: i) the surface adsorption of poly-DADMAC took place in the form of a monolayer and ii) the poly-DADMAC chain has a cylindrical geometry. The surface of poly-DADMAC was initially estimated by calculating the surface of the monomer and its polymerization degree, taking into account the bond distances and assuming a cylindrical monomer (535.87 nm^2/g, being equal to $4.87 \cdot 10^{17}$ $nm^2/\mu eq\text{-}g$).

Moreover, using the values obtained for cationic demand and the carboxyl content it was possible to calculate the CNF's theoretical surface area by the following equation:

$$\sigma_{CNF} = (CD - CC) \cdot S_{Poly-DADMAC} \, {}^{nm^2}/_{\mu eq\ g}$$

Where CD is cationic demand, CC is carboxyl content (both expressed in μeq-g/g) and $S_{Poli-DADMAC}$ is the specific surface of Poly-DADMAC, in ${}^{nm^2}/_{\mu eq\ g}$.

Finally, assuming that nanofibers are perfect cylinders and that cellulose density is 1.5g/cm^3, diameter was estimated.

The yield of nanofibrillation was determined by centrifuging (rotational speed of 4,000 rpm; 3,100 G-force) an aqueous suspension of CNF (50 mL; 0.05 wt%) in a vessel equipped with a nitrocellulose membrane (pore size of 0.22 μm) at the bottom. This concentration was selected to be in the range of the critical networking concentration (Naderi, Lindström et al. 2014). The retained solids were weighted and referred to the total amount of CNF added to the vessel, obtaining the non-fibrillated percentage. The degree of polymerization (DP) of CNF was determined from intrinsic viscosity measurements, according to UNE 57-039-92. The viscosimetric average molecular weight was calculated from the equation: $g = KMa$, where g is the intrinsic viscosity, K = 2.28 and a = 0.76 (Henriksson, Berglund et al. 2008). Transmittance measurements were performed on CNF suspensions with 0.1 % solid content. The sample was introduced into quartz cuvettes and the transmittance measured with a UV–Vis Shimadzu spectrophotometer UV-160A set in the range between 400 and 800 nm. Distilled water was used as reference.

Nanopapers characterization

Density and porosity were calculated from the basis weight and thickness of the obtained nanopapers. Nanopapers were mechanically tested in an Instron universal testing machine provided with a 2.5 kN load cell. The gap between clamps was set at 150 mm and preload was 0.1 N with a cross-head testing velocity of 15 mm/min. The testing specimens were cut down to stripes of 180 mm length and 15 mm width.

Transmittance measurements were performed on nanopapers, introducing the samples in the UV-Vis spectrophotometer without cuvettes. The wavelength was ranged between 400 and 800 nm, using air as reference.

Water contact angles on nanopapers surface were measured using a DSSA24 drop-shape analyzer from Krüss GmbH (Germany) with the Krüss Advance Software. The measurements were performed at room temperature with a frequency of two measurements per second. The total testing time was 30 s for each nanopaper.

Crystallinity degree was calculated from an X-Ray Diffraction (XRD) profile. XRD measurements were performed on small pieces of nanopaper. Cu Kα radiation was generated with a Bruker D8 Advance diffractometer. Sweeps of 5 – 50° 2θ were made with a step size of 0.05 s and the step time measurement was 10 s. The crystallinity was calculated using the diffraction intensities of the crystalline structure and that of the amorphous fraction according to the method reported by Segal, Creely et al. (1959):

$$\%Cr = \left(\frac{I_H - I_L}{I_H}\right) \cdot 100$$

The morphology of the surface of nanopapers was observed by Field Emission Scanning Electron Microscopy (ZEISS SUPRA400) at an accelerating voltage of 2 kV. The samples were bound tothe metal holder using carbon tape and coated with a thin layer of gold.

The thermal behaviour of the nanopapers was evaluated to analyse the thermal stability of fibers, using about 10 mg solid samples and a thermogravimetric analyser Perkin-Elmer Pyris from 20 to 600 °C at a heating rate of 10 °C/min under oxygen condition.

Results and discussion

Cellulose nanofibers characterization

CNF were characterized according to the methodology described in the previous section. The carboxyl content (CC), cationic demand (CD), specific surface (σ), diameter (d), transmittance (T), degree of polymerization (DP) and yield of fibrillation are shown in Table 1.

TABLE 1 GOES AROUND HERE

Those CNF obtained by TEMPO-mediated oxidation showed increasing carboxyl content as the amount of sodium hypochlorite during oxidation was increased. This effect is completely understandable, taking into account that TEMPO/NaBr/NaClO oxidation in water at basic pH selectively oxidizes C6 primary hydroxyls of cellulose to C6 carboxylate groups (Isogai, Saito et al. 2011). This increasing carboxyl content let to higher cationic demand of the CNF suspensions due to the increase on the specific surface thereof. Carboxyl groups, either in its acidic or salt form, have greater volume than hydroxyl groups. Moreover, they impart electrostatic charges between fibers, promoting fibrillation during the homogenization stage. On the other side, enzymatically hydrolyzed CNF presented a constant CC as the amount of enzyme was increased, being the same that for the neat pulp (not shown in the table). Nonetheless, cationic demand was progressively increased, meaning that specific surface was also increased. Hence, Table 1 shows the estimation of the specific surface and average diameters (assuming cylindrical-shaped CNF) of both TEMPO-oxidized and enzymatically hydrolyzed CNF. As reflected, diameter was decreased for increasing amounts of NaClO during TEMPO-mediated oxidation. On the other hand, as the enzyme dosage was increased during enzymatic hydrolysis, no clear effect on CNF diameter was observed from 160 to 320 g/Tn.

The relationship between DP and CNF diameters is interesting to understand the mechanism of each treatment. As reflected in Table 1, in the case of TEMPO-oxidized CNF the obtained DP went from 490 to 197, for 5 to 15 mmols, respectively. On the other hand, those CNF obtained by enzymatic hydrolysis presented slightly lower DP, ranging from 412 to 309 for 80 to 320 g/Tn, respectively. It is true that DP measurement using cooper ethylenediamine (CED) as cellulose solvent may decrease the chain length and, thus, the DP due to β-elimination of adjacent glycoside bonds at the C6-aldehyde units. However, since all the measurements were performed following the same methodology, they can be used to compare the obtained CNF, assuming the same effect of CED on all the samples. Considering that there is a direct relationship between the DP and the length of TEMPO-CNF (Shinoda, Saito et al. 2012), aspect ratio can be calculated. Although in principle this relationship is only valid for TEMPO-CNF, the obtained DP values for enzymatic CNF were also used for the calculation of their lengths, leading to plausible values.

Aspect ratio brings to the light the main difference between the two treatments used for CNF production in this work. On the one side, TEMPO-mediated oxidation is able to fibrillate fibers separating fiber bundles, leading to thinner fibers, at the same time that cuts cellulose chains and decreases the fiber length. On the other, enzymatic hydrolysis acts in the amorphous regions of cellulose, cutting cellulose chain and reducing their length more than their diameter. This is why CNF with similar length (CNF-T/5 and CNF-E/80) present such different diameter and, thus aspect ratio.

The higher diameter that CNF-E have is reflected in their transmittance at 600 nm wavelength and yield of fibrillation. In this sense, it is expectable that nanopapers prepared with CNF-T will present higher transmittance than those obtained from CNF-E, being more suitable for optical applications where transparency is a requirement.

All the XRD patterns of CNF exhibited the typical Cellulose I crystalline structure, with peaks located at 15°, 16° and 23°corresponding to ($\bar{1}10$), (110) and (200) reflection planes, respectively. The CrI values of the nanopaper from CNF-T and CNF-E were around 50% and in the range of 54-64 %, respectively. The higher CrI of nanopaper from CNF-E comes from the higher removal of amorphous regions during the enzymatic treatment.

Physical and morphological characterization of nanopapers

Table 2 shows the evolution of thickness, density, porosity and transmittance both as oxidation degree and enzyme dosage were increased. Basis weight was set at 40 g/m^2. Variations are attributed to errors while measuring CNF consistency and/or the volume taken for nanopaper formation.

TABLE 2 GOES AROUND HERE

Since thickness was decreased and basis weight was kept constant, density was increased with increasing amount of NaClO and enzyme for CNF-T and CNF-E, respectively. Apparently, it seems that there is a direct influence of the specific surface of CNF on the nanopaper density. However, comparing CNF-E/320 with CNF-T/15, it can be seen that the obtained nanopapers presented similar density (and porosity) while their specific surfaces were considerably different (see Table 1). Hence, it appears that there is no direct correlation between specific surface and bulk density, although as treatment severities were hardened bulk density was slightly increased. These values of bulk density are significantly higher than those reported in the literature related to nanopapers prepared from CNF-T (Henriksson, Berglund et al. 2008). These higher densities, and low porosities, are interesting for applications where barrier properties are needed (Österberg, Vartiainen et al. 2013). Nonetheless, the achieved densities are slightly lower than already published values (Wu, Saito et al. 2012). The obtained nanopapers were produced by filtration and then vacuum dried, fact that limited the shrinkage of CNF and subsequent compaction of CNF. In addition, Wu, Saito et al. (2012) used the supernatant of CNF suspension after centrifugation, meaning that the specific surface and aspect ratio of their fibrils was significantly higher to the ones used in this work, enhancing inter-CNF bonding and, thus, promoting compaction as well. However, when the same methodology is followed, similar values can be found (Sehaqui, Liu et al. 2010). Transmittance was enhanced as NaClO content was increased for the case of CNF-T. In the same way, increasing amounts of enzyme reduced the opacity of nanopapers made of CNF-E. This increase on transmittance is explained, on the one hand by the decrease in the amount of non-fibrillated and partially fibrillated fractions responsible for a light-scattering phenomenon, and on the other hand by the decrease in the nanopaper porosity (Besbes, Alila et al. 2011).

Wetting of nanopapers was assessed by water contact angle (WCA) measurement and results are reported in Table 3. Those nanopapers made of CNF-T presented WCA of about 85° when drop was deposited, while those prepared with CNF-E had about 10° less. These values are significantly high compared to those reported in the literature, where WCA are usually in the range of 50 – 55 ° (Sun, Wu et al. 2015). The high density of surface hydroxyl groups both in cellulose and hemicellulose confer to CNF a huge hydrophilic character and, according to Dankovich and Gray (2011), WCA lower than 40° is expected. The higher values obtained in this work may be explained by two effects: i) the presence of residual lignin in the form of a thin layer blocking part of the surface hydroxyl groups (Ferrer et al. 2012) and/or (ii) the presence of residual extractives. Besides, after 30 s of the drop deposition, WCAs were not excessively decreased, fact that might be explained by the low porosity of the nanopapers, hindering water absorption by capillarity.

TABLE 3 GOES AROUND HERE

Mechanical characterization of nanopapers

Nanopapers were tested at tensile, obtaining the tensile strength (σ_t^{NP}), Young's Modulus (E_t^{NP}) and their elongation at maximum stress (ε_t^{NP}).

TABLE 4 GOES AROUND HERE

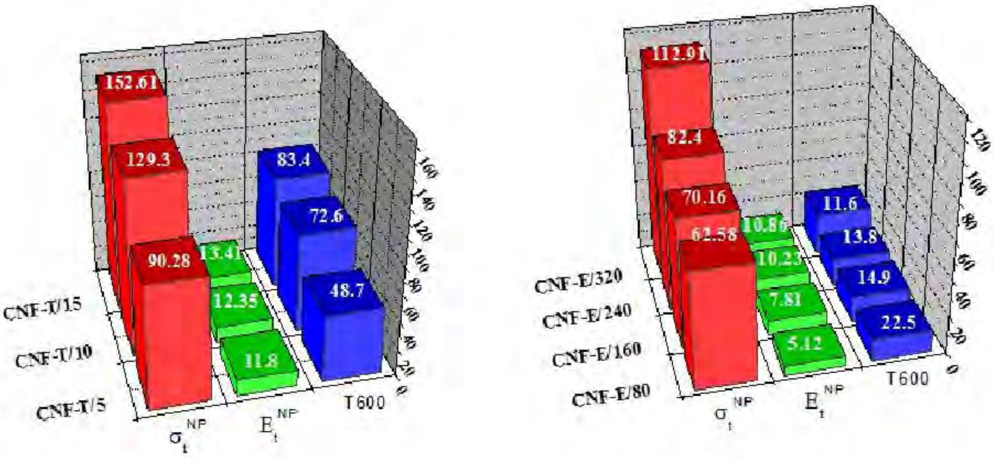

Figure 1. Evolution of strength, modulus and transmittance at 600 nm of nanopapers

Table 4 and Figure 1 bring to the light the influence of the production method, as well as the severity of the treatment on mechanical and optical properties. In the case of CNF-T, tensile strength was increased from 90.3 to 152.6 MPa as the amount of NaClO was increased (from 5 to 15 mmols/g, respectively). On the other hand, the maximum tensile strength that was achieved with enzymatic CNF was 112.9 MPa, being a value in between nanopapers made of CNF-T/5 and CNF-T/10. The differences between the strength of nanopapers from CNF-T and CNF-E are likely due to the differences on the intrinsic properties of each type of CNF. CNF-T presented higher specific surface and yield of fibrillation than CNF-E, fact that provided them higher swelling capacity and, thus, better suitability to form a three-dimension network (Chun, Lee et al. 2011). In addition, higher specific surface implies higher density of inter-CNF bonds that, at the same time, provided improved mechanical performance to the resulting CNF-based structures due to higher density. For an equivalent tensile strength, CNF-T presented higher Young's modulus than CNF-E. This could be explained by the fact that CNF-T were significantly shorter than CNF-E, limiting their plastic behavior under stress and, at the same time, their future applications where flexibility was required.

In addition to the strong dependence of the nanopapers properties on the density (Sehaqui, Zhou et al. 2011), other characteristics are expected to affect them, including: i) intrinsic properties of the nanofibers, ii) number of bonds per volume unit and iii) nature of these bonds (Delgado-Aguilar, Tarrés et al. 2015). In this sense, denser papers will present higher degree of bonds per volume unit and, thus higher mechanical properties. However, as the intrinsic properties of the CNF are much stronger than micro sized cellulose fibers, nanopapers will also present higher mechanical properties compared to common paper. Figure 2 shows the evolution of the tensile strength as density is increased, both for CNF-T and CNF-E.

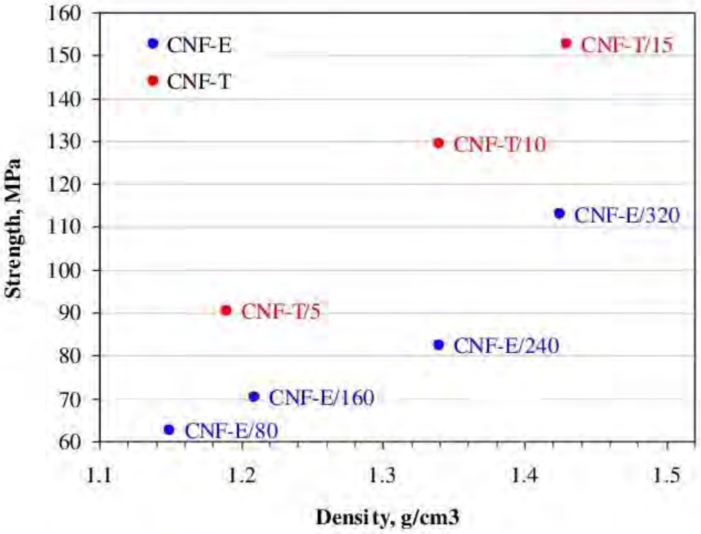

Figure 2. Evolution of the tensile strength as function of density

For a similar value of density (i.e. CNF-T/15 and CNF-E/320), it can be seen that CNF-T nanopaper presented a tensile strength 35 % higher than that made from CNF-E. This difference was even higher when comparing CNF-T/10 and CNF-E/240 (57 %).

However, the increase on the tensile strength of the nanopapers did not merely result from the increase on the density, but also involved the contribution of the intrinsic mechanical properties of the CNFs, as shown in Table 5.

TABLE 5 GOES AROUND HERE

As it is possible to see, as the amount of NaClO (CNF-T) and enzyme (CNF-E) were increased, intrinsic mechanical properties improved. In the case of CNF-T, Young's modulus was increased merely by higher compaction as the oxidation degree was increased, since no significant changes can be observed in the specific E_t^{NP}. On the other hand, for the case of CNF-E, intrinsic CNF modulus was enhanced until an enzyme dosage of 240 g/Tn was achieved, being the same than for CNF-E/320. However, the effect of density was significantly higher on tensile strength than stiffness.

Field-emission scanning electron microscopy (FE-SEM)

Transparency is a property that offers a wide range of applications to nanopapers (Hu, Zheng et al. 2013). This property is driven by the amount of light that is scattered through the nanopaper when light radiates it. Light scattering occurs both at nanopaper surface and along the interfacial area CNF-air, and it depends on the surface roughness and on the morphology of the CNF. In fact, given the strong dependence of light scattering with the particle size ($\propto D^3$) (Althues, Henle et al. 2007) then the main parameter governing the transparency of nanopapers would be the width of CNF, the fraction of unfibrillated or partially fibrillated material and the size of the pore of the nanopaper.

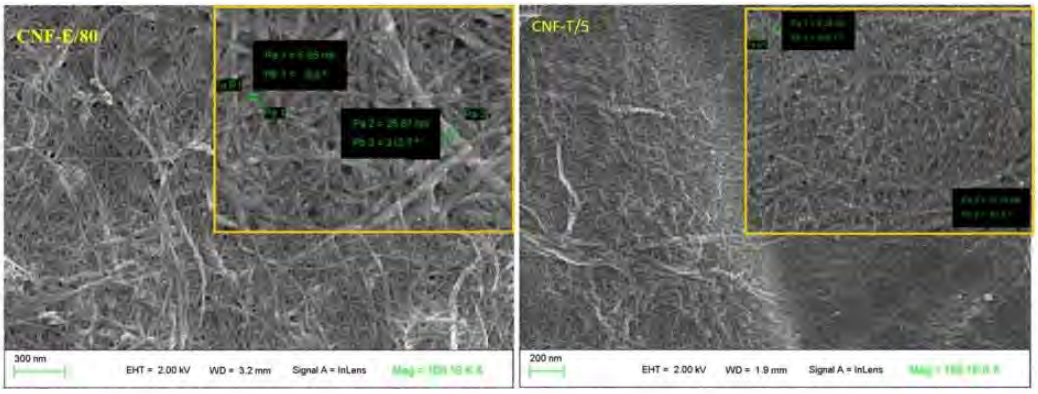

Figure 3. FE-SEM observation of nanopapers surfaces made of CNF-E (left) and CNF-T (right) at high magnification

FE-SEM observation was conducted with the purpose of comparing the morphology of CNF as function of the treatment used for their production. In all cases, nanopapers showed randomly oriented and entangled CNF forming a 3-D structure. In the case of those nanopapers obtained from CNF-E, diameters ranging from 5 to 25 nm were be observed, while those prepared with CNF-T showed a narrower distribution, being of 5 to 10 nm. FE-SEM images revealed the huge capacity that CNF have to set up entangled networks through, presumably, inter-CNF hydrogen bonds. However, it is worth to mention that FE-SEM observation at lower magnification (covering a piece of nanopaper of 100 x 100 µm, Figure 4) revealed the presence of fibrils in the micro domain, arising from the fraction partially fibrillated in both cases (both CNF-T and CNF-E).

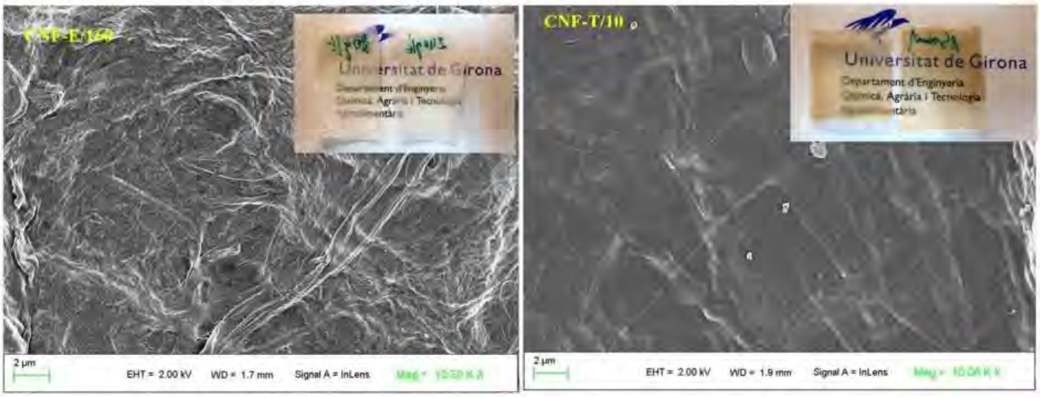

Figure 4. FE-SEM observation of nanopapers surfaces made of CNF-E (left) and CNF-T (right) at low magnification

The lower transparency of nanopapers made from CNF-E compared to those from CNF-T come mainly from the higher presence of non-nanofibrillated material, which scatters the light more than those fibrils in the nano domain. Recovering the data from Table 1, one can see that yield of fibrillation was ranged between 95.6 and 97.1 % in the case of CNF-T and from 20.5 to 38.9 % for CNF-E. The yield of fibrillation was increased with the severity of the treatment, as well as density did. Thus, the higher presence of fibrillated material leads to higher specific surface of the CNF suspension, implying more capability to form inter-CNF bonds and, at the same time, form more compacted entangled networks.

Thermogravimetric analysis (TGA)

There are several application fields where nanopapers have potential to be applied and, at the same time, thermal stability is a requirement. In this sense, samples were submitted to thermogravimetric analysis, leading to the curves shown in Figure 5 with the corresponding peaks (Table 6).

TABLE 6 GOES AROUND HERE

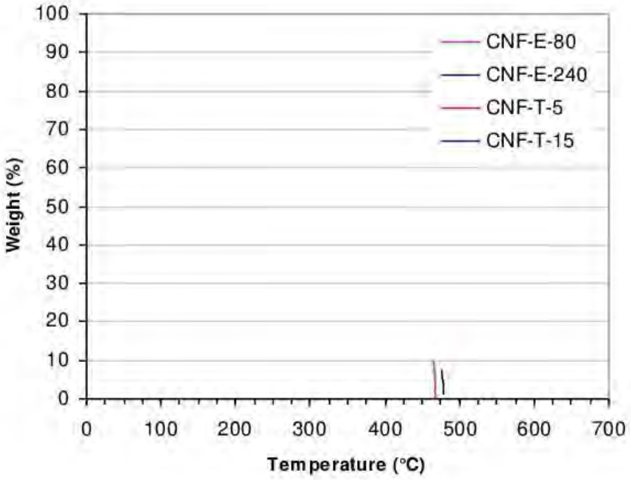

Figure 5. TGA curves of nanopapers made of CNF-E and CNF-T under oxygen atmosphere

As it can be seen in Figure 5, thermogravimetric curves experienced three changes during the heating ramp. First of all, at the temperature of about 100 °C, absorbed water (moisture) was evaporated, experiencing a weight loss of 7 and 10 % for CNF-E and CNF-T, respectively. The higher specific surface and carboxyl content of CNF-T is apparent again, since it contained 3 % more of water at the same environmental conditions. Between 240 and 280 °C, depending on the CNF type, another weight loss can be observed. This stage can be associated to hemicelluloses degradation, which are more sensitive to temperature than cellulose due to its amorphous character and, thus, higher exposition. The presence of carboxyl groups on CNF-T surface clearly affected the thermal stability of such nanopapers, occurring 40 °C before than in the case of CNF-E. The decomposition then continued with a third and last step between 400 and 650 °C, where about 40 % of the total weigh was lost. This third step is more perceptible in the case of CNF-T than in the case of CNF-E. Depending on the carboxyl content of the CNF-T, this weight loss was observed at 450 °C (CNF-T/5) or at 650 °C (CNF-T/15). The shift between the temperatures in this third stage denotes the formation of a carbonaceous layer with its thickness depends on the carboxyl content. This layer may delay the thermal decomposition of the organic material and could explain the higher residue found at 600 °C in CNF-T/15.

Conclusions

In the present work, the effect of CNF production method on the final properties of nanopapers has been investigated. CNFs prepared by TEMPO-mediated oxidation showed higher fibrillation yield, transparency and specific surface than that prepared by enzymatic hydrolysis. According to FE-SEM observation, diameters were in the range of 5 to 10 nm and 5 to 25 nm for CNF-T and CNF-E, respectively. These values were in consonance with that calculated using cationic demand and carboxyl content.

Nanopapers produced from CNF-E presented lower transmittance than those made of CNF-T. This fact limits the applicability of such nanopapers in products where transparency is a requirement. However, even though the tensile strength and transparency of CNF-T nanopapers was higher, stiffness is limiting as well their use for foldable devices. Density was also found to be a key parameter on the final mechanical performance of nanopapers, especially for tensile strength. Overall, good mechanical properties in both cases were obtained, leading to fully bio-based and biodegradable high-performance structures with high potential of applications in several fields.

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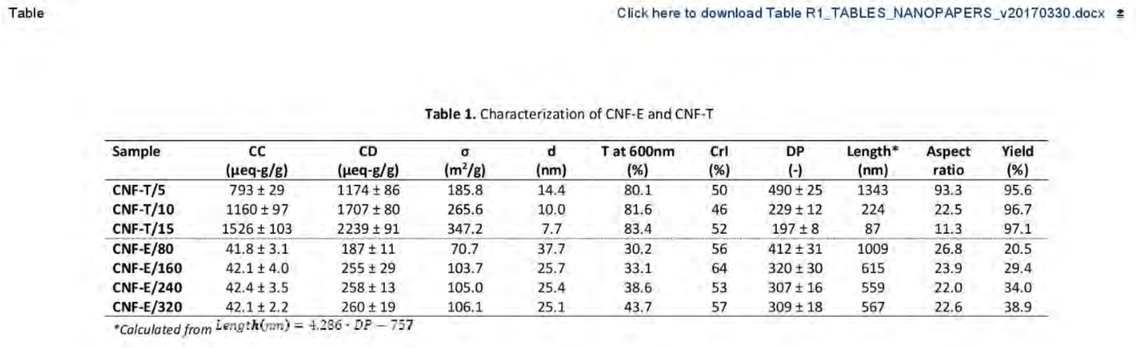

Table

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Table 1. Characterization of CNF-E and CNF-T

Sample	CC (μeq-g/g)	CD (μeq-g/g)	σ (m^2/g)	d (nm)	T at 600nm (%)	CrI (%)	DP (-)	Length* (nm)	Aspect ratio	Yield (%)
CNF-T/5	793 ± 29	1174 ± 86	185.8	14.4	80.1	50	490 ± 25	1343	93.3	95.6
CNF-T/10	1160 ± 97	1707 ± 80	265.6	10.0	81.6	46	229 ± 12	224	22.5	96.7
CNF-T/15	1526 ± 103	2239 ± 91	347.2	7.7	83.4	52	197 ± 8	87	11.3	97.1
CNF-E/80	41.8 ± 3.1	187 ± 11	70.7	37.7	30.2	56	412 ± 31	1009	26.8	20.5
CNF-E/160	42.1 ± 4.0	255 ± 29	103.7	25.7	33.1	64	320 ± 30	615	23.9	29.4
CNF-E/240	42.4 ± 3.5	258 ± 13	105.0	25.4	38.6	53	307 ± 16	559	22.0	34.0
CNF-E/320	42.1 ± 2.2	260 ± 19	106.1	25.1	43.7	57	309 ± 18	567	22.6	38.9

**Calculated from* $Length(nm) = 4.286 \cdot DP - 757$

Table 2. Physical characterization of the CNF-T and CNF-E nanopapers

Nanopaper	Basis weight (g/m^2)	Thickness (μm)	Density (kg/m^3)	Porosity (%)	Transmittance at 600 nm (%)
CNF-T/5	39.75 ± 2.01	33.4 ± 1.1	1,19	20.7	48.7
CNF-T/10	38.92 ± 1.17	27.8 ± 0.6	1,40	6.7	72.6
CNF-T/15	37.90 ± 1.88	26.5 ± 0.8	1,43	4.7	83.4
CNF-E/80	38.79 ± 0.89	33.7 ± 0.3	1,15	26.5	11.6
CNF-E/160	39.06 ± 1.02	32.3 ± 1.2	1,21	13.2	13.8
CNF-E/240	41.86 ± 2.00	31.2 ± 1.0	1,34	5.4	14.9
CNF-E/320	40.33 ± 2.46	28.3 ± 0.4	1,43	5.0	22.5

Table 3. Water Contact Angle at 0 and 30 seconds of drop deposition

Nanopaper	WCA at 0s (°)	WCA at 30s (°)
CNF-T/5	85.5	72.1
CNF-T/10	85.0	71.6
CNF-T/15	85.1	70.7
CNF-E/80	78.3	68.3
CNF-E/160	76.2	67.4
CNF-E/240	75.1	66.0
CNF-E/320	75.0	65.7

Table 4. Evolution of the tensile properties of the obtained nanopapers

Nanopaper	σ_t^{NP} (MPa)	E_t^{NP} (GPa)	ε_t^{NP} (%)
CNF-T/5	90.3 ± 3.2	11.8 ± 0.9	1.89 ± 0.09
CNF-T/10	129.3 ± 6.4	12.4 ± 1.0	2.38 ± 0.15
CNF-T/15	152.6 ± 7.0	13.4 ± 1.1	2.69 ± 0.11
CNF-E/80	62.6 ± 2.3	5.1 ± 0.5	1.96 ± 0.08
CNF-E/160	70.2 ± 5.0	7.8 ± 0.4	2.03 ± 0.15
CNF-E/240	82.4 ± 8.0	10.2 ± 0.9	2.28 ± 0.20
CNF-E/320	112.9 ± 9.8	10.9 ± 0.8	2.31 ± 0.21

Table 5. Specific tensile strength and specific Young's modulus of nanopapers

Nanopaper	**Specific σ_t^{NP} (MPa·cm^3/g)**	**Specific E_t^{NP} (GPa·cm^3/g)**
CNF-T/5	75.87	9.92
CNF-T/10	96.49	9.22
CNF-T/15	106.72	9.38
CNF-E/80	54.42	4.45
CNF-E/160	57.98	6.45
CNF-E/240	61.49	7.63
CNF-E/320	79.24	7.62

Table 6. Characteristic data from TGA analysis of the CNF samples

Sample	**T_{onset} (°C)**	**Residue at 180°C (%)**	**Residues at 500°C (%)**
CNF-T/5	245	10	25
CNF-T/15	240	10.5	30
CNF-E/80	287	7	0
CNF-E/240	285	8	0

3.5. Artículo V

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Magnetic bionanocomposites from cellulose nanofibers: Fast, simple and effective production method

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ABSTRACT

Nanocellulose is becoming a topic of great interest due to its lightweight, huge availability and its interesting properties. Among these properties, it is worthy to distinguish its specific surface and its strength. Both properties allow producing films with great mechanical properties able to retain nanoparticles which can provide the nanopaper of much functionality. Many applications for nanocellulose nanocomposites have been reported, demonstrating the interesting opportunities that this product has in a near future. In this sense, the present work attempts to produce membranes based on cellulose nanofibers (CNF) filled with magnetite nanoparticles with the purpose of developing membranes for loudspeakers. The main advantage of this is the avoiding of the iron core that one can find in any loudspeaker, since the membrane itself acts as that core. Bionanocomposites ranging from 10 to 70% of magnetite nanoparticles were produced by filtration in a nitrocellulose membrane with a pore size of 0,22 μm. Tensile tests showed that mechanical properties were decreased as the amount of magnetite was increased. They were observed by FE-SEM to see the interactions between nanoparticles and CNF. Finally, a loudspeaker prototype was developed in order to evaluate the sonorous efficiency of the resulting membranes.

1. Introduction

In the last years, nanotechnology has experienced remarkable advances in terms of findings, new uses and financial support. Nanotechnology has much to offer in several sectors such as environment –materials development [1–4]–, waste treatment [5]–, energy –improvement on energy storage devices [6–8], thermal insulation [9]–, food industry –rheology modifiers [10], heavy metals and toxins detectors and removers [11]–, medicine –drug dealers [12–15], biosensors [16]–, textile –smart textiles and intelligent fabrics [17]–, building –lighter and stronger structural materials, self-repairing materials [18]– and electronics –nanowires, biosensors [19]–, among others. Among all these interesting and novel applications, nanocellulose can offer a wide range of functionalities, properties and environmental friendly solutions [20–22].

The main attractions of cellulose nanofibers (CNF) are their ability to form 3-D structured networks, their physical and mechanical properties, lightweight, biocompatibility, biodegradability and functionality [20]. Among the most common uses of CNF, it is worthy to distinguish their use as paper additive for mechanical [20,21,23–26] and barrier [27–29] properties enhancement, as thermoplastics reinforcement [30], aerogels [31–33] and hydrogels [34–36] with several purposes and as strong, transparent and flexible supports for electronics [37–42].

Among all the applications of CNF, nanopapers deserve special attention. Nanopapers are sustainable and renewable membranes made of CNF that can be obtained by several methods such as evaporation [43] and filtration [1,3,4]. The main difference with conventional paper is that at least 51% of its weight comes from CNF [1], conferring them improved properties. The main features of nanopapers are their transparency, surface smoothness and low porosity due to the high bonding capacity of CNF and their low dimensions, fact that makes them interesting for printing and packaging applications, among others [44]. Besides, the surface chemical composition of CNF confers them the capability to be functionalized

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in many different ways, allowing thus the obtaining of functional membranes for many applications such as solar cells [45], loudspeakers [46], optoelectronic devices [40], etc.

