

Novel production of lignocellulosic nanofibrils with diverse physical and chemical characteristics from wheat straw

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Abstract

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As the demand for sustainable materials grows, lignocellulosic nanofibrils (LCNFs) have gained attention for their abundance, renewability, and promising properties. However, widespread commercialization remains limited by unsustainable production methods, high costs, and the narrow functionality of currently available nanocellulose, which often lacks tunable properties. Existing LCNF production techniques frequently rely on expensive feedstocks, hazardous chemicals, or lack clarity on how process modifications affect material properties, making it difficult to tailor products for specific applications. This research addresses these challenges by developing process conditions to produce LCNFs with diverse physical and chemical properties from wheat straw. Seven fractionation treatments were conducted, varying sodium hydroxide (7.5–15%), hydrogen peroxide (0–7.5%), and temperature (50–95 °C), followed by chemical composition analysis. Two treatments were oxidized with 7.5% peracetic acid, while three others were oxidized at both 7.5% and 30%. Resulting samples were cast into films and thoroughly

characterized using FTIR, conductometric titrations, SEM, XRD, UV–vis spectroscopy, TGA, contact angle measurements, and tensile testing. Lowering fractionation parameters (temperature and chemical charge) increased hemicellulose and lignin retention, enhancing yields and producing LCNFs with smaller diameters, semi-crystalline structure, low UV transmittance, moderate thermal and mechanical properties, and low wettability. Residual lignin content correlated with several properties: higher lignin led to less uniform fibrils, reduced optical transparency, increased UV absorption and haze, and lower thermal mass loss. Mechanical strength and elastic modulus declined with lignin content, except in samples treated with 30% PAA. Lignin also decreased surface hydrophilicity. Additionally, charge demand and mechanical performance were dictated not only on lignin content but also on whether lignin was primarily removed during fractionation or oxidation. This work contributes to sustainable material development by offering an adaptable platform for producing LCNFs with tunable properties suitable for a wide range of end-use applications.

Table of Contents

Table of Contents	i
List of Figures.....	iii
List of Table	iv
Acknowledgements	v
1. Introduction.....	1
1.1. What are cellulose nanofibrils?.....	1
1.2. What are lignocellulosic nanofibrils?	2
1.3. What are the properties of LCNFs?	3
1.4. What are the applications of LCNFs?.....	4
1.5. What are current methods to change the physical and chemical characteristics of LCNFs?.....	5
1.6. Research objectives.....	7
2. Materials and methods	8
2.1. Materials	8
2.2. Alkaline peroxide fractionation and refining	9
2.3. Peracetic acid oxidation	9
2.4. Lignocellulosic nanofibril production.....	9
2.5. Lignocellulosic nanofibril films.....	10
2.6. Characterization methods.....	10
2.6.1. Mass yield after fractionation and oxidation	10
2.6.2. Chemical composition	10
2.6.3. Fournier-transform infrared spectroscopy (FTIR)	10
2.6.4. Charge density	11
2.6.5. Scanning electron microscopy (SEM)	11
2.6.6. X-ray diffraction (XRD)	11
2.6.7. UV vis spectroscopy	12
2.6.8. Thermogravimetric analysis (TGA).....	12
2.6.9. Mechanical tensile test.....	12
2.6.10. Surface wettability	13

2.7.	Extent of delignification	13
2.8.	Statistical analysis	13
3.	Results and discussion	14
3.1.	Effect of process conditions on chemical characteristics of fibers	14
3.2.	Lignocellulosic nanofibril characterization	18
3.2.1.	FTIR and charge density	18
3.2.2.	Nanofibril morphology and structure.....	19
3.2.3.	Optical properties.....	22
3.2.4.	Thermal properties	24
3.2.5.	Mechanical properties	26
3.2.6.	Wetting behavior and surface free energy	29
3.3.	Effect of process conditions on physical characteristics of LCNFs.....	31
4.	Conclusion	34
5.	Future Work	35
6.	Bibliography	38
7.	Appendix A – Supplementary Information	49

List of Figures

Figure 3.1. (A) FTIR spectra. (B) Scatter plot of the charge density. *Statistically significant at a 95% confidence level.	19
Figure 3.2. SEM images of the LCNF samples with lignin contents ranging from 2.6 wt.% to 11.3 wt.%.	20
Figure 3.3. Nanofibril diameter distributions.....	21
Figure 3.4. XRD patterns and crystallinity index measurements. *Statistically significant at a 95% confidence level.	22
Figure 3.5. (A) Optical transmittance spectra of the LCNF samples. (B) Scatter plot of the haze. *Statistically significant at a 95% confidence level.	24
Figure 3.6. (A) TGA curve. (B) DTG curve. (C) Scatter plot of the thermal mass loss percentage. (D) Scatter plot of the thermal peak degradation temperature. *Statistically significant at a 95% confidence level.	26
Figure 3.7. (A) Scatter plot of the ultimate tensile strength. (B) Scatter plot of the elastic modulus. (C) Scatter plot of the strain at break. *Statistically significant at a 95% confidence level.....	29
Figure 3.8. (A) Scatter plot depicting the water contact angle. (B) Scatter plot depicting the surface free energy. *Statistically significant at a 95% confidence level.	31
Figure 3.9. (A) Bar plot of the charge demand against the delignification ratio. (B) Bar plot of the ultimate tensile strength against the delignification ratio.	34

List of Table

Table 3.1. Chemical composition of the original wheat straw and seven different fractionation treatments, and mass balance results relative to the original wheat straw. *Statistically significant at a 95% confidence level.	15
Table 3.2. Chemical composition of eight different treatments after oxidation and mass balance results relative to the original wheat straw. *Statistically significant at a 95% confidence level.	17
Table 3.3. Nanofibril diameter and percentage of nanofibrils with diameters less than 20 nm for LCNF samples. *Statistically significant at 95% confidence.	21
Table 3.4. TGA summary characteristics for the LCNF samples. *Statistically significant at a 95% confidence level.	26
Table 3.5. Contact angle and surface free energy measurements for the LCNF samples. *Statistically significant at a 95% confidence level.	31
Table 3.6. Extent of delignification values for LCNF samples with lignin contents between 2.6 wt.% and 3.9 wt.%.	34
 Table A.1. Summary of all fractionation process conditions.	 49
Table A.2. Chemical composition of the original wheat straw and seven different fractionation treatments, and mass balance results relative to the original wheat straw. *Statistically significant at a 95% confidence level.	50

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1. Introduction

1.1. What are cellulose nanofibrils?

Cellulose, the most abundant biopolymer in nature, is a linear polysaccharide found in plant cell walls that serves as the major constituent in plants. Cellulose is comprised of glucose monomers linked by β -1,4-glycosidic bonds that extend to degrees of polymerization of up to 10,000 glucose units [1]. Cellulose fibrils comprise of multiple chains of cellulose molecules bound together through covalent and hydrogen bonding. Through intense mechanical or chemical processing, these cellulose fibrils can be deconstructed into cellulose microfibrils and nanofibrils. Broadly, cellulose microfibrils and nanofibrils are considered nanocellulose. However, nanocellulose generally is defined as cellulose nanofibrils that are less than 100 nm wide and a few micrometers long [2]. There are two main types of nanocellulose: cellulose nanofibers (CNF) and cellulose nanocrystals (CNC). Their main difference is that CNFs are longer fibrils that contain crystalline and amorphous regions of cellulose, whereas CNCs are shorter fibrils that lack amorphous regions and are purely crystalline.

Currently, the main feedstock for CNF production is bleached kraft pulp. The pulp undergoes either mechanical, chemical, or enzymatic pretreatment to produce CNFs. However, mechanical pretreatments are energy intensive (specific energy consumptions of up to 30,000 kWh/ton) [3] and enzymatic pretreatments take too much time, so chemical pretreatments are currently favored. The most common chemical pretreatment is TEMPO (2,2,6,6-Tetramethylpiperidin-1-oxyl) oxidation. TEMPO-oxidation produces CNFs with homogenous diameters around 3-4 nm [4], and high carboxyl content since TEMPO selectively oxidizes the C6 primary hydroxyl group into carboxylic acids inducing a large negative surface charge and greater electrostatic repulsion

of CNFs in aqueous solutions [5]. However, there are many disadvantages to TEMPO oxidation and overall CNFs that make it very unsustainable and limit its industrial feasibility. TEMPO has a high chemical cost, toxicity, and needs to be separated from the CNFs through dialysis, which is both expensive and time-consuming [6]. CNFs have high production costs associated with bleached pulp feedstocks and chemical costs that limit large-scale production and are hydrophilic which limits their applications in sustainable nanocomposites and food packaging. Therefore, this study will shift focus from CNFs and center around the production of another sustainable material, lignocellulosic nanofibrils (LCNFs).

1.2. What are lignocellulosic nanofibrils?

Generally, LCNFs are cellulose nanofibrils with lignin and hemicelluloses. Lignin, the second most abundant biopolymer in nature, is a polymer network of phenolic rings that acts as the “glue” in plants. Its primary function is to bind cellulose, hemicellulose, and other plant constituents together to form important structural materials in plants. Hemicelluloses are branched polysaccharides that comprise glucose, xylose, arabinose, galactose, and mannose sugars. Their primary roles are to strengthen plant cell walls by interacting with cellulose and lignin. LCNFs refer to any cellulosic nanofibril that is less than 100 nm wide and has a lignin content greater than 1% [3]. LCNFs, like CNFs, are longer fibrils that contain amorphous and crystalline regions of cellulose. However, the addition of lignin and hemicellulose to LCNFs offers unique properties that can expand its role in various applications. In the production of LCNFs, the original biomass components are preserved, leading to increased yields. By bypassing multiple steps required for producing low-lignin pulp, production costs are notably lowered. Furthermore, utilizing inexpensive feedstocks for nanocellulose production proves to be a far more sustainable approach compared to traditional bleached pulp. Thus, LCNFs represent a

sustainable alternative to conventional CNFs, offering significant economic and environmental benefits.

LCNFs are derived from low-cost lignocellulosic biomass or unbleached pulps using chemical or mechanical pretreatments. Their production typically involves three steps, starting with feedstock selection. Common feedstocks include agricultural residues (e.g., wheat straw [6], [7], [8], sugarcane bagasse, corn stover), wood residues (e.g., sawdust, wood chips) [9], and natural plants (e.g., banana, jute, pineapple) [10], which are often low-value waste materials. Significant efforts have also focused on wood-derived sources, such as Norway spruce [11], switchgrass, poplar, and pine [12]. The second step is fractionation, a chemical treatment that removes lignin, hemicelluloses, extractives, and ash while preserving cellulose and some hemicellulose. The final step is pretreatment, which may be chemical, mechanical, or enzymatic, like CNF production. Most commercially available LCNFs are produced via TEMPO-oxidation or purely mechanical methods, resulting in specific LCNF types suited for a limited range of applications.

