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Preparation And Characterization of Nano Fibrillated Cellulose Derived from Oil Palm Trunks Using Different Enzyme Concentrations

Siti Nur 'Arifah M Anuar[1], Amizon Azizan[1], Rafidah Jalil[2*], Latifah Jasmani[2]

[1]*Faculty of Chemical Engineering, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*
[2]*Forest Products Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor, Malaysia*

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ABSTRACT

Nanocellulose refers to materials derived from cellulose that are reduced to the nanoscale, typically ranging from 1 to 100 nanometres. Nanocellulose is distinguished by its large surface area, mechanical strength, and biodegradability, all of which contribute to its unique properties. However, the production process for nanocellulose is faced with challenges concerning energy efficiency, scalability, and environmental impact. Existing methods such as mechanical fibrillation and acid hydrolysis require high energy input, and excessive chemicals, and often result in low yields. This study explores the synthesis of nano fibrillated cellulose (NFC) from Oil palm trunks (OPT), a sustainable by-product of the palm oil industry. NFC was synthesized by enzyme treatment using cellulase enzyme (Trichoderma reesei) and subsequent mechanical fibrillation and high-pressure homogenization. Two NFC samples, NFC A and NFC B, were synthesized using different enzyme concentrations (0.1% and 1.0%) v/v, respectively, and compared for their chemical and physical properties. Both samples exhibited high total solid contents (95.55% for NFC A and 95.44% for NFC B), indicative of efficient processing. Characterization through Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM) revealed that increasing enzyme concentration led to smaller fibril diameters and shorter fibre lengths, with NFC B (1.0% enzyme concentration) achieving finer, more uniform fibrils compared to NFC A (0.1%). Moreover, NFC B is more stable with a lower polydispersity index (PDI = 0.405) than NFC A (PDI = 0.859). These findings recommend that the development of smaller, more stable NFC fibrils is initiated by higher cellulase concentrations and that NFC B's properties make it the better option for use in hydrogels, drug delivery, and composite materials.

INTRODUCTION

Cellulose is the most prevalent biopolymer and a basic structural material of plant cell walls. It is not only prevalent in terrestrial plants but also found across diverse living organisms, including algae, fungi, and a

[2*] Corresponding author. *E-mail address*: rafidahjalil@frim.gov.my
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variety of bacteria, as well as in marine species such as tunicates (Taylor N. G., 2008). Due to its unique structure, cellulose plays a crucial role in chemical transformations, making it a promising starting material for future applications. Structurally, cellulose is characterized as a linear syndiotactic homopolymer, comprising D-anhydro glucopyranose units that are linked via β-(1→4)-glycosidic bonds (Qiu X. & Hu S., 2013). This unique bonding arrangement contributes to its remarkable properties. Nanocellulose, which refers to cellulose fibers at the nanometric scale, exhibits exceptional mechanical resilience and strength relative to its size, making it an ideal candidate for reinforcing composite materials. The properties of nanocellulose are noteworthy, including its biodegradability, renewability, and biocompatibility. Additionally, nanocellulose displays significant transparency, high tensile strength, and impressive Young's modulus, which enhances its applicability in a variety of fields, from packaging materials to biomedical applications. The large specific surface area, high crystallinity, low density, non-abrasive and non-flammable nature, coupled with its non-toxicity and affordability, further amplify the interest in its production and utilization.

The term lignocellulosic biomass refers to the dry matter derived from plants, recognized as the most abundant raw material on Earth for biofuel production. It consists of two types of carbohydrate polymers, cellulose, and hemicellulose, alongside an aromatic polymer known as lignin Each of these constituents possesses distinct chemical properties, and their composite nature significantly complicates processing due to their inherent resistance to degradation, a phenomenon referred to as recalcitrance. Effectively overcoming this recalcitrance is essential for yielding valuable, high-quality products, which typically requires the implementation of various strategies, including the application of heat, chemical treatment, enzymatic processes, and microbial action. Among the various sources of lignocellulosic biomass, the oil palm trunk (OPT), sourced from the *Elaeis guineensis* species, stands out as a significant by-product of the palm oil industry, particularly in Malaysia and Indonesia. Oil palm trees have a productive life span of approximately 25 to 30 years, during which they yield economically viable outputs; post this period, replanting becomes necessary. This cycle generates substantial quantities of OPT biomass. The oil palm industry in Malaysia produces an estimated 70 million tonnes of biomass residue annually (Shuit et al., 2009), underscoring the potential for resource recovery and utilization. The core principles of effective waste management include reducing waste, recycling resources, and maximizing energy recovery. The remaining waste, including ash, can either be repurposed as fertilizer or disposed of appropriately. Palm oil production results in biomass residue at both plantation and mill sites. This biomass residue is categorized into six types: oil palm fronds (OPF) and oil palm trunks (OPT) from plantations, empty fruit bunches (EFB), palm kernel shells, mesocarp fiber, and palm oil mill effluent (POME) from mill sites. These diverse categories of biomass highlight the opportunities for improving sustainability and resource efficiency within the palm oil production sector.