Following under the umbrella of nanotechnology, magnetite (Fe_3O_4) nanoparticles are also playing a key role on the development of new products and technologies. These nanoparticles behave as superparamagnetic material, which is a form of magnetism that appears in small ferromagnetic or ferromagnetic nanoparticles. In the case of magnetite nanoparticles, in the absence of an external magnetic field, their magnetization appears to be in average zero, like paramagnets. However, their susceptibility is much larger than that of paramagnets [47]. The use of these nanoparticles on membranes could improve the current loudspeakers (electrodynamic loudspeakers), which are based on the movement of a magnet due to the induction of a magnetic field when a coil is electrically excited. Prior to this study, a nanocellulose-based loudspeaker has been already developed [46], but the experimental procedure was long and it required in situ synthesis of the magnetite nanoparticles. This long and sophisticated procedure makes the development of these novel loudspeakers unfeasible from an industrial point of view.

The development of plane speaker systems has been widely studied through history due to plenty of reasons, from an acoustic quality and convenience point of view. The convenience of plane speaker systems is rather obvious, they have a nearly zero deep design and they usually don't need bulky and heavy magnets. The aforementioned makes the plane speaker systems lighter and slender than other loudspeaker types. It also have to be told that usually plane speaker systems require much more surface area to operate than box speakers based on moving coil drivers which may be inconvenient in order to accommodate the speaker to the room, but at the same time this more surface usually leads to a better soundstage of the speaker.

Advances in magnets make that moving coil drive mounts in box speakers solve part of the bulk and heavy problems, even partially the need of deep space.

From an acoustic point of view, plane speaker systems often give a better sound quality. Apart from the better soundstage aforementioned, moving coil drivers need a light and stiff diaphragm membrane. These two requirements are simplified in the most plane speaker systems since stiffness isn't required, so low mass is the only main objective.

Also, plane speaker systems usually are closer to the ideal of perfect speaker. Theoretically, loudspeakers should use light diaphragm membrane drivers suspended in free space with no box, magnet structure, or frame around the drivers. Absolutely most conventional box speakers are far from this ideal. The mass of the driver is high and the rear wave is contained within the box or magnet structure. While the frequency response can be adjusted out in a box speaker, the character of the sound is inevitably affected. In the worst cases, the music sounds a box-constricted and unnatural.

There are many plane speaker systems, most of them require a conductor diaphragm membrane, like magnetostatic or ribbon loudspeakers.

In this article a new approach to plane speaker system is presented, using superparamagnetic Fe_3O_4 nanoparticles filled nanocellulose membranes for the diaphragm. In this approach, there is no need to require a conductor diaphragm membrane, and it's need a coil of insulated wire in the form of a solenoid to create the magnetic field that moves the magnetic responsive nanopaper diaphragm. This speaker system most resemble to classic moving iron speakers, but better them since the diaphragm is much more light (nanopaper) and thus the quality of sound reproduction is much better. The difficulty in this approach is the creation of the magnetic field to be uniform to all the surface of the magnetic responsive nanopaper diaphragm which can limit the total surface of the loudspeaker and thus their advantages as a plane speaker system.

2. Experimental

2.1. Materials

Bleached kraft eucalyptus pulp (BKHP) was kindly provided by Ence – Energía y Celulosa (Spain) and was used as raw material for the production of the CNF. The pulp was used in its dry form. Magnetite nanoparticles, with diameters ranging between 50 and 100 nm and a density of 4.8 g/cm^3, were provided by Sigma-Aldrich (Spain). Chemicals and materials for each treatment will be described in the corresponding sections.

2.2. Methods

2.2.1. Preparation of cellulose nanofibers

BKHP was subjected to enzymatic hydrolysis. The process was carried out according to the methodology reported by Tarrés, Saguer, Pèlach, Alcalà, Delgado-Aguilar and Mutjé [25]. BKHP was dispersed at 1.5 wt% in water in a laboratory pulper for 30 min at 3000 rpm. Then, the fibers were filtered until 10 wt% consistency and refined in a PFI mill for 4000 revolutions. This process was carried out to swell the fibers and thus to promote the activity of enzymes. Briefly, refined fibers were suspended in water again (until reaching a pulp consistency of 5 wt%), and 0,1 N HCl was dropped until achieving a pH of 5. Then, the suspension was heated until 50 °C under constant stirring to avoid temperature gradients. At this step, the enzyme cocktail was dropped into the suspension and stirred for 4 h. The enzymatic process was stopped by heating the suspension to 80 °C for 15 min, where the enzyme used suffers its denaturation. Enzyme dosage was ranged between 80 and 320 g/Tn. The enzymatically hydrolyzed pulp then was washed with distilled water and kept at 4 °C. The process was performed using the commercial enzyme cocktail Novozym 476, kindly provided by Novozymes A/S (Denmark), that contains 2% of *endo*-β-1,4-glucanases with an activity factor of 4500 CNF-CA/g of cellulose (tested over a CMC substrate).

Once cellulose fibers were oxidized, they were subjeted to high-pressure homogenization in a homogenizer Panda Plus 2000 from Gea Niro Soavi (Italy) 3 times at 300 bar, 3 times at 600 bar and 3 times at 900 bar.

2.2.2. Characterization of cellulose nanofibers

Cationic demand was determined by colloidal titration using a Mütek PCD-04 charger analyzer from BTG, S.A. (UK). This titration was performed through surface absorption of poly-DADMAC and the excess was titrated with PES-Na, an anionic standard polymer, both from BTG. The carboxyl content was determined by ionic exchange between two defined pHs. This methodology is based on the ionic exchange that takes place between carboxylic groups from cellulose and zinc cations from an aqueous suspension. The yield of nanofibrillation was determined by centrifuging an aqueous suspension of CNF in a vessel equipped with a nitrocellulose membrane (pore size of 0.22 μm) at the bottom. The retained solids were weighted and referred to the total amount of CNF added to the vessel, obtaining the non-fibrillated percentage [24].

Diameter and specific surface were estimated from the cationic demand and carboxyl content, taking into account that the absorption of poly-DADMAC occurred in monolayer and considering a cylindrical geometry of CNF, as reported elsewhere [21,25,48].

2.2.3. Preparation of the nanopapers

Prior to nanopapers formation, CNF and Fe_3O_4 nanoparticles were dispersed in water at the desired proportions. Suspensions

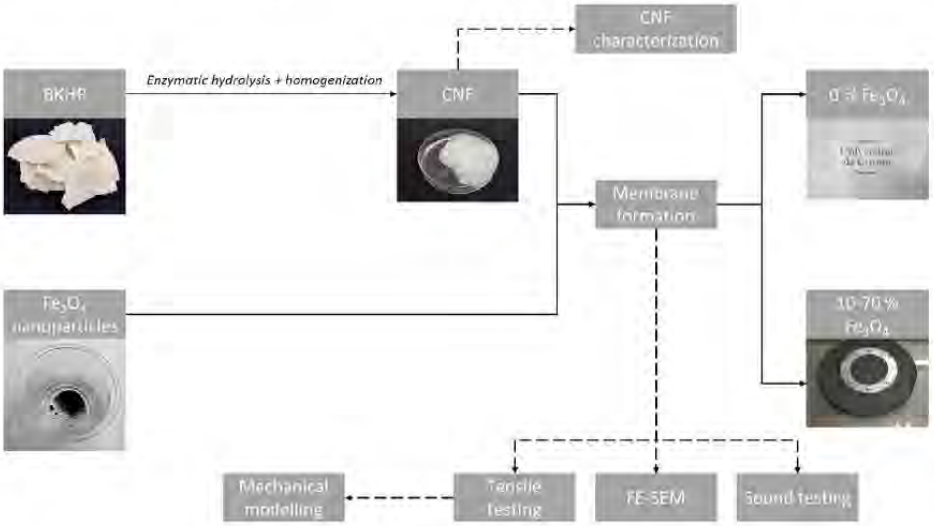

Fig. 1. Experimental procedure.

were kept under gentle stirring for 10 min. The amount of magnetite nanoparticles was ranged from 0 to 70% with regard to the amount of CNF, leading to an experimental batch of 8 samples. The amount of magnetite nanoparticles was not further increased, since CNF distribution was extremely hindered and membranes did not present dimensional stability. In order to ensure a good retention both of CNF and magnetite nanoparticles, 2% of cationic starch (Cargill HR 05947). Slurries were then vacuum-dewatered in a Rapid Köthen-like sheet former equipment. The total amount both of CNF and magnetite nanoparticles was set to form 60 g/m^2 nanopapers. The screen in the stock container was provided with a 0.22 μm nitrocellulose filter membrane to retain both CNF and nanoparticles. The sheet former operated at −0.7 bar of pressure. The slurries were left to dewater until a mat of CNF and Fe_3O_4 nanoparticles was observed at the bottom of the stock container. The thus obtained nanopaper sheets were then put between two absorbing sheets and vacuum dried during 15–20 min in a Rapid-Köthen sheet former (ISP mod. 786 FH) according to ISO 5269-2 until constant weight. Finally, the handsheets were conditioned in a climatic chamber at 23 °C and 50% of relative humidity for 24 h before testing. The whole experimental procedure is shown in Fig. 1.

2.2.4. Physical characterization

The characterization of the obtained nanopapers was performed by determining basic properties such as basis weight (ISO 536), thickness (ISO 534) and density.

2.2.5. Mechanical testing

Nanopapers were mechanically tested at tensile in an Instron universal testing machine provided with 2.5 kn load cell. Distance between clamps was set at 150 mm. Preload was 0.1 N and cross-head speed 20 mm/min. The testing specimens were cut down to stripes of 200 mm length and 15 mm width. The obtained results were the average of at least 5 tested samples.

2.2.6. Scanning electron microscopy (SEM)

A Hitachi S-3000 variable pressure SEM microscope (Hitachi High-Technologies Corporation, Japan) was used. SEM surface images were acquired at ×1,500 and ×50,000 magnifications, in secondary electron imaging mode. The acceleration voltage and working distance were 7.0 kV and 12 mm, respectively.

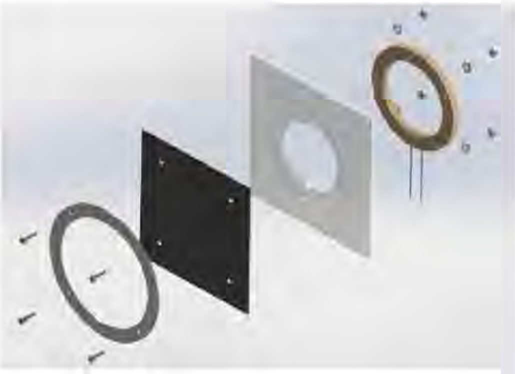

Fig. 2. Loudspeaker prototype design with SolidWorks.

2.2.7. Loudspeaker design and manufacturing

The first steep was the conceptualization of the loudspeaker. Different ideas were hand-sketched. The concept was evolved until a final design was agreed on. Then, a virtual mock-up was designed by computer-aided design software SolidWorks (Fig. 2). The product was based on standard and commercial parts availability (magnets, nuts and bolts. . .), and the dimensions of the nanopaper.

Then, all the commercial elements were purchased and the loudspeaker was manufactured.

2.2.8. Sound testing

Sound measurements were performed with sound level meter CEVSA SC310 equipment, which can perform third octave band measurements. Membranes containing from 10% to 70% of magnetic nanoparticles were tested, used as a diaphragm of the loudspeaker prototype. The measurements were carried out in a quiet reverberant room, in a two regular plane geometric envi-

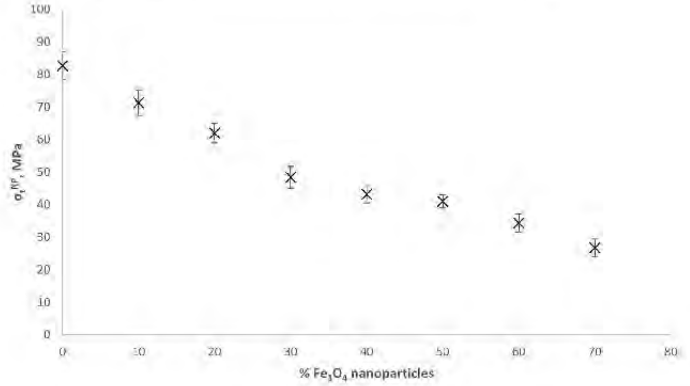

Fig. 3. Evolution of the tensile strength (σ_t^{NP}) of the obtained nanopapers as the amount of Fe_3O_4 nanoparticles is increased.

Table 1
Characterization of the CNF.

Yield (%)	38.9
Transmittance at 600 nm (%)	43.7
Degree of polymerization	309
Cationic demand (μeq-g/g)	260
Carboxylic content (μeq-g/g)	42.1
Specific Surface (m^2/g)	106.1
Diameter (nm)	25.1

ronment with an estimated directivity coefficient of 4. This sound testing methodology doesn't allow to fully characterizing the loudspeaker performance, but is sufficient to assess the influence of the amount of magnetic nanoparticles in the nanopaper diaphragm, by direct comparison of the sound radiation in every case. The sound pressure level measurements, in third octave band mode, were carried out by placing the developed loudspeaker prototype in the edge of the two plane geometric environment and placing the microphone of the sound level meter at 25 cm of the loudspeaker's diaphragm in the direction of maximum sound radiation (directivity), orthogonal to the surface of the diaphragm. Prior to every sound level measurement the sound level meter was calibrated using a CESVA CB5 device. Despite all efforts, there could be residual noise in the reverberant room, so this residual sound was firstly and lastly measured, and finally averaged to be subtracted from the main measurement.

The loudspeaker prototype was then connected to a computer and pink noise was played. Pink noise is a signal with a frequency spectrum such that the power spectral density is inversely proportional to the frequency of the signal. In this type of noise, each octave carries an equal amount of noise energy. This kind of signal has been picked instead of white noise to adapt the measurement to the sound level meter used, and to ease the comparison among third octave bands.

3. Results and discussion

3.1. Characterization of CNF

As described above, CNF were characterized prior to nanopaper synthesizing. In this sense, Table 1 shows the overall properties of the CNF used for the present study.

It can be seen that the obtained CNF presents a transmittance at 600 nm of 43.7%, while other types of CNF such as TEMPO-oxidized present transmittance near to transparency [21]. Transmittance of CNF suspensions are strongly related to the CNF dimensions and, thus, to the obtained yield of fibrillation (38.9%). Comparatively, those CNF obtained by oxidative methods present higher yields, near to 100%. This high yield comes from the introduction of carboxylic and aldehyde groups into native cellulose, maintaining though its fibrous morphology [49]. The introduction of such groups in cellulose C6 eases further homogenization and, thus, nanofibers individualization. However, since transparency is not a requirement in Fe_3O_4 filled nanopapers due to the opacity of such nanoparticles, enzymatic hydrolysis was found to be as effective as TEMPO-mediated oxidation is [25]. Since no carboxylic groups were introduced, the carboxyl content accounted for 42.1 μeq-g, as reflected in Table 1. Moreover, the cationic demand was 260 μeq-g/g. Both values can be used for the estimation of the specific surface area of the obtained CNF and, thus, their diameter, according to a methodology reported elsewhere [48]. Even though the obtained values of specific surface and diameter come from estimations based on cationic demand and carboxylic content, it has been previously demonstrated that the obtained values are plausible. Briefly, this methodology is based on the relationship between cationic demand and specific surface area of cellulosic suspensions. Following this reasoning, and considering poly-DADMAC (*P+*)'s monomer diameter, degree of polymerization (662) and monolayer adsorption, the specific surface area and average diameter were calculated [21,25,42]. This monolayer adsorption can be assured because it has the high molecular weight (M_W) of 107 kDa. Indeed, low-M_W polymers could penetrate into the cellulose fiber cell walls, while high-M_W polymers could be used for fiber surface charge measurement [42,50,51]. The estimated specific surface area of single *P+* molecule was $4.87 \cdot 10^{17}$ $nm^2/\mu eq$-g (σ^{P+}). After this value, the specific surface area of CNF (σ^{CNF}) was calculated from the next equation, taking into account the differential between cationic demand and carboxyl content, which equals to that amount of poly-DADMAC adsorbed due to Van der Waals forces:

$$\sigma^{CNF} = (CD - CC) \cdot \sigma^{P+} \quad (1)$$

Where CC is the carboxylic content and CD the cationic demand. Afterwards, it was possible to estimate the average diameter of a single CNF when they are considered perfect cylinders, according to the next equation:

$$d^{CNF} = 4/\left(\rho_{cellulose} \cdot \sigma^{CNF}\right) \quad (2)$$

Thus, the obtained average diameter for a single CNF was 25.1 nm, corresponding to a specific surface area of 106.1 m^2/g, being values similar to those reported in the bibliography for enzy-

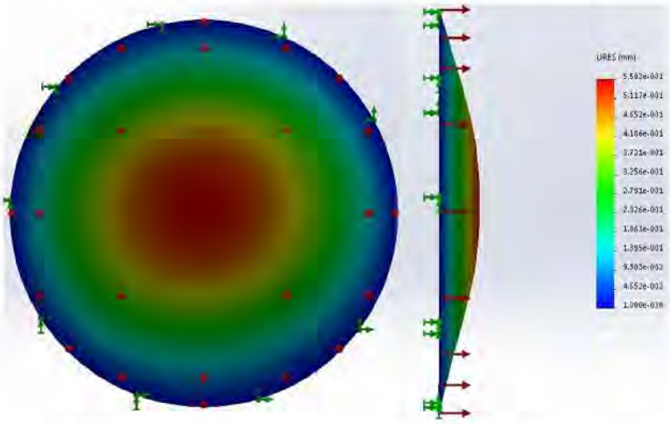

Fig. 4. Displacement modelling of the nanopapers to a homogeneous perpendicular stress.

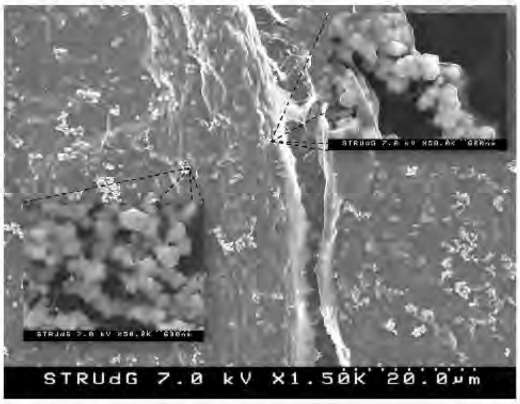

Fig. 5. SEM images of the 60% Fe_3O_4 filled bionanocomposites.

matically hydrolyzed CNF [25], and quite distant to those reported for TEMPO-oxidized CNF [21,42].

At first sight, taking into account the obtained properties of CNF, the interaction of Fe_3O_4 nanoparticles with CNF is expected to be satisfactory, mainly due to their huge specific surface and amount of hydroxyl groups on their surface and, thus, their capability to form hydrogen bonds. Moreover, taking into account their diameter, the obtained CNF will lead to high density nanopapers with really low porosity and surface roughness.

3.2. Physical characterization

Nanopapers were characterized according to the methodology described above. In this sense, Table 2 shows the obtained basis weights for each nanopaper, their thickness and density.

The obtained basis weights were around 60 g/m^2, as described above. The main reason of producing 60 gsm (g/m^2) basis weight nanopapers falls on ensuring their durability after several tests in the loudspeaker prototype. As it is reflected in Table 2, the obtained thicknesses were between 38 and 46 µm. Thickness strongly depends on the amount of material (basis weight), so density was calculated in order to assess the effect on the physical properties of adding heavier nanoparticles on nanopapers. As described above, Fe_3O_4 nanoparticles, according to the supplier, have a density of 4.8 g/cm^3 (three times higher than cellulose, which is about 1.6 g/cm^3). At first sight, one can expect that the addition of such heavier component should increase the final density of the obtained membranes. However, while this effect appears for 10 and 20% filled nanopapers, density is decreased at higher Fe_3O_4 nanoparticles concentration. This decrease on the density comes mainly from their lower flexibility against CNF and, thus, their lower compaction capacity, leading to higher thicknesses at constant basis weights.

Table 2
Basis weight, thickness and density of the obtained nanopapers.

%Fe_3O_4	Basis weight (g/m^2)	Thickness (µm)	Density (g/cm^3
0	59.37 ± 0.68	41 ± 1	1.45
10	62.01 ± 1.02	39 ± 0	1.59
20	60.28 ± 0.73	38 ± 1	1.59
30	58.36 ± 0.51	41 ± 1	1.42
40	58.91 ± 0.19	42 ± 0	1.40
50	60.39 ± 0.87	45 ± 2	1.34
60	61.51 ± 1.13	46 ± 0	1.34
70	60.09 ± 0.98	44 ± 0	1.37

3.3. Tensile properties

Loudspeaker membranes are continuously subjected to vibrations. These vibrations are more or less extensive depending on the frequency of the magnetic field that, at the same time, is function of the current flowing through the coil. Due to this movement, membranes have to be stiff enough to emit sound but not excessively, otherwise it would break. Thus, the obtained membranes were tested at tensile with the purpose of assessing their stiffness, tensile strength and strain at maximum strength. Fig. 3 shows the evolution of tensile strength (σ_t^{NP}) as the amount of Fe_3O_4 was increased.

The tensile strength of the neat nanopaper was 82.7 MPa. As expected, the tensile strength was decreased as the amount of nanoparticles was increased. This behaviour comes from the low aspect ratio of the Fe_3O_4 nanoparticles that, according to the technical data sheet provided by the supplier, they are spherical. In addition, the decrease on the density of nanopapers as the amount of nanoparticles was increased is also reflected on such mechanical properties drop. This effect was also observed by other authors [46,52,53]. Thus, the amount of magnetite nanoparticles will be

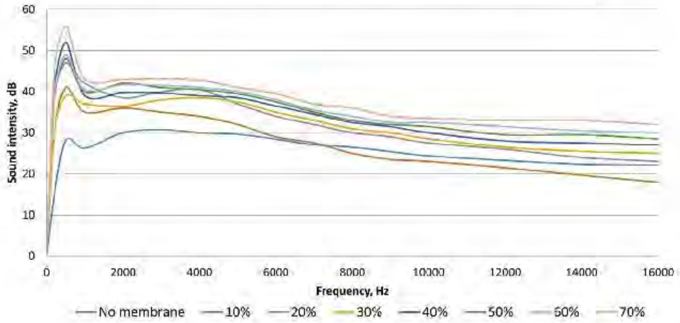

Fig. 6. Sound intensity of each membrane as frequency is increased.

Table 3
Young Modulus (E_t^{NP}) and strain at maximum stress (ε_t^{NP}) of the obtained nanopapers.

%Fe_3O_4	E_t^{NP} (GPa)	ε_t^{NP} (%)
0	11.03 ± 0.63	1.69 ± 0.13
10	10.87 ± 0.77	1.73 ± 0.08
20	9.85 ± 0.29	1.96 ± 0.07
30	9.03 ± 0.71	2.15 ± 0.19
40	8.56 ± 0.42	2.31 ± 0.21
50	7.12 ± 0.33	2.68 ± 0.10
60	5.39 ± 0.48	2.81 ± 0.16
70	4.89 ± 0.31	3.12 ± 0.26

limited by the mechanical requirements of the final application of the developed membranes. Young modulus and strain at maximum strength were also determined. As explained above, stiffness is an important parameter to be controlled if a good sound emission wants to be ensured. In this sense, Table 3 shows the obtained results of Young Modulus and strain at maximum stress for each developed membrane.

Surprisingly, Young modulus was decreased as the amount of Fe_3O_4 nanoparticles was increased, being the neat nanopaper the stiffest (11.03 GPa) and the 70% filled nanopaper the most flexible (4.89 GPa). This can be also observed in the evolution of the strain at maximum stress of nanopapers, where neat nanopapers experienced an elongation of 1.69% and, on the other hand, the reached value of the 70% magnetite filled was 3.12%. This decrease can be explained by the fact that high Fe_3O_4 nanoparticles content can limit the interaction between CNF and, thus, limiting the creation of a continuous network. In this sense, in consonance with tensile strength, the addition of such nanoparticles created weak points where the resulting membrane can break. The decrease on the Young Modulus can be advantageous for the final application. However, it must be taken into account that as the amount of nanoparticles is increased, the tensile strength of the membrane is decreased. A well-balanced membrane should assure the integrity thereof and a good sound quality, avoiding undesirable noise due to vibrations. In order to further understand to which stresses the membrane will be subjected, Fig. 4 shows the stress modelling of the 70% nanoparticles filled membrane during vibrations.

As it can be seen in Fig. 4, the maximum displacement occurs in the central region of the membrane, as expectable. This displacement was quantified in 0.5 mm approximately. However, the outer part of the membrane is the most stressed region when it is subjected to a magnetic field. It must be borne in mind that this modelling was performed in static conditions, and the pressure was supposed only in one direction. In its real application, the obtained membranes will be repeatedly subjected to bidirectional pressures. Nonetheless, after simulating the effect of displacing 0.5 mm the central part of the membrane, a minimum factor of safety of 27 was obtained, which brings to the light the suitability and feasibility of using nanocellulose-based membranes in such application. In addition, the simulated membrane was the 70% charged, which was also the weaker one in terms of tensile strength.

3.4. SEM observation

With the purpose of further understanding how magnetite nanoparticles were distributed, nanopapers were observed by SEM (Fig. 5). Magnetite nanoparticles, according to the supplier, were spherical with diameters ranging between 50 and 100 nm. This size was corroborated by SEM observation.

As expected, Fe_3O_4 nanoparticles were homogeneously distributed along the obtained membranes. Considering the final application of these membranes, a good distribution of the nanoparticles must be ensured, allowing thus a homogeneous vibration of the membrane itself. Fe_3O_4 nanoparticles in water, as most of fibrous materials, presented anionic surface charge. In this sense, SEM images corroborate that the presence of cationic starch helped nanoparticles retention, getting retained by the CNF network. CNF are strongly anionic, as Fe_3O_4 nanoparticles are. The presence of cationic starch helped the interaction between the cellulosic and mineral nanostructured components, in the same extend that occurs with, for instance, paper and mineral fillers during papermaking process, where cationic starch is also used as retention agent [54].

3.5. Sound testing

The result of the sound testing show an uniform behaviour of all magnetic responsive nanopaper diaphragm from 10% to 70% of magnetic nanoparticles content. The general behaviour of the different membranes shows that the loudspeaker doesn't reproduce all frequencies the same. The highest sensitivity it's found in frequencies contained in the 500 Hz third band. This behaviour could have been conditioned to the mounting stress and the diameter of the diaphragms tested. Apart from the aforementioned behaviour at 500 Hz third octave band, the results show a decrement sound pressure reproduction level efficiency with higher frequencies. It's difficult to say if this behaviour is most conditioned by the mass of the membranes (which is very similar despite the amount of magnetic nanoparticles content) or the characteristics of the solenoid itself. Anyway, in this aspect, the loudspeaker prototype should be improved in order to reach a minimum hi fidelity sound reproduction quality (Fig. 6).

Among the different magnetic nanoparticles content the behaviour is clear, the sensitivity of the loudspeaker increases with the magnetic nanoparticles content. It can be easily seen as the membrane with 70% content of magnetic nanoparticles reproduces higher sound pressure levels making the loudspeaker louder with the same quality acoustic reproduction. This behaviour is explained by the fact that the membrane reacts stronger at the same magnetic field if the amount of magnetic nanoparticles is higher. The fact that the quality acoustic reproduction maintains almost the same despite the magnetic nanoparticles content is because of the basis weight of the membrane almost doesn't change. In that sense, the most desirable scenario is the addition of the maximum content of magnetic nanoparticles, making the loudspeaker louder and not deteriorating the quality of the acoustic reproduction.

4. Conclusions

In this work, different nanocellulose-based membranes filled with Fe_3O_4 nanoparticles have been obtained through an effective and simple method. Fe_3O_4 were successfully dispersed in the cellulose nanofibers matrix, as corroborated by SEM observation and finally during sound testing. A decrease on the mechanical properties was observed due to the lower aspect ratio of the added nanoparticles, while stiffness was increased. Thanks to computer-aided design software, the maximum displacement of the membrane was calculated, limiting thus the amount of magnetite nanoparticles. The highest sensitivity was found in frequencies contained in the 500 Hz third octave band, being increased as the amount of magnetite was increased.

Stiffness was found to limit the amount of magnetite nanoparticles. As future work, the incorporation of plasticizers (i.e. glycerol) or common cellulose fibers should be considered if nanocellulose-based loudspeakers wanted to be produced.

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3.6. Artículo VI

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Review

Nanofibrillated cellulose as an additive in papermaking process: A review

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ABSTRACT

During the last two decades, cellulose nanofibres (CNF) have emerged as a promising, sustainable reinforcement with outstanding potential in material sciences. Though application of CNF in papermaking is recent, it is expected to find implementation in the near future to give a broader commercial market to this type of cellulose. The present review highlights recent progress in the field of the application of cellulose nanofibres as additives in papermaking. The effect of CNF addition on the wet end process is analysed according to the type of pulp used for papermaking. According to the literature consulted, improvement in paper's overall properties after CNF addition depended not only on the type and amount of CNF applied, but also in the pulp's origin and treatment. Bulk and surface application of CNF also presented significant differences regarding paper's final properties. This review also revises the mechanisms behind CNF reinforcing effect on paper and the effect of chemically modified CNF as additives.

Contents

1. Introduction

The increasing interest in nanomaterials from renewable origins and their unique properties have led to intensive research in the area of nanocelluloses produced from resources available worldwide (Kalia, Boufi, Celli, & Kango, 2014; Zhang, Batchelor, Varanasi, Tsuzuki, & Wang, 2012). More specifically, nanofibrillar cellulose, nanosized cellulose fibrils produced by the fibrillation of the cell wall of cellulose fibres through intensive mechanical action, have gained increasing attention in papermaking as a wet-end additive,

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with potential applications as a dry and wet strength agent but also as a coating to improve the barrier properties of paper (Lavoine, Bras, & Desloges, 2012; Lindström & Aulin, 2014; Shatkin, Wegner, & Bilek, 2014).

Different reasons motivated the interest of CNF as a new family of paper component: (i) The nanoscale lateral dimension of CNF expands the specific surface, (ii) their lengths in the micrometer, (iii) their semi-crystalline structure composed of extended cellulose chains, (iv) their high intrinsic mechanical strength along with good flexibility, (v) their high potential to interact with cellulosic fibres through hydrogen bonding, and (vi) their inherent tendency to form strong entangled network accounted. Although the application of CNFs as additives in papermaking is quite recent, it is promising in the near future and is likely to give a broader commercial market to sustainable reinforcement.

Several reviews on topics about cellulose nanofibres have been already published. Most of them deal with properties and application of cellulose nanofibres in composite materials based on polymeric matrices (Eichorn et al., 2010; Khalil, Bhat, & Yusra, 2012; Moon, Martini, Nairn, Simonsen, & Youngblood, 2011; Siró & Plackett, 2010). Other reviews analysed the current techniques used for CNF production and characterization (Kangas et al., 2014; Khalil et al., 2014), barrier properties (Lavoine, Desloges, Dufresne, & Bras, 2012) or general properties and possible applications of CNFs (Klemm et al., 2011). To the best of the authors' knowledge, there are only two publications (Brodin, Gregersen, & Syverud, 2014; Osong, Norgren, & Engstrand, 2015) that present advances of CNF application into papermaking slurries. The present review further highlights recent progress in the field of the usefulness of CNF as an additive for papermaking including wood pulps, agricultural wastes and recycled paper. This review also includes a brief introduction to nanocellulose, the application of CNFs as additives in pulps according to their origin, their reinforcing effect mechanism, and their application as a coating material.

2. From cellulose to nanocellulose

Cellulose is a semi-crystalline polysaccharide appearing in nature in the form of fibres with 0.5 to up several mm in length and is organized in four hierarchical levels (Klemm et al., 2011). At the molecular level (~Å), cellulose is composed of β-D-glucopyranose units linked by 1-4-β glycosidic bonds with alternately rotated glucose units. At the supramolecular level, cellulose chains aggregate together in the form of elementary fibrils, each one about 3 nm in diameter, formed by alternated crystalline and amorphous domains. These elementary fibrils are aligned and further aggregate into larger microfibrils or macrofibrils with diameter of 10–25 nm. At the structural level the microfibrils stick together in a spiral manner within a three layer cell wall structure to form the cellulose fibres (Fengel & Wegener, 1984).

Given the hierarchical structure of cellulose, it is possible to break down the cell wall into nanoscale cellulose known as nanocellulose with different morphology according to the extraction mode. Cellulose nanocrystals (CNC) and cellulose nanofibres (CNF) constituted the two main families of nanocelluloses. The terms microfibrillated cellulose (MFC) and nanofibrillated cellulose (NFC) are still frequently found in literature to describe cellulose nanofibres suspensions. However, the present review will only use the term CNF to describe suspensions containing cellulose fibres of nanometric size. Cellulose nanocrystals are extracted from fibres after a complete dissolution of the non-crystalline fractions, while the cellulose nanofibres are produced mainly through an intensive mechanical shearing action to break out the cell wall of fibres and release the cellulose fibrils in the form of bundles of elementary fibrils. CNF is the main type of nanocellulose that has attracted attention for papermaking applications.

Since the introduction of CNF in 1983 by Turbak, Snyder, and Sandberg (1983), the high pressure homogenization and the microfluidization are still the main methods currently used to effectively produce CNF (Davoudpour et al., 2015). Nevertheless, in the last years the use of grinders and refiners has increased as preferred methods for CNF fabrication because they can operate at higher CNF concentrations, fibre pre-treatment is optional and clogging problems are non-existent.