1.3. What are the properties of LCNFs?

LCNFs offer a range of desirable characteristics, including renewability, non-toxicity, biocompatibility, biodegradability, and recyclability [13]. Their high aspect ratio results in a large surface area, low density, and excellent mechanical strength [3]. The presence of lignin further enhances thermal stability, UV absorption, antioxidant activity, and hydrophobicity. The high thermal degradation temperature of lignin—greater than that of cellulose and hemicellulose—contributes to the improved thermal stability of LCNFs [14]. Its polymeric structure, rich in phenolic rings, is responsible for increased UV absorption, antioxidant capacity, and hydrophobicity. Espinosa et al. demonstrated that the UV absorption and antioxidant activity of

LCNF-reinforced polyvinyl alcohol (PVA) films increased with lignin content, attributed to the aromatic ring electrons in lignin [5]. Similarly, Rojo et al. found that the water contact angle of LCNF nanopapers rose with lignin content, reflecting greater hydrophobicity due to the hydrophobic aryl groups in lignin's phenolic structure [11].

Current production methods often prioritize maximizing cellulose yield during delignification and oxidation, leading to reduced hemicellulose content. However, hemicellulose plays a vital role in enhancing LCNF properties. It contributes to stronger inter-fiber bonding and increased bonding area, resulting in improved mechanical performance of fiber networks [15]. Additionally, hemicellulose enhances colloidal stability in aqueous media by imparting a negative surface charge, which promotes electrostatic repulsion and reduces aggregation—improving optical transparency [16]. It also inhibits microfibril coalescence, aiding the nanofibrillation process [17]. These benefits underscore the importance of retaining hemicellulose in LCNFs.

1.4. What are the applications of LCNFs?

LCNFs have a wide range of applications owing to their unique properties. These properties make LCNFs a promising biomaterial for various applications, including bio-based composites [5], [6], papermaking [8], food packaging [18], hydrogels [19], aerogels [20], filtration membranes [21], and electronics [22]. However, different applications often require diverse physical and chemical properties. LCNFs with high lignin content exhibit enhanced thermal stability [14], greater ultraviolet light absorption [23], and reduced hydrophilicity [11] compared to low lignin-containing LCNFs, which offer superior tensile strength, elastic modulus [11], and optical transmittance in the visible region [18]. For applications such as food packaging, which require excellent mechanical properties, high transparency, and excellent water and oxygen

barrier properties [24], neither high-lignin nor low-lignin LCNFs offer a perfect solution.

Therefore, tailoring the physical and chemical characteristics of LCNFs for specific applications is crucial to expand their range of potential uses.

1.5. What are current methods to change the physical and chemical characteristics of LCNFs?

Our research group at the University of Washington developed a simple, scalable process for producing LCNFs, enabling their use as sustainable materials across various applications. The method involves a two-step chemical treatment—alkaline hydroxide fractionation followed by peracetic acid oxidation—paired with intensive mechanical pretreatment using a microfluidizer to produce a uniform LCNF product [6]. This process was designed to offer a cost-effective alternative to TEMPO-oxidized nanocellulose, which dominates the market at approximately \$1000 USD/kg. TEMPO-oxidized nanocellulose is known for its small fibril size, strong electrostatic repulsion, excellent colloidal stability, and large specific surface area [25]. Pascoli et al. conducted a techno-economic analysis of the process, estimating a minimum selling price of \$4.60 USD/kg at a 15% discount rate [26], significantly lower than the ~\$1.00 USD/g cost of commercially available nanocellulose [27]. They also assessed the robustness of the process by applying it to other agricultural residues, including corn stover, reed canary grass, and industrial hemp. The resulting LCNFs showed similar morphology, crystallinity, and visible light transmittance [28]. However, a key knowledge gap remains whether LCNFs with tailored physical and chemical properties can be produced by systematically adjusting process conditions.

One common approach to modify the physical and chemical properties of LCNFs is surface adsorption of charged additives. For example, Xhanari et al. reduced the hydrophilicity of TEMPO-oxidized CNFs by adsorbing varying concentrations of cetyltrimethylammonium

bromide (CTAB), increasing the water contact angle from 41.7° to 71.7° as CTAB concentration rose from 0 to 0.51 mmol/g [29]. However, CTAB is highly toxic if ingested and poses serious environmental risks [30], as does TEMPO [31], limiting their commercial viability. Korhonen et al. improved the surface hydrophobicity of cellulose-composite films by coating them with cationic starch and carnauba wax particles, raising the contact angle from 43° to 128° while preserving mechanical strength [32]. Despite its effectiveness, carnauba wax is expensive due to labor-intensive harvesting and purification processes [33] and remains in high demand for applications such as automotive waxes [34]. These factors contribute to elevated operating costs, presenting a challenge for large-scale implementation.

Another method to tailor the physical and chemical properties of LCNFs involves incorporating lignin as an additive. By adding external lignin to cellulose nanofibrils (CNFs), researchers can replicate LCNF-like structures and impart specific functionalities. For instance, Hai et al. modified lignin nanoparticles with citric acid and octadecyl amine and applied them as coatings on TEMPO-oxidized CNF films, enhancing hydrophobicity, antioxidant activity, and UV shielding for potential food packaging applications [35]. Similarly, Farooq et al. studied nanocomposite films made from CNFs and four types of modified softwood kraft lignin. As lignin content increased, tensile strength dropped from 132.0 MPa to 38.2 MPa, the elastic modulus from 5.7 GPa to 2.9 GPa, and water permeability improved from 2023 to 101 L/h/m² [36]. Despite these benefits, this approach faces scalability challenges. Lignin, a byproduct of the pulp and paper industry, is often burned for electricity generation, supporting mill operations, earning renewable energy credits [37], or contributing to biogenic carbon capture efforts [38]. This competition limits consistent, large-scale lignin availability. Additionally, Hai et al. used TEMPO-oxidized CNFs, which pose health risks [31], [39], and bleached hardwood kraft pulp,

priced at \$1490 USD/ton [40], further increasing production costs. These factors hinder commercial scalability.

The physical and chemical properties of LCNFs can be tuned by adjusting processing conditions. Rojo et al. varied the temperature and duration of the SO₂–ethanol–water (SEW) fractionation process to produce three LCNF samples with different lignin contents from Norway spruce. As lignin content increased from 0 wt.% to 13.5 wt.%, nanofibril diameter decreased from 44 nm to 16 nm, tensile strength declined from 164 MPa to 116 MPa, elastic modulus dropped from 14.3 GPa to 12.2 GPa, and water contact angle rose from 35° to 78° [11]. In contrast, Espinosa et al. produced LCNFs from wheat straw using various pretreatment methods—TEMPO-oxidation, mechanical, and enzymatic—resulting in lignin contents ranging from ~0 wt.% to 7.7 wt.%. As lignin increased, nanofibril diameter grew from 6.8 nm to 14.0 nm, and light transmittance fell from 90% to 75% [8]. While both approaches yielded LCNFs with varied properties, neither study explained how specific process changes affected the resulting chemical characteristics. Rojo et al. emphasized lignin content variation without detailing the impact of fractionation parameters, and Espinosa et al. focused on final LCNF properties rather than the mechanisms behind different pretreatments. Additionally, both used high-pressure homogenization—a method with high energy demands and operating costs—limiting scalability [41]. Espinosa et al. also used TEMPO, a toxic chemical with known genotoxic and cytotoxic effects [31], [39], and costly enzymes [42], [43], further complicating commercial viability.

1.6. Research objectives

Current methods for modifying LCNF characteristics often overlook how process conditions influence their physical and chemical properties. Parameters such as chemical charge and temperature significantly affect the chemical composition of treated biomass, which in turn

impacts key physical traits relevant to specific applications. However, most studies focus on comparing LCNF properties without examining how process conditions shape those characteristics. Limited research has explored intentionally adjusting process parameters to tailor chemical composition and, consequently, physical performance. Few studies investigate whether different process conditions yielding similar chemical compositions result in different physical properties. Additionally, many existing methods rely on hazardous chemicals or costly feedstocks and processes, posing challenges for large-scale commercialization. Although Pascoli et al. [6] demonstrated commercial viability and robustness, their approach does not address the potential for tuning LCNF characteristics through process modification. This study aims to fill that gap by establishing process conditions for producing LCNFs with varied physical and chemical characteristics from wheat straw. Seven fractionation treatments were applied, varying sodium hydroxide (7.5–15%), hydrogen peroxide (0–7.5%), and temperature (50–95°C), followed by chemical composition analysis. Two treatments underwent peracetic acid oxidation at 7.5%, while three additional treatments were oxidized at 7.5% and 30%, respectively. All samples were formed into films and comprehensively characterized using FTIR, conductometric titrations, SEM, XRD, UV–vis spectroscopy, TGA, contact angle measurements, and tensile testing.

2. Materials and methods

2.1. Materials

Wheat straw bales were sourced from Snohomish County, WA. The feedstock was air-dried to a moisture content of 7.0% and stored in sealed containers at room temperature until use.

Other reagents include: 50% (w/w) sodium hydroxide (VWR Chemicals BDH), 50% (w/w) hydrogen peroxide (Cascade Columbia Distribution), diethylenetriamine pentaacetic acid (DTPA) 98+% (Acros Organics, Fisher Scientific), 32% (w/w) peracetic acid in dilute acetic acid (Sigma Aldrich).

2.2. Alkaline peroxide fractionation and refining

Wheat straw was subjected to seven different treatments, varying in sodium hydroxide (NaOH) and hydrogen peroxide (H₂O₂) charges (7.5–15% and 0–7.5%, respectively, based on oven-dry biomass) and fractionation temperatures ranging from 50 to 95 °C. Specific conditions are detailed in Appendix A, Table A.1. After fractionation, the pulped fibers were washed with deionized (DI) water and homogenized to produce wheat straw pulp, which was then refined using a laboratory-scale PFI mill for 30,000 revolutions.

2.3. Peracetic acid oxidation

Refined wheat straw pulp was treated with peracetic acid (PAA) at either 7.5% or 30% w/w (based on oven-dry biomass), following a modified protocol from Pascoli et al. [6]. The pulp was mixed with a PAA solution (pH 4.8) in an IKA HB 10 rotary evaporator, and the reaction proceeded at 85 °C for 50 minutes. After oxidation, the pulp was vacuum filtered and subjected to a three-step wash with a caustic solution, followed by multiple washes with deionized (DI) water.