In the processing of lignocellulosic biomass, enzymatic pretreatment is a crucial step that enhances cellulose accessibility, thereby streamlining subsequent processes such as fibrillation and hydrolysis. The increasing demand for sustainable and renewable resources contributed to recent developments in the extraction of cellulose from biomass. To enhance extraction procedures, several chemical, mechanical, and enzymatic techniques have been developed and improved (Manzano et al., 2025). Depending on the type of biomass and the cellulose's purpose, each technique offers specific advantages and disadvantages. For example, cellulose enzymes such as *Trichoderma reesei* cellulase act on specific glycosidic bonds to breaks down cellulose into smaller pieces, making it easier to produce nano-fibrillated cellulose (NFC) (Jourdier et al., 2013; Hansini et al., 2025). Additionally, xylanase targets hemicelluloses, thereby aiding in the separation of these compounds from lignin and improving accessibility to cellulose fibers (Liu et al., 2024). Laccase and peroxidase, which are ligninolytic enzymes, effectively remove lignin, a tough substance that can hinder cellulose fibrillation (Mohotloane et al., 2024). Moreover, pectinases are employed to disintegrate the pectin matrix present in pectin-rich

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biomass, thereby facilitating better access to cellulose. When combined with cellulases, beta-glucosidase breaks down cellobiose into glucose, further helping to break down cellulose (Srivastava et al., 2019). The enzyme xyloglucanase also enhances cellulose accessibility by degrading xyloglucans, which hold plant cell walls together, thus facilitating fibrillation (Cosgrove et al., 2023). Together, these enzymes enhance NFC production, increasing the efficiency of processing various lignocellulosic biomass sources.

Previous research has shown the successful production of NFC using enzyme-assisted pre-treatment with various lignocellulosic materials. For instance, rice straw, which is considered agricultural waste, has been employed to produce NFC, where cellulase enzymes such as Trichoderma reesei are utilized to break down cellulose before mechanical fibrillation (Zhou et al., 2017). Chemical, enzymatic, and mechanical processes can all be used for producing NFC (Rajesh et al., 2025). This enzymatic treatment improves fiber separation and enhances the properties of the resulting NFC, leading to increased surface area and superior mechanical characteristics. Similarly, sugarcane bagasse, a by-product of sugarcane processing, has also been used to make NFC with enzyme treatments like cellulases and xylanases (Kane et al., 2023). These enzymes break down lignin and hemicelluloses, thus preparing cellulose fibers for effective fibrillation. NFC derived from sugarcane bagasse exhibits remarkable tensile strength and transparency, making it ideal for biodegradable films and composites (Gond et al., 2021). Other materials, such as cotton and wood, have also been explored for NFC production through enzyme-assisted pre-treatment (Jimat et al., 2024). Enzymes like cellulases and laccases are used to remove lignin and hemicelluloses, improving the quality and fibrillation of the NFC (Dias et al., 2022). These studies have shown that enzyme-assisted pre-treatment can efficiently produce high-quality NFC from a range of lignocellulosic biomass sources, offering a sustainable alternative to traditional materials (Olaiya et al., 2022). This approach is especially beneficial due to its efficiency and environmental friendliness. For example, enzyme and chemical pre-treatment methods for cellulosic fibers have significantly reduced energy consumption, from 20,000–30,000 kWh/ton to just around 1,000 kWh/ton (Lee et al., 2023). Enzymes primarily target and break down the lignin and hemicellulose components while leaving the cellulose fraction intact. This research aims to compare the nano fibrillated cellulose (NFC) produced from oil palm trunk (OPT) using enzymatic pretreatment methods. By evaluating the efficiency and quality of NFC production through the application of different enzyme concentrations, this study seeks to establish a sustainable and economically viable approach to biomass utilization in bioproduct production.