Albeit the promising potential uses of CNFs in multitude applications, their scale-up production is still limited and below expectation. One major obstacle to this development is the high energy consumption involved during the mechanical disintegration of the fibres into nanofibres (Josset et al., 2014; Naderi, Lindström, & Sundström, 2015). Another challenge is the capacity to produce CNF with uniform size. The energy consumption is strongly dependent on the fibres pre-treatment method. Values ranging from 10 up to 100 kW h per Kg of dry CNF have been reported (Chaker, Mutjé, Rei Vilar, & Boufi, 2014; Klemm et al., 2011). This high energy demand results from the necessity to operate under high pressure ranging from 200 to 800 bar with multiple passes until a high fibrillation degree is reached. The fibres pre-treatment prior to the disintegration process is another approach currently adopted to strongly decrease the energy demand. Chemical pre-treatment has emerged as one of the most efficient and popular pre-treatment strategies to facilitate the break-up of the fibres network by generating ionic or ionisable groups within the internal structure of the fibres. This can be achieved via a TEMPO-mediated oxidation (Saito, Nishiyama, Putaux, Vignon, & Isogai, 2006), carboxymethylation (Siró and Plackett, 2010; Wågberg et al., 2008) via sulfonation with sodium bisulphate (Buzała, Przybysz, Rosicka-Kaczmarek, & Kalinowska, 2015), periodate oxidation (Liimatainen, Visanko, Sirviö, Hormi, & Niinimäki, 2013), or quaternization (Chaker & Boufi, 2015; Ho, Zimmermann, Hauert, & Caseri, 2011). However a critical content in ionic groups is needed to effectively facilitate the release of the cellulose nanofibres and break down the cell wall of the fibres (Besbes, Alila, & Boufi, 2011; Besbes, Rei Vilar, & Boufi, 2011). These surface charges will have a key role in papermaking wet-end chemistry. Another merit of the pre-treatment is the reduction of the risk of clogging during the homogenization or microfluidization processes. In addition to the fibres pre-treatment approach, the delignification mode was recently shown to affect the fibrillation efficiency of the bleached cellulose fibres (Chaker, Alila, Mutjé, Rei Vilar, & Boufi, 2013). Though chemical pre-treatment can drastically reduce the energy consumption of CNF fabrication, in many cases the price of some chemicals clearly surpasses the energy costs saved (Delgado-Aguilar, González et al., 2015).

3. Application of CNF into papermaking pulps

3.1. Chemical pulps

Table 1 resumes some results reported by several authors on the effect that CNF produce when used as additives in papermaking suspensions. The columns indicate, from left to right, the author and year of publication of the consulted work, type of CNF in reference to the type of pretreatment used before homogenization/microfluidization, pulp used for paper sheet production, maximum amount of CNF added, type of retention agent used (if any) and the maximum increase in strength produced by the maximum CNF content. According to literature consulted, most of the published works on CNF applied to fibrous slurries were based on experiments performed on chemical pulps. Chemical pulps are made by cooking (digesting) the raw materials, using the kraft

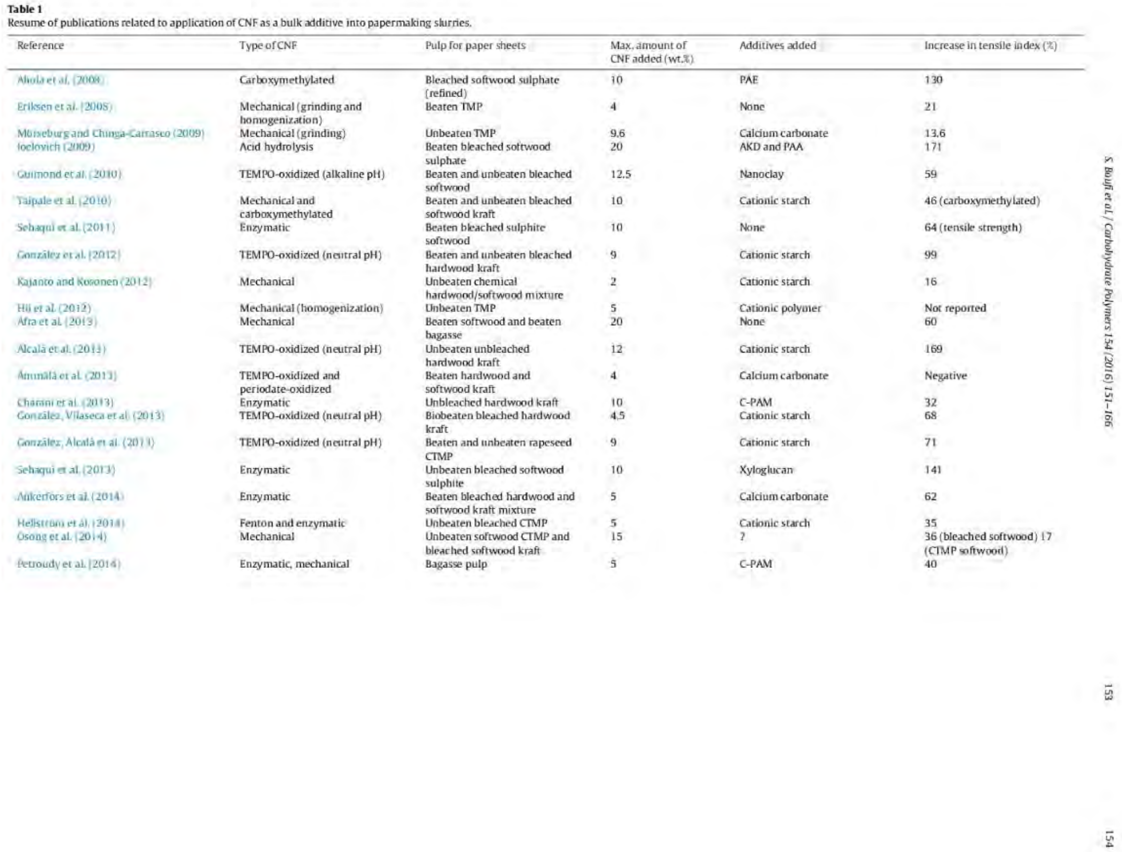

Table 1
Resume of publications related to application of CNF as a bulk additive into papermaking slurries.

Reference	Type of CNF	Pulp for paper sheets	Max. amount of CNF added (wt.%)	Additives added	Increase in tensile index (%)
Ahola et al. (2008)	Carboxymethylated	Bleached softwood sulphate (refined)	10	PAE	130
Eriksen et al. (2008)	Mechanical (grinding and homogenization)	Beaten TMP	4	None	21
Mörseburg and Chinga-Carrasco (2009)	Mechanical (grinding)	Unbeaten TMP	9.6	Calcium carbonate	13.6
Ioelovich (2009)	Acid hydrolysis	Beaten bleached softwood sulphate	20	AKD and PAA	171
Guimond et al. (2010)	TEMPO-oxidized (alkaline pH)	Beaten and unbeaten bleached softwood	12.5	Nanoclay	59
Taipale et al. (2010)	Mechanical and carboxymethylated	Beaten and unbeaten bleached softwood kraft	10	Cationic starch	46 (carboxymethylated)
Sehaqui et al. (2011)	Enzymatic	Beaten bleached sulphite softwood	10	None	64 (tensile strength)
González et al. (2012)	TEMPO-oxidized (neutral pH)	Beaten and unbeaten bleached hardwood kraft	9	Cationic starch	99
Kajanto and Kosonen (2012)	Mechanical	Unbeaten chemical hardwood/softwood mixture	2	Cationic starch	16
Hii et al. (2012)	Mechanical (homogenization)	Unbeaten TMP	5	Cationic polymer	Not reported
Afra et al. (2013)	Mechanical	Beaten softwood and beaten bagasse	20	None	60
Alcalá et al. (2013)	TEMPO-oxidized (neutral pH)	Unbeaten unbleached hardwood kraft	12	Cationic starch	169
Ämmälä et al. (2013)	TEMPO-oxidized and periodate-oxidized	Beaten hardwood and softwood kraft	4	Calcium carbonate	Negative
Charani et al. (2013)	Enzymatic	Unbleached hardwood kraft	10	C-PAM	32
González, Vilaseca et al. (2013)	TEMPO-oxidized (neutral pH)	Biobeaten bleached hardwood kraft	4.5	Cationic starch	68
González, Alcalá et al. (2013)	TEMPO-oxidized (neutral pH)	Beaten and unbeaten rapeseed CTMP	9	Cationic starch	71
Sehaqui et al. (2013)	Enzymatic	Unbeaten bleached softwood sulphite	10	Xyloglucan	141
Ankerfors et al. (2014)	Enzymatic	Beaten bleached hardwood and softwood kraft mixture	5	Calcium carbonate	62
Hellström et al. (2014)	Fenton and enzymatic	Unbeaten bleached CTMP	5	Cationic starch	35
Osong et al. (2014)	Mechanical	Unbeaten softwood CTMP and bleached softwood kraft	15	?	36 (bleached softwood) 17 (CTMP softwood)
Petroudy et al. (2014)	Enzymatic, mechanical	Bagasse pulp	5	C-PAM	40

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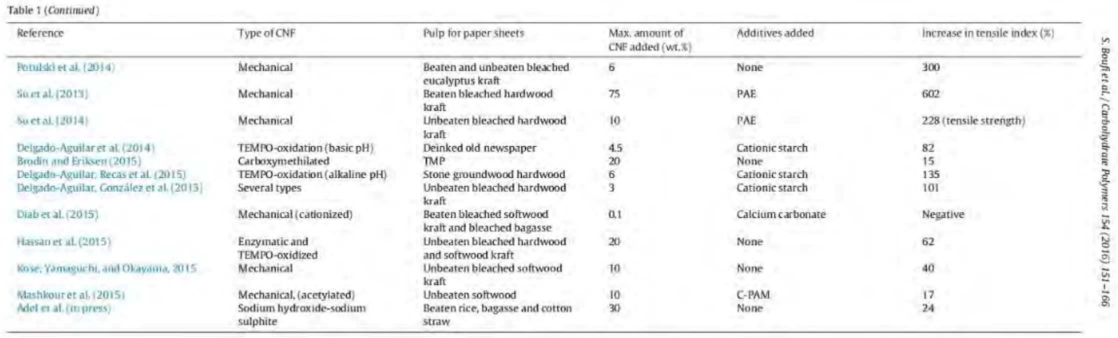

Table 1 (*Continued*)

Reference	Type of CNF	Pulp for paper sheets	Max. amount of CNF added (wt.%)	Additives added	Increase in tensile index (%)
Potulski et al. (2014)	Mechanical	Beaten and unbeaten bleached eucalyptus kraft	6	None	300
Su et al. (2013)	Mechanical	Beaten bleached hardwood kraft	75	PAE	602
Su et al. (2014)	Mechanical	Unbeaten bleached hardwood kraft	10	PAE	228 (tensile strength)
Delgado-Aguilar et al. (2014)	TEMPO-oxidation (basic pH)	Deinked old newspaper	4.5	Cationic starch	82
Brodin and Eriksen (2015)	Carboxymethilated	TMP	20	None	15
Delgado-Aguilar, Recas et al. (2015)	TEMPO-oxidation (alkaline pH)	Stone groundwood hardwood	6	Cationic starch	135
Delgado-Aguilar, González et al. (2015)	Several types	Unbeaten bleached hardwood kraft	3	Cationic starch	101
Diab et al. (2015)	Mechanical (cationized)	Beaten bleached softwood kraft and bleached bagasse	0.1	Calcium carbonate	Negative
Hassan et al. (2015)	Enzymatic and TEMPO-oxidized	Unbeaten bleached hardwood and softwood kraft	20	None	62
Kose, Yamaguchi, and Okayama, 2015	Mechanical	Unbeaten bleached softwood kraft	10	None	40
Mashkour et al. (2015)	Mechanical, (acetylated)	Unbeaten softwood	10	C-PAM	17
Adel et al. (in press)	Sodium hydroxide-sodium sulphite	Beaten rice, bagasse and cotton straw	30	None	24

S. Boufi et al. / Carbohydrate Polymers 154 (2016) 151–166 (sulphate) and sulphite processes to remove lignin wood components leaving intact the cellulose fibres in the form of individualized fibres. The kraft (sulphate) process is the most dominating chemical pulping process worldwide. Chemical pulps are generally used for fabrication of high-quality papers with high mechanical properties and better brightness in comparison to mechanical pulps (Smook, 2003).

The effect that CNFs have on bleached chemical pulps has been extensively studied. Both kraft and sulphite pulps from pine or eucalyptus are the main target of CNF additives to improve wet and dry strength properties. The addition of CNF into papermaking slurries presents several advantages over other traditional methods such as mechanical beating used to improve wet and dry strength of pulps. For example CNFs induce enhancement in tensile strength even at low concentrations (lower than 5% with respect to pulp); CNFs can be produced from almost any cellulose source and since they are usually present as aqueous suspensions, they can be added either into the pulp suspension at the beginning of the papermaking process or as a coating onto the final paper. CNFs can also be mixed with fibres, fillers, cationic polymers and other additives in order to modify paper's properties. The main advantage that CNFs offer over mechanical beating is that nanofibres do not damage the overall structure of fibres. In maintaining their original properties, fibres can extend their lifetime which is very interesting for paper recycling. By eliminating or reducing mechanical beating the formation of fines is also reduced. Though the reduction of dewatering is an issue that seemed to be a major drawback for CNF implementation at industrial levels, recent publications indicate that this problem can be overcome (Brodin & Eriksen, 2015).

In general, the presence of CNF improves the overall strength of paper, reduces its porosity and increases density. This enhancement in mechanical and physical properties is a direct consequence of the increase in specific surface area that CNF add when combined with papermaking suspensions. The boost in specific surface area promotes the formation of fibre–fibre bonds which consolidate the paper structure, increases density and overall tensile strength, rigidity and Z-strength. Light scattering is also modified after CNF addition. However, the enhancement effect depends on several factors such as amount of CNF added, degree of fibrillation of the CNF (available specific surface area), use and addition strategy of retention agents and pulp's refined degree (Brodin & Eriksen, 2015).

The presence and correct dosing of retention agents becomes necessary to ensure the adsorption of nanofibres onto the fibre's surface and to reduce drainage deterioration (Ahola, Österberg, & Laine, 2008; Su, Zhang, Batchelor, & Garnier, 2014; Taipale, Österberg, Nykänen, Ruokolainen, & Laine, 2010). The decrease in drainability is particularly high in CNF with high fibrillation degrees such as nanofibres obtained by TEMPO-mediated oxidation or carboxymethylation. The detrimental effect on drainage of CNF addition to pulp suspension is one of the major shortcomings limiting the use of CNF in papermaking. Drainage is a critical parameter in the paper manufacturing process since it limits the production efficiency of a paper machine. Assuming an inverse dependence between the drainage resistance and the square of the specific surface area (Hubbe & Heitmann, 2007), drainage rate is expected to drop at a faster rate than the linear one with the content of CNFs. Plugging of interfibre pores and reduction of sheet's permeability are the dominant mechanisms by which CNFs impact dewatering (Rantanen & Maloney, 2013).

One way to reduce the negative effect on drainage is to add an appropriate amount of retention aid to the pulp suspension. However, the effect is strongly dependent on the type and dosage of retention aid. In addition to its effect on drainage, retention aids were shown to have a crucial influence on the mechanical properties of paper. This effect was explained by the increase in the viscosity of the fibrous slurry and the decrease in the paper porosity during formation. Ahola et al. (2008) used a combination of CNF and a cationic polyelectrolyte (poly(amideamine)-epichlorohydrin (PAE)) to enhance the wet and dry strength of pulps. The addition strategy of the nanofibres and PAE did not affect the total adsorbed amount of PAE, but it had a strong effect on the distribution of the substances in the paper matrix which had a crucial effect on wet and dry strength properties of pulp. It was also shown that, by adding first PAE to the pulp slurry followed by CNF, a bilayer system forms where PAE adsorbs first on the fibre's surface followed by the nanofibres adsorption (Ahola et al., 2008). This mechanism induced improvements of up to 130% in tensile index of bleached pine kraft pulp. However, when PAE and CNF were mixed together before being added into the papermaking slurry, increase in tensile index was less significant. This was attributed to the formation of anionic nano-aggregates that did not adsorb well on the fibre's surface due to the lack of electrostatic attraction and uneven distribution on the surface.

Similarly, Taipale et al. (2010) investigated the effect that different types of CNFs and fines have on the drainage of kraft pulps and the strength of papers made thereof. Cationic poly-(diallyldimethylammonium) chloride (poly-DADMAC), cationic starch and three different types of polyacrylamides (C-PAM) were used this time as retention agents. Pulp drainage in the presence of CNF was carefully studied and the results demonstrated a linear increase of drainage time with respect to the amount of nanofibres added to the slurry. This fact was explained as the result of the increase in specific surface area due to the presence of CNF which boosted the formation of hydrogen bonds with water; decrease in the available pore area as well as lengthening of the capillaries required for water flow. The effect of pH was also considered as crucial in the water retention. Indeed, the ionization degree of carboxylated CNF strongly depended on pH and under pH 4, CNF became uncharged reducing the possibility of interaction with cationic retention agents. A pH over 6 was needed to favour the binding of the retention agents with CNF. The same author demonstrated that drainage rate in the presence of CNF could be optimized by modifying the CNF/retention agent composition and media conditions such as pH, salt concentration and type of CNF/retention agent used. It was found that a content of 30 mg CNF/g pulp is an optimum to ensure a significant improvement in paper strength without severe loss in drainage rate. Nevertheless, this optimum concentration is a system specific value, and depends on degree of beating, properties of CNF, ionic strength, pH and type of polyelectrolyte. The reinforcing effect of CNF was also confirmed by the improvement in the tensile index and Scott bond (fig. 1). A meaningful decrease in air permeability of paper along with an increase in its density was also noted in agreement with results reported elsewhere (Eriksen, Syverud, & Gregersen, 2008).

The properties of CNF, in terms of fibrillation degree, morphology and surface charges have an important effect on the final properties of the reinforced paper. CNF with high fibrillation degree, i.e: a high percentage of fibrillated material with width lower than 20 nm, brought a substantial improvement in paper's strength. Delgado-Aguilar, González et al. (2015) prepared CNF with a high pressure homogenizer using different pre-treatment methods (chemical, mechanical and enzymatic). The resulting CNF had different yields of fibrillation and specific surface area depending on the type of pre-treatment. These CNFs were added to bleached hardwood kraft pulp and the tensile tests of the ensuing paper revealed that the best improvements in strength were observed in CNF with high yield of fibrillation (>90%). However, the authors also demonstrated that CNF with lower yield of fibrillation and accordingly lower energy cost, are also able to bring about significant increase in tensile strength. In a similar experiment, Su, Mosse, Sharman, Batchelor, and Garnier (2013) also compared the effect of cellulose microfibrils (CMP) prepared by cryogenic milling

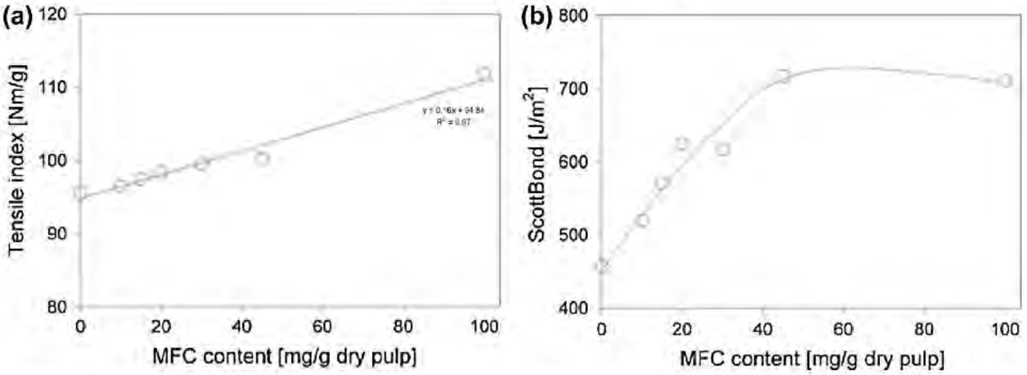

Fig. 1. Effect of CNF (MFC) content on the strength properties of paper, a tensile index and b out-of-plane strength. 30 min refined pulp was used and 15 mg/g dry pulp of cationic starch was added to retain the MFC (from Taipale et al., 2010).

and commercial nanofibres. CNF of nanometric scale produced better improvement in tensile strength and density in comparison to its micrometric counterpart. SEM pictures of the resulting papers are presented in Fig. 2.

Controlled refining of pulps is another way to amplify the effect of CNF addition on paper properties. Refining is a key step in paper engineering to impart to pulps the appropriate characteristics for papermaking (Gharehkhani et al., 2015). The main target of refining is to improve the bonding ability of fibres so that they form strong and smooth paper sheets with good printing properties. After refining, the fibres become collapsed, more flexible, shortened lengthwise, swollen in diameter and softened. Their surface area is increased to facilitate the binding of fibres in subsequent stages of the papermaking process.

The effect of refining treatment on the reinforcing potential of CNF in paper was investigated in several recent papers. González et al. (2012) prepared papers from refined and unrefined bleached eucalyptus kraft pulp reinforced with varying amounts of highly-fibrillated TEMPO-oxidized CNF. Refined and unrefined pulps showed similar increases in tensile index after addition of 3 wt% of CNF. However, by adding 6 wt% of CNF, unrefined pulps had higher increase in tensile index than refined ones.

Guimond, Chabot, Law, and Daneault (2010) also noted a similar trend in bleached pine pulps, suggesting that the increase in inter-fibre bonding potential of CNFs was somehow hindered by the increased influence of fibre fibrillation produced by mechanical refining. The presence of fines in refined pulps also might play a significant role in the interactions between CNF and fibres. For example, Ahola et al. (2008) reported increases of 130% with addition of CNF into a refined, fines-free pine pulp. In the same way, Potulski, Bolzon, Klock, and Sulato (2014) mentioned that bleached eucalyptus kraft pulp with a refining degree of 15°SR had an increase of 258% in tensile index, whereas pulps with 25°SR presented improvement of only 41% with the same amount of added CNF. The author ascribed these differences to the amount of fines formed in relation to the refining intensity, suggesting also that the combination of both refining and CNF addition should be carefully studied to find an optimum. Afra, Yousefi, Hadilam, and Nishino (2013) reported that CNF obtained from softwood using a disk grinding process produced similar increases in tensile strength when applied to bleached softwood and bagasse fibres with different beating degrees. Sehaqui, Zhou, and Berglund (2013) offered a different explanation on modest improvement of tensile strength when CNF are used on refined pulps. In his work, CNF was used as reinforcing additive for bleached softwood pulps refined at 1000, 2000 and 4000 PFI-revolutions in order to produce high-density papers. Refined pulps were added with 10 wt% CNF and some samples were also mixed with 5 wt% of xyloglucan. Mechanical tests demonstrated that refined pulps also developed an increase in tensile index after the addition of CNF. However, a plateau was found at approximately 100 N m/g, that is, pulps refined at 4000 PFI-revolutions did not show improved tensile index after the addition of CNF. According to the authors, this plateau represents the amount of bonded area when the paper sheet is so strongly bonded and that bonds are not broken when the paper is subjected to tensile forces but the fibres themselves are broken. The increase in density was also assessed and a good correlation between density and tensile strength increase was found.

González, Vilaseca et al. (2013) added TEMPO-oxidized CNF to previously enzymatically treated (biorefined) bleached eucalyptus kraft pulp. Cationic starch was used as a retention agent. Enzymatic treatment induced external fibrillation without fines production, which kept the drainage rate almost unmodified. This allowed the addition of CNF to the pulp slurry without strong deterioration of °SR but with important improvement in paper's strength (fig. 3). Even though biorefined pulp did not present significant amounts of fines, the increase after addition of 3 wt% of CNF was 53%, whereas unrefined pulps developed up to 73% of increase after the same addition of CNF. These results confirm that the rate of increase in tensile strength due to CNF addition is less significant in refined pulps than unrefined ones not only because of the presence of fines, which might form aggregates with nanofibres in the presence of cationic polymers, but also because the fibrillated surface in refined pulps does not interact. Overall, results from authors indicate that the increase in tensile strength is more significant in unrefined, fines-free pulps than in previously refined, chemical bleached pulps with high content of fines.

The incorporation of mineral fillers, such as calcium carbonate, kaolin or talc in paper has been a common practice for many years in order to change properties as texture, opacity, brightness, basis weight, dimensional stability and printability. The amounts of fillers vary from none to at least 30% of the whole furnish. Due to the surface charge of mineral filler and their low particle size, the presence of CNF in pulp suspension was shown to greatly affect the filler retention and the reinforcing potential of added CNF. The interaction of cellulose nanofibres and the filler during sheet forming was investigated by Ämmälä, Liimatainen, Burmeister, and Niinimäki (2013) and Diab et al. (2015). The first author studied the interactions between TEMPO and periodate-chlorite oxidized CNF with refined eucalyptus kraft pulp filled with ground calcium carbon-

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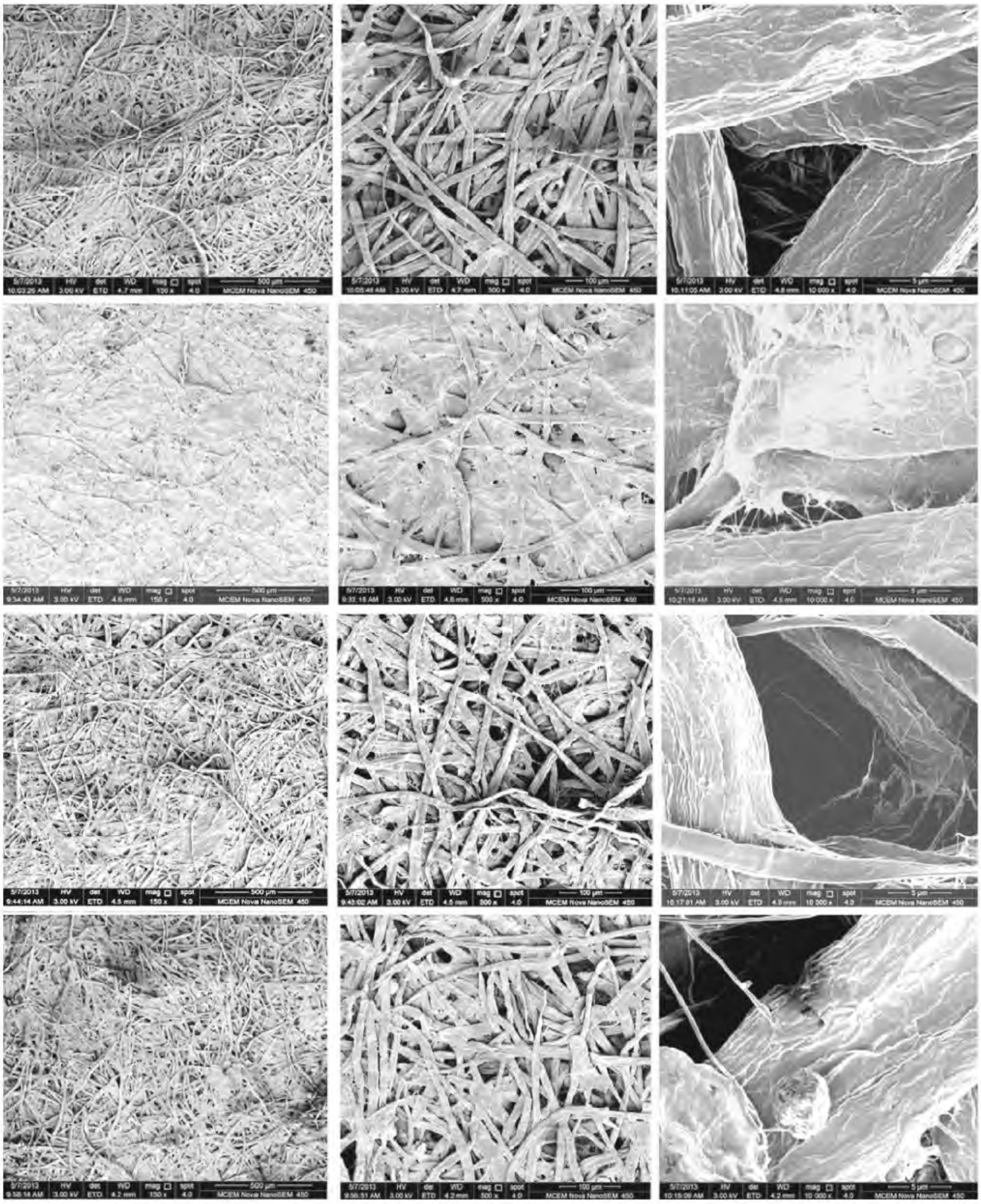

Fig. 2. Morphologies of paper engineered by cellulosic additives, examined by SEM of three different magnification (×150, ×500, ×10000). The bar represents a length of 500, 100 and 5 μm. From up to the bottom line: control sheet (hardwood fibre); addition of 100 mg MFC2/g hardwood fibre; addition of 100 mg MFC1/g hardwood fibre; addition of 100 mg CMPs/g hardwood fibre (from Su et al., 2014).

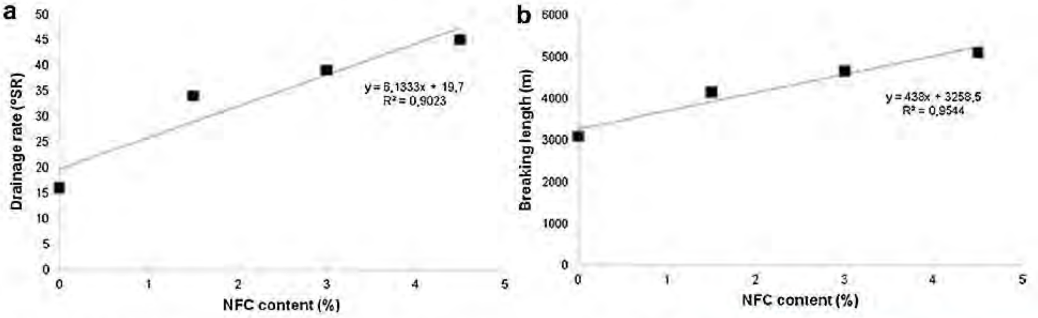

Fig. 3. Evolution of (a) drainage rate and (b) breaking length in enzyme-treated pulps reinforced with CNF (from González, Vilaseca et al., 2013; González, Alcalá et al., 2013).

ate (GCC). Results indicated that CNF caused agglomeration of GCC under slightly alkaline conditions, improving filler retention during sheet formation. The agglomeration tendency is postulated to arise from soluble divalent Ca^{2+} ions in solution due to the partial solution of GCC, which will assist the anionized CNFs in attaching cationic Ca^{2+} patches to the GCC surfaces and interconnecting particles to form larger agglomerates, all of which will increase filler retention. Thus CNFs seem to be potential retention aids for use in fine paper production instead of traditional polymers. However, though retention was improved, no increase in mechanical properties could be achieved, suggesting that, at such alkaline conditions, CNF might form aggregates with refined fibres which do not favour good sheet formation. Diab et al. (2015) developed cationized CNF by reaction of nanofibres with B-chloroethyldiethylamine followed by quaternization using methyl iodide. The authors used this cationized CNF as retention agents in softwood and bagasse pulps. Though cationized CNF improved retention of fillers without reducing drainage rate, its efficiency was still below that of cationic polymers such as cationic polyacrylamide (CPAM).

The effect of CNF on pulps run in the paper machine has also been assessed. Kajanto and Kosonen (2012) applied two different grades of CNF into machine-chest refined chemical pulp from a paper mill. The pulp was formed by a mixture of softwood and hardwood with no filler and cationic potato starch was used for retention. Under set conditions, good runnability, formation and total retention were reported for the trials. In general, density and strength properties increased significantly with CNF addition; a negative effect on opacity was measured, though this parameter depends upon final application. In a similar experiment, Ankerfors, Lindström, and Söderberg (2014) studied at pilot scale the behaviour of CNF-reinforced pulps with high filler content (up to 35 wt%). Cationic starch was also used as a retention agent. Similarly to what Kajanto and Kosonen reported, no runnability major issues were detected; pulp's drainability was reduced with CNF addition. However, the dry content after pressing was still higher for all CNF points and for the CNF/cationic starch point than the reference point. This was attributed to the high filler content that compensated the reduction of dewatering produced by CNF. Tensile properties were improved, mainly Z-strength and fracture toughness, which would allow an increase in the amount of filler.

Su et al. (2013) prepared papers from bleached hardwood pulp highly refined in a PFI-mill at 500, 1000, 2000, 5000 and 10000 revolutions; intense beating was supposed to produce extensive surface fibrillation of fibres in a way that most of the nanofibres would remain attached to the main fibres. Besides, unrefined pulps were reinforced with CNF at different ratios (5, 10, 25, 50 and 75 wt%); a set of samples was also added with polyamideamine-epichlorohydrin (PAE). Drainage rate, quality formation and mechanical tests were analysed. The intense beating effectively produced tethered CNF induced a reduction in paper's thickness at a constant basis weight. Porosity was also decreased. As other authors have reported, the addition of CNF brings about deterioration of the drainage rate, as well as increase in paper's density. Either free or tethered, CNF in combination with polyamideamine-epichlorohydrin (PAE) increased the dry strength and wet strength of papers by an order of magnitude.