2.4. Lignocellulosic nanofibril production

PAA-treated pulp was diluted to a 0.4% consistency and mechanically fibrillated using a commercial blender, yielding LCNFs with uniform morphology. The resulting nanofibrils met the standard definition of lignocellulosic nanofibrils, with fiber diameters below 100 nm and

lignin content above 1% [3]. All samples were stored in glass bottles and refrigerated at 3 °C until use.

2.5. Lignocellulosic nanofibril films

LCNF films were cast in 100 mm diameter glass crystallizing dishes using a simple casting method. The samples were dried in an NRTL-certified vacuum oven at 60 °C and -25 mmHg for approximately 48 hours. The oven was equipped with a digital controller and connected to a 240 L/min rotary vane vacuum pump with an exhaust filter, KF25D adapter, and clamp. After drying, the films were hot-pressed using a Rosin Tech heat press at 145 °C for 15 hours. All films were then conditioned at 23 °C and 30% relative humidity for at least 12 hours before testing.

2.6. Characterization methods

2.6.1. Mass yield after fractionation and oxidation

Total mass yield after fractionation and oxidation was determined gravimetrically by comparing the oven-dry mass of the final fibers to that of the original wheat straw feedstock.

2.6.2. Chemical composition

The chemical composition of the original wheat straw, post-fractionation pulp, and post-oxidation pulp were analyzed to evaluate the impact of chemical treatments. Cellulose, hemicellulose, and both acid-soluble and acid-insoluble lignin were quantified using the Klason method [44], [45], [46], [47]. Ash content was measured gravimetrically following Sluiter et al. [48], and total extractives were determined via water-based Soxhlet extraction with a 12-hour reflux, as previously described [49].

2.6.3. Fournier-transform infrared spectroscopy (FTIR)

Infrared spectra were collected using a Shimadzu FT-IR Prestige-21 spectrometer equipped with a DLATGS detector and a MIRacle ATR accessory (PIKE Technologies). Data were acquired under ambient conditions over the 500–4000 cm⁻¹ range, with a resolution of 4 cm⁻¹ and 40 accumulated scans.

2.6.4. Charge density

Charge density was measured by conductometric titration following a previously reported method [50] with minor modifications. A 0.5 mL aliquot of 0.1 M HCl was added to 50 mL of a 0.1 wt.% sample suspension and stirred for 10 minutes. Titration was performed by adding 0.01 M NaOH in 100 µL increments, while conductivity was recorded using a conductivity meter. Charge density (µmol COOH/g) was calculated using Equation 2.1, with V₁ and V₂ representing the volumes of NaOH required to neutralize excess HCl and carboxylic acids, respectively.

$$\text{Charge density} = \frac{(V_1 - V_2) \times [\text{NaOH}]}{\text{Sample Mass Oven-Dry}} \quad (\text{Eq. 2.1})$$

2.6.5. Scanning electron microscopy (SEM)

SEM imaging was performed using an Apreo S microscope (Thermo Fisher Scientific) equipped with an ETD in-chamber and T2 in-column SE detector. Imaging was conducted under high vacuum at 2 kV and 13 pA. Samples were prepared by bath sonicating a 0.01 wt.% LCNF suspension for 3 minutes, then drop-casting 10 µL onto a clean SiO₂ wafer (Ted Pella Inc.). Before imaging, samples were sputter-coated with a 2 nm platinum layer using a Leica EM ACE600 coater. Fiber diameter was analyzed by measuring 500 individual fibrils using ImageJ software.

2.6.6. X-ray diffraction (XRD)

Crystallinity was evaluated using X-ray diffraction (XRD) on a Bruker D8 Discover system with a Pilatus 100K 2D detector and Cu K α radiation, operated at 50 kV and 1 mA.

Diffraction patterns of neat films were collected over a 2θ range of 10° – 50° in 5.5° increments. The crystallinity index (CI) was calculated using the Segal method, where I_t is the intensity of the crystalline (200) peak at $2\theta = 22.7^\circ$, and I_a is the intensity of the amorphous (110) peak at $2\theta = 18^\circ$, as shown in Equation 2.2 [51].

$$CI (\%) = \frac{I_t - I_a}{I_t} \times 100 \quad (\text{Eq. 2.2})$$

2.6.7. *UV vis spectroscopy*

The optical transmittance was measured using a PerkinElmer UV/VIS/NIR Lambda 750 spectrometer across a wavelength range of 250–800 nm. Visible light transmittance was evaluated by recording percent transmittance at 660 nm, while values at 280 nm and 320 nm were used to assess transmittance in the UV-B and UV-A regions, respectively. Optical haze was also measured using the same spectrometer over a range of 380–780 nm.

2.6.8. *Thermogravimetric analysis (TGA)*

Thermal properties were assessed using a Mettler Toledo TGA/DSC 3+ thermogravimetric analyzer. Samples were heated from room temperature to 600 °C at a rate of 10 °C/min under a nitrogen flow of 60 mL/min.

2.6.9. *Mechanical tensile test*

Mechanical properties were tested using an EJA Vantage-10 Tensile Tester with a 50 mm gauge length, following conditioning at 25 °C and 50% relative humidity for at least 12 hours.

Films were cut into 70 mm × 15 mm specimens, and thickness was measured at ten points using a Digimatic Micrometer.

2.6.10. Surface wettability

Surface wettability was assessed by measuring the static contact angles of water and diiodomethane on film surfaces using a Krüss Drop Shape Analyzer. Images were captured immediately after droplet deposition. Surface free energy was calculated using the Owens-Wendt-Rabel-Kaelble (OWRK) model within the Krüss software. Each sample was analyzed with a minimum of four replicates.

2.7. Extent of delignification

The extent of delignification was calculated using Equation 2.3, where L_i denotes the initial lignin content before treatment and L_f represents the final lignin content after treatment.

$$\text{Extent of delignification} = \frac{L_i - L_f}{L_i} \times 100 \quad (\text{Eq. 2.3})$$

The delignification ratio was calculated as the proportion of lignin removal during PAA oxidation relative to the total extent of delignification.

2.8. Statistical analysis

Analysis of variance (ANOVA) was performed on all physical and chemical properties to evaluate statistical significance. A p-value below the alpha level of 0.05 (95% confidence) was considered statistically significant.

3. Results and discussion

3.1. Effect of process conditions on chemical characteristics of fibers

Wheat straw was subjected to seven fractionation treatments with varying NaOH (7.5%–15%) and H₂O₂ (0%–7.5%) charges, based on oven-dry biomass, and temperatures ranging from 50°C to 95°C. Table 3.1 summarizes the chemical composition of the original feedstock and post-fractionation samples, along with corresponding mass balances. Mass yields ranged from 66.1% to 87.0% across treatments. Cellulose recovery remained consistently at 100%, confirming no cellulose loss, while hemicellulose recovery varied between 82.6% and 97.3%. At constant chemical charges, increasing temperature reduced hemicellulose recovery. Conversely, at fixed temperatures (50°C and 90°C), decreasing chemical charges increased hemicellulose retention. Pascoli et al. reported similar cellulose and hemicellulose recoveries (99.9% and 79.6%) after delignifying wheat straw at 90°C with 15% NaOH and 7.5% H₂O₂ [6]. Decreasing temperature from 95°C to 50°C at constant chemical charges reduced lignin removal, thereby increasing mass yield. This trend aligns with findings by Selig et al., who observed increased mass yield and lignin content in corn stover when pretreatment temperature was decreased from 65°C to 25°C [52]. Similarly, at constant temperature, decreasing NaOH and H₂O₂ charges increased lignin content and mass yield. For example, lignin content increased from 10.3% to 16.3% at 50°C and from 7.0% to 12.5% at 90°C as chemical charges decreased. Gould observed a similar trend in kenaf, where increasing H₂O₂ concentration from 0.5% to 2.0% enhanced lignin solubilization by 10% under alkaline conditions at 25°C [53].

	Chemical composition (%)					Mass yield	Mass balance (%)		
	Cellulose*	Hemicellulose*	Lignin*	Ash*	Extractives*		Cellulose recovery	Hemicellulose recovery	Lignin recovery
Wheat straw	36.4 ± 1.1	23.3 ± 0.6	22.7 ± 0.7	11.5 ± 0.1	13.6 ± 0.7	-	-	-	-
95°C, 15% NaOH, 7.5% H ₂ O ₂	56.7 ± 2.5	29.1 ± 1.3	6.5 ± 0.2	1.8 ± 0.2	4.1 ± 0.0	66.1	100.0	82.6	18.9
90°C, 15% NaOH, 7.5% H ₂ O ₂	58.4 ± 1.2	28.9 ± 0.5	7.0 ± 0.1	2.1 ± 0.2	3.4 ± 0.3	68.0	100.0	84.4	20.9
70°C, 15% NaOH, 7.5% H ₂ O ₂	54.9 ± 1.3	29.0 ± 0.7	7.0 ± 0.4	2.0 ± 0.3	3.6 ± 0.4	68.4	100.0	85.1	21.0
50°C, 15% NaOH, 7.5% H ₂ O ₂	49.4 ± 2.9	27.6 ± 1.7	10.3 ± 1.2	2.6 ± 0.1	6.0 ± 0.1	77.0	100.0	89.9	34.5
50°C, 15% NaOH, 0% H ₂ O ₂	50.1 ± 0.3	27.8 ± 0.3	13.1 ± 0.2	4.1 ± 0.1	3.8 ± 0.0	75.9	100.0	89.0	42.5
90°C, 7.5% NaOH, 0% H ₂ O ₂	49.8 ± 0.8	29.3 ± 0.4	12.5 ± 0.1	5.6 ± 0.5	3.8 ± 0.7	75.4	100.0	91.7	40.5
50°C, 7.5% NaOH, 0% H ₂ O ₂	43.1 ± 0.0	26.0 ± 0.3	16.3 ± 0.2	7.8 ± 0.4	4.1 ± 0.5	87.0	100.0	97.3	57.6

Table 3.1. Chemical composition of the original wheat straw and seven different fractionation treatments, and mass balance results

relative to the original wheat straw. *Statistically significant at a 95% confidence level.