EXPERIMENTAL

Raw Materials

Oil palm trunk (OPT) fibers were obtained from Opteraz Sdn Bhd., located in Kluang, Johor. These samples were dried in an oven at 60 °C for 24 hours until a constant weight was achieved, ensuring that the moisture content remained below 10%.

Production of Oil Palm Trunk (OPT) Pulp

OPT fibers underwent a chemical pulping process utilizing a rotary digester at the Forest Research Institute Malaysia (FRIM) under controlled conditions of 170 °C for a duration of 4 hours. The pulping process was performed using an 18% (v/v) sodium hydroxide (NaOH; Chemiz, Malaysia) solution, which facilitated the breakdown of lignin. Then, the OPT pulp (Figure 1a) was thoroughly washed with distilled water to eliminate any residual lignin and other impurities.

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Production of Oil Palm Trunk (OPT) Bleached Pulp

Following the pulping process, a comprehensive five-step bleaching protocol was implemented to enhance the purity and brightness of the OPT pulp. This procedure comprised sequential extractions employing a variety of chemicals, specifically 3% (v/v) chlorine dioxide (ClO_2; R&M Chemicals, Malaysia), 3% (v/v) acetic acid (CH_3COOH; Chemiz, Malaysia), and 1% (v/v) sodium hydroxide (NaOH; Chemiz, Malaysia). The bleaching was conducted in a controlled water bath (Memmert, Germany) maintained at a temperature of 70 °C for a duration of 7 hours to produce OPT bleached pulp (Figure 1b).

Fig. 1. OPT pulp (a) and OPT bleached pulp (b)

Production of Nano fibrillated Cellulose (NFC) from OPT Bleached Pulp

An enzymatic pre-treatment was conducted on 26 g of OPT bleached pulp using a cellulase enzyme, specifically *Trichoderma reesei* (ATCC 26921, USA), at concentrations of 0.1% (NFC A) and 1% v/v (NFC B). The enzyme was mixed with the pulp and 100 mL of 0.05 M sodium citrate buffer (pH 4.8) in a 250 mL Duran bottle at a solid loading of 0.26% (w/v). This pre-treatment process was carried out in an incubator shaker (Medical Supply Co. Ltd., China), which was set to maintain a constant temperature of 40°C with 51 hours of incubation time (Figure 2a). The shaker facilitated uniform mixing by operating at a constant agitation speed of 150 rpm, ensuring consistent enzyme activity and optimal conditions for the breakdown of the pulp's cellulose structure. Fibrillation was subsequently performed using high-pressure homogenization with a speed of 25,000 rotations per minute (IKA, Malaysia) to produce a cellulose slurry representing the NFC (Figure 2b). Centrifugation was then carried out using a centrifuge (DJB Labcare, United Kingdom) at 60 rpm for 10 minutes to separate the NFC solid from the slurry. This was followed by several washes with distilled water.

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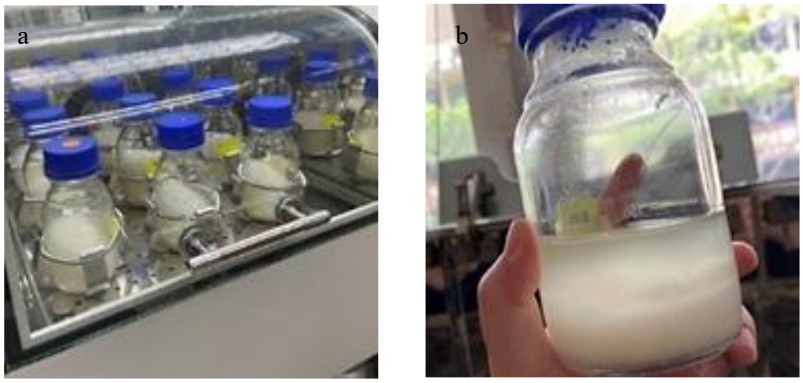

Fig. 2. Enzymatic pre-treatment setup of OPT bleached pulp in an incubator shaker (a) Nano fibrillated cellulose (NFC) (b).