Another interesting approach for CNF/wood pulp based papers was presented by Fang et al. (2013) who developed a bilayer hybrid, writable paper using unrefined wood fibres and CNF; the ensuing papers possessed high optical transmittance and superior smoothness and these properties depended on the amount of CNF added during the paper preparation. Furthermore, carbon nanotubes were deposited on the smoother side of a 60% CNF-reinforced paper, thus fabricating a transparent and conductive paper electrode with applications on flexible optoelectronic devices such as touch screens displays and solar cells. CNF-reinforced papers have also been covered as composites and their properties studied under the traditional parameters used in material sciences. Sehaqui, Allais, Zhou, and Berglund (2011) used softwood bleached pulp as a base for fabricating CNF-reinforced paper. CNF was prepared by enzymatic hydrolysis followed by microfluidization. Papers with 2, 5, 7 and 10% content of CNF were mechanically tested; the results demonstrate decrease in porosity and improved density as well as significant increases in tensile strength, strain to failure and Young's modulus in relation to the amount of CNF added. The most important increases were found in samples with 2 wt% of CNF and further additions showed more discrete improvement. The structure of these CNF-reinforced papers (named as biocomposites by the authors) was studied by FE-SEM microphotography and the images indicated that the presence of CNF led to smoother surfaces in comparison to non-reinforced papers; porosity was also reduced, which gave place to denser papers. Stress-strain curves indicated an improvement in ultimate tensile strength and strain to failure. Finally, thermomechanical properties showed increase in the storage modulus at very low temperatures, indicating that wood fibre/CNF composites can be very stiff under conditions of low molecular mobility.

3.2. Thermomechanical pulps (TMP)

Thermomechanical pulps are produced by heating woodchips with steam under high pressure followed by mechanical disintegration (Buzała et al., 2015). TMPs have more lignin and hemicellulose than kraft pulps and their composition is very similar to that of woodchips (Mörseburg & Chinga-Carrasco, 2009). TMP also contain some proteins and fatty acids (Buzała et al., 2015). Thermome-

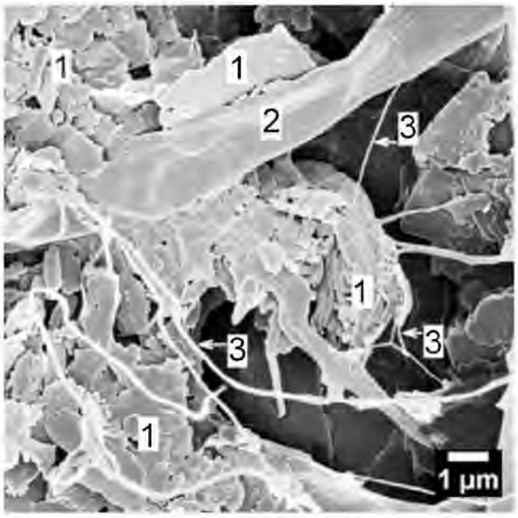

Fig. 4. Surface field emission-SEM image of the uncalendered sample showing the nano-sized fibrils interacting with the clay and fibrous material. The image shows 1 clay particles, 2 fibrillar material from the TMP and 3 nanofibres (from Mörseburg and Chinga-Carrasco, 2009).

chanical pulps present, in general, superior mechanical properties than mechanical pulps, still preserving the high-yield of mechanical pulps. Production of TMP consumes much more energy than mechanical pulping, but TMP shows longer fibres and lower fine content than mechanical pulps. As a result, papers fabricated from TMP are stronger than those from mechanical pulp.

The addition of CNF into TMP has been already assessed by several authors. Eriksen et al. (2008) studied CNF from kraft pulp as strength enhancer for TMP. The particle size of the CNF was mostly micrometric, so the term microfibrillated cellulose fits better the type of material used in that study. Handsheets with a 4% content of MFC were fabricated and mechanically tested; the tensile strength improved after the addition of MFC in comparison to samples without MFC. Further increase was achieved by raising the intensity of the mechanical disintegration of MFC. Besides, air resistance was also increased, whereas light scattering coefficient was reduced. Results for energy consumption during MFC preparation showed high energy consumption levels due to the lack of any adequate pretreatment of cellulose fibres before the homogenization/grinding process. Mörseburg and Chinga-Carrasco (2009) fabricated TMP-based sheets added with clay and CNF as fillers. The components were added in a way that TMP fibres constituted mostly the centre layer while fillers were deposited on the surface of the sheet. Formed sheets were subsequently calendered. Mechanical characterization indicated that the expected reduction in paper strength after clay addition can be compensated by appropriate CNF addition to specific layers in the z-direction. CNF was shown to bind mutually to clay filler and fibres. Furthermore, the studies on optical properties showed a significant increase in gloss relative to the purely fibre-based layered sheets. The presence of CNF clearly strengthened the adhesion between the clay filler and the fibres by developing a high number of particle contacts and potential bonding sites, while simultaneously maintaining a pore structure suitable for scattering light as shown in Fig. 4.

Hii, Gregersen, Chinga-Carrasco, and Eriksen (2012) analysed how CNF affects the pressability and properties of paper made of TMP and ground calcium carbonate. The negative effect that CNF has on the drainage rate of pulps was also reported, and this negative effect was increased when CNF with intense mechanical treatment was used as filler. However, mechanical properties showed important enhancement after the addition of CNF even when the amount of GCC was near 30%. The same authors also reported an increase in the z-direction strength when compared to neat TMP paper sheets or only GCC-filled ones. Long Gurley porosity test times where registered for CNF-reinforced papers, demonstrating that the presence of nanofibres improves packing and bonding of TMP fibres which leads to high air-pass resistance.

Osong, Norgren, and Engstrand (2014) compared the effect that ligno-cellulose nanofibres (LCNF) and cellulose nanofibres have on CTMP. LCNF are cellulose nanofibres that still contain small amounts of lignin and hemicelluloses that facilitate mechanical disintegration of cellulose fibres. The authors reported that LCNF improve overall paper's mechanical properties without significantly modifying sheet density. The maximum increase in tensile index (around 7%) for CTMP was achieved in papers with 15 wt% of LCN. Higher increases were observed in bleached kraft pulp (36% of improvement) with the same amount of LCNF. This relatively low increase in CTMP regarding the amount of LCN added was attributed by the author to the presence of fines in CTMP and the presence of lignin and hemicelluloses in the LCNF.

Hellström, Heihjnesson-Hultén, Paulsson, Hakansson, and Germgard (2014) fabricated CNF by various pre-treatments, namely Fenton's reagent, monocomponent endoglucanase and acidic hydrogen peroxide. The pulp used was bleached birch kraft pulp. After pre-treatment, the fibres were passed through a high-pressure homogenizer to release the nanofibres. The target fibre was spruce chemithermomechanical pulp to represent the middle ply of paperboard. Cationic starch and anionic colloidal silica solution were used as retention agents. The addition of 5% of Fenton pre-treated CNF produced the highest increase in mechanical properties, namely 35% in tensile index, 50% in Z-strength and 25% in tensile stiffness in comparison to non-reinforced papers. The authors also reported that this increase presented a very good correlation with paper's density and surface area and charge. Brodin and Eriksen (2015) also prepared LCNF from fractionated TMP and applied it to peroxide bleached, printing-grade TMP; either carboxymethylation or sulfonation was used as pre-treatment followed by high-pressure homogenization. Paper sheets were prepared in a sheet former with white water recirculation; LCNF-reinforced papers were formulated to contain 20 wt% of LCNF. Carboxymethylated LCNF produced more increase in dewatering times than sulfonated LCNF; LCNF-reinforced papers also became denser and less permeable to air. Both sulfonated and carboxymethylated LCNF fabricated from the TMP fines fraction improved tensile strength and light scattering; authors also found that carboxymethylated LCNF rendered the papers more brittle than its sulfonated counterpart.

3.3. *Mechanical pulps*

Mechanical pulping consists in reducing woodchips to fibres by mechanical grinding the fibres against a revolving stone, which grinds the woodchips into pulp by abrasive action. This method removes very small amounts of lignin which results in papers of low quality compared with papers from chemical pulps. Newer mechanical pulp methods include refiner mechanical pulping where wood chips are passed between two revolving discs. Pulps obtained by mechanical pulping are usually destined to cardboard, newsprint, paper towels, tissues. This process, along with thermomechanical pulping, yields about 90% of the starting material on a solids basis (Hellström et al., 2014). The effect that CNF as either bulk or coating additive has on stone ground wood (SGW) pulp was investigated by Delgado-Aguilar, Recas et al. (2015). In his work, SGW from hardwood was added to CNF fabricated through TEMPO-mediated oxidation, using cationic starch as a retention agent. Paper sheets were fabricated with a Rapid-Köthen sheet former and contained a maximum of 6 wt% of CNF. Authors discovered that addition of 4.5–6 wt% of CNF increased °SR from originally 34–58, equivalent to the same pulp refined at 1500 PFI-revolutions. However CNF-reinforced papers showed superior tensile strength compared to their refined counterparts. On the other hand, CNF applied in coating also improved paper's strength and rigidity, whereas porosity was significantly reduced.

3.4. Unbleached pulps

Suspensions of unbleached pulps and CNF have not been thoroughly investigated. Alcalá, González, Boufi, Vilaseca, and Mutjé (2013) analysed the effect of CNF prepared through TEMPO-mediated oxidation when applied as a bulk additive in papers based on unbleached eucalyptus pulp and using cationic starch and colloidal silica as retention agents. The amount of CNF varied from 0 to 3, 6, 9 and 12 wt%. The results indicated that tensile strength increase from 19.75 MPa in non-reinforced papers up to 53.20 MPa for samples with 12 wt% of CNF. When the rate of increase between samples is analysed, it was found that the most intense increase was seen in samples with 3 wt% of CNF (65% of increase). However, the rate of increase between samples with 3 and 6 wt% of CNF is lower (26%), finding its lowest value between 9 and 12 wt% of CNF (6.3% of increase). These results may indicate that, as more CNF are added, less specific surface area is available for CNF to be adsorbed onto the fibre's surface. As most of the available area is already bonded, tensile failure depends not only on the amount of bonds but also on the ultimate tensile strength of the fibres. Charani et al. (2013) produced CNF from unbleached kraft Kenaf bast fibre and unbleached Scotch pine fibre to add it into unbleached kraft hardwood pulp either as a bulk additive or as a coating. CNF were prepared by enzymatic pre-treatment followed by high-pressure homogenization. It is interesting to note that the authors reported that kenaf bast fibres suspensions could be passed at consistencies slightly superior than 5%, compared to 1–2% reported by most of the literature for wood fibres. Both kenaf and Scotch pine CNF produced similar increases in tensile strength when applied as bulk additives and the rate of increase was similar for all the samples.

3.5. Pulps from agricultural wastes

The use of agricultural and forest residues such as sugar cane bagasse, wheat, rapeseed and rice straw are interesting alternatives to wood as a source for the fabrication of paper and paperboard, especially in those countries where forest resources are less abundant; however, few works detail the effects that CNF produce in this type of fibres when they are used for papermaking applications. González, Alcalá, Arbat, Vilaseca, and Mutjé (2013) used CNF from TEMPO-oxidized eucalyptus fibres to reinforced chemithermomechanical pulp (CTMP) from rapeseed wastes for the manufacturing of fibreboard and liner paper grades. The addition of CNF varied from 0 to 3, 6 and 9 wt%, resulting in increase of tensile index up to 43.85 Nm/g for the 9 wt% sample with a °SR of 88. In order to overcome the deterioration of drainability, the authors opted to first apply a mild PFI-beating to the rapeseed CTMP followed by addition of small amounts of CNF, in an attempt to reduce the increase in °SR. The results indicated that, as in chemical bleached pulps, previously refined pulps developed minor increases in tensile strength compared to unrefined ones. Bagasse pulp has also been the target for CNF as a strengthening additive. Hassan et al. (2015) reported that the addition of CNF on enzyme-treated, bleached bagasse pulps positively affected both wet and dry strength of the pulp, but burst resistance and tear strength decreased with growing amounts of CNF. Authors observed positive effects on compactness and decrease in porosity (Fig. 5). Petroudy, Syverud, Chinga-Carrasco, Ghasemain, and Resalati (2014) also studied the effect of CNF on bagasse-based paper. In that work, small addition of CNF along with correct dosing of retention agent maintain the drainage rate without significant variations, whereas mechanical strength was improved. The main advantage in the addition of CNF into papermaking pulps from agricultural wastes lies on their lower cost and high availability.

Adel et al. (in press) produced nanofibres from rice, bagasse and cotton stalks through mechanical disintegration in a Masuko grinder and applied the resulting CNF into refined papermaking slurries from the same types of agricultural residues. The amount of CNF applied in bulk varied from 5 to 30 wt%. The reinforcing effect of the nanofibres was compared to that of bleached softwood fibres. In that work the pulps were previously refined before CNF addition in order to improve interactions between nanofibres and fibres. The authors reported that the addition of 30 wt% of CNF induced higher increase in breaking length in comparison to softwood fibres which are commonly used as reinforcing in Egypt's papermaking industry.

3.6. Deinked pulp

Delgado-Aguilar et al. (2014) reported on the addition of CNF from TEMPO-oxidized bleached fibres into deinked pulps fabricated from deinked old magazines and newspapers. Results indicated that only 1.5 wt% of CNF was necessary to achieve higher tensile strength and stiffness than paper from refined pulp with similar freeness and water retention value. The addition of more CNF further improved mechanical strength, allowing the recovery of some of the strength properties of the original pulp. Papers also became stiffer, denser and less porous with the presence of CNF. One of the main advantages of adding CNF to recycled paper is that they allow eliminating the beating process traditionally applied to recycled pulps to recover some of their properties. This process damages the fibre, reducing its lifetime.

4. Mechanism of CNF strenghtening

The strength of paper is largely dependent on the number of fibre to fibre bonds during consolidation and drying of the fibre network. During the drying process, fibres come close together under the effect of Laplace pressure arising from the curvature of the meniscus of the liquid bridge in fibre boundary. The resulting hydrogen bonding between the bounded areas under molecular contact increase the cohesion between fibres in contact. Different factors are known to affect the paper strength such as (i) Fibre strength and length, (ii) specific bond strength and strength of bonded area, (iii) sheet formation, and (iv) distribution-residual stresses. Any intervention favouring one of these factors is likely to enhance the strength of the paper.

Currently this can be done in several ways such as refining (Hubbe, 2006; Molin & Daniel, 2004), the addition of wet or dry strengthening agents (Ahola et al., 2008; Taipale et al., 2010) and chemical modification of the fibre (Minor, Atalla, & Harten, 1991). In all of these approaches, a chemical additive is added or a physical treatment is applied to promote the bonding capacity of fibres. The strengthening contribution of CNF may be explained through two possible mechanisms; in the first one, CNF act as an adhesion promoter by bridging adjacent fibres and favouring the fibre–fibre bonding and increasing the bonded area (fig. 6); in the second one, CNF may generate a different network embedded among larger fibres that contributes to boost the load-bearing capacity of the paper. In fact since hydrogen bonding requires close proximity between neighbouring hydroxyl groups less than 0.35 nm before

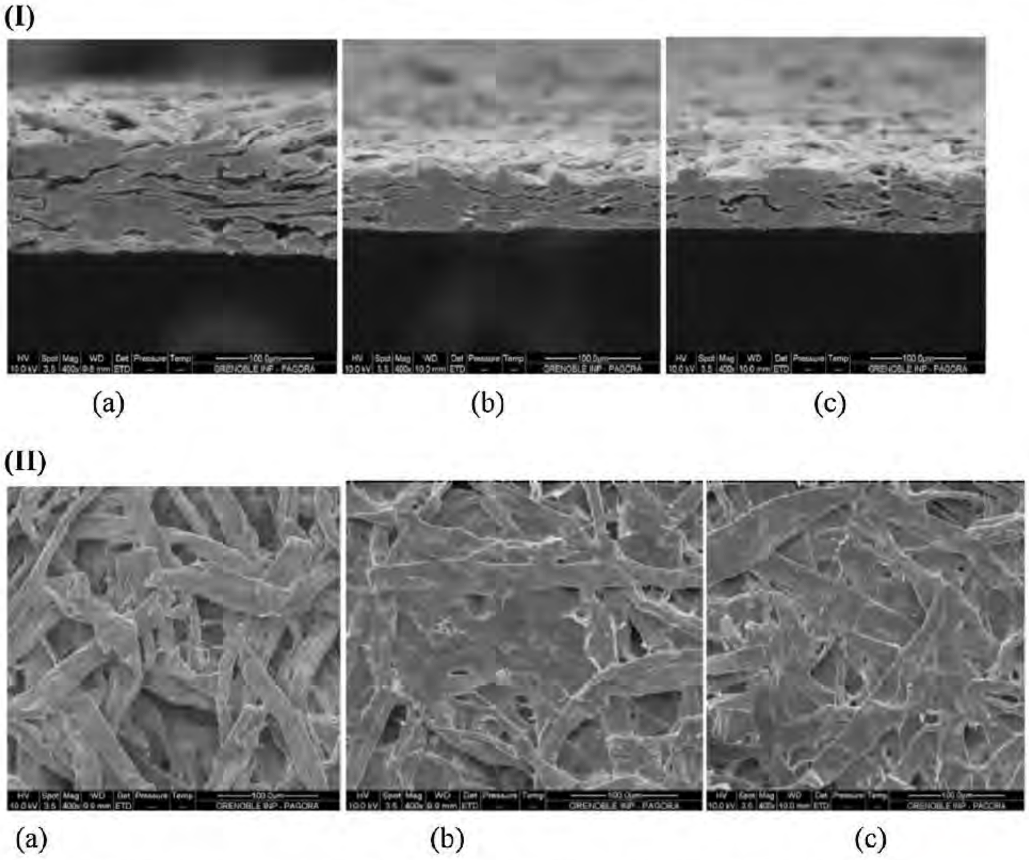

Fig. 5. SEM of paper sheet edges (I) and surfaces (II) prepared using: (a) softwood fibres, (b) softwood + 20% MFC, and (c) softwood + 20% TMFC (from Hassan et al., 2015).

such bonding can develop, the presence of CNF with micro sized length contributed to increase the bonded area by bridging adjoining fibres and via entangled network formed by the nanosized cellulose fibrils. Moreover, given the similar structure between fibres and CNF, we expect a strong affinity between fibres and CNF networks. As a result, the final paper strength is a contribution of both fibres and CNF network in an additive manner. The effect of CNF addition might be viewed as similar to the effect produced by beating.

5. Modified CNF as additive in papermaking

The chemical modification of CNF is a useful approach to change the properties of cellulose and to impart new properties to the nanofibres in terms of hydrophobicity, wettability, compatibility and possibility of interaction with a host matrix. So far, the usefulness of modified CNF in papermaking was tackled only in two recent papers (Mashkour, Afra, Resalati, & Mashkour, 2015; Missoum, Martoïa, Belgacem, & Bras, 2013). Missoum et al. (2013) studied the effect of addition of Alkyl Ketene Dimer (AKD) modified CNF on the mechanical and barrier properties of paper. CNF was prepared by enzymatic pre-treatment of bleached wood pulp (Spruce and Pinus in 60/40% proportion) followed by mechanical disintegration in a Masuko Grinder (10 passes between the rotating and the static stones at 1500 rpm). The chemical modification consisted in grafting AKD using an environmentally friendly approach based on nanoemulsion concept CNF. The resulting modified CNF was added to the pulp slurry in quantities ranging from 5 to 50% of treated and untreated CNF. Interestingly, no retention agent was used to avoid the loss of CNF during the filtration process. CNF retention rate was studied and results indicated that beyond a given concentration, CNF cannot retain in the pulp slurry, regardless of CNF type used. Highest retention was found in samples with only 5% CNF content and samples with modified CNF presented the highest retention rate. Density was also improved, particularly in papers with unmodified CNF. Mechanical and water absorption properties were also improved in comparison with papers without any kind of CNF, and samples with unmodified nanofibres presented the best results. The main finding of this work is to highlight the possibility of application of AKD-modified CNF as an internal sizing agent to produce paper with good barrier property against water. The paper reinforced with 50% of CNF or with 50% of modified CNF, the fibres seem to be more packed together thanks to the nanofibres acting as a binder in paper (Fig. 7).

Hubbe (2006) evaluated the effect of adding acetylated CNF and fibres into a bleached softwood pulp. CNF was produced using an ultra-fine friction grinder. The fibres were modified using heterogeneous acetylation process. C-PAM was used as retention

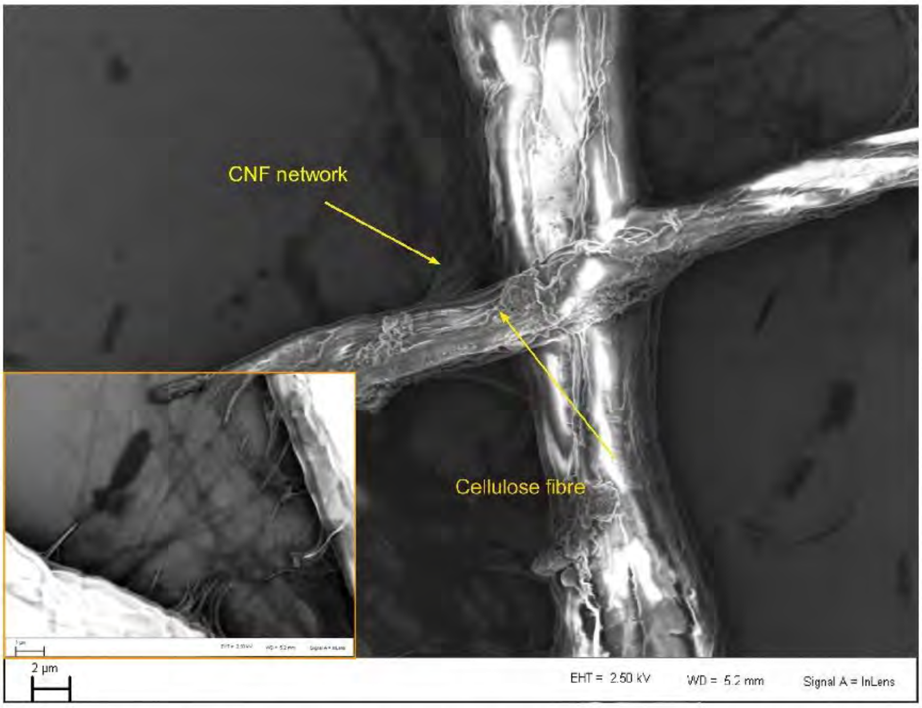

Fig. 6. FE-SEM microphotography showing how CNF are bounded to larger cellulose fibres. The smaller picture represents a zoom in of two adjacent fibres with a CNF network partially filling up the gap between the fibres (from Boufi, González and Mutjé, unpublished data).

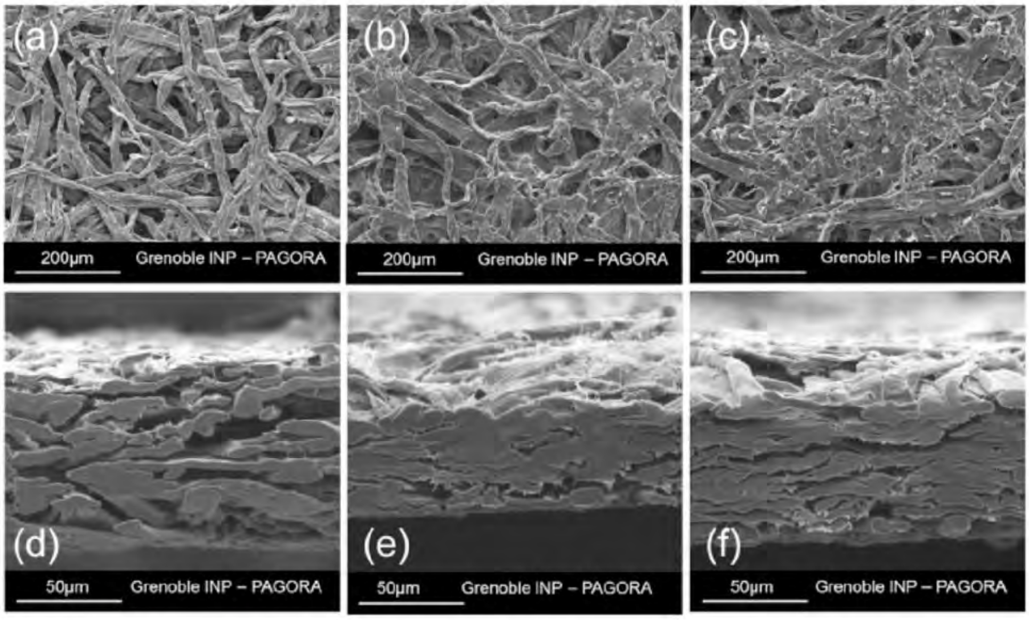

Fig. 7. SEM images of surface paper reinforced (a) without CNF (reference), (b) with CNF, (c) with modified CNF and cross section of (d) without CNF, (e) with CNF and (f) with modified CNF (from Missoum et al., 2013).

agent. The results indicated that non-acetylated CNF improved density, burst strength, tensile strength and air resistance of paper without considerably affecting the water absorption of paper, whereas acetylated CNF, when used as pulp additive instead of CNF, decreased water absorption about 23%. Moreover, acetylated CNF also increased air and water barrier properties.

6. CNF as a coating material in papermaking

The CNF films and coatings are promising for potential applications as transparent and biodegradable packaging films with high barrier properties. One of the first advantages of this approach is that dewatering issues can be completely avoided when coatings are added after paper fabrication. Coatings in paper can be applied by several techniques such as bar coating, roll coating, spray coating and size press coating. Such techniques allow varying the amount of coating material applied to paper: Lavoine, Desloges, and Bras (2011) studied the application of CNF on paper's surface through bar coating and size press coating. Results indicated that ten layers of CNF applied by bar coating reached a coating weight of 14 g m^{-2}, whereas size press allowed only 3 g m^{-2} of CNF. Aulin, Gällstedt, and Lindström (2010) and Aulin and Ström (2013) have reported a huge improvement in barrier properties (oxygen permeability and oil resistance) when paper was coated with a thin layer of microfibrillated cellulose (MFC) using a rod coater for sheets. The air permeability decreased from 69 000 to 4.8 nm/Pa for the unbleached paper and from 660 to 0.2 nm/Pa for greaseproof paper. The coat weight was about 1.3 and 1 g m^{-1} for the unbleached and greaseproof paper, respectively.

Based on FE-SEM observation, it was proposed that the reduced surface porosity induced by fibrils was on the origin of the superior oil barrier properties (Fig. 8). Syverud and Stenius (2009) compared the tensile index and barrier properties of fibre-oriented papers from unrefined softwood pulp coated with different amounts of CNF (up to 8%) prepared only by shearing disintegration. Tensile index was studied, showing a discreet increase from 35 N m/g for the uncoated sample to 40 N m/g for papers with 8% of CNF coat. However, the barrier properties presented a significant boost after the application of CNF, varying from 6.5 to 360 nm Pa^{-1} s^{-1} in samples with 0 and 8% CNF respectively. This reduction in air permeability was explained as a consequence of the reduced surface porosity induced by nanofibres. In a similar line, Hult, Iotti, and Lenes (2010) used CNF in combination with shellac to form a coat on paper and paperboard based substrates using a bar coater or a spray coating technique. The air permeability of both paper and paperboard was substantially decreased with a multilayer coating of MFC and shellac. Oxygen transmission rate and water vapor transmission also presented important decreases, making these new coated papers interesting for high barrier packaging.

Lavoine, Bras, and Desloges (2012) applied CNF on cardboard using bar coating technique and analysed the effects on bending stiffness, compressive strength and barrier properties. Authors found that the coating technique has two opposite consequences: it negatively affected the structure cohesion of cardboard which led to decreasing in compressive strength. However, the presence of CNF layers improved bending stiffness due to an increase in cardboard's thickness. In a way, CNF counterbalanced the negative effect that the coating process had on cardboard since CNF induced enhancement of 30% in bending stiffness and compressive strength in machine direction. Very low enhancement was detected in barrier properties of cardboard after CNF coating. Water absorption was also found to be higher in CNF-coated cardboard.

Surface application of CNF foams has also been proposed. The main advantage of applying foam is that it allows obtaining low coating weights by spreading a thicker coating layer. The preparation of CNF foams has been investigated by Kinnunen, Hjelt, Kenttä, and Forsström (2016) who prepared foams from a mixture of 2.9% CNF and an anionic surfactant mixed in a foam generator with compressed air, producing thus stable foams with a content of 80–95% of air. After applying this foam on paper, surface properties were modified such as lower air permeability, increased smoothness and lower contact angle.

Yungchang F. Chin and Yan Feng published in 2014 the patent US 20140050922 A1 for coating and coated paper. In that patent authors developed a paper coating based on CNF, pigment, latex and an auxiliary additive such as starch, lubricants, defoamers, etc. in water suspension. The coat formed on the paper's surface if reported to improve the fracture resistance of the paper, reduce the risk of heavy coat cracking and mechanical failure of the coat such as spallation, blistering, tearing, and peeling away from the paper substrate. Furthermore, the coating composition containing CNF is reported to also reduce the roughness of the coated paper and make the surface of the coated paper much smoother. Authors also informed that papers with such coating as high-grade printing paper and wrapping paper for food and cosmetics.

7. Conclusions

The use of CNFs as additives for papermaking slurries has demonstrated to be an effective alternative to improve paper's overall properties. After CNF addition, paper's strength and density are highly increased, whereas porosity and opacity are reduced and these modifications can be tuned by controlling the amount and degree of fibrillation of CNFs added. CNF content between 1 and 10 wt% in paper sheets already present enhancement in mechanical properties comparable to what is achieved by traditional mechanical beating. The use of cationic polymers as retention agents guarantees the fixation of CNF onto the fibre's surface, helping also to balance the expected deterioration in drainability at high CNF content. The addition of CNF into pulp slurries increases the bonding capacity of fibres by adding more specific surface area to the suspension which translates into a boost of hydrogen bonds between fibres. CNFs have been also investigated as a way to compensate the loss in strength in papers containing mineral fillers.

Chemical hardwood and softwood pulps have been the main target for CNFs as strength enhancer. The best reinforcing effect is observed in fines-free, unrefined pulps, using cationic polymers as retention agents. CNF-reinforced TMP has also been extensively studied; the addition of CNF in this type of pulps did not produce significant improvements in paper's strength when compared to chemical pulps. Probably, the higher content of fines in TMP in comparison to chemical pulps somehow diminishes the effect of CNF in TMP. The use of CNF in agricultural wastes also stands as an alternative for increasing the strength of papers fabricated from such wastes, mainly in countries without access to large forest resources. In the case of deinked pulps, the addition of CNF can reduce or completely eliminate the need of applying mechanical refining to already deteriorated fibres. The use of CNF as coatings applied to already formed sheets of paper has not been comprehensively explored in comparison to bulk application. However, results indicate that paper's strength is also improved and porosity strongly decreases too when CNF are applied by bar coating or size pressing. The application of CNF suspensions as sprays or foams has also been proposed. The production of films fully made of CNF has given rise to papers with mechanical, optical and barrier properties that surpass those of ordinary paper. CNF films are usually prepared after methodologies similar to those used for papermaking at laboratory scale. Casting and evaporation is also extensively used. This type of papers present high tensile strength and stiffness and can show high transparency which makes them suitable for applica-

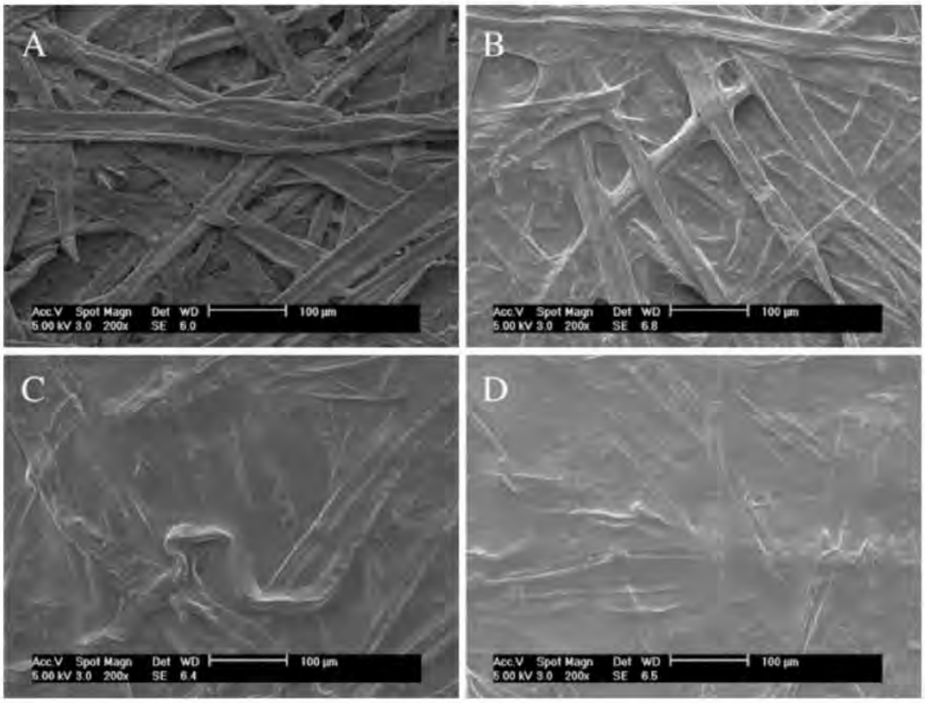

Fig. 8. FE-SEM micrographs of uncoated (a) and CNF-coated unbleached papers with coat weights of ca. 0.9 (b), 1.3 (c) and 1.8 g/m2 (d), respectively. The scale bar is 100 lm (from Aulin et al., 2010).

tions in optical and electronic devices. They have also proved to be promising materials where high barrier properties are required which makes them suitable for packaging applications and filter systems.