PAA oxidation treatments were selected based on post-fractionation lignin content to ensure a broad range of lignin contents. This included treatments at 90°C with 15% NaOH and 7.5% H₂O₂ (7.0 wt.% lignin) and 50°C with 7.5% NaOH and 0% H₂O₂ (16.3 wt.% lignin). Additionally, three treatments with similar lignin content (~12 wt.%)—50°C with 15% NaOH and 7.5% H₂O₂, 50°C with 15% NaOH and 0% H₂O₂, and 90°C with 7.5% NaOH and 0% H₂O₂—were oxidized using both 7.5% and 30% PAA to investigate the impact of oxidation conditions on LCNF physical and chemical properties. Table 3.2 presents the post-oxidation chemical composition and mass balance relative to the original wheat straw. Overall mass yields ranged from 60.5% to 80.7%. Cellulose recovery remained at 100.0%, confirming no cellulose loss during oxidation, while hemicellulose recovery ranged from 73.8% to 97.3%. These findings are consistent with Pascoli et al., who reported 99.7% cellulose recovery but lower hemicellulose retention (61.3%) at 30% PAA [6]. As the PAA charge increased from 7.5% to 30%, lignin recovery decreased, likely due to the mild delignification effect of PAA during oxidation [6].

	Chemical composition (%)					Mass balance (%)			
	Cellulose*	Hemicellulose*	Lignin*	Ash*	Extractives*	Mass yield	Cellulose recovery	Hemicellulose recovery	Lignin recovery
50°C, 15% NaOH, 7.5% H ₂ O ₂ , 30% PAA	63.6 ± 0.9	29.6 ± 0.5	2.6 ± 0.1	-	-	65.3	100.0	82.9	7.5
90°C, 15% NaOH, 7.5% H ₂ O ₂ , 7.5% PAA	62.9 ± 0.1	28.4 ± 0.2	3.1 ± 0.2	-	-	60.5	100.0	73.8	12.7
90°C, 7.5% NaOH, 0% H ₂ O ₂ , 30% PAA	61.8 ± 0.4	30.5 ± 0.1	3.9 ± 0.1	-	-	68.2	100.0	89.3	11.7
50°C, 15% NaOH, 0% H ₂ O ₂ , 30% PAA	61.6 ± 0.5	28.8 ± 0.2	3.9 ± 0.1	-	-	66.2	100.0	81.8	11.3
50°C, 15% NaOH, 7.5% H ₂ O ₂ , 7.5% PAA	58.4 ± 1.0	27.9 ± 0.7	5.6 ± 0.3	-	-	67.0	100.0	80.3	16.5
90°C, 7.5% NaOH, 0% H ₂ O ₂ , 7.5% PAA	55.9 ± 0.7	28.1 ± 0.4	7.7 ± 0.4	-	-	64.5	100.0	77.8	21.8
50°C, 15% NaOH, 0% H ₂ O ₂ , 7.5% PAA	57.8 ± 0.2	29.6 ± 0.1	8.0 ± 0.5	-	-	73.3	100.0	88.9	25.8
50°C, 7.5% NaOH, 0% H ₂ O ₂ , 7.5% PAA	50.3 ± 0.6	28.8 ± 0.3	11.3 ± 0.2	-	-	80.7	100.0	97.3	40.0

Table 3.2. Chemical composition of eight different treatments after oxidation and mass balance results relative to the original wheat

straw. *Statistically significant at a 95% confidence level.

3.2. Lignocellulosic nanofibril characterization

Eight treatments were chosen for characterization: 50°C, 15% NaOH, 7.5% H₂O₂, 30% PAA; 90°C, 15% NaOH, 7.5% H₂O₂, 7.5% PAA; 90°C, 7.5% NaOH, 0% H₂O₂, 30% PAA; 50°C, 15% NaOH, 0% H₂O₂, 30% PAA; 50°C, 15% NaOH, 7.5% H₂O₂, 7.5% PAA; 90°C, 7.5% NaOH, 0% H₂O₂, 7.5% PAA; 50°C, 15% NaOH, 0% H₂O₂, 7.5% PAA; and 50°C, 7.5% NaOH, 0% H₂O₂, 7.5% PAA. Samples will be labelled according to their residual lignin content as 2.6 wt.%, 3.1 wt.%, 3.9 wt.%, 3.9 wt.%, 5.6 wt.%, 7.7 wt.%, 8.0 wt.%, and 11.3 wt.% LCNF samples.

3.2.1. FTIR and charge density

The FTIR spectra of the LCNF samples are shown in Figure 3.1A. The sharp peak at 1027 cm⁻¹ in all LCNF samples corresponds to the C-O-C stretching of glycosidic bonds in cellulose chains [54]. The spectral band at 3335 cm⁻¹ is attributed to O-H stretching in aliphatic hydroxyl groups on the cellulose backbone and phenolic groups in lignin [8], [55]. The peaks at 2918 and 2849 cm⁻¹ are associated with symmetric and asymmetric C-H stretching of methyl and methylene groups in cellulose and hemicellulose [55]. The sharp peak at 1740 cm⁻¹ corresponds to C=O stretching of carboxyl groups found in p-coumaric acids of hemicellulose and lignin or in uronic and acetyl ester groups of hemicellulose [6], [55]. The peak at 1645 cm⁻¹ is attributed to conjugated carbonyl groups in lignin [55]. Sharp peaks at 1510 and 1422 cm⁻¹ are associated with aromatic ring vibrations in the lignin backbone [8], [55]. The peak at 1367 cm⁻¹ corresponds to asymmetric C-H deformation and C-O stretching in syringyl groups of lignin [55].

The charge density of the LCNF samples, measured by conductometric titration, is presented in Figure 3.1B. The charge density ranged from 144.1 µmol/g for the 3.1 wt.% LCNF sample to 212.5 µmol/g for the 11.3 wt.% LCNF sample. These results align with previous findings in our

research group, as Pascoli et al. reported charge densities between 105 $\mu\text{mol/g}$ and 266 $\mu\text{mol/g}$ for their PAA-pretreated samples [6], while Maerz observed an average charge density of 138.8 $\mu\text{mol/g}$ across all PAA-treated samples [56]. Galland et al. reported similar charge densities of 193.4 and 206.8 $\mu\text{mol/g}$ for the peracetic acid treatment of aspen and spruce chips, respectively [[57]]. The peracetic acid oxidized the reducing ends of cellulose and hemicellulose [57], resulting in the relatively low charge densities observed in this study and contributing to the sharp peak at 1740 cm^{-1} in the FTIR spectra.

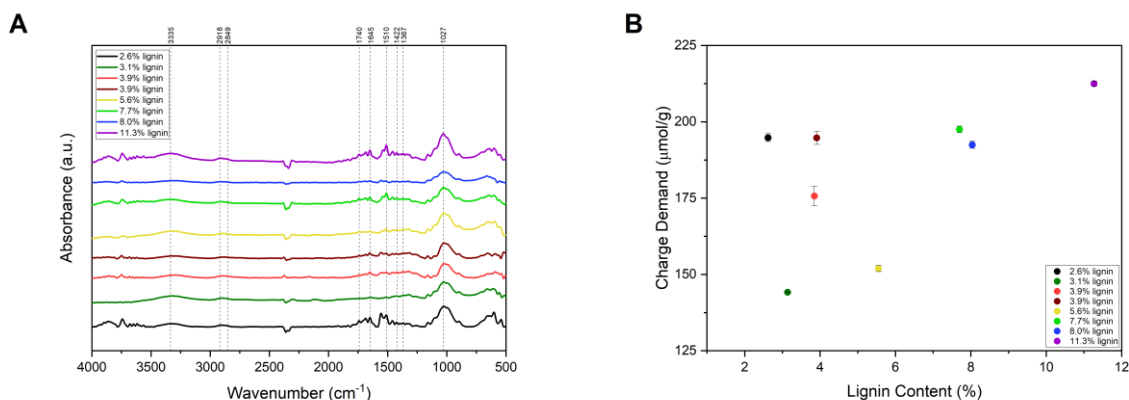


Figure 3.1. (A) FTIR spectra. (B) Scatter plot of the charge density. *Statistically significant at a 95% confidence level.

3.2.2. Nanofibril morphology and structure

The morphology of the LCNF samples was examined using SEM, shown in Figure 3.2, revealing a heterogeneous mixture of nanofibrils and microfibrils. Size distribution curves are shown in Figure 3.3, and Table 3.3 summarizes average nanofibril diameters and the percentage of nanofibrils below 20 nm. The proportion of nanofibrils under 20 nm ranged from 77.4% to 97.0%, decreasing with higher lignin content. This suggests that increased lignin contributes to greater heterogeneity by hindering fibril separation, as lignin binds fibrils tightly and raises the

energy needed for defibrillation [58]. Despite the heterogeneity, average fiber diameters remained similar across samples. The high presence of sub-20 nm nanofibrils contributed to the small average diameters, while larger microfibrils accounted for the broad standard deviations. These average diameters are consistent with literature values, even though the samples were not homogenized or microfluidized. For example, Rojo et al. produced LCNFs with diameters ranging from 16 to 44 nm using delignified Norway spruce followed by microfluidization [11], while Espinosa et al. achieved 6–14 nm diameters from wheat straw treated through various pretreatments and microfluidization [8]. Differences in nanofibril diameters across studies are attributed to feedstock type, processing conditions, and fibrillation methods [3], [58]. Although Rojo et al. suggested lignin may aid defibrillation [11], our findings differ, likely due to the limited mechanical treatment in the absence of microfluidization.

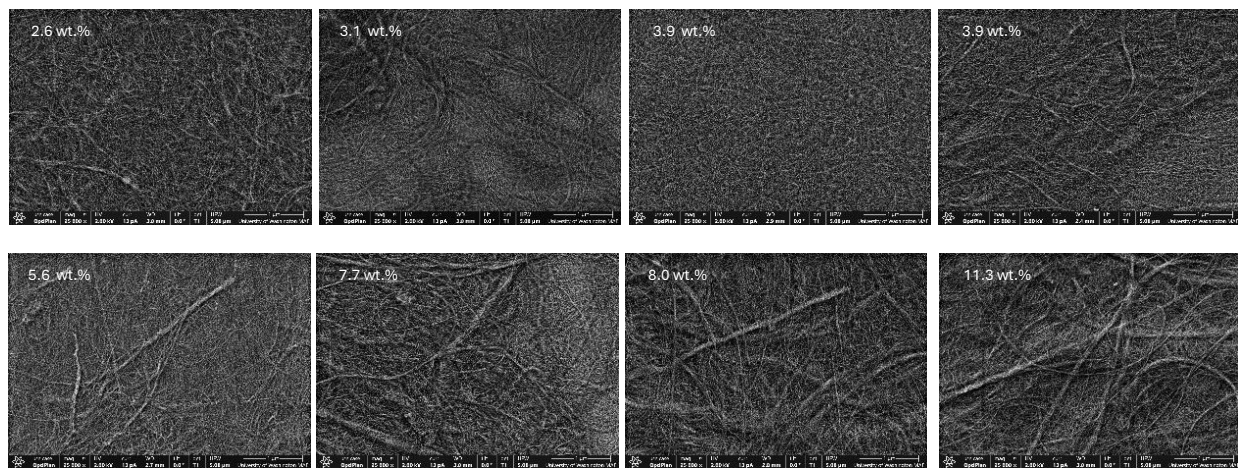


Figure 3.2. SEM images of the LCNF samples with lignin contents ranging from 2.6 wt.% to 11.3 wt.%.