Characterization

Total Solid Content (TSC)

About 1 g of oven-dried NFC was weighed and dried in a convection oven at 103˚C for 24 hours until a steady weight was achieved. Then, the final weight after drying was measured and calculated by using Equation 1.

$$\text{Total Solid content (\%)} = W_f/W_i \times 100 \quad (1)$$

Where,

W_f = Weight of NFC sample after drying

W_i = Weight of NFC sample before drying

Atomic Force Microscopy (AFM)

To determine the size of the produced NFC-OPT, an Atomic Force Microscope (AFM) (Bruker Innova, Germany) was used. The NFC-OPT was first dissolved in distilled water at a concentration of 0.01% by weight (NFC-OPT: H_2O). A sample of the resulting NFC-OPT solution was then placed onto mica sheets, which were allowed to air-dry before analysis using AFM in tapping mode. NFC length and diameter size were measured from AFM images using image-analyzing software (XEI 4.3.4). While aspect ratio was calculated using Equation 2.

$$\text{Aspect Ratio} = \text{Length/Diameter} \quad (2)$$

Scanning Electron Microscopy (SEM)

NFC samples need to be well dried before being analyzed using Scanning Electron Microscopy (SEM) (Hitachi TM4000Plus, Japan). Next, a thin layer of the desiccated NFC is applied to a SEM specimen, typically using conductive tape. The sample must be sputter-coated with a small coating of gold, platinum, or carbon to avoid charging during imaging as cellulose is non-conductive. Depending on the size of the fibrils, a suitable magnification for SEM imaging of NFC between 1,000× and 50,000× has

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been chosen. Lower voltages have been used to capture surface characteristics without damaging the sample with an accelerating voltage between 1 and 15 kV.

Polydispersity Index (PDI)

Polydispersity index measurements were performed using dynamic light scattering (DLS) with a concentration of 0.1% by weight (NFC-OPT: H_2O) was sonicated for a period of 5 min. The sample should next be measured using a DLS instrument that calculated particle size distribution based on light scattering intensity. Dividing the standard deviation by the mean particle size yields the PDI, the width of the size distribution. A PDI near zero implies a tight, homogeneous size distribution, while a greater PDI indicates a wider distribution with more variation in particle sizes. The measurement was repeated multiple times to ensure accuracy.

RESULTS AND DISCUSSION

The concentration of the enzyme significantly affects the level of cellulose fibrillation that can be achieved in the production of nano-fibrillated cellulose (NFC). Specifically, cellulase, which is a key enzyme utilized in this process, is responsible for hydrolyzing cellulose fibers into nanoscale fibrils. An increase in the concentration of cellulase tends to enhance fibrillation because a higher enzyme load leads to a more thorough breakdown of the cellulose structure.

Effect of Enzyme Concentration on Total Solid Content (TSC)

At enzyme concentrations of 0.1% and 1.0% (v/v), both samples exhibited high total solid content (TSC), with 95.55% of NFC A and NFC B at 95.44%. This indicates that both samples have negligible water content. The minimal difference of TSC between NFC A and NFC B is only 0.11%. NFC A has a slightly higher solid content, which could in the end provide a somewhat denser product, even if the small difference has no apparent impact on the outcome of the solid content of NFC. The efficacy of cellulase in breaking down cellulose into its nanoscale fibrils is well documented. For instance, Nagl et al. (2021) reported that the enzymatic activity involving endoglucanases plays a significant role in the fibrillation process, highlighting the importance of enzyme combinations in optimizing cellulose degradation. Moreover, Gao et al. (2017) illustrated that cellulase production from effective fungal strains such as *Trichoderma reesei* can significantly improve cellulose degradation, supporting the notion of enzymatic concentration positively affecting the TSC in NFC production. The high concentration of cellulase leads to a more complete hydrolysis of cellulose fibers, resulting in a denser NFC product. This aligns with the findings reported by Kan et al. (2012) that cellulase treatment can enhance the crystallinity and fiber structure, ultimately contributing to a refined solid product.

Effect of Enzyme Concentration on Diameter Size, Length and Aspect Ratio using AFM

The NFCs produced using different enzyme concentrations were observed by AFM to determine their diameter, length, and aspect ratio. Representative AFM images are shown in Figure 3 and the average values of length and diameter as well as aspect ratios are listed in Table 1.