In spite of all the potential that CNF show for papermaking applications, some crucial issues remain to be clarified. For example, though pilot-scaled papermaking reports indicate no major complications in runnability of CNF-reinforced pulps, more research at this level becomes necessary to fully understand the implications of incorporating CNF into industrial-scaled papermaking. Another major point of interest is the cost of CNF manufacturing. Though modern pre-treatments of fibres have strongly reduced the cost of CNF in comparison with the first reports on their fabrication, it still should be decisively demonstrated that CNF can be competitive regarding production costs and product requirement in front of traditional methodologies used in papermaking, mainly mechanical refining. The investment of incorporating new equipment for CNF production and application in the paper machine should also be considered.

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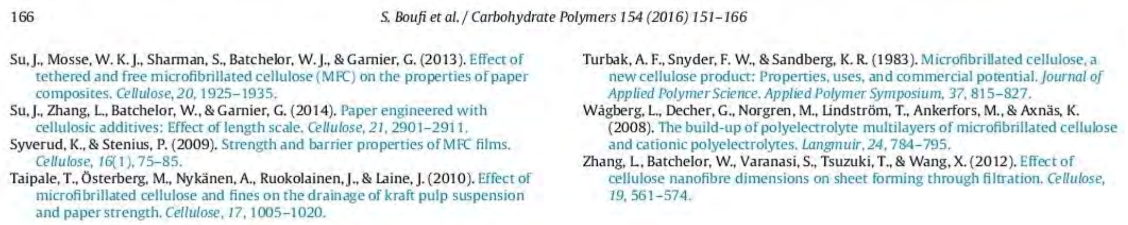

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3.7. Artículo VII

Cardboard boxes as raw material for high-performance papers through the implementation of alternative technologies: more than closing the loop

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Abstract

The aim of this work is to develop high-performance papers from recovered cardboard boxes. To tackle this objective, cardboard boxes were enzymatically refined and subsequently reinforced with CNF. Results revealed that although cardboard boxes are usually used for test-liner paper production, they could be used for high-performance applications. In this sense, stronger papers than common test-liner were produced through the use of harmless techniques for fibers. In terms of circular economy, the proposed combined approach goes further than closing the loop: new markets can be achieved or lighter papers could be used, leading to significant resources savings.

Keywords: enzymatic refining; enzymatic hydrolysis; cellulose nanofibers; recycled fibers; mechanical properties

1 Introduction

Paper recycling is becoming one of the main activities of paper mills (Delgado-Aguilar, et al., 2015a; Delgado-Aguilar, et al., 2015b; Hubbe, 2014). In this sense, 54 % of the worldwide fibers used for paper production come from recovered papers (Delgado-Aguilar, et al., 2015a; Hubbe, 2014; Pöyry, 2011), and in some paper grades, such as containerboard and newsprints, the rate of recycled fibers can be even higher (Delgado-Aguilar, et al., 2015d). Moreover, several strategies to enhance mechanical properties, decrease the paper basis weight, or replace fibers for fillers, are currently under investigation. The environmental awareness of society, together with petroleum depletion, has resulted in an intensive use of paper as substitute of oil-based materials in several sectors during last years. Although cellulose is a renewable and abundant raw material, society is not consuming it sustainably (M. a. Hubbe, 2014), and this is why recycling is gaining interest and prominence. However, some processes involved in paper recycling cause fiber deterioration due to hornification phenomena, reducing thus life span of paper products. Traditionally, this loss is compensated submitting the fibers to a mechanical refining stage, promoting again swelling and, thus, recovering bonding capacity. However, this process causes irreversible structural damages that, in the long term, also reduces paper products' life span (M. A. Hubbe, Venditti, & Rojas, 2007). The deterioration of fibers and the increase of fines fraction involve in turn a reduction on pulp suspension dewatering. Therefore, the search of new fiber treatments to reduce or replace mechanical refining has been one of the main challenges during the last years. This search is aimed by giving to recycled slurries the capacity to produce papers with the technical requirements by different approaches such is the case of using virgin fibers from agroforestry waste (Hurter, 2002a, 2002b), enzymatic refining, adding nanocelluloses, or new chemical-based bonding strategies (M. a. Hubbe, 2014).

One of the most relevant methodologies has been the incorporation of cellulose nanofibers (CNF) into the pulp suspensions (Boufi et al., 2016; Brodin, Gregersen, & Syverud, 2014; González et al., 2012). Several investigations proved the incorporation of cellulose nanofibers as an effective treatment to improve mechanical properties of paper (Ahola et al., 2008; Brodin et al., 2014; Delgado-Aguilar et al., 2015c; Taipale et al., 2010; Tarrés et al., 2016b). Moreover, cellulose nanofibers do not cause morphological damages on fibers, fact that leads to an increase on the number of the recycling cycles (Afra et al., 2013; Delgado-Aguilar, et al., 2015d). Many of the studies have used TEMPO-mediated CNF (Saito, Kimura, Nishiyama, & Isogai, 2007) but, however, recent works have shown the effectivity of enzymatic hydrolysis or mechanical fibrillation as an alternative to produce CNF in terms of paper reinforcing potential (Henriksson et al., 2007; Pääkko et al., 2007; Tarrés et al., 2016b; Delgado-Aguilar, 2016c; Espinosa et al., 2015).

Another approach is the addition of endo-β-1,4-glucanases in paper slurries to increase the swelling of fibers and, thus, their bonding capacity. Enzymes were already known in paper industry for pulping or deinking purposes. Several previous studies (Delgado-

Aguilar, et al., 2015a; González et al., 2013) showed that enzymatic refining significantly increased the mechanical properties of paper with no negative effects on pulp drainability.

For all the above, the aim is to develop high-performance papers from recovered cardboard boxes by means of producing recycled pulp, enzymatically refine it and, finally, to incorporate cellulose nanofibers in bulk. The effects on ultimate paper properties and pulp drainability will be studied. In addition, the obtained results will be extrapolated to a real papermaking process using existing data from a specific paper mill.

2 Materials and methods

2.1 Materials

Saica Natur Noroeste S.A. (Zaragoza, Spain) kindly provided different papers and pulps from their manufacturing process (Figure 1), including: pulp from the head box (3), paper from before the size press (2) and end product (liner-grade paper at pope, 1). These materials were used to determine the process parameters and the minimum requirements of the produced papers.

Figure 1 around here

Used cardboard boxes were kindly supplied by Gros Mercat S.A. (Spain). Enzymatic refining was carried out with a commercial endo-β-1,4-glucanase enzyme cocktail (Serzym 50), supplied by SERTEC-20 S.L. (Spain), with an activity of 84,000 CMU/g at 60 °C and at pH 4.8 over a carboxymethylcellulose (CMC) substrate. Cellulose nanofibers were prepared from bleached kraft hardwood pulp (BKHP), kindly supplied by Torraspapel S.A. (Spain), and its enzymatic hydrolysis was performed with another enzyme cocktail (Novozyme 476) provided by Novozymes A/S (Denmark), containing 2 % of endo-β-1,4-glucanases with an activity factor of 4500 CNF-CA/g of cellulose (tested over a CMC substrate). All the reagents required for the experimentation of this work were acquired at Sigma Aldrich (Spain) and were used as received.

2.2 Cellulose nanofibers production

Enzymatic hydrolysis was carried out according to the methodology reported by Tarrés et al. (2016b). BKHP was dispersed at 1.5 wt% in water in a laboratory pulper for 30 minutes at 3,000 rpm. Then, the fibers were filtered until 10 wt% consistency and refined in a PFI mill for 4,000 revolutions. This process was carried out to swell the fibers and thus to promote the activity of enzymes. Briefly, refined fibers were suspended in water again (until reaching a pulp consistency of 5 wt%), and 0,1 N HCl was dropped until achieving a pH of 5. Then, the suspension was heated until 50 °C under constant stirring to avoid temperature gradients. At this step, the enzyme cocktail was dropped into the suspension and stirred for 4 hours. The enzymatic process was stopped by heating the suspension to 80 °C for 15 minutes, where the enzyme used suffers its denaturation. Enzyme dosage was set at 320 g/Tn. The enzymatically hydrolyzed pulp then was washed with distilled water and kept at 4 °C.

The treated suspension (1 wt% consistency) was gradually homogenized in a PANDA Plus laboratory homogenizer (Gea Niro Soavi, Italy) following the sequence of three passes at 300 bar, three passes at 600 bar and three passes at 900 bar. The main reason of increasing gradually the pressure was to avoid clogging in the pressure chambers.

2.3 Cardboard boxes pulping

Used cardboard boxes were first torn in small pieces to be disintegrated in a pilot scale pulper equipped with a helicoidal rotor, with a maximum capacity of 50 L of suspension. In our experiment, 2 kg (dry weight) were disintegrated at 10 wt% consistency at 60 °C during 20 minutes in presence of 1 % of sodium hydroxide to further promote fiber individualization. The rotational speed of the rotor was set at 1100 rpm. Finally, excess water was removed by centrifugation until achieving a consistency of 25 wt% and the pulp was stored in hermetic plastic bags at 4 °C.

2.4 Mechanical refining

Cardboard boxes pulp at 10 wt% was refined in a PFI mill (NPFI 02 Metrotec S.A.) at 500, 1000 and 1500 revolutions.

2.5 Enzymatic refining

Enzymatic refining was performed according to Delgado-Aguilar et al., 2015d, taking advantage of the process optimization reported in that work. In this sense, the enzyme dosage was set at 350 g/Tn of dry pulp at 5 wt% consistency at pH 4.8 and stirred for 30 minutes, maintaining the temperature at 60 ± 3 °C.

2.6 Cellulose nanofibers addition

Enzymatically refined pulp was dispersed in water in a pulp disintegrator for 90 minutes at 3000 rpm. CNF were added to the slurry during this disintegration process. The amount of CNF was calculated to obtain 1.5, 3 and 4.5 % CNF-reinforced paper sheets. After disintegration, pulps were stirred for 30 minutes at 500 rpm in presence of 0.5 % and 0.8 % of cationic starch and colloidal silica, respectively. These two last additives were added as retention agents. The neat paper, without CNF, did not contain neither cationic starch nor colloidal silica.

2.7 Pulps characterization

All cardboard boxes pulps, including neat, mechanically refined, enzymatically refined and containing CNF were characterized in terms of morphology and drainability.

The morphological analysis was carried out using a MorFi Compact analyzer (TechPap, France) equipped with a CCD video camera. About 30,000 fibers were analyzed by the software MorFi v9.2. Among other parameters, this software was able to calculate mean fiber length, mean diameter and fines percentage (fibers shorter than 76 μm). All characterizations were performed in triplicate.

Drainability was measured by means of Schopper – Riegler degree (°SR), which was determined in a Schopper – Riegler Tester (mod. 95587 PTI) following ISO standard 5267-1.

2.8 Paper production and characterization

Paper sheets were prepared in a Rapid-Köthen sheet former (ISP mod. 786 FH) according to ISO 5269-2 and conditioned at 23 °C and 50 of relative humidity for 48 hours before testing, following ISO 187. Nonetheless, basis weight was set at 120 g/m^2 to make the laboratory papers comparable to those supplied by Saica Natur Noroeste S.A.

Breaking length and Young's modulus were obtained from tensile testing. Testing experiments were carried out in a Hounsfield 42 universal testing machine equipped with 2.5 kN load cell and at constant elongation rate (ISO 1924-2). The gap between clamping jaws was 150 mm and cross head speed was set at 20 mm/min. The test started with a preload of 0.1 N. Breaking length was calculated as described by Tarrés et al. (2017) and Young's modulus as the relationship between stress and strain at the elastic region of the material, automatically calculated by the software. In the case of commercial papers, these tests were performed both at machine and cross direction.

Air permeability was obtained with a Gurley porosimeter (Papelquímia). Testing was performed according to ISO 5636-5. Results were expressed in seconds, meaning the required time to pass through the paper 100 cm^3 of air at certain pressure.

Internal bonding was determined in an internal bond tester (mod. IBT 10A IDM). Both samples dimensions and testing conditions were set according to TAPPI standard T569.

3 Results and discussion

3.1 Process parameters and minimum requirements

It is well known that there are several differences between paper manufacturing process at laboratory scale and large scale. Among others, fiber orientation and starch coating at the size press are two parameters strongly influencing the ultimate paper strength. This effect cannot be observed at laboratory scale with an isotropic paper sheet former, which works in static conditions, and fibers are randomly oriented and distributed. In this sense, as described above, three samples from the manufacturing process were taken and characterized in terms of some physic-mechanical properties. Thus, taking into account the properties of paper produced with the pulp from the head box (stage 3, Figure 1), one can set the minimum requirements that the pulp produced at laboratory scale must accomplish.

The first sample was the end product (stage 1, Figure 1), a test-liner paper grade. Breaking length (BL), Young's modulus (E), internal bond (IB) and air permeability (AP) were measured. The two tensile-depending properties were measured both at machine and cross direction.

Table 1 around here

Table 1 shows that there is a great difference if both the breaking length and the Young's modulus are measured at machine and at cross direction. As mentioned above, this difference comes from the fiber orientation during paper formation due to the speed differential between the inlet flow (from the head box) and the cloth (dewatering stage). Thus, the breaking length of the paper measured at machine direction is 2.25 times higher than at cross direction. The same trend is observed in the values of the Young's modulus. When papers are tested at cross direction, fibers are not performing all their strength and paper tends to break at lower stress (but higher strain and thus, lower Young's modulus) than when they are tested at machine direction (Delgado-Aguilar et al., 2015a).

Internal bonding and air permeability were measured as well to set the target values to be achieved.

The effect of the starch coating at the size press was determined by comparing the papers at pope (stage 1, Figure 1) with the papers just after formation and before the size press (stage 2, Figure 1).

The obtained breaking length at machine direction was 4943 m and at cross direction, 2194 m. If these two values are compared, one can see that the relation between machine and cross direction is approximately the same. The same effect can be observed in the Young's modulus values. Comparing the obtained values of this uncoated papers with the commercial test-liner paper, one can see that the effect of starch can be quantified in approximately an increase of 12%. The rest of the properties were affected in the same sense. Air permeability, as expected, was increased (less time during testing). When paper is coated, starch not only remains on its surface, but also penetrates into it closing/covering some pores of the paper. This effect on the porosity has been previously reported and it was quantified as well at laboratory scale (Tarrés, Delgado-Aguilar, et al., 2016).

Finally, in order to assess the effect of fiber orientation on tensile properties of paper, pulp from the head box (stage 3, Figure 1) was used to produce paper sheets at laboratory scale. The obtained papers were tested in the same way and results are shown in Table 1.

As it is possible to see, the uncoated paper reached a breaking length value at machine direction 1.45 times higher than the one obtained at laboratory scale with the fibers randomly oriented. Regarding the rest of the properties, with the exception of air permeability, experienced the same effect. However, is widely known that the orientation factor is associated to each paper machine and the paper production conditions (differential speed between pulp inlet and the cloth, flowrate and pulp consistency at the head box, etc ...) and, of course, on the pulp composition. As has been reported by several authors (Laine, Mattsson, & Swerin, 2004; Lindström & Swerin, 2015; Tanaka, Swerin, & Ödberg, 1993), paper properties can also affected by fibers flocculation due to the addition of polyelectrolytes. The addition of retention agents, together with cellulose nanofibers, can create fiber bundles, which directly affects to the slimness of the compounds, hindering thus orientation. In this sense, the increase of 45% of breaking length between isotropic paper sheets and paper machine papers is only indicative and cannot be used as a benchmark.

Paper products, once recycled, present lower properties than the original one. These lower properties come mainly from hornification phenomena during recycling loops (Fernandes Diniz, Gil, & Castro, 2004). According to Delgado-Aguilar et al. (2015b), this loss can be quantified in 11 % for recycled pulps.

3.2 Effect of mechanical refining on cardboard boxes

Cardboard boxes are usually made of test-liner and fluting paper. If these boxes, at the end of their life, are wanted to be reintroduced in the loop, some strategies must be adopted to recover the original mechanical properties. One way to do this after paper recycling involves a stage of mechanical refining of the pulp. Depending on the intensity of this treatment, higher mechanical properties can be achieved but, at the same time, fibers suffer severer structural damages. Table 2 shows the results of applying different refining intensities to the disintegrated cardboard boxes.

Table 2 around here

As the refining intensity was increased, mechanical properties were improved. However, in terms of morphology, the amount of fines was increased and, at the same time, the mean fiber length was decreased due to shearing during the treatment. Besides this, the drainability of the pulp suspension dramatically worsened at higher PFI revolutions; exemplarily, when pulp was refined at 1500 PFI revolutions, the loss on drainability was 17 °SR, while the increase on breaking length was 42 % (from 3064 to 4356 m). The internal bonding strength and the Young's modulus were also noticeably upgraded. The increase of the mechanical properties comes from the fiber morphology modification, external fibrillation and swelling. These changes on fibers morphology also produce a significant increase on the specific surface of fibers, leading to higher bonding capacity thereof and, thus, decreasing the porosity of paper sheets due to higher fibers compaction.

When pulp was refined at 500 PFI revolutions, 3438 m of breaking length were achieved. Taking into account that there is no fiber orientation, together with the anisotropic factor found above (1.45) and the starch contribution (12 %), one could predict a breaking length of 5570 m of the final paper at the pope (step 1, Figure 1). Therefore, this refining intensity would be enough to bring the mechanical properties to the commercial requirements. Thus, one could admit that higher levels of mechanical refining would allow to play with the reduction of the basis weight or the incorporation of mineral fillers in order to obtain the same mechanical requirements with fewer amounts of fibers, leading to raw material savings and, thus, contributing both to the economy and environment.

Another consequence of mechanical refining is the fiber deterioration and fiber morphology changes during the process. This damage on fibers limits the recycling loops of paper. The less recyclability of paper produced by mechanical refining, would lead to a reduction of fiber permanence on technosphere and so increasing the consumption of natural resources (Delgado-Aguilar et al., 2015b).

3.3 Effect of enzymatic refining and cellulose nanofibers (CNF) addition on cardboard boxes

New strategies are needed to avoid the deficiencies caused by mechanical refining and to improve the properties of recycled papers. One possibility is based on enzymatically refining the pulps followed by the addition of CNF into the cardboard slurry. It is assumed that the use of enzymes will prevent fiber deterioration during the refining process (Delgado-Aguilar et al., 2015c; González et al., 2013). In the same trend, CNF prepared by enzymatic hydrolysis are aimed to help in promoting mechanical properties while preventing other negative effects. The effects of enzymatically refining and adding CNF to the recycled cardboard boxes are reflected in Table 3.

Table 3 around here

The first finding, as compared to untreated slurry, is that enzymatic refining produced an increase on the mechanical properties of the same magnitude than a refining intensity of 1500 PFI revolutions, while losing only 2.5 degrees of drainability and keeping intact the mean fiber length and the percentage of fines in the recycled suspension. This is remarkable considering that the classic mechanical refining produced a loss of 17°SR to get this mechanical performance (breaking length and Young’s modulus). In this sense, the effect on the fiber morphology and fiber damaging caused by mechanical refining becomes apparent. Contrarily to what shown for mechanical refining (Table 2), enzyme treatment neither caused any shortening of the mean fiber length nor generated significant amount of fines. The morphology of the original and recycled fibers after mechanical refining and enzymatic refining was observed by FE-SEM (Figure 2). On the other hand, González et al., 2013 reported the effect of enzymatically refining virgin bleached kraft eucalyptus suspensions, where an increase of 64 % on breaking length was achieved. This improvement is significantly higher than the obtained in the present work, which accounted for 46 %. This difference is understandable if the results reported by Delgado-Aguilar et al., 2015d are considered. In that case, one-to-one deinked old newspapers and magazines pulp was subjected to enzymatic refining with the same conditions, obtaining an increase on breaking length of 34 %. As it is possible to see, origin of the fibers and the structural damages that already have due to recycling processes are key in the properties enhancement that enzymes can impart. However, the enzyme dosage was found to have lower impact on the resulting properties of paper, since 300 g/Tn increased the breaking length on 26 % and 400 g/Tn, in 47 %, being just 1 % higher than in the case of 350 g/Tn.

Figure 2 around here

Comparing Figure 2a and Figure 2b, the effect of mechanical refining can be clearly seen. After 1000 revolutions in the PFI mill, even this equipment is less harmful than large scale refiners, higher external fibrillation can be observed, leading to rougher fiber surface and promoting bonding between fibers. However, as explained above, mechanical refining causes irreversible structural damages, fact that in the following recycling cycles will limit the strength of paper. On the other hand, endoglucanases usually act on the amorphous part of the cellulose chain, breaking the beta-1-4 bonds, generating external fibrillation and fibrils in the paper slurry. Momeni 2014 proposed a mechanism of interaction between fibers and endoglucanases, which consists on cellulose chain surrounding, accessing thus the amorphous part thereof. In this sense, pronounced external fibrillation may hinder the interactions between fibers and enzymes.

Comparatively, Figure 2c shows the paper made of enzymatically refined cardboard pulp. Apparently, there is minimal effect on fiber morphology after enzymatic treatment and the fibers seem to preserve their surface smoothness. However, denser papers can be observed, fact that indicates that fibers are more compacted and, thus, more strongly bonded.

Together with enzymatic refining, the effect of adding CNF was also investigated. Different amounts of enzymatic CNF were added into the enzymatically treated cardboard slurry. This combination has been previously reported both for virgin and recycled fibers (Delgado-aguilar et al., 2015a; González et al., 2013). As Table 3 shows, 6689 m of breaking length (isotropic formation) can be achieved by the addition of 4.5 % of CNF with a drainability of 64 °SR. Although this drainability is 3 °SR worse than the 1500 revolutions refined pulp, the breaking length is significantly higher (1.54 times higher). In addition, comparing this value with those reported by Delgado-Aguilar et al. (2015a), where old newspapers and old magazines were used, a pulp with such drainability presented the half of the tensile strength. Something interesting of adding CNF to paper slurries is the fact that mechanical properties are significantly increased but no structural damages are caused. In this sense, the combination of enzymatic refining and CNF addition are a clear alternative to those conventional processes, where less properties are achieved, life span is significantly lower and drainabiltiy is of the same magnitude.

Taking into consideration the process parameters calculated above, the obtained results can be used to predict results at large scale. Therefore, it is possible to estimate the theoretical values of breaking length that a paper produced with the treatments reported above would have at the pope (stage 1, Figure 1). Results are shown in Figure 3.

Figure 3 around here

With these assumptions, the breaking length of the papers made of enzymatically refined pulp at pope 7264 m, about 31% over the commercial requirements (5534 m), used as reference. Thus, the use of enzymatic refining as a way to improve/recover the mechanical properties of paper is a strong alternative to mechanical refining, since similar mechanical performance can be achieved, with better drainability and without structural damages on fibers. In addition, if 4.5 % of CNF are added in combination with enzymatic refining, almost 11000 m of breaking length can be achieved. The use of this combined technique could allow to produce paper for applications with higher requirements or to adopt strategies for resources saving, such as decreasing basis weights and/or increasing the amount of mineral fillers in paper products, facts that would decrease the production costs of paper. However, there are still some issues that may be addressed. On the one hand, the poor drainability of the enzymatically treated pulp with 4.5 % of CNF must be solved through the introduction of drainage agents, fact that, at the same time, may slightly decrease the mechanical performance of papers. On the other, upscaling of these processes must be studied, taking into account residence times, operational costs and efficient retention systems at large scale.

4 Conclusions

In this work, a recycling strategy based on enzymatic refining and the use of enzymatic-CNF has been successfully used for the recovery of cardboard boxes avoiding any mechanical refining. Hereinafter are the main conclusions:

- The positive effect of enzymatic refining on recycled slurries is remarkable; it improves the mechanical properties of recycled paper, with low impact on pulp drainability and keeps the mean fiber length and fines in the recycled slurry. In the applied conditions.
- Enzymatic refining and CNF addition have been successfully combined. The combination of enzymatic refining and 4.5 % CNF addition could lead to papers with almost 11000 m of breaking length (MD), with 64 °SR.
- The current recycling strategy allows tailoring papers of desired characteristics depending of market requirements or runnability of the industrial process.
- The implementation of this process can contribute to extend the life span of recovered fibers and to reduce the use of virgin fibers; according the this, thinner papers (lower basis weight), or papers with higher amount of mineral fillers can be designed. All these would contribute to increase the recycling loops of recovered fibers and to the preserve fresh natural resources.

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Table

Table 1. Physic-mechanical properties of the commercial test-liner at different points of paper machine

	G (g/m^2)	BL_{MD} (m)	BL_{CD} (m)	E_{MD} (GPa)	E_{CD} (GPa)	IB (J/m^2)	AP (s)
Final paper	120	5534 ± 54	2457 ± 81	4.7 ± 0.1	1.8 ± 0.1	245 ± 12	25.7 ± 3
Uncoated	116	4943 ± 42	2194 ± 65	4.1 ± 0.2	1.6 ± 0.1	223 ± 18	21.2 ± 2
Isotropic	120	3401 ± 29		2.2 ± 0.3		202 ± 13	20.1 ± 1

G: basis weight; MD: machine direction; CD: cross direction

Table 2. Physic-mechanical properties of paper made of cardboard boxes at different refining intensities.

R. Intensity (REV)	°SR	BL (m)	E (GPa)	IB (J/m^2)	AP (s)	l_w^f* (μm)	Fines** (%)
0	44 ± 1	3064 ± 41	4.3 ± 0.1	165 ± 15	14 ± 1	755 ± 21	45.9 ± 0.4
500	47 ± 0	3438 ± 51	4.7 ± 0.1	208 ± 18	26 ± 3	711 ± 31	52.0 ± 0.6
1000	53 ± 1	3877 ± 39	5.0 ± 0.2	311 ± 26	34 ± 1	672 ± 22	55.8 ± 0.2
1500	61 ± 1	4356 ± 48	5.8 ± 0.1	397 ± 37	71 ± 4	603 ± 30	58.2 ± 0.3

** Mean fiber length weighted in length; **Percentage of fines, in length*

Table 3. Physic-mechanical properties of paper made of cardboard boxes after enzymatic refining and CNF addition

	CNF (%)	°SR	BL (m)	E (GPa)	IB (J/m^2)	AP (s)	l_w^f* (μm)	Fines** (%)
Untr	0	44 ± 1	3064 ± 41	4.3 ± 0.1	165 ± 15	14 ± 1	755 ± 21	45.9 ± 0.4
Enz.Tr	0	47 ± 1	4473 ± 55	5.8 ± 0.1	372 ± 21	62 ± 3	752 ± 33	46.2 ± 0.2
	1.5	52 ± 1	5123 ± 37	5.8 ± 0.1	425 ± 33	98 ± 2	751 ± 24	46.1 ± 0.2
	3.0	59 ± 1	6038 ± 61	5.8 ± 0.1	467 ± 24	124 ± 2	751 ± 22	46.4 ± 0.3
	4.5	64 ± 1	6689 ± 60	5.9 ± 0.2	502 ± 18	177 ± 4	748 ± 19	46.7 ± 0.4

** Mean fiber length weighted in length; **Percentage of fines, in length*

Figure

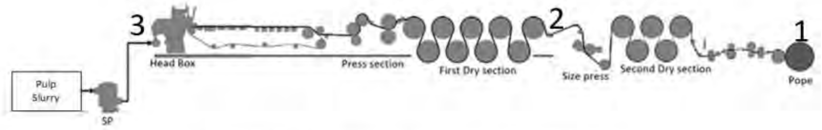


Figure 1. Liner paper manufacturing process

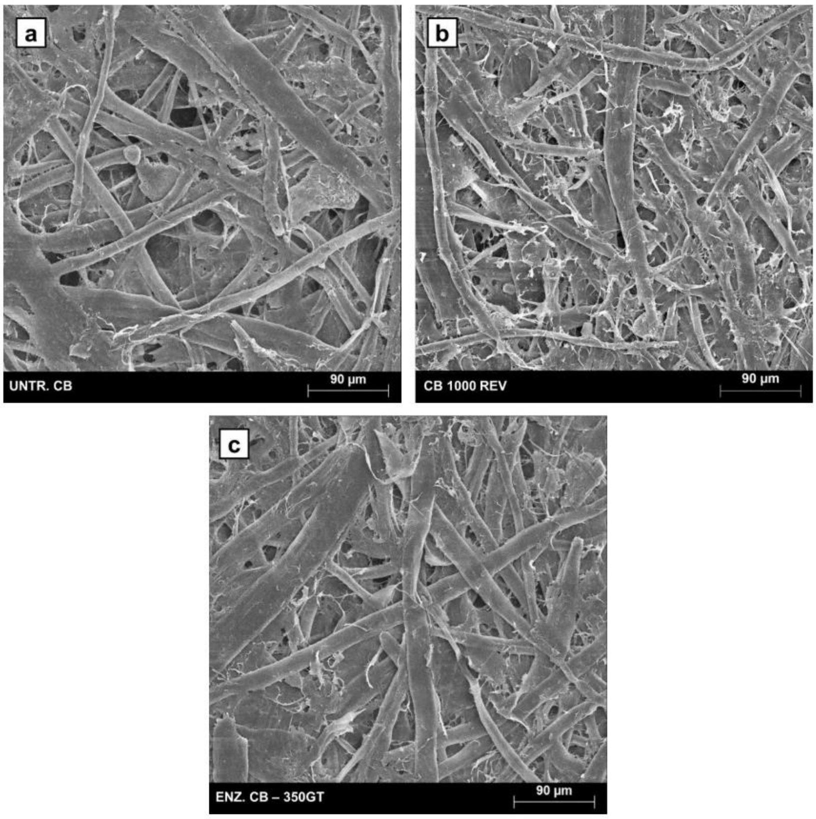


Figure 2. FE-SEM micrographs of paper made of (a) untreated recycled cardboard pulp; (b) refined cardboard pulp, and (c) enzymatically refined cardboard pulp

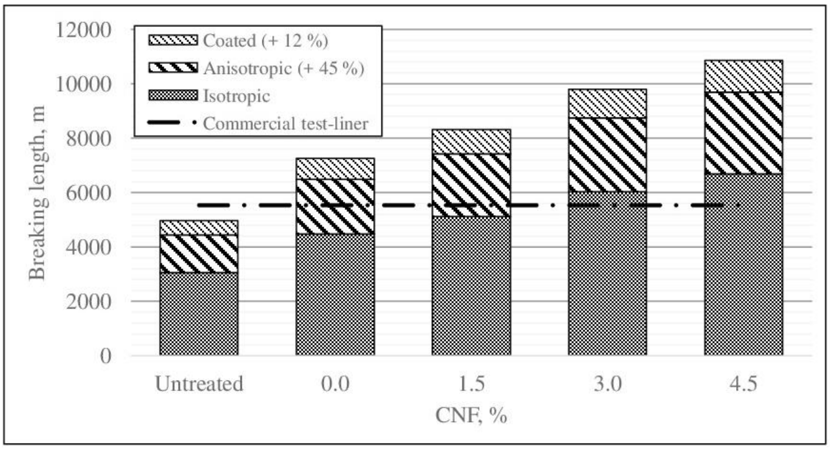
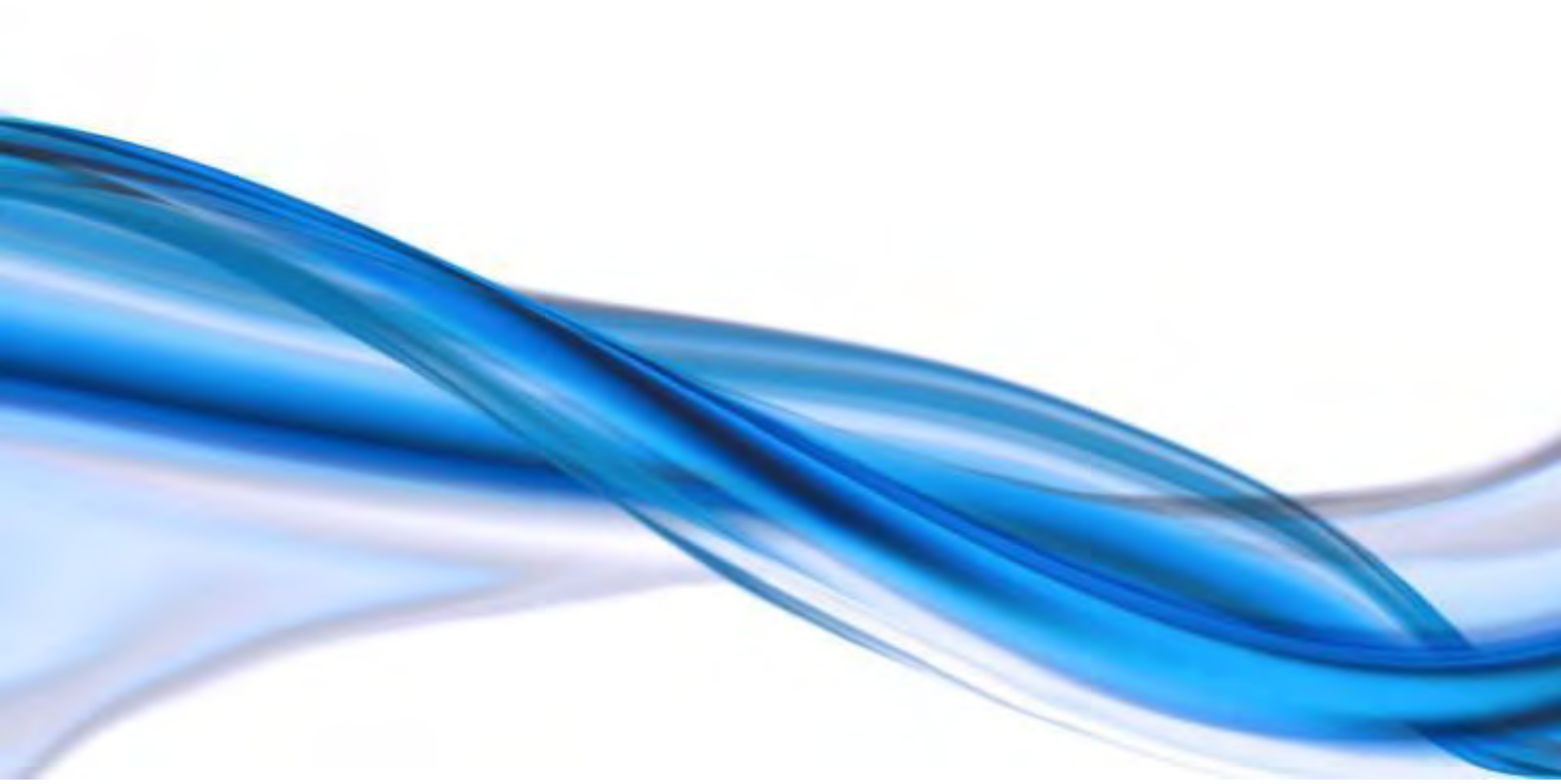


Figure 3. Estimated breaking length of papers at pope

Discusión general de resultados



4. Discusión general de resultados

4.1. Optimización de las CNF enzimáticas

El primer artículo publicado para la presente tesis investiga la optimización en la fabricación de nanofibras de celulosa a través de un proceso de hidrólisis enzimática seguida de homogenización a alta presión. El rendimiento de estas nanofibras aplicadas como refuerzo para pastas papeleras es posteriormente estudiado. La modificación de las condiciones de pretratamiento de las CNF permitió producir nanofibras con propiedades optimizadas. La optimización de las nanofibras se definió como su capacidad para inducir mejoras en las propiedades mecánicas de las hojas de papel donde se aplicaron. La tabla 1 muestra los cambios de pH y consistencia de las pastas durante el tratamiento y el incremento en longitud de ruptura inducido en el papel donde se aplicó. Las mejoras más significativas se observaron cuando las fibras fueron pretratadas a una consistencia del 5 %, en particular con un pH de 5, lo cual produjo un incremento en la longitud a ruptura del 86 %.