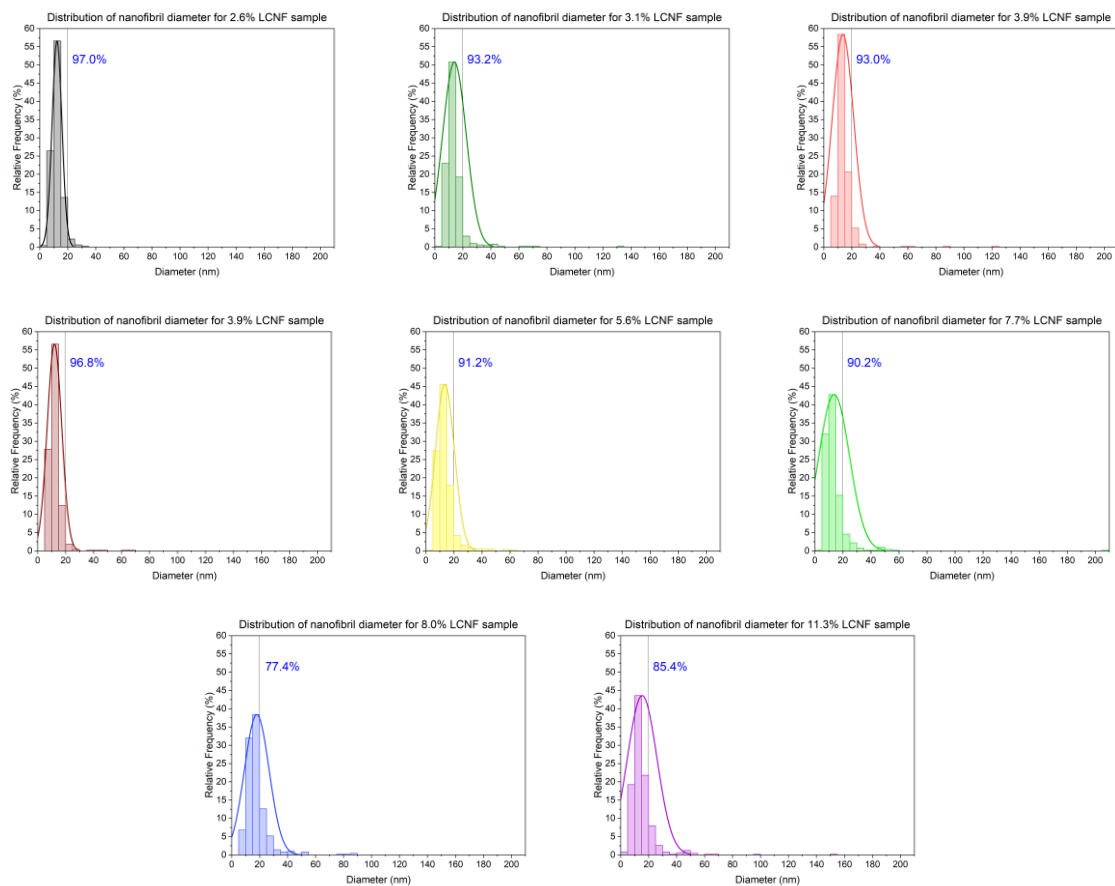


Figure 3.3. Nanofibril diameter distributions.

Lignin content (%)*	Fiber diameter (nm)*	Nanofibrils with diameters less than 20 nm (%)*
2.6	12.3 ± 3.5	97.0
3.1	14.0 ± 8.5	93.2
3.9	13.7 ± 7.8	93.0
3.9	12.0 ± 5.2	96.8
5.6	13.6 ± 6.7	91.2
7.7	13.8 ± 11.0	90.2
8.0	18.0 ± 8.8	77.4
11.3	15.4 ± 10.6	85.4

Table 3.3. Nanofibril diameter and percentage of nanofibrils with diameters less than 20 nm for

LCNF samples. *Statistically significant at 95% confidence.

XRD analysis was conducted to examine the crystalline structure of the LCNF film samples. All samples displayed the characteristic cellulose I crystalline structure, with prominent diffraction peaks observed at 2θ angles of approximately 16.4° and 22.4° , corresponding to the (1 1 0) and (2 0 0) crystalline planes of cellulose, respectively [59], shown in Figure 3.4. The crystallinity index for the LCNF samples is also presented in Figure 3.4. The crystallinity index ranged from 57.8% to 64.3%. The amorphous nature of lignin [60] leads to lower crystallinity index values and contributes to the semi-crystalline structure of LCNF samples. Bian et al. reported comparable crystallinity index values, ranging from 69.0% to 76.2% for unbleached hardwood LCNFs with lignin contents ranging from 4.0 wt.% to 17.2 wt.% [61]. Similarly, Yuan et al. observed crystallinity index values between 46.8% and 62.1% in their LCNF samples as the lignin content ranged from 2.8 wt.% to 23.8 wt.% [14].

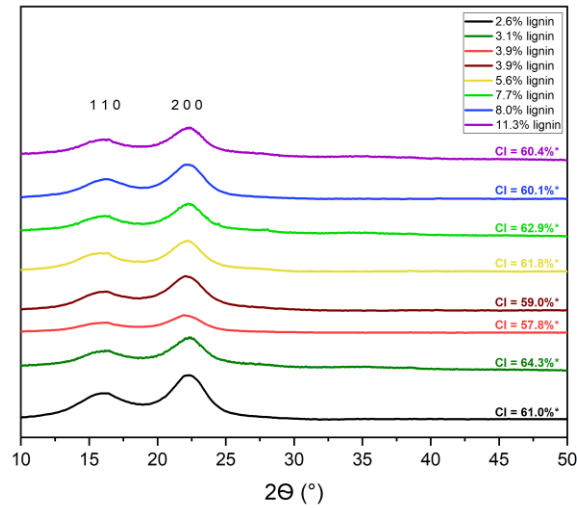


Figure 3.4. XRD patterns and crystallinity index measurements. *Statistically significant at a 95% confidence level.

3.2.3. Optical properties

The optical transmittance of all LCNF samples was measured across the visible region (400–800 nm), the UV-A region (315–400 nm), and the UV-B region (280–315 nm), shown below in Figure 3.5A. Generally, the optical transmittance in the visible region decreased with increasing lignin content. The optical transmittance at 660 nm ranged from 78.2% for the 3.9 wt.% LCNF sample to 53.8% for the 11.3 wt.% LCNF sample. This reduction in transmittance is due to the golden-brown color of lignin that reduces the transparency of the medium and high sample films. Similarly, the optical transmittance in the UV-A region decreased with increasing lignin content, particularly down to 350 nm. At 320 nm, LCNF samples with lignin contents between 2.6 wt.% and 3.9 wt.% exhibited light transmittance ranging from 2.7% to 8.3%, while samples with lignin contents between 5.6 wt.% and 11.3 wt.% showed transmittance below 1.0%. All LCNF samples exhibited minimal optical transmittance in the UV-B region. The 2.6 wt.% LCNF sample showed the highest transmittance at 280 nm, measuring 2.0%, while the 7.7 wt.%, 8.0 wt.%, and 11.3 wt.% LCNF samples recorded values of 0.1%, 0.2%, and 0.2%, respectively. The low transmittance in the UV-A and UV-B regions for the LCNF samples with lignin contents between 5.6 wt.% and 11.3 wt.% is attributed to the UV-blocking properties of lignin. Lignin contains numerous aromatic rings and phenolic groups that interact with UV light, absorbing the radiation. This absorption leads to reduced light transmittance in the UV region as lignin content increases.

The optical haze of the LCNF samples was assessed within the visible spectrum (400–800 nm), revealing values ranging from 82.6% in the 2.6 wt.% LCNF sample to 92.5% in the 11.3 wt.% LCNF sample. A positive correlation was observed between the lignin content in the LCNF samples and their optical haze, as illustrated in Figure 3.5B. Lignin in the films scatters light, which leads to haze [62]. As the lignin content increases, the scattering of light intensifies,

resulting in greater haze. Trifol and Moriana observed similar trends in optical transmittance and haze, noting a decrease in transmittance in the visible region and an increase in haze from approximately 45% to 90% at 400 nm as they increased the LCNF content, and consequently, the lignin content of their CNF-LCNF composites [18].

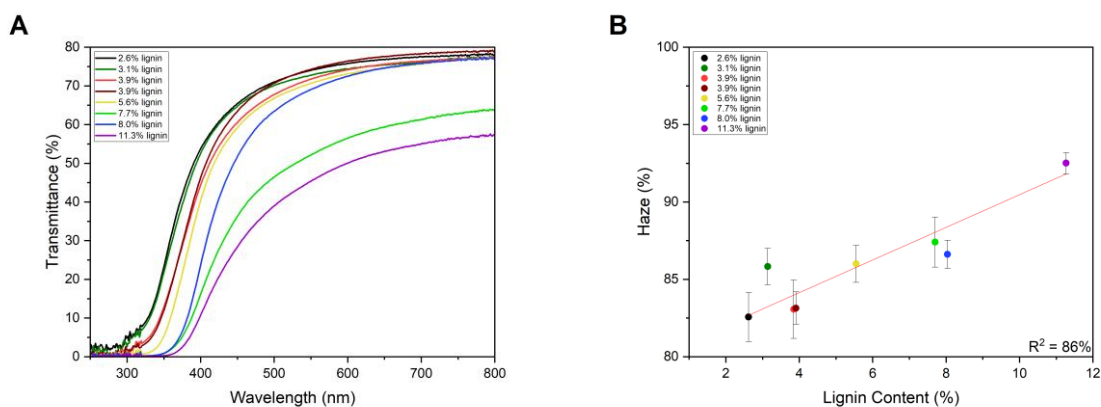


Figure 3.5. (A) Optical transmittance spectra of the LCNF samples. (B) Scatter plot of the haze.

*Statistically significant at a 95% confidence level.

3.2.4. Thermal properties

The thermal stability and degradation behavior of the LCNF film samples were examined using thermogravimetric analysis (TGA), shown below in Figures 3.6A and 3.6B. The characteristics of TGA and DTG (derivative thermogravimetry) are summarized in Table 3.4. All LCNF samples exhibited the same onset thermal degradation behavior. Initially, between 50 and 150°C, all samples showed a mass loss of approximately 10% due to the evaporation of moisture. Between 200 and 350°C, the largest mass loss of 50-60% was observed. This is a result of hemicellulose, cellulose, and lignin pyrolysis within this temperature range [63]. After 350°C, a mass loss of 10% was observed for all samples, which can be attributed to the charring of aromatic lignin [64]. Pascoli et al. [6] and Yuan et al. [14] reported similar thermal degradation

behavior, observing a 5% to 10% mass loss from 20°C to 100°C due to water evaporation, an immediate onset of mass loss at 275°C continuing to 375°C, followed by a 20% mass loss between 400°C and 600°C for their LCNF samples. The thermal percent mass loss decreased from 80.2% for the 3.1 wt.% LCNF sample to 75.7% for the 11.3 wt.% LCNF sample, as shown by the negative correlation in Figure 3.6C. At temperatures above 400°C, lignin undergoes charring due to the presence of abundant aromatic rings in its structure. This char acts as a barrier to heat and oxygen, enhancing thermal stability and flame retardancy [65]. The peak thermal degradation temperature ranged between 342.2°C and 347.5°C. There was no observed correlation between the lignin content of the LCNF samples and the peak thermal degradation temperature, as shown in Figure 3.6D.

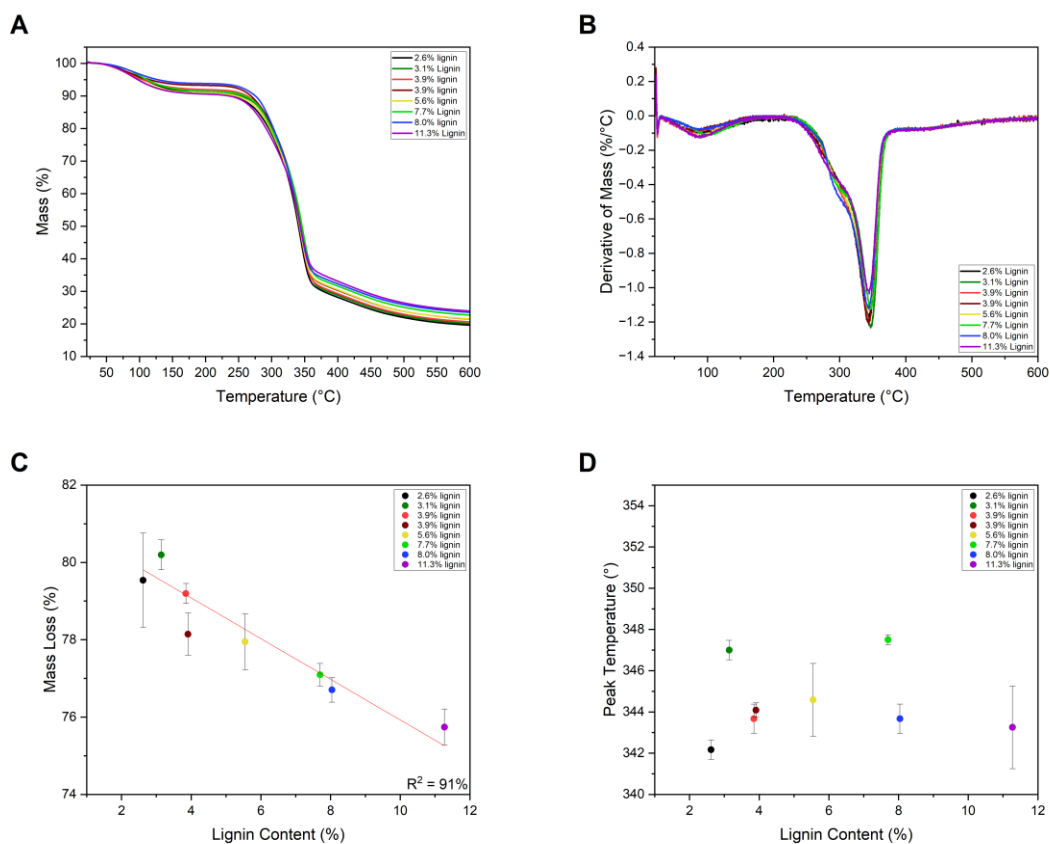


Figure 3.6. (A) TGA curve. (B) DTG curve. (C) Scatter plot of the thermal mass loss percentage. (D) Scatter plot of the thermal peak degradation temperature. *Statistically significant at a 95% confidence level.

Lignin content (%) [*]	Peak temperature (°C)	Overall mass loss (%) [*]	Residual mass at 110°C (%)	Residual mass at peak temperature (%)	Residual mass at 600°C (%) [*]
2.6	342.2 ± 0.5	79.5 ± 1.2	94.8 ± 0.2	48.5 ± 0.0	20.5 ± 1.2
3.1	347.0 ± 0.5	80.2 ± 0.4	94.1 ± 0.4	47.0 ± 0.7	19.8 ± 0.4
3.9	343.7 ± 0.7	79.2 ± 0.3	94.8 ± 0.3	47.7 ± 0.0	20.8 ± 0.3
3.9	344.1 ± 0.4	78.1 ± 0.5	95.5 ± 0.3	48.4 ± 0.1	21.9 ± 0.5
5.6	344.6 ± 1.8	78.0 ± 0.7	94.6 ± 1.1	49.8 ± 0.2	22.1 ± 0.7
7.7	347.5 ± 0.2	77.1 ± 0.3	95.3 ± 0.1	49.1 ± 1.4	22.9 ± 0.3
8.0	343.7 ± 0.7	76.7 ± 0.3	95.7 ± 0.4	49.8 ± 1.3	23.3 ± 0.3
11.3	343.3 ± 2.0	75.7 ± 0.5	94.1 ± 0.6	50.4 ± 2.4	24.3 ± 0.5

Table 3.4. TGA summary characteristics for the LCNF samples. *Statistically significant at a 95% confidence level.

3.2.5. Mechanical properties

Tensile tests were performed on LCNF film samples with varying lignin content to assess their mechanical properties. Excluding the lignin samples with 2.6 wt.%, 3.9 wt.%, and 3.9 wt.%—which were produced using 30% PAA during oxidation—the ultimate tensile strength was observed to decrease as lignin content increased, as shown in Figure 3.7A. This reduction can be attributed to lignin disrupting hydrogen bonding between individual nanofibrils, leading to diminished tensile strength [66]. A similar trend was reported by Trifol and Moriana, who found that increasing the LCNF concentration in cellulose nanofibril (CNF)-LCNF composite films (and thereby the lignin content) reduced the ultimate tensile strength from 182.8 MPa for 75/25 CNF/LCNF films with approximately 4.7% lignin to 122.6 MPa for pure LCNF films with about 15.9% lignin [18]. The low-lignin LCNF samples treated with 30% PAA exhibited significantly lower ultimate tensile strength than the other LCNF samples due to differences in

process conditions. Specifically, the increase in PAA charge from 7.5% to 30% not only solubilized different lignin but also altered the bonding mechanisms during film formation [67]. This might have resulted in localized and delocalized bonding areas, increasing the likelihood of failure even under low stress loads.

The overall elastic modulus decreased as the lignin content increased, from 6.1 GPa in the 3.1 wt.% LCNF sample to 3.6 GPa in the 11.3 wt.% LCNF sample, as shown by the negative correlation in Figure 3.7B. The 41% reduction in elastic modulus is attributed to the inherent difference between cellulose and lignin. Since lignin has a significantly lower elastic modulus than pure cellulose, its presence leads to the observed decrease [68]. A similar trend was observed by Trifol and Moriana when they found that the elastic modulus of their CNF-LCNF composite films decreased from 7.2 GPa for 75/25 CNF/LCNF films to 3.8 GPa for pure LCNF films [18]. In contrast, Rojo et al. reported that the elastic modulus increased from 9.5 GPa to 13.4 GPa when increasing the lignin content from 2 wt.% to 4 wt.% but then decreased to 12.2 GPa when further increasing the lignin content to 14 wt.% [11]. The differing trends observed by Rojo et al., compared to those in this study and by Trifol and Moriana, may be attributed to variations in process conditions and film preparation methods. Rojo et al. hot-pressed their LCNF films at 100°C and 220 bar for five minutes, causing lignin to soften and fill the gaps between cellulose nanofibrils, acting as a binder and enhancing bonding throughout the films [11]. Although the LCNF samples in this study were hot-pressed, the heterogeneous size distribution of the LCNFs—caused by limited mechanical treatment without microfluidization or homogenization—diminished interbonding strength. This reduction in bonding led to the observed decrease in modulus with increasing lignin content.

In contrast to the clear decreasing trends observed in ultimate tensile strength and elastic modulus, the strain at break exhibited a different pattern. As the lignin content increased from 2.6 wt.% to 7.7 wt.%, the strain at break increased from 0.8% to 3.2% . However, beyond this point, the strain at break declined to 2.4% as lignin content further increased to 11.3%, as illustrated in Figure 3.7C. A similar trend was observed by Rojo et al., who reported an increase in strain at break from 2.9% to 3.5% as the lignin content increased from 0 wt.% to 2 wt.%, followed by a decrease to 1.7 wt.% when the lignin content was further increased to 14% [11]. This behavior is attributed to lignin acting as a bonding agent, effectively transferring stress and providing some flexibility up to a certain threshold [18]. Beyond this point, excessive lignin disrupts fiber-fiber bonding, reducing the material's stress-handling capacity resulting in less strain before failure.