Tabla 1: Efecto de la consistencia y pH de la reacción sobre la calidad de las CNF, la cual fue determinada como incremento en la longitud de ruptura. Tiempo de contacto 2 H y carga enzimática de 160 gr/t.

Número de tratamiento	**Consistencia de la pasta** (%)	**pH**	**Incremento longitud a ruptura** (%)
1	3	7	55
2	3	5	65
3	5	7	66
4	5	5	86

A partir de estos resultados, la optimización de las CNF se continuó mediante la modificación de la cantidad de enzima añadida y el tiempo de contacto entre esta y las fibras. La tabla 2 muestra las propiedades de las CNF obtenidas mediante distintos tiempos de contacto y dosis de enzimas.

Tabla 2: Caracterización de las CNF obtenidas tras diferentes tiempos de contacto y carga enzimática.

Dosis de enzima (g/t)	**Tiempo** (h)	**Grado de polimerización**	**Demanda catiónica** (μeq·g/g)	**Rendimiento** (%)	**Transmitancia a 800nm** (%)
80	2	450	175	14.2	28.7
	3	431	179	19.5	30.4
	4	412	187	20.5	31.5
160	2	419	183	16.3	28.7
	3	346	215	20.1	30.0
	4	320	255	29.4	34.5
240	2	317	252	27.9	30.0
	3	315	253	28.9	33.6
	4	307	258	34.0	38.9
320	2	324	246	29.2	31.9
	3	318	250	28.7	32.1
	4	309	255	38.9	36.1

En la tabla 2 se observa que la evolución del grado de polimerización (DP) está estrechamente relacionado tanto con el tiempo de contacto entre la fibra y la enzima así como con la concentración de la primera. En general los resultados indican que, a una misma carga enzimática, el DP depende del tiempo de contacto con la enzima. Los DP más altos se observaron en fibras con cargas enzimáticas de 80 g/t y tiempos de contacto que variaron entre 2 y 4 horas. Al duplicar la carga enzimática y mantener los mismos tiempos, el DP se redujo aún más; sin embargo, el incremento de la carga enzimática a partir de este punto ya no produjo reducciones significativas en el DP. La demanda catiónica mostró una evolución similar, siendo menor en muestras con poca carga enzimática y tiempos de contacto de 2 horas; a partir de cargas enzimáticas de 240 g/t la demanda catiónica se mantiene con pocas variaciones significativas, siendo la más alta la obtenida en muestras tratadas con 240 g/t de enzima y 4 horas de contacto. En cuanto al rendimiento de nanofibrilación, nuevamente las muestras CNF-80/2, 80/3 y 80/4 ofrecieron los porcentajes más bajos de todo el conjunto. Mayores cargas indujeron mejores rendimientos, siendo los más altos los ofrecidos por la muestra CNF-320/4 con casi un 40 %. La mínima transmitancia a 800 nm se registró en la muestra CNF-80/2 (28.7 %) mientras que la muestra CNF-240/4 mostró valores de casi 40 % de transmitancia. Es interesante señalar que una mayor carga enzimática ya no produjo posteriores incrementos en la transmitancia con los mismos tiempos de contacto.

Aunque no se muestra en la tabla, los análisis para determinar la cantidad de grupos carboxílicos indicaron un valor alrededor de 76 μeq g/g para todas las muestras preparadas. Si se tiene en cuenta que la literatura consultada indica que altos rendimientos de fibrilación y demanda catiónica son indicios de una alta superficie específica, entonces es de esperar que un estudio de este parámetro indique una evolución positiva del mismo al aumentar la carga enzimática y los tiempos de contacto. La figura 5 muestra los resultados de superficie específica para cada muestra de CNF.

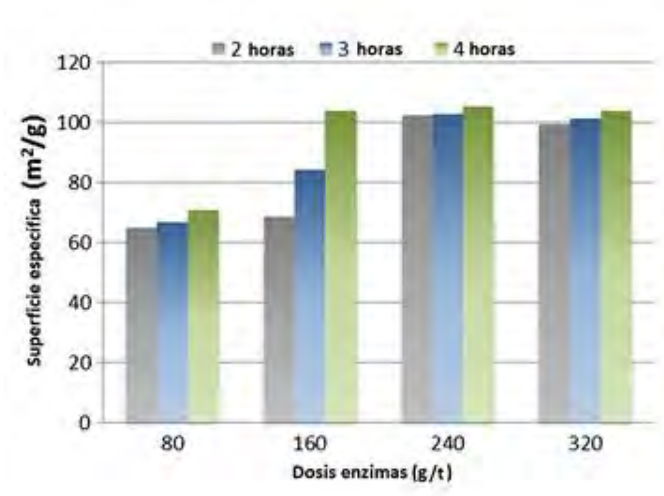


Figura 5: Evolución de la superficie específica de las suspensiones de CNF en función de la dosis enzimática y el tiempo de contacto.

La reducción del diámetro promedio de las CNF es una consecuencia esperada con el incremento de la superficie específica. Los cálculos realizados al respecto indicaron que las CNF con superficies específicas de unos 100 m^2/g presentan diámetros de unos 24 nm aproximadamente.

4.2. Aplicación de las CNF enzimáticas en suspensiones papeleras

Incremento en la superficie específica de las suspensiones de fibras celulósicas es una propiedad deseada en pastas papeleras donde se requieren mejoras en las propiedades mecánicas del papel. Por lo tanto, es de esperar que la adición de nanofibras de celulosa con alta superficie específica induzca un incremento en la resistencia de un papel. La figura 6 muestra como la longitud a ruptura evoluciona en relación con el incremento en la carga enzimática y el tiempo de contacto.

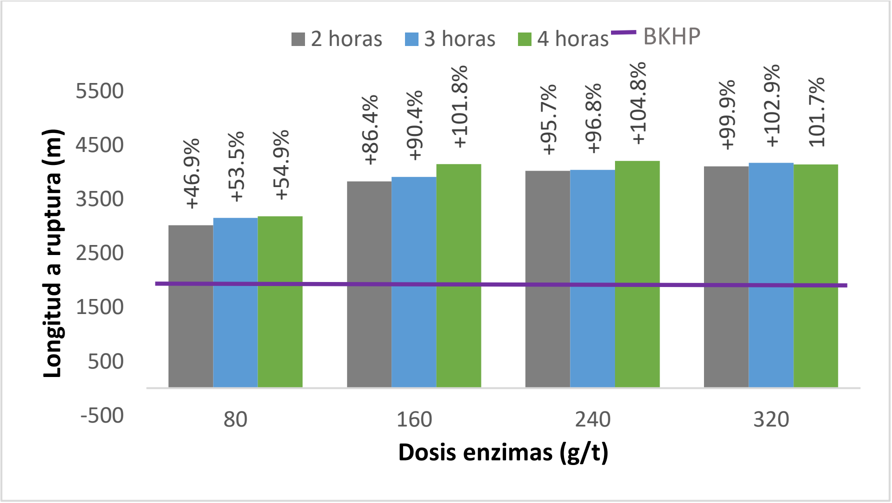


Figura 6: Evolución de la longitud a ruptura de papeles reforzados con CNF tratadas con distintas cargas enzimáticas y tiempos de contacto. Los porcentajes indican el incremento en relación con el papel original con una longitud a ruptura inicial de 2054 m.

El incremento en el tiempo de contacto y la carga enzimática producen efectivamente mayor superficie específica en las CNF y, por tanto, un aumento en la cantidad de puentes de hidrógeno que pueden formarse entre fibras. La formación de dichos puentes es el principal mecanismo responsable de la resistencia del papel. La figura 5 muestra que los incrementos más importantes dependen mucho más de la carga enzimática que el tiempo de contacto, ya que las mejoras en longitud de ruptura son menos significativas que cuando se comparan cargas distintas. No obstante a partir de 160 g de enzima por tonelada de pasta, los incrementos se mantienen más o menos constantes. Esto se debe a que, a partir de cierto punto, el aumento del área enlazada entre fibras (relative bonded area) ya no se incrementa ya que toda el área disponible ha sido ya enlazada; esto conlleva a un estancamiento en la progresión de la longitud a ruptura. Es por esto que las CNF preparadas a partir de la oxidación TEMPO, las cuales suelen tener superficies específicas mayores, aumentan de manera más significativa la resistencia del papel. No obstante, es bien sabido el fuerte deterioro que las CNF TEMPO producen en la capacidad de drenaje de las pastas papeleras, mientras que en el caso de las CNF enzimáticas esta reducción de la drenabilidad es mucho más moderada.

Las propiedades de las CNF las convierten en una alternativa interesante al refino mecánico que la industria papelera aplica a las pastas celulósicas como método para incrementar la resistencia del papel. Dicho refino, dependiendo de su intensidad, incrementa de manera considerable la superficie específica de las fibras al provocar fibrilación externa de las mismas, a la vez que incrementa su flexibilidad. Sin embargo, el refino mecánico presenta también inconvenientes tales como reducción en la longitud de las fibras, producción de finos y en general deterioro de las pastas, lo que puede reducir de manera significativa las propiedades originales de la fibra y por tu tanto su reaprovechamiento. Esto se hace más crítico si se tiene en cuenta que en el futuro se exigirá un aprovechamiento más sostenible de los recursos forestales que son la principal fuente de pastas celulósicas para la producción de papel. Es por estas razones que el uso de las CNF representa una alternativa interesante al refino mecánico tradicional ya que pueden ayudar a reducir la cantidad de refino aplicado, lo que ayudaría a preservar las propiedades originales de las fibras y por tanto incrementar la vida útil de las mismas.

Para evaluar la efectividad de las CNF en pastas papeleras a nivel industrial, se procedió a la aplicación de un 3 % de CNF en pastas refinadas industrialmente a dos distintas intensidades: 75 y 135 kW/t de pasta. La influencia sobre la longitud a ruptura del papel fabricado a partir de las pastas reforzadas se muestra en la figura 7.

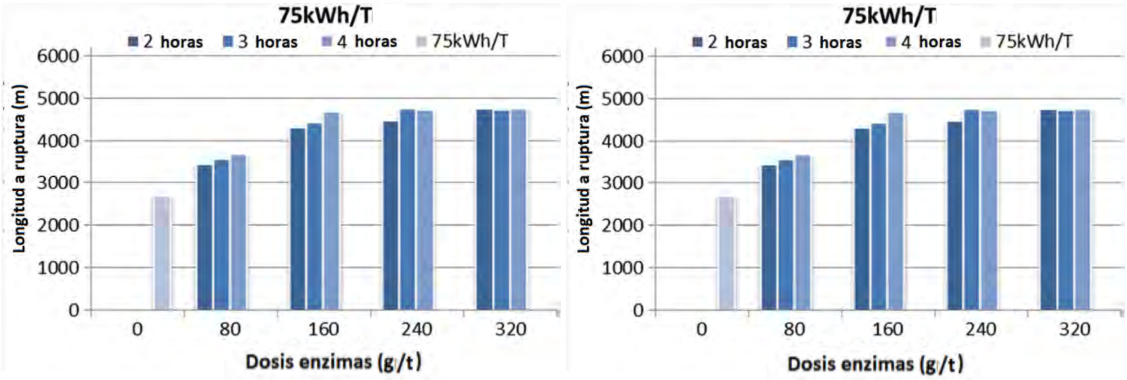


Figura 7: Evolución de la longitud a ruptura de diferentes papeles refinados y reforzados con CNF pretratadas con distintas dosis de enzimas y tiempos de contacto variables.

La adición de CNF enzimática indujo mejoras en la resistencia del papel en ambos tipos de pastas refinadas; los incrementos más altos se logran a añadiendo CNF que han sido tratadas con dosis enzimáticas de 160 g/t y tiempos de contacto de 4 horas. A partir de aquí, el incremento en la dosis no se traduce en más incremento en la longitud de ruptura y esto ocurre en los dos tipos de pastas utilizadas. La longitud a ruptura más alta se acercaba a los 6000 m y se observó en las pastas refinadas más intensamente (135 kwh/t). La figura 6 también demuestra que la pasta refinada a 75 kwh/t y adicionada con un 3 % de las CNF-320/4 alcanza una longitud a ruptura ligeramente superior a la registrada en la pasta únicamente refinada a 135 kWh/t sin CNF añadidas. Estos resultados indican que la adición de CNF permite efectivamente reducir la cantidad de refino a aplicar sobre una pasta papelera sin perder las propiedades requeridas. Además, la reducción en la cantidad de refino permitiría preservar en mayor medida las propiedades originales de las fibras, lo que permitiría alargar la vida útil de las mismas. La correcta combinación de adición de CNF y refino mecánico permitiría obtener pastas papeleras con las propiedades deseadas sin deteriorar de manera intensa las fibras. Se debe destacar que las longitudes a ruptura se han obtenido corresponden a papeles donde las fibras presentan orientación anisotrópica, por lo tanto es de esperarse que en condiciones de fabricación industrial donde las fibras del papel están orientadas en el mismo sentido, la longitud a ruptura sea aún superior a los resultados presentados aquí.

Un fenómeno interesante que se observa a partir de los resultados es que el incremento en longitud a ruptura en pastas con mayor grado de refino es inferior que en aquellas con menos refino o sin refinar del todo. Por ejemplo, si tomamos la muestra CNF-320/4 vemos que produjo un aumento de 102 % en la longitud a ruptura de la pasta sin refinar, del 76 % en la pasta refinada a 75 kWh/t y de tan solo el 39 % aproximadamente en la pasta refinada a 135 kWh/t. La principal explicación ofrecida por diversos autores ante este fenómeno se basa en que, en el caso de las fibras refinadas, la interacción entre estas mismas también aumenta en comparación con las fibras no refinadas, provocando que las CNF no tengan tanta superficie específica disponible para enlazarse.

Otro aspecto importante a tener en cuenta antes de considerar a las CNF como alternativa real para su uso, ya sea como aditivos en otras aplicaciones, es el costo de fabricación, así como las ventajas ambientales delante de otras alternativas. Para el presente trabajo, la muestra CNF-160/4 fue la más balanceada en términos de incremento en la longitud de ruptura y costo de producción, el cual fue de 1.93 € por kilogramo seco de nanofibra, de los cuales 0.33 céntimos corresponden a los reactivos usados y 1.598 € al costo de la energía utilizada (contabilizando tanto la usada para la hidrólisis enzimática como para la homogenización). A partir de este costo podemos calcular que, adicionar un 3 % de CNF a una pasta papelera sin refinar vale 57.9 € por tonelada de papel. Por otro lado, para alcanzar la misma longitud a ruptura inducida por las CNF mediante la aplicación de refino mecánico, se necesitan 135 kWh/t, lo que equivale a 10.8 € por tonelada (considerando que el kW/h vale 0.08 €), es decir, aproximadamente 10 veces menos que al aplicar CNF. Es necesario recalcar que, si bien en la comparativa las CNF parecen una alternativa poco atractiva, el costo de los reactivos utilizado en el cálculo corresponde a precios de pequeñas cantidades a nivel de laboratorio; asimismo, la tecnología de la fabricación de las CNF como aditivo en papel es relativamente nueva delante del refino mecánico, el cual es una técnica industrial establecida y optimizada desde hace varias décadas. Por lo tanto, es de esperar que posteriores investigaciones acerca de la optimización de los costos de producción de CNF para su uso como aditivos en la industria papelera mejoren sensiblemente las perspectivas de aplicación a gran escala.

El segundo artículo de la presente tesis continuó con el estudio de las CNF enzimáticas como aditivos del papel, pero en esta ocasión aplicadas en combinación con CNF fabricadas vía oxidación TEMPO. En el momento de su publicación, dicho artículo fue el primero en explorar la utilización de dos tipos distintos de CNF sobre un mismo soporte papelero. El objetivo principal era obtener incrementos significativos en la resistencia del papel sin utilizar ningún tipo de refino mecánico. Para esta ocasión, las CNF enzimáticas usadas fueron las CNF-240/4 siguiendo los mismos criterios de optimización desarrollados en el artículo anterior. En el caso de las CNF TEMPO, se fabricaron de dos tipos: TEMPO/5-CNF y TEMPO/15-CNF, donde los números corresponden a la cantidad de NaClO en milimoles por gramo de celulosa oxidada. Los distintos tipos de CNF fueron caracterizados y comparados entre sí para comprender mejor su interacción con las fibras papeleras. Los resultados de dicha caracterización están resumidos en la tabla 3. Las diferencias observadas entre las CNF enzimáticas y las oxidadas con 5 mmoles de NaClO son notorias en prácticamente todas las propiedades analizadas excepto en el desempeño estándar al ser aplicadas en una pasta papelera (3 %). En general, las CNF TEMPO presentaron un mayor rendimiento de nanofibrilación (93 %), indicando que la mayor parte de las fibras estaban dentro del rango nanométrico. Esto se tradujo también en que las suspensiones de las CNF-TEMPO tuvieran una mayor transmitancia a 600 nm de casi 80 %. Es interesante observar que en contra de lo esperado, las CNF enzimáticas presentaron un grado de polimerización inferior a las TEMPO. La demanda catiónica fue considerablemente más alta en las CNF TEMPO, producto también de su alto contenido carboxílico.

Tabla 3: Caracterización de los diferentes tipos de CNF usados en combinación sobre un mismo soporte papelero.

Tipo de CNF	**Desempeño estándar Δ(%)**	**Rendimiento (%)**	**Transmitancia a 600nm (%)**	**Consumo energético (kW·h/kg)**	**Grado de polimerización**
Enz-CNF	108.52	34.0	37.1	19.11	333
TEMPO/5-CNF	109.15	93.0	78.6	7.61	488

Tipo de CNF	**Demanda catiónica (µeq·g/g)**	**Contenido carboxílico (µeq·g/g)**	**Superficie específica* (m^2/g)**	**Diámetro* (nm)**	**Resistencia a tracción (MPa)**
Enz-CNF	258	42	105.2	23.8	82.4
TEMPO/5-CNF	1295	881	201.6	12	90.3

*Valor estimado

En cuanto a sus propiedades físicas, las CNF TEMPO mostraron una superficie específica que aproximadamente doblaba a la de su contraparte enzimática y la mitad del diámetro. En cuanto a la resistencia a tracción de los nanopapeles fabricados con ambos tipos de CNF, las CNF TEMPO fueron más resistentes que las CNF enzimáticas, lo que está en concordancia con su mayor grado de polimerización.

Las CNF caracterizadas fueron aplicadas primero en masa sobre una pasta papelera sin refinar con el fin de analizar las diferencias en las propiedades del papel resultante con respecto al tipo de CNF utilizada. Los resultados (presentes en la tabla 3) indican que las propiedades mecánicas de los papeles no presentan grandes variaciones con respecto al tipo de CNF utilizada. Sin embargo, sí que se aprecian diferencias importantes en la drenabilidad de las pastas. En la tabla 4 se observa que, a una misma cantidad de CNF, las nanofibras enzimáticas inducen un deterioro de la drenabilidad mucho más moderado en comparación con las CNF TEMPO.

Tabla 4: Propiedades mecánicas y drenabilidad de papeles reforzados con CNF.

Contenido de CNF	**Tipo de CNF**	**°SR**	**Longitud a ruptura (m)**	**Reventamiento (kPa·m²/g)**	**Cohesión interna (J/m²)**
0	-	15	2054±95	0.87±0.1	61.4±7.2
1.5	TEMPO/5-CNF	25	3407±89	1.64±0.3	117.4±9.8
	Enz-CNF	21	3384±101	1.60±0.2	112.4±10.1
3	TEMPO/5-CNF	33	4296±79	2.12±0.5	161.4±13.4
	Enz-CNF	24	4283±91	2.09±0.3	151.5±8.7
4.5	TEMPO/5-CNF	41	4521±108	2.24±0.4	168.9±16.7
	Enz-CNF	29	4578±110	2.30±0.6	164.7±10.2

Este resultado debe tenerse en consideración también al comparar la factibilidad en el uso de un tipo determinado de nanofibras ya que un deterioro significativo en el °SR no es deseado en la fabricación del papel. Como complemento del estudio de las propiedades mecánicas, las propiedades físicas de los papeles reforzados con CNF también se estudió y los resultados se presentan en la tabla 5.

Tabla 5: Propiedades físicas de los papeles reforzados con CNF.

Contenido CNF	Tipo de CNF	Grosor (µm)	Densidad (g/cm^3)	Mano (cm^3/g)	Opacidad (%)	Porosidad (%)	Porosidad Gurley (s)
0	-	145.52	0.524	1.91	88.6	65.07	1.1
1.5	TEMPO/5-CNF	142.31	0.541	1.85	88.1	63.93	1.8
	Enz-CNF	138.64	0.551	1.81	88.2	63.27	2.1
3	TEMPO/5-CNF	140.17	0.553	1.81	87.5	63.13	3.4
	Enz-CNF	133.88	0.570	1.76	87.6	62.00	3.0
4.5	TEMPO/5-CNF	138.06	0.566	1.77	87.1	62.27	5.2
	Enz-CNF	129.34	0.587	1.70	87.0	60.87	3.8

El grosor de los papeles se reduce conforme aumenta la cantidad de CNF; esto conlleva un incremento de la densidad y reducción en el parámetro de la mano. Es interesante notar que las mayores densidades se observaron en los papeles reforzados con CNF enzimáticas. En lo que respecta a la opacidad se observó una reducción muy ligera de la misma. Esto en parte puede explicarse si consideramos que las CNF tienen dimensiones por debajo de la longitud de onda visible, por lo que no se espera que dispersen la luz visible. El ligero descenso en la opacidad puede atribuirse más bien a la disminución en el grosor del papel a un mismo gramaje.

La porosidad medida como el porcentaje de espacio vacío en el papel se redujo también, aunque las reducciones más significativas fueron vistas en las muestras reforzadas nuevamente con CNF enzimáticas. En lo que respecta a la porosidad Gurley, las CNF TEMPO provocaron un mayor aumento en el tiempo de paso del aire durante el ensayo. En general los resultados indican que la adición de CNF modifican sensiblemente las características físicas del papel al hacerlo más compacto y por tanto más denso. Esto puede deberse a que, durante la formación del papel, las nanofibras de celulosa que flotan libremente en la suspensión de fibras se comportan como finos que reducen el radio del menisco que aparece durante el drenado del agua cuando se forma el papel, lo cual incrementa la diferencia de presión entre la fase acuosa y los alrededores; esto ayuda a aproximar más a las fibras unas con otras, lo cual incrementa el número de puentes de hidrógeno que se forman entre las fibras cuando se seca el papel. Este mecanismo también ayuda a reducir el diámetro de los poros entre fibras, lo que influencia directamente en los resultados del porcentaje de espacio vacío y porosidad Gurley.

Los papeles reforzados con CNF fueron posteriormente tratados con una suspensión de CNF aplicadas superficialmente con una varilla. Solamente se utilizaron CNF-TEMPO para aplicación superficial ya que experimentos anteriores demostraron que las CNF enzimáticas no inducían mejores destacables en el papel seco cuando eran aplicadas de manera superficial en comparación con las CNF TEMPO. Una posible razón para este comportamiento es las diferencias de tamaño y rendimiento de fibrilación entre ambos tipos de CNF. En concreto, el mayor tamaño de las CNF enzimáticas puede impedir que las fibras penetren en la estructura poco porosa de los papeles reforzados previamente con CNF en masa. En cambio, las CNF TEMPO, gracias a su menor diámetro y longitud pueden penetrar con mayor facilidad entre los poros del papel y enlazarse con otras fibras en el interior de la estructura del papel.

Dos tipos de CNF TEMPO se usaron para aplicación superficial, identificadas como TEMPO-5/CNF y TEMPO-15/CNF, donde los números indican la cantidad de NaClO en milimoles aplicada por gramo de celulosa durante la oxidación TEMPO. Es bien sabido que la cantidad de NaClO usada durante la oxidación determina la longitud y diámetro promedios de las CNF. Por lo tanto, es de esperar que CNF muy oxidadas penetrarán más fácilmente en el papel. A partir de estos dos tipos de CNF se produjeron dos suspensiones denominadas A/5 y A/15; la primera solución estaba compuesta de 0.45 % en peso de TEMPO/5-CNF y 2.5% de almidón, mientras que la solución A/15 estaba compuesta por un 0.45 % en peso de TEMPO/5-CNF y la misma cantidad de almidón que en la solución anterior. Mediante el cálculo de la densidad del papel seco tras la aplicación superficial fue posible determinar la cantidad de CNF retenidas durante la aplicación. La figura 8 muestra la evolución de esta propiedad en función de la cantidad de CNF enzimáticas aplicadas en masa y el tipo de solución aplicada en superficie.

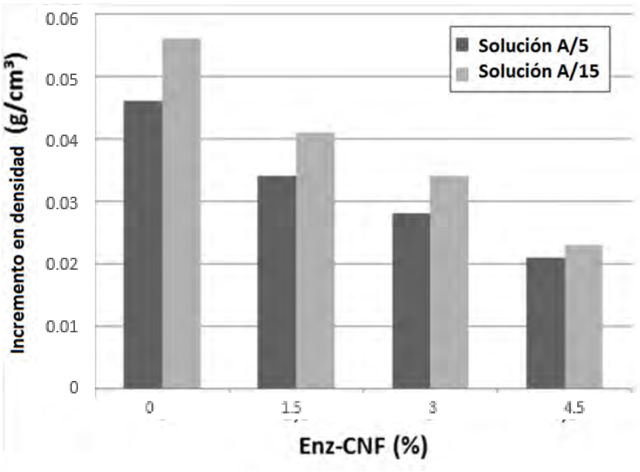


Figura 8: Incremento en la densidad tras la aplicación de las soluciones contra el contenido de CNF enzimáticas en el papel.

Un aspecto interesante que muestra la figura 8 es que el mayor aumento en densidad inducido por la aplicación superficial se presentó en los papeles no reforzados con nanofibras; además, los incrementos fueron disminuyendo conforme el contenido de CNF en el papel era mayor. Es también destacable que la solución A/15 produjo siempre los incrementos más altos en densidad. Este comportamiento confirma que las CNF con un mayor contenido carboxílico y por tanto menor diámetro y longitud penetran mejor en la estructura del papel. Estos resultados también confirman la fuerte dependencia de la longitud de las CNF con el grado de oxidación.

En cuanto a las propiedades mecánicas, los papeles tratados con la suspensión A/15 presentaron las mejores propiedades mecánicas. Los resultados de toda la caracterización mecánica se encuentran en la tabla 6. Los resultados también indican que, de manera similar a lo que se observó en la densidad, el incremento en la longitud a ruptura disminuyó en aquellos papeles con mayor cantidad de CNF aplicadas en masa. Esta tendencia pueda estar relacionada con la menor porosidad de los papeles reforzados con mayores cantidades de CNF, lo cual impide la penetración de las nanofibras. Sin embargo, si tenemos en cuenta el incremento global de la longitud a ruptura del papel reforzado con respecto al que no lo está, notamos que el aumento es del 270 % en relación con las propiedades originales. También se observa un aumento importante en la cohesión interna para todas las muestras, aunque los incrementos más amplios se dieron tras la aplicación en masa de las CNF.

Tabla 6: Propiedades físicas y mecánicas de papeles reforzados con CNF enzimáticas y tratados superficialmente con CNF TEMPO.

Solución	**Contenido CNF (%)**	**Longitud a ruptura (m)**		**Incremento longitud a ruptura (%)**	**Cohesión interna (J/m^2)**	**Densidad (g/cm^3)**
		Antes de aplicación superficial	**Después de aplicación superficial**			
A/5	0	2054±95	3834±92	86.7	88.3±6.2	0.570
	1.5	3384±101	5177±101	52.9	120.8±4.8	0.585
	3	4283±91	6062±87	41.5	170.5±3.8	0.598
	4.5	4578±110	6093±63	33.1	193.4±9.2	0.608
A/15	0	2054±95	4327±110	110.7	87.1±7.7	0.580
	1.5	3384±101	6144±105	81.6	127.2±8.6	0.592
	3	4283±91	7658±85	78.8	177.4±10.1	0.604
	4.5	4578±110	7672±72	67.6	205.5±11.8	0.610

Para entender cómo la presencia del almidón influencia la fijación de las nanofibras sobre el papel, un tercer tipo de suspensión fue preparada la cual contenía únicamente 0.45% en peso de TEMPO/15-CNF y se aplicó sobre el mismo tipo de soporte papelero que se utilizó en los experimentos anteriores. Los resultados mostraron que la aplicación superficial de CNF sin almidón también mejora la densidad del papel en proporciones similares a las observadas en papeles tratados con las soluciones que contienen almidón. Sin embargo, el aumento de las propiedades mecánicas fue inferior en los papeles tratados con la solución sin almidón en comparación con los papeles tratados con soluciones que sí contienen almidón. Este comportamiento demuestra que la presencia del almidón debe jugar un rol importante en el proceso de fijación de las CNF sobre las fibras del papel, contribuyendo de manera decisiva en el aumento de las propiedades mecánicas del papel. La figura 9 resume claramente el efecto que los tres tipos de suspensiones ejercen sobre la longitud a ruptura del papel tratado.

La figura muestra que, en general, la aplicación superficial de pequeñas cantidades de nanofibras efectivamente aumenta la longitud a ruptura del papel, pero la mejora se vuelve más significativa cuando la suspensión contiene almidón; los mejores resultados se observaron en las muestras tratadas con la suspensión A/15 aplicada sobre papeles reforzados previamente con 3 y 4.5 % en peso de nanofibras, lo cual dio como resultado longitudes a ruptura cercanas a las 8000 m. Estos resultados son destacables si recordamos que no se aplicó ningún tipo de refino mecánico previo a las fibras.

La microscopía electrónica de barrido permitió observar asimismo el aspecto superficial que los papeles tratados tienen (figura 10). Las microfotografías muestran la estructura del papel original sin ningún tipo de tratamiento superficial ni adición de CNF en masa.

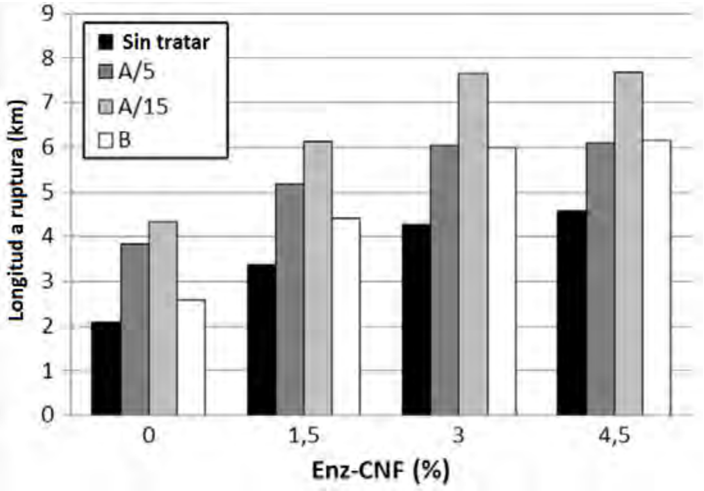


Figura 9: Efecto sobre la longitud a ruptura de papeles cubiertos superficialmente con los tres tipos de suspensiones preparadas.

En este tipo de papel, se observan los grandes espacios y poros existentes entre fibras. En cambio la muestra tratada que corresponde a un papel tratado muestra una estructura mucho más compacta donde los espacios y poros entre fibras han sido parcialmente ocupados por las CNF, tanto las aplicadas en masa como superficialmente.

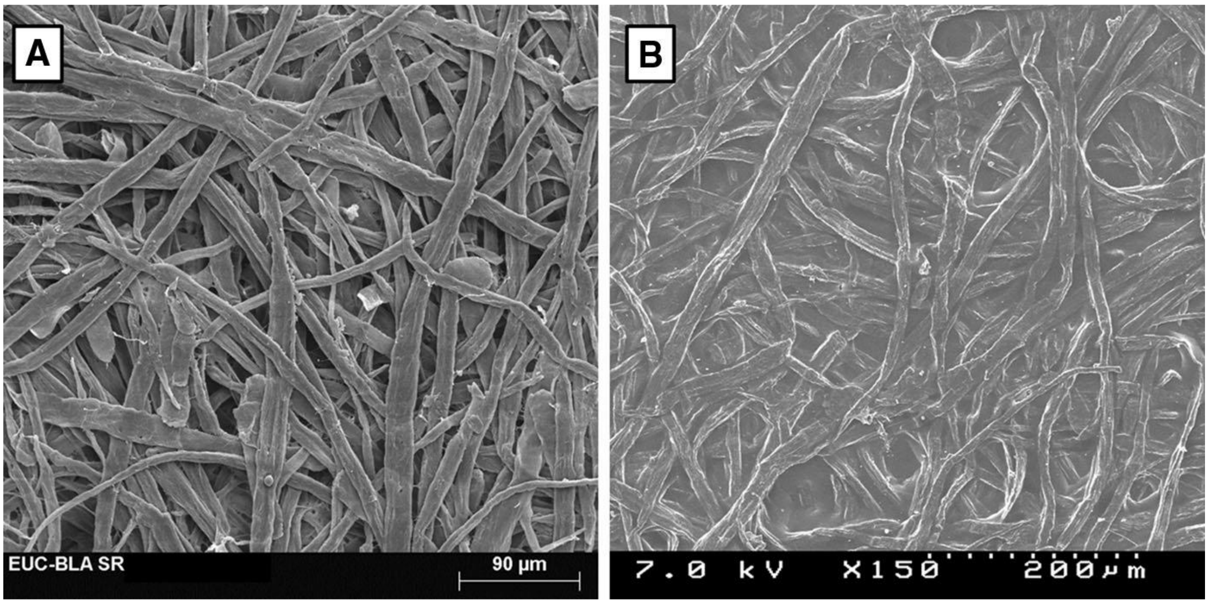


Figura 10: Microfotografía electrónica de barrido de A) un papel sin ningún tipo de tratamiento, y B) un papel con CNF aplicadas en masa y superficialmente.

En ambas fotografías puede verse también que los papeles en cualquier caso presentan fibras bien definidas donde su superficie se preserva en buena manera en comparación a lo que suelen presentar las fibras refinadas mecánicamente.

Si bien la combinación de CNF enzimáticas adicionadas en masa junto con CNF TEMPO aplicadas superficialmente ofrece mejoras significativas en las propiedades a resistencia del papel, es importante señalar que la aplicabilidad de esta técnica a nivel industrial aún debe ser estudiada a profundidad con tal de entender las posibles modificaciones en los equipos y procesos de fabricación del papel a gran escala.

El siguiente paso en el uso de CNF enzimáticas en soportes papeleros consistió en su aplicación sobre papeles fabricados a partir de residuos de cajas de cartón los cuales fueron refinados enzimáticamente previo a la adición de las CNF. Este estudio se realizó, con el objetivo de fabricar papeles para altos desempeños, con longitudes a ruptura superiores a las vistas en papeles liner comerciales. Para este caso, se usaron refinos tanto mecánicos como enzimáticos de las fibras, para poder realizar una comparativa sobre el efecto que ambos procesos tienen sobre las propiedades de los productos finales. Las CNF fueron fabricadas utilizando el mismo proceso de hidrólisis enzimática que el usado en los experimentos anteriores.

Debido a que los papeles fabricados a nivel industrial presentan en general mejores propiedades mecánicas que los hechos en laboratorio, debido a la orientación de las fibras (homogénea para los industriales), las propiedades mecánicas de los papeles industriales se tomaron en cuenta como referencia para los papeles a fabricar en laboratorio. La caracterización mecánica y física de estos papeles comerciales arrojó los resultados presentes en la tabla 7.

Tabla 7: Propiedades físico-mecánicas de papeles test-liner comerciales.

G (g/m^2)	**LR_{DM} (m)**	**LR_{CD} (m)**	**E_{DM} (GPa)**	**E_{CD} (GPa)**	**CI (J/m^2)**	**PA (s)**
120	5534 ± 54	2457 ± 81	4.7 ± 0.1	1.8 ± 0.1	245 ± 12	25.7 ± 3

G: gramaje; LR_{DM}: Longitud a ruptura dirección máquina; LR_{CD}: Longitud a ruptura contra dirección; CI: cohesión interna; PA: permeabilidad al aire.

Los resultados indican la gran diferencia existente en la longitud a ruptura y el módulo de Young cuando estos son medidos en dirección máquina y transversalmente. Estas diferencias se deben a la orientación de las fibras, la cual unidireccional en el sentido máquina; esto está provocado por la diferencia de velocidad entre el flujo de entrada de la pasta y la etapa de drenado. Esto provoca que la resistencia sea más del doble en los papeles con fibras orientadas en comparación a las que no lo están. Si bien estos valores pueden ser modificados por muchas otras variables durante el proceso de fabricación tales como diferencia de velocidad, consistencia de la pasta, uso de aditivos y tipo de fibra utilizado, son una buena referencia de las propiedades del papel fabricado a escala industrial. Para entender el efecto que el reciclado tiene sobre las propiedades de las fibras, se desintegraron cajas de cartón y la pasta resultante fue refinada y caracterizada. Los resultados están resumidos en la tabla 8.

Tabla 8: Propiedades físico-mecánicas de papeles fabricados a partir de cajas de cartón recicladas y refinadas a distintas intensidades en un molino PFI. LR=longitud a ruptura; CI=cohesión interna; PA=permeabilidad al aire; lfw=longitud de la fibra ponderada en longitud.

Intensidad de refino (REV PFI)	**°SR**	**LR (m)**	**E (GPa)**	**CI (J/m^2)**	**PA (s)**	**l_w^f (µm)**	**Finos* (%)**
0	44 ± 1	3064 ± 41	4.3 ± 0.1	165 ± 15	14 ± 1	755 ± 21	45.9 ± 0.4
500	47 ± 0	3438 ± 51	4.7 ± 0.1	208 ± 18	26 ± 3	711 ± 31	52.0 ± 0.6
1000	53 ± 1	3877 ± 39	5.0 ± 0.2	311 ± 26	34 ± 1	672 ± 22	55.8 ± 0.2
1500	61 ± 1	4356 ± 48	5.8 ± 0.1	397 ± 37	71 ± 4	603 ± 30	58.2 ± 0.3

**Porcentaje de finos en longitud.*

El refinado mecánico, aunque podría considerarse ligero, provoca un gran deterioro de la drenabilidad de la pasta. Si bien la longitud a ruptura, rigidez y cohesión interna se incrementan, los resultados obtenidos mediante este método no se acercan a los observados en el papel liner fabricado industrialmente. Si bien un refino mecánico más intenso podría aplicarse para recuperar las propiedades originales de la fibra, el descenso en la drenabilidad de la pasta sería más allá de lo considerado como aceptable en la industria papelera. El deterioro de la drenabilidad viene explicado en buena parte por el aumento de finos y la reducción en la longitud de las fibras como demuestran los resultados observados en la tabla 8. Es interesante comparar estos resultados con los obtenidos a partir de las mismas fibras pero refinadas enzimáticamente y adicionadas con CNF enzimáticas (tabla 9). Se observa que, si bien existe un importante deterioro de la drenabilidad de la pasta, la longitud a ruptura de los papeles tratados enzimáticamente es muy superior en comparación con pastas con un °SR similar. Por ejemplo, las pastas refinadas a 1500 revoluciones en molino PFI desarrollaron una longitud a ruptura de 4356 m y un °SR de 61; en cambio, el refino enzimático produjo papeles con una longitud a ruptura ligeramente superior y con un °SR de 47, bastante similar al observado en la pasta original sin refinar.

Tabla 9: Propiedades físico-mecánicas de papeles fabricados a partir de cajas de cartón recicladas y refinadas enzimáticamente. LR=longitud a ruptura; CI=cohesión interna; PA=permeabilidad al aire; lfw=longitud de la fibra ponderada en longitud.

	CNF (%)	**°SR**	**LR** (m)	**E** (GPa)	**CI** (J/m^2)	**PA** (s)	l_w^f* (μm)	**Finos*** (%)
Sin tratar	0	44 ± 1	3064 ± 41	4.3 ± 0.1	165 ± 15	14 ± 1	755 ± 21	45.9 ± 0.4
Tratamiento Enzimático	0	47 ± 1	4473 ± 55	5.8 ± 0.1	372 ± 21	62 ± 3	752 ± 33	46.2 ± 0.2
	1.5	52 ± 1	5123 ± 37	5.8 ± 0.1	425 ± 33	98 ± 2	751 ± 24	46.1 ± 0.2
	3.0	59 ± 1	6038 ± 61	5.8 ± 0.1	467 ± 24	124 ± 2	751 ± 22	46.4 ± 0.3
	4.5	64 ± 1	6689 ± 60	5.9 ± 0.2	502 ± 18	177 ± 4	748 ± 19	46.7 ± 0.4

** Porcentaje de finos en longitud.*

La adición de CNF enzimáticas produjo incrementos importantes en la longitud a ruptura del papel y la cohesión interna, si bien la rigidez se mantuvo prácticamente constante independientemente de la cantidad de nanofibras añadidas y en valores muy similares a los vistos en las pastas refinadas en molino. Como era de esperarse, la porosidad se redujo en función de la cantidad de CNF añadidas. En cuanto a las propiedades físicas destaca la baja presencia de finos tras el tratamiento enzimático en comparación con el mecánico, mientras que la longitud de las fibras se reduce moderadamente. Estas dos últimas propiedades explican por qué el °SR se mantiene a niveles inferiores a las observadas en las pastas refinadas mecánicamente: la menor producción de finos y la preservación en buena medida de la longitud original de la fibra hacen que la reducción en la capacidad de drenaje de la fibra sea menos drástica que la vista utilizando refinos mecánicos. En general estos resultados son destacables ya que si los comparamos con las propiedades de los papeles liner comerciales, vemos que las pastas tratadas enzimáticamente y reforzadas con CNF tienen desempeños que superan con creces los ofrecidos por las pastas comerciales estudiadas como ejemplo.

A partir de estos resultados es posible predecir el comportamiento que las pastas tratadas enzimáticamente y reforzadas con CNF tendrían en una máquina de papel a nivel industrial donde las fibras estarían orientadas en una sola dirección. Los resultados de dichas predicciones se presentan en la figura 11.

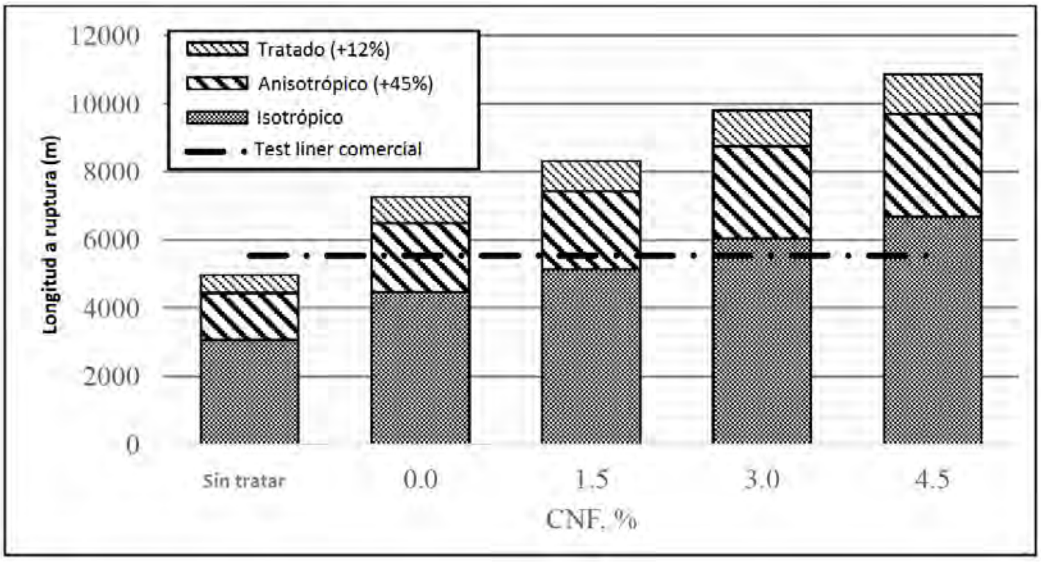


Figura 11: Longitud a ruptura estimada de las pastas a nivel del pope.

De acuerdo a estas estimaciones, la longitud a ruptura de los papeles refinados enzimáticamente sería de hasta 7264 m al nivel del pope, esto es un 31 % por encima de los requerimientos comerciales para papeles liner/fluting usados como referencia para el presente trabajo. Esto demuestra que los tratamientos enzimáticos representan una importante alternativa al refino mecánico ya que se obtienen altas prestaciones de resistencia sin comprometer de manera importante la capacidad de drenaje de la fibra, al mismo tiempo que se preservan en buena medida las propiedades originales de la fibra, lo que permitía alargar los ciclos de reaprovechamiento de los residuos de papel y cartón. Asimismo, la adición de un 4.5 % en peso de CNF enzimática en combinación con el biorefino haría posible conseguir longitudes a ruptura de casi 11000 m. Estos niveles de longitud a ruptura permitirían fabricar papeles para aplicaciones con altos requerimientos de resistencia; asimismo, el incremento en la longitud a ruptura permitiría reducir el gramaje de ciertos papeles o incrementar la cantidad de cargas minerales que suelen aplicarse al papel para reducir costos sin comprometer los requerimientos de resistencia de acuerdo a su aplicación.

4.3. Exploración de nuevas aplicaciones para las CNF: aerogeles y nanopapeles magnéticos

Una vez demostrada la viabilidad de las fibras de celulosa como aditivos que aumentan la resistencia en las pastas papeleras, la siguiente parte de la tesis consistió en expandir las posibles aplicaciones de las CNF en campos distintos al papelero aprovechando varias de las propiedades más destacables de las nanofibras de celulosa, en particular su alta superficie específica. Para ello, se realizaron estudios sobre la factibilidad en la fabricación de aerogeles y nanopapeles. Los aerogeles son producidos cuando suspensiones acuosas de nanofibras son congeladas y liofilizadas para obtener una estructura esponjosa con una gran superficie específica y cierta estabilidad dimensional. Para la presente tesis, se propuso la fabricación de aerogeles a partir de CNF tratadas con AKD como absorbentes del petróleo y otros hidrocarburos que son derramados accidentalmente en el mar.

Si bien las CNF enzimáticas han sido presentadas hasta ahora como alternativa viable y verde a las CNF obtenidas con pretratamientos químicos, es importante señalar que para la fabricación de aerogeles se utilizaron tanto CNF obtenidas vía TEMPO (debido a la alta superficie específica propia de este tipo de nanofibras gracias a su alto nivel de nanofibrilación) así como CNF obtenidas enzimática y mecánicamente, esto con el fin de analizar como diferentes nanofibras con distintas propiedades influencian en las propiedades finales de los aerogeles. Previo a la fabricación de los aerogeles, ambos tipos de CNF fueron tratadas con AKD, un reactivo altamente utilizado en la industria papelera para conferir propiedades hidrofóbicas a las fibras de celulosa. Mediante variaciones en el pretratamiento y en la cantidad de AKD añadido a las CNF, se fabricaron diferentes tipos de aerogeles con el fin de observar como la calidad de las CNF y la dosificación del AKD, afectan las propiedades de absorción de hidrocarburos.

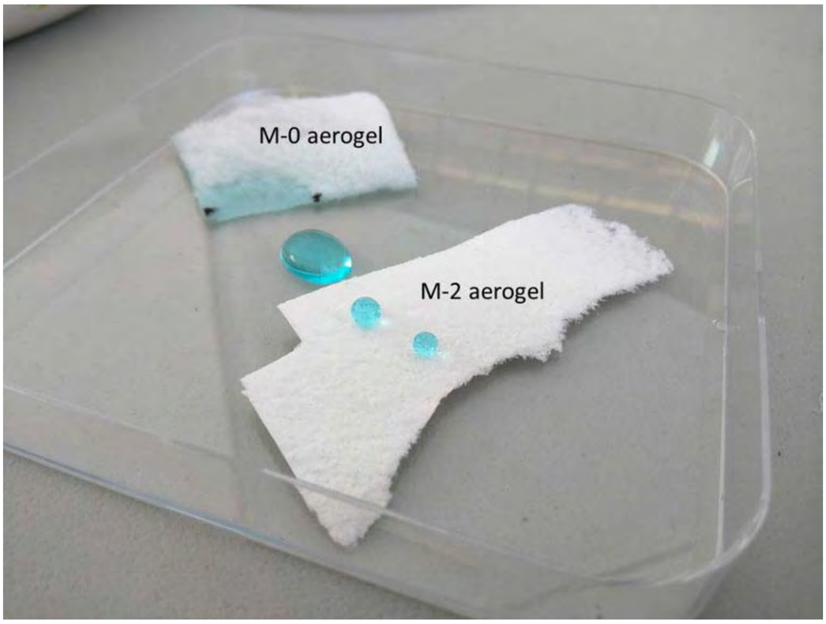


Figura 12: Comportamiento de una gota de agua sobre un aerogel sin AKD (M-0) y otro con AKD (M2).

Visualmente, los aerogeles no presentaron ninguna diferencia destacable entre todas las muestras preparadas, tanto enzimáticas como TEMPO. La parte del aerogel que había estado en contacto con el recipiente era más suave y homogénea en aspecto que la parte que estaba en contacto con el aire, debido seguramente a la compactación por gravedad que sufren las CNF en el fondo del recipiente. Una primera prueba cualitativa sobre la hidrofobicidad de los aerogeles tratados con AKD se muestra en la figura 11. La caracterización de los tres tipos de CNF utilizadas se resume en la tabla 10. Se observan grandes diferencias entre las CNF obtenidas químicamente y el resto de tipos de nanofibras fabricadas.

Tabla 10: Caracterización de las CNF previo al tratamiento con AKD.

Tipo de CNF	Contenido carboxílico (meq-g/g)	Demanda catiónica (meq-g/g)	Rendimiento (%)	Superficie específica (m^2/g)	Diámetro (nm)
TEMPO	1376.6	2021.6	96.12	293.48	9.09
EENZIMÁTICAS	75.2	272.9	41.28	88.72	30.06
MECÁNICAS	76.4	216.2	22.13	62.73	42.51

El contenido carboxílico y por tanto la demanda catiónica son mucho más altas en las CNF obtenidas por oxidación TEMPO, mientras que son bastante similares entre las enzimáticas y las mecánicas. En cuanto al rendimiento de nanofibrilación, las CNF TEMPO tienen un 96 %, seguidas de las enzimáticas y las mecánicas con un 41 y 22 % respectivamente. Como ya se ha mencionado, un aspecto importante es la superficie específica que pueden aportar los distintos tipos de CNF y en este apartado destacan las CNF TEMPO con una superficie de 293 m^2/g. De la misma manera, las CNF TEMPO presentaron los diámetros teóricos más pequeños. Una vez que las CNF fueron modificadas con diferentes cantidades de AKD, se procedió a la caracterización de los aerogeles. La propiedad más importante a considerar es la porosidad del aerogel. La figura 13 muestra la porosidad (como porcentaje de espacio vacío) para todas las muestras producidas. En general, todos los aerogeles presentaron altas porosidades, entre el 95 y el 99 %.

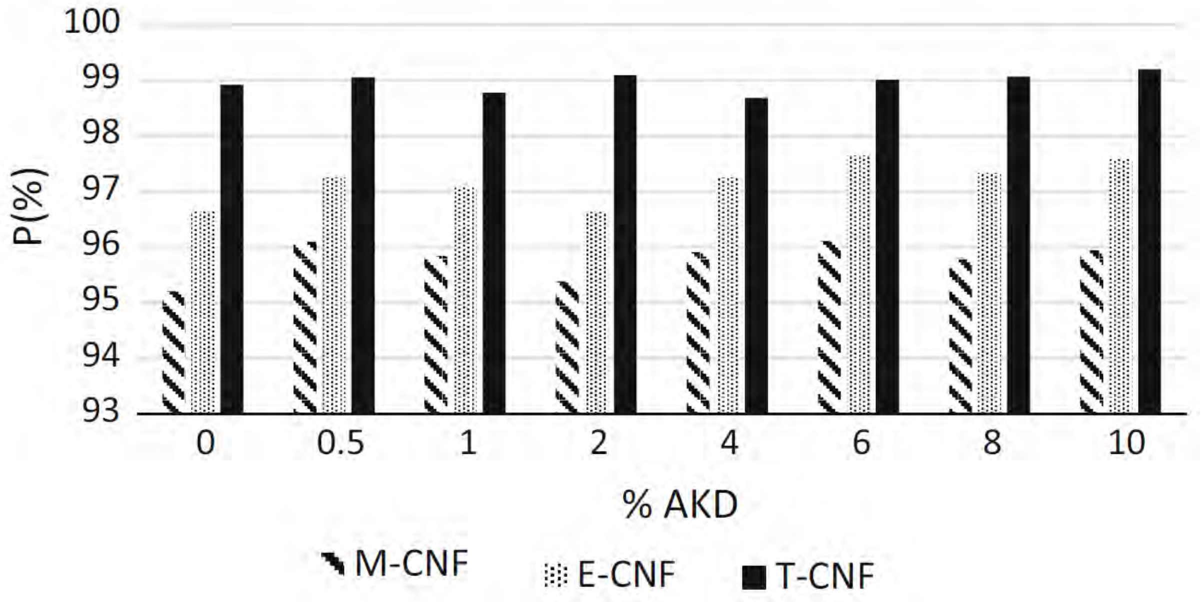


Figura 13: Evolución de la porosidad de los aerogeles en función de la cantidad de AKD.

Los aerogeles preparados a partir de CNF TEMPO presentaron los valores de porosidad más altos, seguido por las CNF enzimáticas y las mecánicas. Estos resultados eran de esperarse una vez vista la caracterización previa de las CNF. Una de las ventajas encontradas con la adición de AKD es que se puede observar un ligero aumento de la porosidad de algunas de las muestras conforme se aumenta la cantidad de AKD. Está claro que la presencia del AKD reduce la hidrofilicidad de las fibras y bloquea parcialmente la formación de puentes de hidrógeno. Si bien esto bloquea la capacidad de hinchamiento de las CNF debido a la absorción de agua, el AKD también reduce el encogimiento de la estructura del aerogel, probablemente debido a la reducción en las fuerzas de atracción entre fibras cuando el agua es eliminada durante la liofilización. Si bien una alta porosidad debería implicar una alta capacidad de absorción de hidrocarburo, otros factores como estabilidad dimensional de los aerogeles, influencian la capacidad de absorción de hidrocarburos o agua en condiciones dinámicas.

Las mediciones del ángulo de contacto de los diferentes aerogeles se muestran en la figura 14. Los resultados indican que tan sólo con la presencia de cantidades cercanas al 1 % ya se pueden obtener incrementos significativos en el ángulo de contacto para todos los tipos de CNF utilizados. Sin embargo los incrementos más destacados se observan en las CNF TEMPO y mecánicas. El aumento en el ángulo de contacto es continuo hasta un 6 %, a partir del cual se observa una estabilización del ángulo de contacto para todas las muestras. El valor más alto se observa en las CNF mecánicas con un 6 % de AKD (142º). En general los aerogeles preparados a partir de CNF enzimáticas mostraron ángulos de contacto más bajos que el resto de muestras, especialmente cuando la cantidad de AKD oscilaba entre el 1 y el 4 %. Durante la preparación de los aerogeles con CNF enzimáticas se observó una especie de laminación en el aerogel, lo cual pudo haber afectado de manera negativa a la dispersión del AKD.

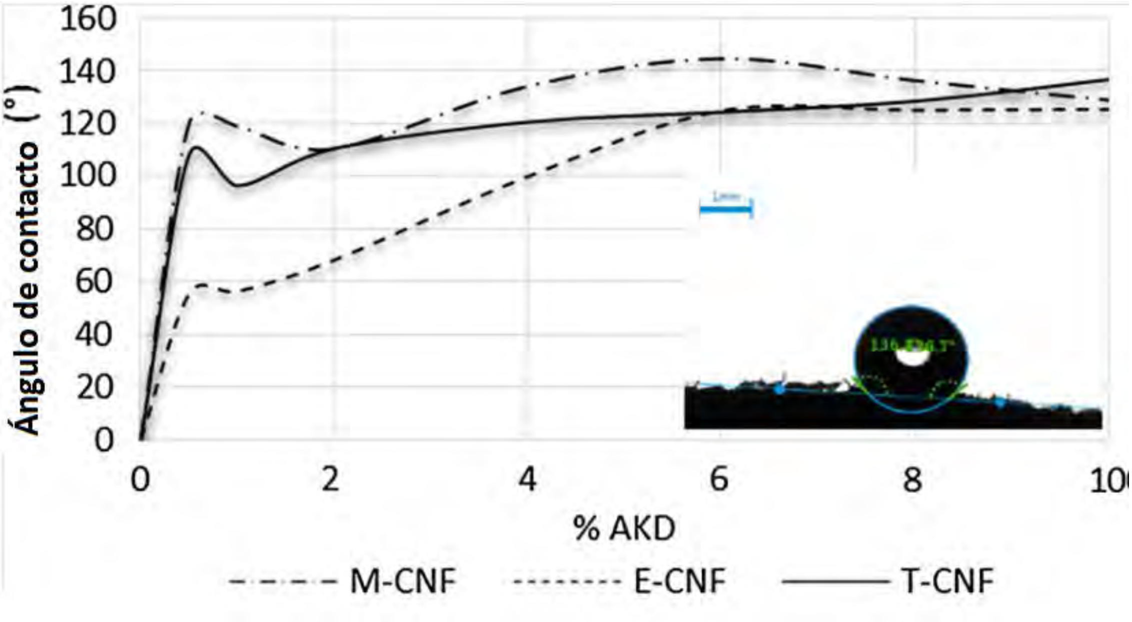


Figura 14: Evolución del ángulo de contacto en relación con la cantidad de AKD aplicada.

La microfotografía electrónica de rastreo permitió observar la estructura del aerogel a base de CNF TEMPO antes y después del tratamiento con AKD (figura 15). Las imágenes muestran que los aerogeles están formados por una red de nanofibras con múltiples enlaces y puntos de unión entre ellas formando así una red tridimensional. El pequeño recuadro de la fotografía superior muestra asimismo las dimensiones nanométricas de las fibras. Las estructuras de mayor grosor observados corresponden a agregados de nanofibras que suelen formarse durante la eliminación del agua durante el proceso de liofilización. En la misma figura, en la parte inferior derecha, se observa el efecto que el AKD tiene sobre las fibras. Básicamente, el AKD forma una barrera sobre las fibras que bloquea su interacción con el agua, lo que genera la hidrofobicidad de los aerogeles.

Es importante recalcar que los diámetros de las nanofibras que aparecen en las microfotografías son mayores a los estimados teoréticamente. Esto se debe a diversos factores. Primero, los diámetros teóricos son calculados a partir de la superficie específica teórica, la cual a su vez es estimada a partir de la cantidad de grupos carboxílicos y la demanda catiónica. Estos dos análisis se realizan en suspensión acuosa donde las fibras están individualizadas y forman puentes de hidrógeno con el agua circundante y no entre ellas. Al eliminar el agua (congelada) durante el proceso de liofilización, las fibras tienden a establecer puentes de hidrógeno entre ellas, lo que las compacta formando estructuras más gruesas compuestas por haces de nanofibras.

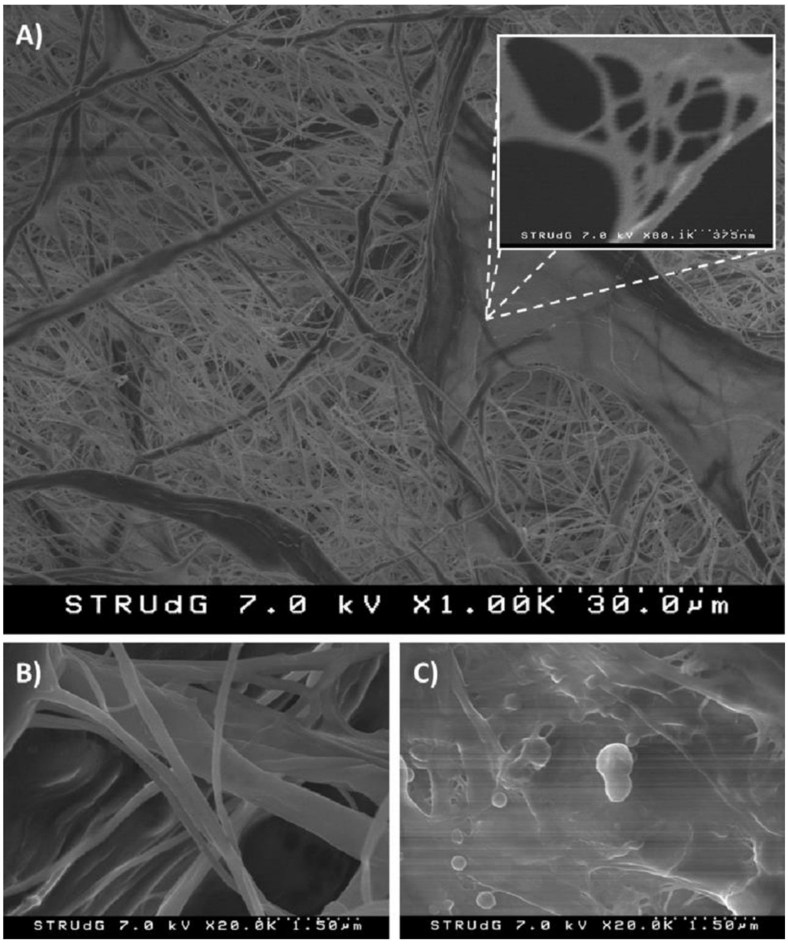


Figura 15: Microfotografía electrónica de rastreo de a), b) aerogel a base de CNF TEMPO sin modificar con AKD; c) aerogel modificado con 6% de AKD.

La capacidad de absorción de hidrocarburos se determinó bajo condiciones estáticas y dinámicas. La figura 16 muestra la evolución de la absorción de agua y aceite de motor de coche (como ejemplo de hidrocarburo) por parte de los aerogeles en condiciones estáticas.

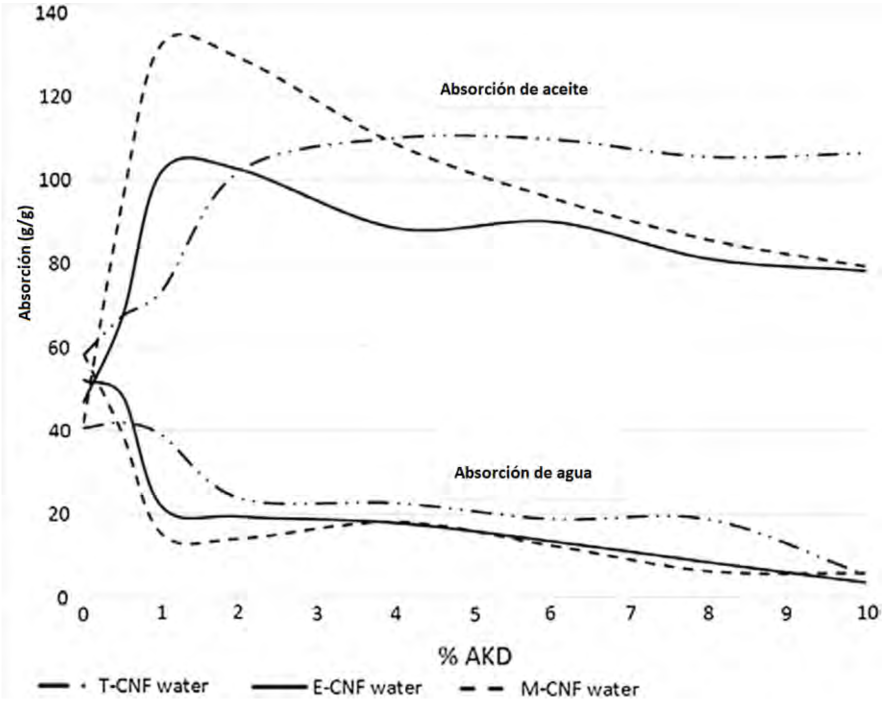


Figura 16: Absorción de agua y aceite de los aerogeles en condiciones estáticas.

Los resultados indican que, a mayor cantidad de AKD presente en el aerogel, se incrementan los gramos de aceite absorbido por gramo de celulosa. Por otro lado, el aumento en la cantidad de AKD reduce la capacidad de absorción del agua. De manera similar a lo visto en la gráfica de la evolución del ángulo de contacto, la mayor capacidad de absorción de aceite se dio en las muestras con un contenido de AKD de alrededor del 1 %. La mayor absorción se observó en los aerogeles de CNF mecánicas, con una absorción de casi 140 g/g, mientras que los otros dos tipos de CNF llegaron a máximos de alrededor de 110 g/g. Es interesante observar que mayores cantidades de AKD redujeron la capacidad de absorción de los aerogeles de CNF mecánicas y enzimáticas, mientras que las CNF TEMPO los valores se mantuvieron más o menos constantes a partir de 2 % de AKD. Es probable que un 1 % de AKD sea suficiente para saturar la superficie de las CNF, lo que hace que mayores cantidades de AKD ya no induzcan incrementos en la hidrofobicidad de las fibras. Los geles sin modificar, en cambio retuvieron aproximadamente la misma cantidad de aceite y agua. En condiciones estáticas, el aceite permanece en la superficie durante el ensayo debido a su baja densidad respecto al agua; esto facilita la absorción del aceite, lo que conduce a que los resultados deban ser considerados con cierta precaución ya que dichas condiciones no se suelen observar en situaciones reales. Asimismo, la densidad y viscosidad de los hidrocarburos, tamaño de poro del aerogel y turbulencias en el medio pueden influir de manera decisiva en la capacidad de absorción de los aerogeles. Por lo tanto, se procedió a realizar los mismos experimentos de absorción pero esta vez bajo condiciones dinámicas. Los resultados son mostrados en la figura 16.