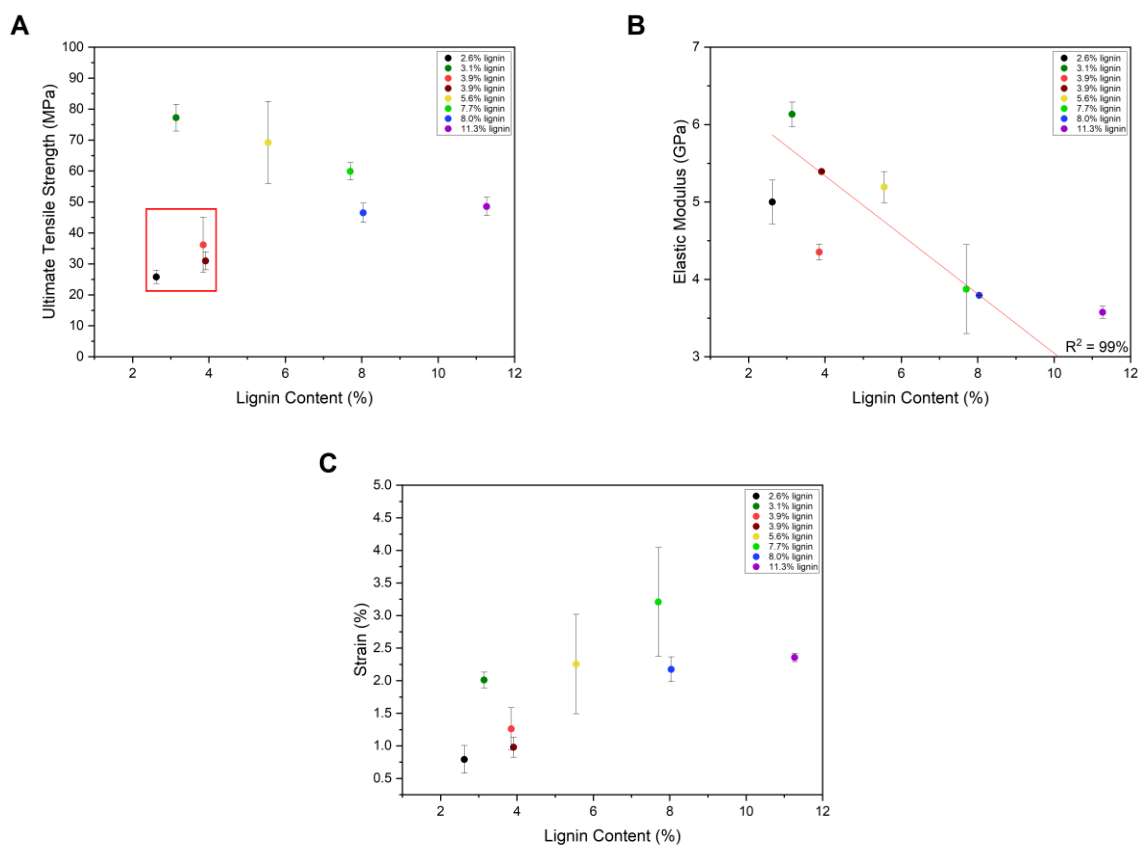


Figure 3.7. (A) Scatter plot of the ultimate tensile strength. (B) Scatter plot of the elastic modulus. (C) Scatter plot of the strain at break. *Statistically significant at a 95% confidence level.

3.2.6. *Wetting behavior and surface free energy*

The average water contact angles (WCA) and surface free energy were measured on the LCNF film samples, summarized below in Table 3.5. As the lignin content in the LCNF samples increased from 2.6 wt.% to 11.3 wt.%, the observed WCA increased from 80.2° to 92.3°, represented by the positive correlation shown in Figure 8A. As expected, the observed WCA increased as the lignin content increased. Lignin consists of a polymer network of phenolic rings, and its aromatic structure imparts a degree of water repellency to the samples [69]. A similar trend was reported by Rojo et al., where the observed WCA for their LCNF nanopapers ranged from 35.4° to 77.7° as the lignin content increased from 0 wt.% to 13.5 wt.% [11]. Likewise, Gu et al. found that their LCNFs with 8 wt.%, 12 wt.%, and 19 wt.% lignin exhibited WCA values of 59.3°, 84.6°, and 95.1°, respectively [70]. The differences in the observed WCA between this study and the values reported by Rojo et al. and Gu et al. can be attributed to differences in the LCNF samples and the film preparation methods. In this study, LCNFs derived from wheat straw using alkaline peroxide delignification and peracetic acid oxidation were cast onto glass dishes to form films. The LCNF films were hot-pressed for 15 hours at 145°C, above the glass transition temperature of lignin [71], allowing lignin to flow and fill surface pores. This process enhanced the film smoothness, leading to higher observed WCA values at comparable lignin content in contrast to Gu et al., who also produced LCNFs from wheat straw through soda fractionation but did not apply hot-pressing to their films. The lower WCA values reported by Rojo et al. are due

to the SEW fractionation process, which sulfonated the lignin in their LCNF nanopapers, increasing the hydrophilicity of the lignin [72].

The surface free energy of the LCNF film samples was calculated using the Owens, Wendt, Rabel, and Kaelble (OWRK) method, which involved taking contact angle measurements with two probing liquids: water and diiodomethane, shown below in Figure 8B. Using this method, the total surface free energy can be divided into two components: the polar component, which represents surface interactions from dipoles and hydrogen bonding, and the dispersive component, which accounts for interactions from London dispersion forces. The total surface free energy ranged from 45.1 mJ/m² for the 2.6 wt.% LCNF sample to 37.1 mJ/m² for the 11.3 wt.% LCNF sample, as shown in Figure 8B. The dispersive component ranged from 41.5 mJ/m² to 3.6 mJ/m², while the polar component varied from 3.67 mJ/m² to 1.0 mJ/m². As the lignin content increased, the total surface free energy decreased, while the dispersive component remained constant, and the polar component also decreased. A similar trend for both the total surface free energy and the polar component was reported by Rojo et al., where the total surface free energy and polar component decreased from 53.9 and 8.5 mJ/m² in the 2 wt.% LCNF sample to 46.7 and 3.5 mJ/m² in the 13.5 wt.% LCNF sample. However, this trend did not apply to the dispersive component, which also decreased with increasing lignin content, from 45.5 mJ/m² in the 2 wt.% LCNF sample to 43.2 mJ/m² in the 13.5 wt.% LCNF sample [11]. The differences in the surface free energy values are due to different fractionation processes. Rojo et al. sulfonated the lignin in their LCNFs, which increased their hydrophilicity, as evidenced by the larger polar component of the total surface free energy. The differences in the dispersive component can be attributed to variations in LCNF functionalization between our samples and those of Rojo et al., resulting from differences in the starting feedstock and fractionation process.

Lignin content (%)*	Water contact angle (°)*	Surface free energy (mN/m)*
2.6	80.2 ± 2.2	45.1 ± 0.7
3.1	80.7 ± 2.0	41.3 ± 1.3
3.9	82.4 ± 0.8	41.2 ± 0.7
3.9	81.6 ± 0.8	42.5 ± 0.9
5.6	85.4 ± 1.6	39.8 ± 1.1
7.7	88.9 ± 1.7	39.1 ± 1.0
8.0	86.3 ± 1.4	37.1 ± 0.3
11.3	92.3 ± 1.5	37.1 ± 0.7

Table 3.5. Contact angle and surface free energy measurements for the LCNF samples.

*Statistically significant at a 95% confidence level.

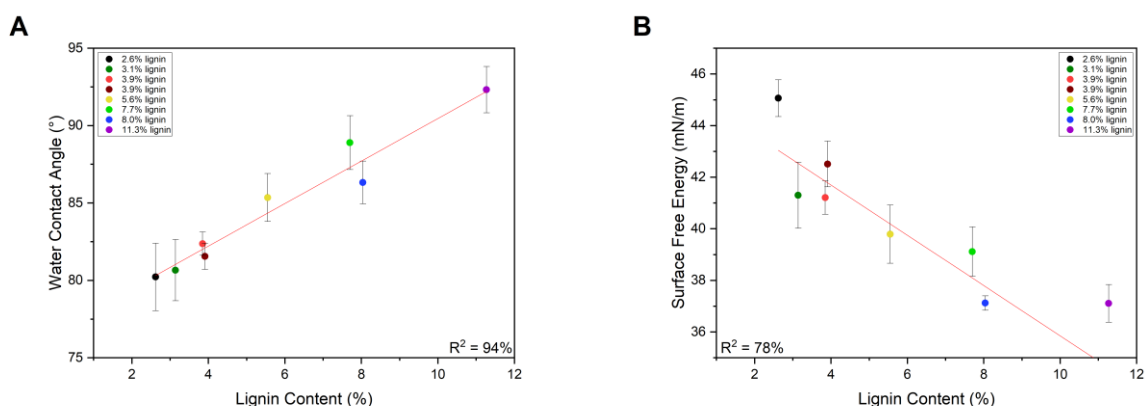


Figure 3.8. (A) Scatter plot depicting the water contact angle. (B) Scatter plot depicting the surface free energy. *Statistically significant at a 95% confidence level.

3.3.Effect of process conditions on physical characteristics of LCNFs

LCNF characteristics can be tailored by adjusting fractionation or oxidation process conditions to achieve specific lignin contents for various applications. During fractionation, modifying the temperature and the chemical charges of NaOH and H₂O₂ influences the extent of lignin and hemicellulose removal, thereby affecting LCNF properties, as previously shown in Tables 3.1 and 3.2. Lowering the temperature or chemical charges reduces lignin removal, leading to greater lignin retention in the LCNFs after oxidation. This increased lignin content

results in larger nanofibrils, decreased homogeneity in nanofibril diameter, lower optical transmittance in the visible range but increased optical haze, reduced thermal mass loss, lower ultimate tensile strength, decreased stiffness, and reduced surface hydrophilicity, as observed in the 90°C, 15% NaOH, 7.5% H₂O₂, 7.5% PAA treatment. Conversely, increasing the temperature or chemical charges enhances lignin removal, reducing lignin retention in the LCNFs after oxidation and producing the opposite effects on these properties, as seen in the 50°C, 7.5% NaOH, 0% H₂O₂, 7.5% PAA treatment. Similarly, increasing the PAA charge during oxidation further promotes lignin removal, leading to lower lignin retention in the LCNFs and similar characteristics, such as reduced nanofibril diameter, increased homogeneity in nanofibril diameter, greater optical transmittance in the visible range but decreased optical haze, increased thermal mass loss, decreased stiffness, and enhanced surface hydrophilicity, as observed in the 50°C, 15% NaOH, 7.5% H₂O₂, 30% PAA treatment. By understanding how process conditions influence LCNF characteristics, we can tailor the fractionation and oxidation processes to produce LCNFs with specific properties for specific end use.

However, for LCNFs with similar lignin contents produced from this process, the method used to reach the final lignin content may differ. Lignin removal can be achieved either by applying higher temperatures and increased NaOH and H₂O₂ charges during fractionation to remove lignin before oxidation, followed by minimal PAA usage, or by using lower temperatures and chemical charges during fractionation while increasing the PAA charge during oxidation to enhance lignin removal and reach the desired lignin content. This pattern is evident in LCNF samples with lignin contents ranging from 2.6 wt.% to 3.9 wt.%. For the 90°C, 15% NaOH, 7.5% H₂O₂, 7.5% PAA treatment, which resulted in a lignin content of 3.1 wt.%, lignin removal was maximized during the fractionation stage, achieving a 69.4% extent of delignification, followed

by 54.8% extent of delignification during oxidation, presented below in Table 3.6. In contrast, the other three treatments exhibited lower extents of delignification during the fractionation stage, ranging from 46.7% to 54.5%, but achieved larger extents of delignification during oxidation, ranging from 67.7% to 74.6%.