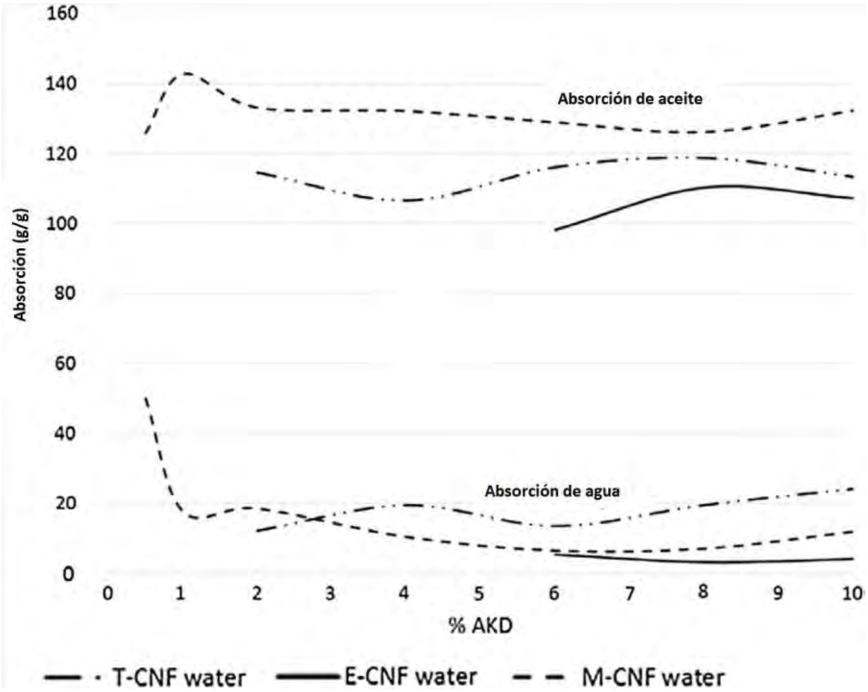


Figura 17: Evolución de la absorción de agua y aceite en condiciones dinámicas de los aerogeles tratados con AKD.

Se puede observar que no se presentan datos para los aerogeles sin tratamiento de AKD y en algunos casos solo se pudieron obtener resultados para aerogeles tratados con un 6 % o más de AKD. Esto se debe a que, bajo condiciones dinámicas, algunos aerogeles se desintegraron en el agua y no pudieron ser recuperados para poder medir la cantidad de agua/aceite absorbida. Esta desintegración pudo deberse a que en estos aerogeles los enlaces entre nanofibras no eran lo suficientemente fuertes o eran demasiado pocos como para mantener la estabilidad dimensional de la estructura del aerogel. Los peores resultados en este sentido se observaron en los aerogeles fabricados a partir de CNF enzimáticas, las cuales sólo pudieron ser testadas a partir de un 6 % de AKD añadido, cantidades que permitieron que el aerogel preservara su forma y peso original. Las CNF enzimáticas no sólo presentaron la menor estabilidad dimensional, sino que también tuvieron la absorción de aceite más baja de todo el conjunto de muestras. En cambio, las CNF mecánicas volvieron a mostrar los mejores resultados como ya hicieron en condiciones estáticas. Es importante destacar también que el máximo peso de aceite absorbido se observó en condiciones dinámicas, concretamente en la muestra de CNF mecánicas con un 1 % de AKD (poco más de 140 g/g). Estas diferencias son provocadas por las turbulencias en condiciones dinámicas que fuerzan la entrada de los líquidos del exterior hacia el seno del aerogel. De hecho, se puede constatar que en el caso de las CNF mecánicas sólo es necesario 0.5 % de AKD para mantener la estabilidad dimensional y la forma del aerogel bajo condiciones dinámicas.

Una tendencia observada en ambas condiciones es que los aerogeles fabricados a partir de CNF TEMPO presentan siempre la capacidad de absorción de agua más alta de todas las muestras con independencia de la cantidad de AKD añadida. Esto es de esperar ya que el AKD bloquea los grupos OH de la superficie de las fibras mediante la introducción de grupos β-ketoester, por lo tanto nanofibras con una alta superficie específica tendrán una mayor capacidad de fijar AKD que las enzimáticas y las mecánicas, que poseen una menor superficie específica. Esto significa que los aerogeles de CNF TEMPO podrían ser capaces de fijar mayores cantidades de AKD lo que aumentaría su capacidad de absorción de hidrocarburos.

Un aspecto importante a tener en cuenta en los aerogeles es su capacidad para ser reutilizados una vez se han saturado de hidrocarburos. Para esto los aerogeles fueron sumergidos en tolueno para remover el aceite absorbido y fueron colocados nuevamente en un recipiente con agua y aceite. La figura 18 muestra los resultados de los experimentos de reciclabilidad en aerogeles tratados con un 10 % de AKD.

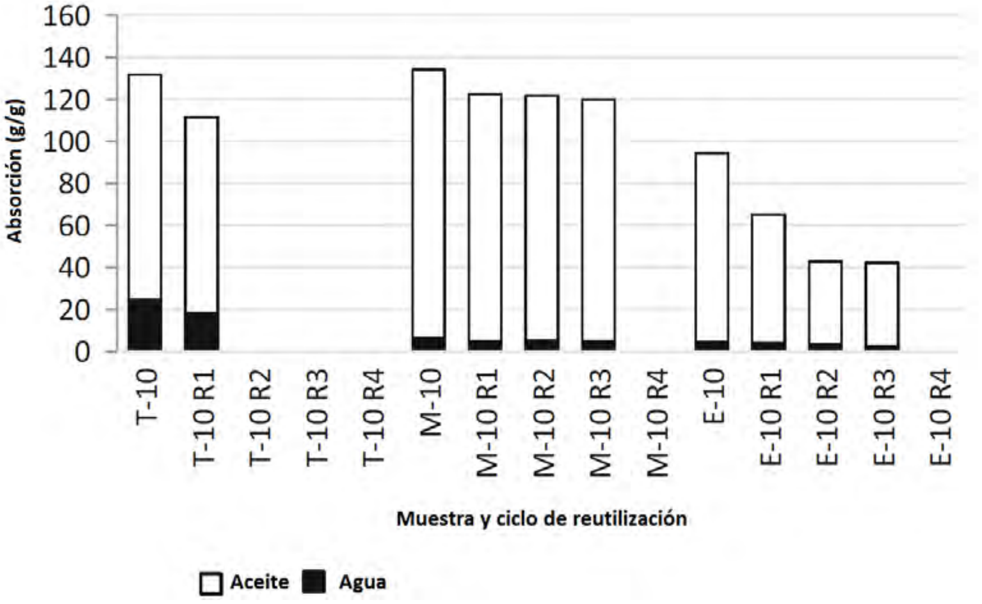


Figura 18: Reciclabilidad de aerogeles tratados con 10% de AKD.

Se eligieron solamente los aerogeles con un 10 % de AKD ya que fueron los que mostraron la mejor estabilidad dimensional y mantuvieron su peso tras la absorción de aceite. Los aerogeles fabricados con CNF TEMPO solamente resistieron un ciclo de reutilización, mientras que el resto resistieron hasta 3 ciclos, si bien mostraron diferentes grados de absorción de agua y aceite con cada ciclo. Los aerogeles a base de CNF TEMPO mostraron la menor capacidad de absorción de aceite tras un primer ciclo de reutilización. Por su parte, los aerogeles de CNF mecánicas exhibieron un buen comportamiento de absorción de aceite con cada ciclo, mientras que los aerogeles enzimáticos sufrieron una pérdida en su capacidad de absorción con cada ciclo de reutilización. En general los resultados demuestran que las CNF modificadas con AKD son una solución viable para la fabricación de materiales absorbentes de hidrocarburos en el agua. En este caso las CNF obtenidas por métodos puramente mecánicos parecen ofrecer las mejores prestaciones en comparación con los otros tipos analizados, no sólo en la capacidad de absorción sino también en su reutilización y costo, ya que este tipo de CNF en particular es relativamente económico comparado con las CNF TEMPO y enzimáticas.

Otro tipo de estructura que puede ser producida a partir de las CNF son los nanopapeles, los cuales consisten en membranas o films formados cuando el agua es evaporada de una suspensión de nanofibras. Los nanopapeles suelen presentar gran transparencia, altas propiedades mecánicas y ligereza, propiedades interesantes para una amplia gama de aplicaciones.

Inicialmente, fue estudiada la producción de nanopapeles con las nanofibras obtenidas a distintos porcentajes de enzima y comparados con los valores obtenidos en nanopapeles producidos con nanofibras de celulosa obtenidas mediante oxidación TEMPO a pH básico con distintos niveles de oxidación. Los resultados obtenidos de las propiedades mecánicas de los distintos nanopapeles se muestran en la figura 19.

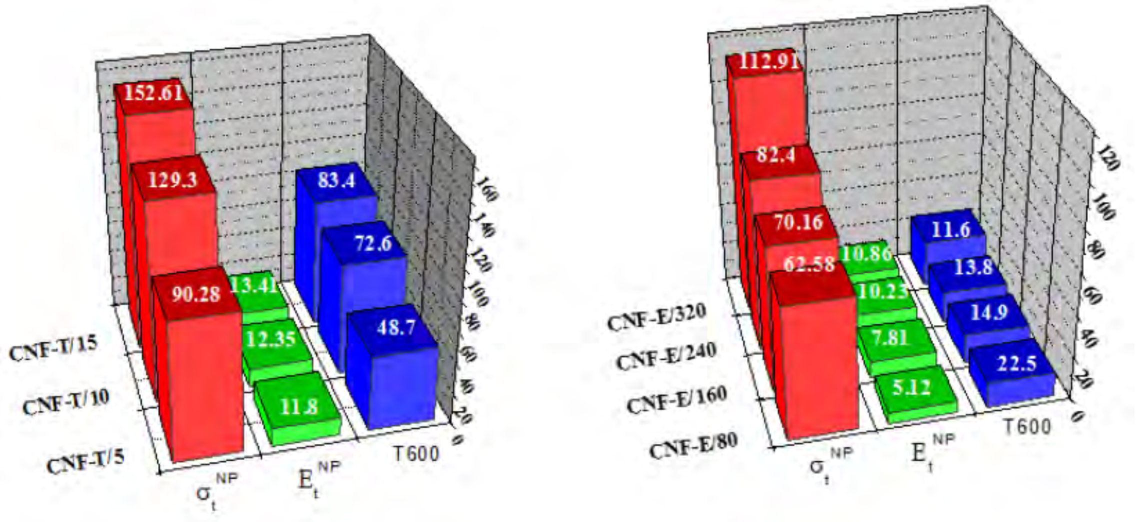


Figura 19: Evolución de la resistencia, módulo de Young i transmitancia a 600 nm de los distintos nanopapeles

La figura 19, muestra la influencia del pretratamiento utilizado para la producción de las distintas nanofibras, así mismo como la severidad de éste, en las propiedades mecánicas y ópticas. En este sentido, la diferencia presentada entre los nanopapeles obtenidos mediante CNF de oxidación TEMPO y CNF de hidrolisis enzimática son producidas por las diferencias en las propiedades intrínsecas de las mismas. Siendo, crucial en las propiedades de los nanopapeles resultantes la superficie específica y el rendimiento de nanofibrilación de las mismas.

Por otro lado la producción de nanopapeles con la adición de nanopartículas de otros materiales, por ejemplo metales, confiere nuevas propiedades a los nanopapeles, lo que amplía la variedad de usos posibles para este tipo de membranas. Para la presente tesis se desarrollaron membranas para altavoces a partir de nanopapeles dotados con partículas de ferrita. La figura 20 resume la metodología seguida para la fabricación de los nanopapeles a base de CNF enzimáticas modificados con ferrita y su caracterización.

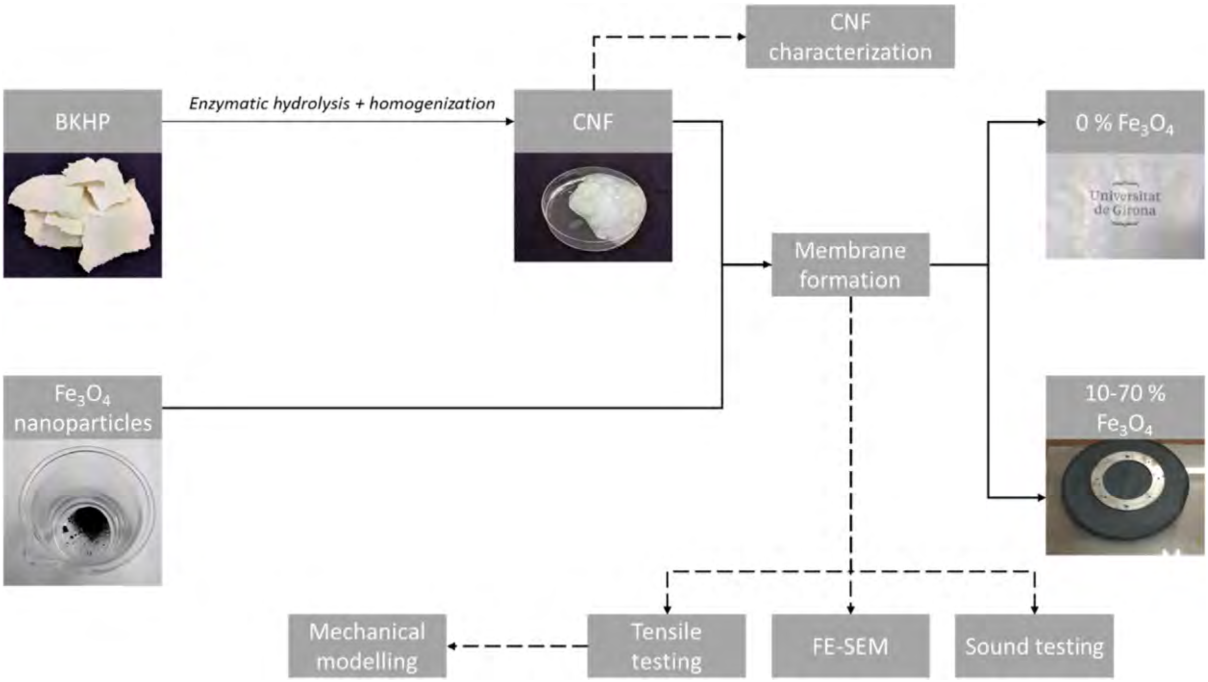


Figura 20: Procedimiento experimental para la fabricación de nanopapeles modificados con ferrita.

El contenido de ferrita varió de un 10 hasta un 70 % en peso en relación con la cantidad de CNF del nanopapel. En cuanto a las propiedades físicas de los nanopapeles (tabla 11) el gramaje se mantuvo alrededor de unos 60 g/m^2. Se eligió este gramaje con la intención de contar con nanopapeles lo suficientemente resistentes para poder asegurar su durabilidad durante las pruebas de sonido.

Tabla 11: Propiedades físicas de los nanopapeles en función de la cantidad de ferrita añadida.

% Fe_3O_4	**Gramaje** (g/m^2)	**Espesor** (µm)	**Densidad** (g/cm^3)
0	59.37±0.68	41±1	1.45
10	62.01±1.02	39±0	1.59
20	60.28±0.73	38±1	1.59
30	58.36±0.51	41±1	1.42
40	58.91±0.19	42±0	1.40
50	61.39±0.87	45±2	1.34
60	61.51±1.13	46±0	1.34
70	60.09±0.98	44±0	1.37

En cambio, el espesor aumentó conforme se incrementaba la cantidad de ferrita, llegando a ser de hasta unos 46 µm en las muestras con un 60 % en peso de ferrita. Por su parte la densidad de los nanopapeles se redujo progresivamente a medida que se añadía más ferrita. Esto se debe a que, si bien la ferrita es mucho más densa que la celulosa (4.8 g/cm^3 contra 1.5), la densidad del nanopapel se reduce a partir de contenidos del 20 % en ferrita ya que esta no tiene la misma capacidad de compactación y flexibilidad que las CNF, lo que provoca mayores espesores mientras el gramaje se mantiene constante.

El análisis de las propiedades mecánicas de los nanopapeles adicionados con ferrita se muestra en la figura 21. Se observa un descenso más o menos lineal de la resistencia a tracción en función de la cantidad de ferrita adicionada.

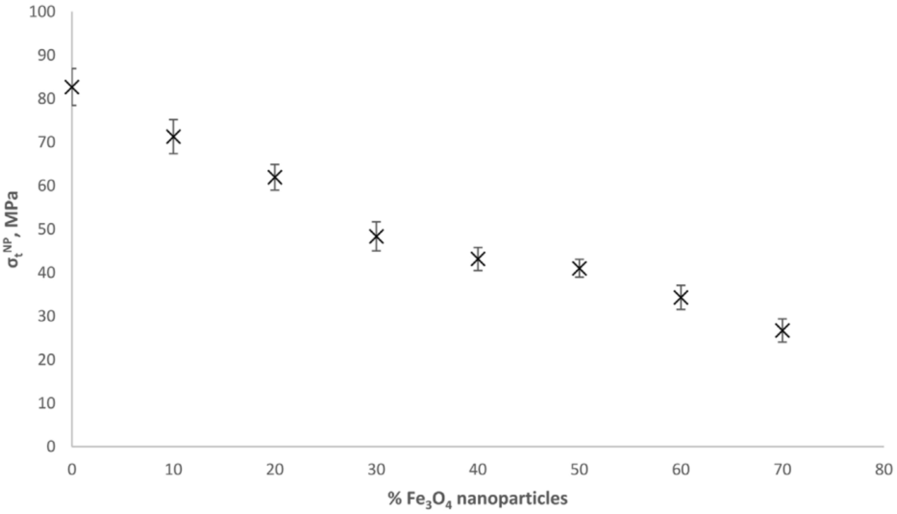


Figura 21: Evolución de la resistencia a tracción de los nanopapeles en relación con la cantidad de ferrita presente.

El descenso en la resistencia del nanopapel se debe a la introducción de partículas que interrumpen la continuidad de la matriz de nanopapel, lo cual disminuye la capacidad de transmisión de carga entre las nanofibras, comprometiendo la resistencia del nanopapel. Las membranas de los altavoces, cuando son usadas, están en constante movimiento vibratorio; la intensidad de las vibraciones depende de la frecuencia del campo magnético el cual, a su vez, está en función de la corriente que fluye a través de la bobina. La rigidez de los nanopapeles es un parámetro importante a ser controlado si se quiere asegurar una buena emisión de sonido. La presencia de la ferrita produjo un descenso en la rigidez original del nanopapel y por ende un aumento en la deformación del mismo. Si bien un descenso en la rigidez del nanopapel podría ser deseable para su uso en altavoces, la pérdida en resistencia a tracción puede comprometer la integridad del nanopapel cuando esté en vibración. Por lo tanto, un buen equilibrio entre flexibilidad y resistencia es deseable para lograr un buen sonido y eliminar ruidos indeseables en la medida de lo posible. Para poder valorar el desempeño de los nanopapeles, se realizó una modelización del funcionamiento de los mismos con un 70 % en peso de ferrita. Como se puede observar en la figura 22, el máximo desplazamiento ocurre en el centro del nanopapel. Este desplazamiento se cuantificó en 0.5 mm aproximadamente. Sin embargo, las partes más externas del nanopapel fueron las zonas sometidas a mayor estrés durante la simulación. Se debe recordar, sin embargo, que en situaciones reales, los nanopapeles estarían sometidos repetidamente a desplazamientos bidireccionales. Sin embargo, la modelación permite establecer un factor mínimo de seguridad de 27, lo que demuestra la factibilidad de utilizar nanopapeles reforzados con ferrita como membranas de altavoces.

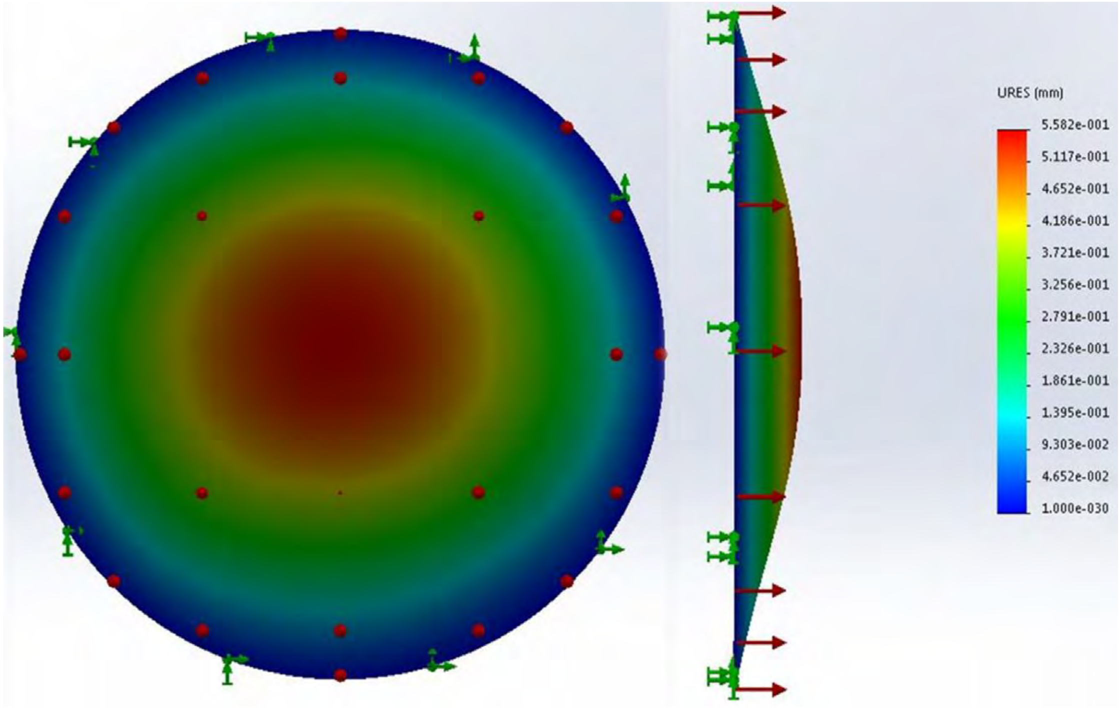


Figura 22: Modelaje del desplazamiento de los nanopapeles ante tensión perpendicular.

Los resultados de las pruebas de sonido están presentes en la figura 23. La gráfica muestra un comportamiento uniforme de todos los nanopapeles modificados con ferrita. En general, las frecuencias no son reproducidas de la misma manera por los altavoces. La sensibilidad más alta se observó en frecuencias contenidas dentro de la tercera banda de 500 Hz.

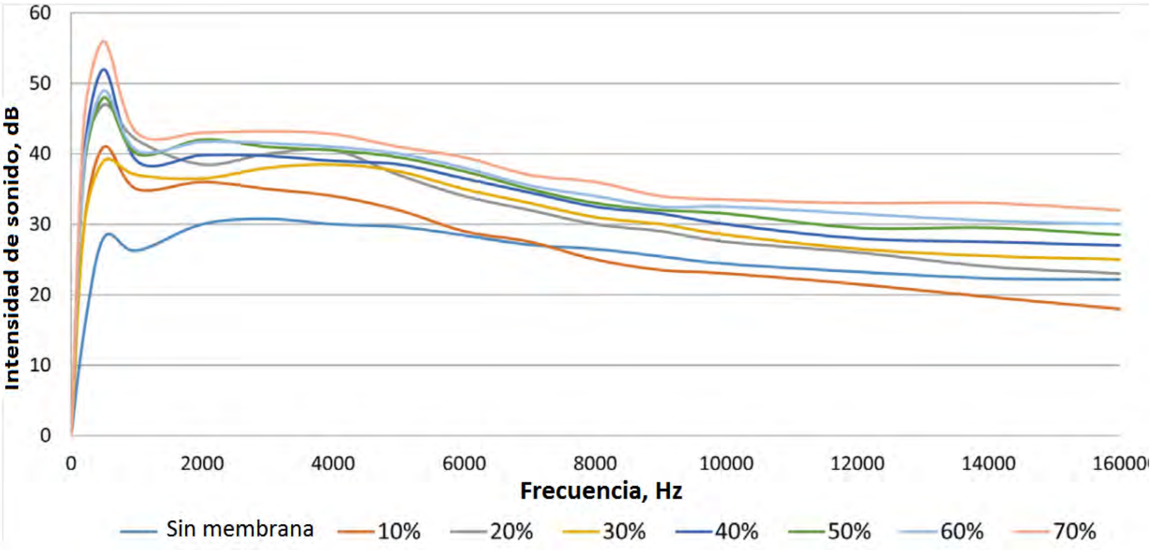


Figura 23: Intensidad del sonido para cada membrana en función de la frecuencia.

Este comportamiento podría haber estado condicionado por la tensión acumulada en el nanopapel y el diámetro de los diafragmas testados. Los resultados muestran un decremento en el nivel de eficiencia de reproducción del sonido conforme se incrementa la frecuencia. Es difícil determinar si esta pérdida en la calidad del sonido está condicionada por la masa de los nanopapeles (la cual es muy similar entre uno y otro nanopapel a pesar de las cantidades variables de ferrita) o a las características del mismo solenoide utilizado. Cualquiera que sea el caso, los resultados muestran que el prototipo de altavoz debe ser mejorado para alcanzar una mínima calidad de sonido reproducido. Un aspecto que queda demostrado es que la cantidad de ferrita presente en los nanopapeles influencia favorablemente la reproducción y fidelidad del sonido; esto se debe a que el nanopapel responde de mejor manera al mismo campo magnético si la cantidad de ferrita es mayor. El hecho de que la calidad de reproducción acústica se mantiene casi constante a pesar del contenido de ferrita se debe a que el gramaje no varía significativamente entre una muestra y otra. Por lo tanto, el escenario ideal sería la adición de un contenido máximo de ferrita para que el altavoz no pierda calidad en la reproducción del sonido. Una posible solución de futuro para este tipo de aplicaciones podría ser la aplicación de plastificantes en los nanopapeles con el fin de reducir la rigidez de los mismos y permitir así la adición de más ferrita, o bien substituir parcialmente las nanofibras de celulosa por fibras de dimensiones micrométricas.

Conclusiones generales

5. Conclusiones generales

La fabricación de nanofibras de celulosa a partir de fibras madereras pretratadas mediante hidrólisis enzimática es un método viable para la obtención de CNF con una importante capacidad de refuerzo sobre pastas papeleras. Tras un proceso de optimización de la hidrólisis enzimática, las CNF obtenidas de esta manera ofrecen una capacidad de refuerzo comparable a la observada en CNF obtenidas mediante métodos químicos que pueden ser costosos a escala industrial.

La adición de las CNF enzimáticas en suspensiones papeleras produce un aumento en la resistencia del papel al promover un mayor número de puentes de hidrógeno entre fibras. Esto también hace disminuir la porosidad y el grosor del papel a un mismo gramaje, lo que incrementa la densidad del mismo. La opacidad también disminuye ligeramente, debido probablemente a la reducción en el espesor del papel.

La aplicación de las CNF enzimáticas puede combinarse con otros métodos de refino tales como el mecánico y el biorefino para producir papeles con altas prestaciones de resistencia. En particular, el uso combinado de CNF juntamente con el biorefino permitió obtener longitudes a ruptura muy superiores a las obtenidas mediante sólo refino mecánico en papeles tipo liner/fluting. Una de las principales ventajas de esta metodología combinada es que las fibras papeleras conservan en gran medida sus propiedades originales, lo que puede alargar la vida útil de las mismas y permitir más ciclos de reciclado.

Las CNF pueden ser añadidas en masa directamente a la suspensión de fibras o bien aplicadas superficialmente en combinación con almidón sobre el papel ya formado. Este método puede ser interesante en el caso de que el deterioro en la capacidad de drenaje de las pastas originada por la presencia de las CNF sea un problema. La combinación de distintos métodos de aplicación juntamente con el biorefino o el refino mecánico permiten la producción de papeles con altas prestaciones de resistencia al tiempo que se podrían ajustar las propiedades deseadas, ya sea añadiendo más o menos nanofibras o ajustando la intensidad del refino.

Las CNF en general presentan propiedades que las hacen interesantes para su aplicación en otros campos a parte del papelero. Los experimentos realizados demostraron que es posible la fabricación de aerogeles, de baja densidad y gran capacidad de absorción, y nanopapeles de altas propiedades mecánicas. Los aerogeles resultaron ser materiales interesantes para su uso como absorbentes de hidrocarburos derramados en el agua cuando las nanofibras son hidrofobizadas con AKD, el cual no sólo reduce la afinidad de las CNF por el agua, sino que mejora la estabilidad dimensional de los aerogeles en el agua bajo condiciones de agitación. Es importante mencionar que pare este tipo de aplicación las CNF preparadas exclusivamente con métodos mecánicos presentaron la mejor absorción de hidrocarburo y estabilidad dimensional, permitiendo incluso su reutilización una vez el aceite era limpiado del aerogel.

Por su parte, las CNF mezcladas con nanopartículas de ferrita fueron utilizadas para fabricar membranas conductoras para altavoces. La ferrita proporcionó conductividad al nanopapel al tiempo que la rigidez del mismo se vio reducida. Se comprobó que la calidad de sonido y potencia del mismo están ligados directamente a la cantidad de ferrita añadida, de tal manera que mayores cantidades de la misma mejoran la calidad del sonido.

En general, se puede concluir que las CNF obtenidas por vía enzimática pueden encontrar aplicaciones en una gran variedad de campos científicos y tecnológicos gracias a su gran versatilidad y disponibilidad, lo que justifica que el futuro se continúe con su investigación sobre nuevas aplicaciones.

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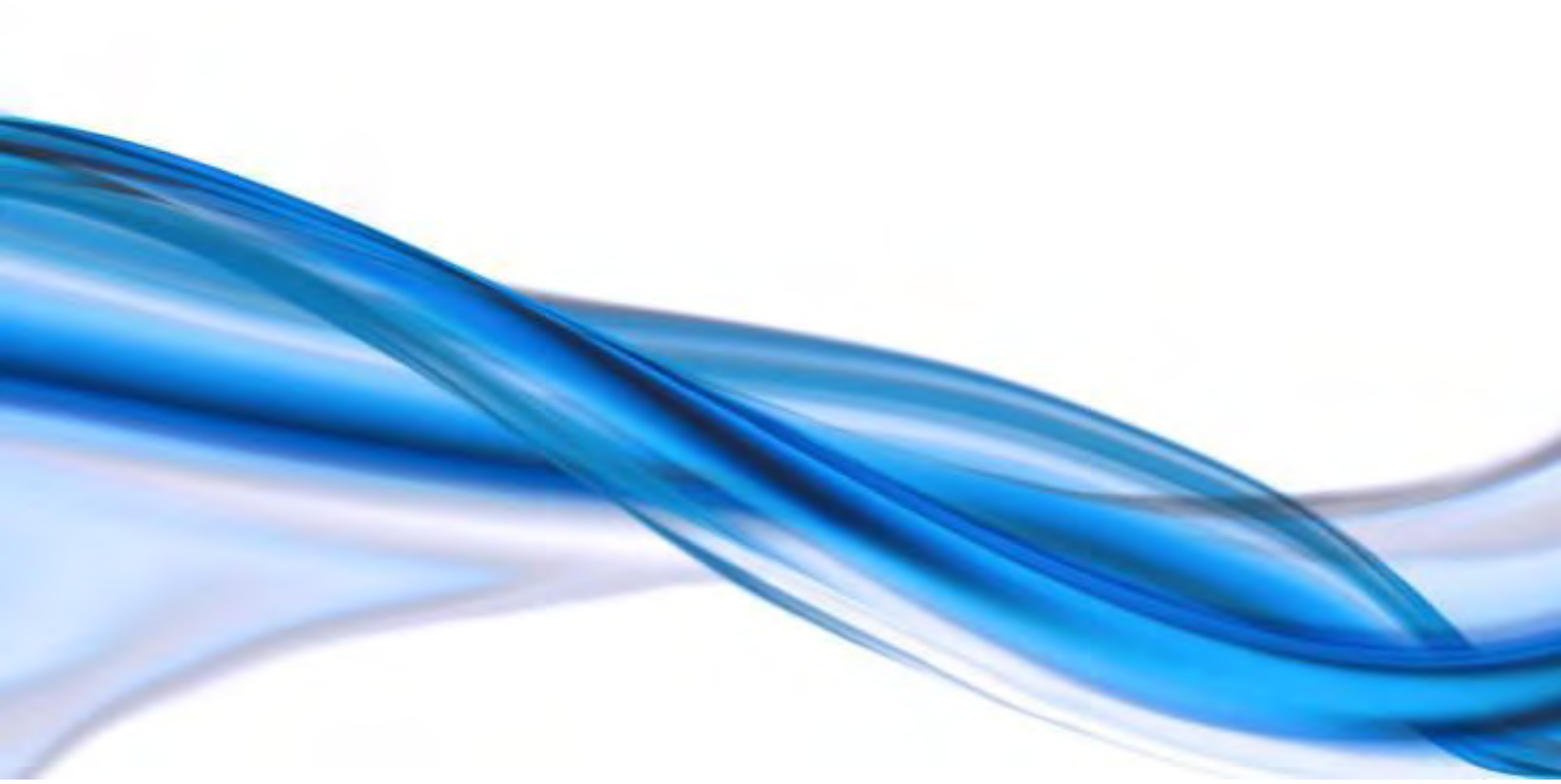

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