The stage at which primary lignin removal occurs—whether during the NaOH/H₂O₂ fractionation or PAA oxidation process—can significantly impact certain LCNF characteristics, even when the total lignin content is similar. This effect is particularly evident in LCNF samples with lignin contents ranging from 2.6 wt.% to 3.9 wt.%. The average charge density for the 90°C, 15% NaOH, 7.5% H₂O₂, 7.5% PAA treatment with 3.1 wt.% lignin was 144.1 $\mu\text{mol/g}$, whereas the average charge density for the 2.6 wt.%, 3.9 wt.%, and 3.9 wt.% treatments, where 30% PAA was used during oxidation, ranged between 175.7 $\mu\text{mol/g}$ and 194.8 $\mu\text{mol/g}$, as shown in Figure 3.9A. The differences in the observed charged demand may result from enhanced oxidation of wheat straw lignin and the reducing ends of cellulose and hemicellulose due to the higher PAA charge [6]. Additionally, ultimate tensile strength is another property significantly influenced by the processing method. The 3.1 wt.% lignin sample exhibited an ultimate tensile strength of 77.2 MPa, substantially higher than the 2.6 wt.% and 3.9 wt.% samples, which ranged from 25.8 MPa to 36.2 MPa, as presented in Figure 3.9B. Though not to the same extent as ultimate tensile strength, similar decreases in the strain at break and elastic modulus were observed in the samples with lignin contents that ranged from 2.6 wt.% to 3.9 wt.% vs. the 3.1 wt.% sample. The increased PAA charge alters lignin solubility, leading to variations in bonding mechanisms within the film samples [67]. These differences can create localized and delocalized bonding regions, potentially causing rapid failure under lower stress conditions. Shifting primary lignin removal to the oxidation stage instead of the fractionation stage affects certain LCNF characteristics,

particularly mechanical properties, which may limit their suitability for applications requiring strong mechanical performance, such as sustainable bioplastics, packaging, and composites.

Treatment	Extent of delignification during fractionation (%)	Extent of delignification during PAA oxidation (%)	Overall extent of delignification (%)	Delignification ratio
90°C, 15% NaOH, 7.5% H ₂ O ₂ , 7.5% PAA	69.4	54.8	86.2	0.64
50°C, 15% NaOH, 0% H ₂ O ₂ , 30% PAA	46.7	67.7	82.8	0.82
90°C, 7.5% NaOH, 0% H ₂ O ₂ , 30% PAA	46.5	68.3	83.0	0.82
50°C, 15% NaOH, 7.5% H ₂ O ₂ , 30% PAA	54.5	74.6	88.5	0.84

Table 3.6. Extent of delignification values for LCNF samples with lignin contents between 2.6 wt.% and 3.9 wt.%.

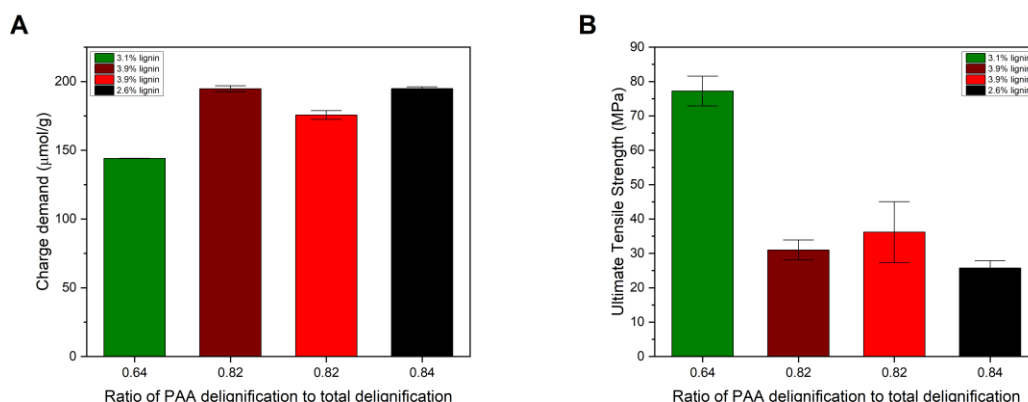


Figure 3.9. (A) Bar plot of the charge demand against the delignification ratio. (B) Bar plot of the ultimate tensile strength against the delignification ratio.

4. Conclusion

This study presents a novel and scalable approach for producing lignocellulosic nanofibrils (LCNFs) with diverse physical and chemical properties from wheat straw by adjusting process conditions. By lowering fractionation parameters—such as temperature and chemical charge—

hemicellulose and lignin retention increased, which boosted yields and produced fibers with distinct characteristics such as small nanofibril diameters, semi-crystalline structure, low UV-light transmittance, moderate thermal stability, moderate mechanical properties, and low surface wettability. The residual lignin content following fractionation and oxidation was found to correlate with several LCNF characteristics. Increased lignin contents led to less uniform nanofibril diameters, decreased optical transmittance, increased UV absorption and haze, and decreased thermal mass loss due to char formation. Mechanical properties, including tensile strength and elastic modulus, also decreased with increased lignin content, likely due to interference with hydrogen bonding—though samples treated with 30% PAA showed an exception. Additionally, increased lignin content reduced the surface hydrophilicity of the LCNFs. Certain LCNF characteristics such as charge demand and mechanical performance were found to heavily depend on whether primary lignin removal occurred during the fractionation or oxidation stage, even when lignin content is similar. For example, increasing the PAA charge from 7.5% to 30% during oxidation raised charge demand but significantly reduced tensile strength. Although elastic modulus and strain at break also declined, the reduction was less severe, limiting the suitability of these LCNFs for applications requiring high mechanical strength. This study presented a variety of novel processes to produce LCNFs with diverse properties from wheat straw, which could be utilized as a platform biomaterial with tunable properties tailored to specific end-use requirements.

5. Future Work

The work shown in this thesis dissertation explored how modifying the process conditions of alkaline peroxide fractionation and peracetic acid oxidation can produce lignocellulosic

nanofibrils (LCNFs) with distinct physical and chemical characteristics from wheat straw. The findings demonstrate the potential to tailor LCNF properties to meet specific application or consumer needs by adjusting fractionation and oxidation parameters. Instead of generating a single type of LCNF with fixed properties, this approach enables the production of a range of LCNFs with tunable characteristics through controlled process modifications. The following future work is recommended:

- Investigate the barrier and antioxidant properties of the eight LCNF samples. Oxygen and water barrier performance is critical for applications such as bioplastics and food packaging, while antioxidant properties are valuable for medical and hygienic uses. Evaluating these properties will help validate the suitability of LCNFs for such targeted applications.
- Evaluate the potential of LCNF samples as additives in pulp and paper applications, such as molded-fiber food packaging, as a sustainable alternative to PFAS. Characterize the resulting materials to assess their physical properties and suitability for commercial use.
- Adjust the process conditions of alkaline peroxide fractionation and peracetic acid oxidation to produce LCNFs with lignin contents exceeding 11.3%—the highest observed in this study—and characterize their physical properties.
- Investigate whether modifying process conditions during alkaline peroxide fractionation and peracetic acid oxidation can produce LCNFs with similar lignin contents at a different peracetic acid charge (e.g., 15%) and assess whether comparable changes in charge demand and ultimate tensile strength are observed.
- Investigate whether changing the process conditions during alkaline peroxide fractionation, followed by peracetic acid oxidation at a 30% charge, yields LCNFs with

higher lignin content that exhibit similar ultimate tensile strength and charge demand behavior as the three previously tested LCNF samples treated under the same peracetic acid charge.

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7. Appendix A – Supplementary Information

Treatment	Temperature (°C)	NaOH charge (%)	H ₂ O ₂ charge (%)	Time (min.)
1	95	15	7.5	150
2	90	15	7.5	150
3	70	15	7.5	150
4	50	15	7.5	150
5	50	15	0	150
6	90	7.5	0	150
7	50	7.5	0	150
8	90	0	0	150

Table A.1. Summary of all fractionation process conditions.

	Chemical composition (%)					Mass balance (%)			
	Cellulose*	Hemicellulose*	Lignin*	Ash*	Extractives*	Mass yield	Cellulose recovery	Hemicellulose recovery	Lignin recovery
Wheat straw	36.4 ± 1.1	23.3 ± 0.6	22.7 ± 0.7	11.5 ± 0.1	13.6 ± 0.7	-	-	-	-
95°C, 15% NaOH, 7.5% H ₂ O ₂	56.7 ± 2.5	29.1 ± 1.3	6.5 ± 0.2	1.8 ± 0.2	4.1 ± 0.0	66.1	100.0	82.6	18.9
90°C, 15% NaOH, 7.5% H ₂ O ₂	58.4 ± 1.2	28.9 ± 0.5	7.0 ± 0.1	2.1 ± 0.2	3.4 ± 0.3	68.0	100.0	84.4	20.9
70°C, 15% NaOH, 7.5% H ₂ O ₂	54.9 ± 1.3	29.0 ± 0.7	7.0 ± 0.4	2.0 ± 0.3	3.6 ± 0.4	68.4	100.0	85.1	21.0
50°C, 15% NaOH, 7.5% H ₂ O ₂	49.4 ± 2.9	27.6 ± 1.7	10.3 ± 1.2	2.6 ± 0.1	6.0 ± 0.1	77.0	100.0	89.9	34.5
50°C, 15% NaOH, 0% H ₂ O ₂	50.1 ± 0.3	27.8 ± 0.3	13.1 ± 0.2	4.1 ± 0.1	3.8 ± 0.0	75.9	100.0	89.0	42.5
90°C, 7.5% NaOH, 0% H ₂ O ₂	49.8 ± 0.8	29.3 ± 0.4	12.5 ± 0.1	5.6 ± 0.5	3.8 ± 0.7	75.4	100.0	91.7	40.5
50°C, 7.5% NaOH, 0% H ₂ O ₂	43.1 ± 0.0	26.0 ± 0.3	16.3 ± 0.2	7.8 ± 0.4	4.1 ± 0.5	87.0	100.0	97.3	57.6
90°C, 0% NaOH, 0% H ₂ O ₂	34.5 ± 0.4	25.7 ± 0.3	21.5 ± 0.3	6.7 ± 0.3	2.3 ± 0.2	81.4	77.1	89.8	76.9

Table A.2. Chemical composition of the original wheat straw and seven different fractionation treatments, and mass balance results

relative to the original wheat straw. *Statistically significant at a 95% confidence level.