Table 1. Relationship between diameter, length, and aspect ratio of NFC from OPT

NFC	Diameter (nm)	Length (nm)	Aspect Ratio
NFC A (0.1%)	96.96±27.04	2789.20±162.77	28.77±10.52
NFC B (1.0%)	64.74±11.73	845.18±45.36	13.06±2.35

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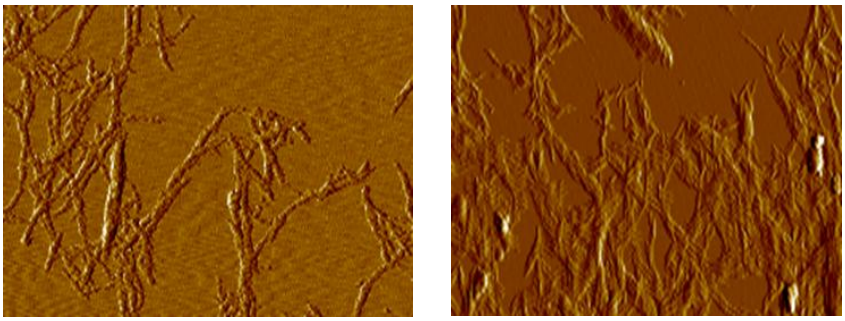

Fig. 3. (left) NFC A with 0.1% enzyme concentration; (right) NFC B with 1.0% enzyme concentration

These results indicate that an increase in enzyme concentration correlates with a reduction in the diameter and length of nano fibrillated cellulose (NFC), aligning with earlier studies. NFC A, produced at a 0.1% enzyme concentration, demonstrates a diameter and length of 96.96 nm and 2789.20 nm respectively. NFC B, generated at a 1.0% enzyme concentration, has a diameter and length of 64.74 nm and 845.18 nm respectively. This indicates that higher enzyme concentrations enhance the breakdown of cellulose fibers into smaller nanofibrils, thereby decreasing the diameter and length size. At reduced enzyme concentrations, the enzyme activity may be insufficient to completely break down the cellulose fibers, leading to the formation of larger and longer fibrils. A higher enzyme concentration enhances fiber breakdown, leading to smaller and shorter NFC fibrils. Smaller fibrils, like those present in NFC B, are advantageous for applications requiring a large surface area, including composite reinforcement, drug delivery, and hydrogels. According to previous research, increasing enzyme loading during the processing of bamboo kraft pulp (BKP) fibers decreases fiber length while increasing fiber diameter. For example, increasing enzyme loading from 0.1% to 5.0% reduced fiber length by 74.1%, while increasing fiber diameter by 9.6%. This shows that higher enzyme concentrations assist in the breaking down of cellulose microfibrils, resulting in shorter, slightly wider fibers (Jo et al., 2020). Furthermore, investigations have demonstrated that enzymatic pretreatments may produce NFCs with much lower energy use. By restricting contacts between microfibrils, enzymes such as cellulases may efficiently break down cellulose, resulting in the formation of nanofibrils ranging in diameter from 5 to 20 nm. The specific diameter obtained is determined by the enzyme type, concentration, and length of treatment (Missoum et al., 2013). Moreover, the relationship between fiber size and physical properties is well-established in material science. Studies have shown that these unique materials with smaller diameters and shorter lengths generally exhibit an increased surface area, and unique chemical, physical, and biological properties which can enhance interactions at the molecular level as compared to their particles at higher scales (Mekuye & Abera et al., 2023). For instance, smaller fibrils are associated with greater surface reactivity and can play a crucial role in applications requiring enhanced bonding capabilities, such as in composite materials (Adib et al., 2024). This increased surface area often leads to improved transparency, making NFC A potentially more suitable for applications in optics and coatings, where aesthetics and clarity are essential.

In addition, the relationship between length and diameter on the aspect ratio of nano fibrillated cellulose (NFC) shows a decrease when enzyme concentration increases. This occurs when enzymes break down the cellulose fibers, affecting both their length and width, thereby decreasing the aspect ratio (Henríquez-Gallegos et al., 2021). As shown in Table 1, the aspect ratio for NFC A and NFC B were 28.77 and 13.06 respectively. These findings are in agreement with Henríquez-Gallegos et al. (2021)

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where high enzyme concentration (1.0% v/v) has produced a lower aspect ratio (13.06) NFC. Moreover, the breaking of the cellulose chains, mainly in the amorphous region, produces a decrease in the aspect ratio (Sanchez-Salvador et al., 2022).

Effect of Enzyme Concentration on Diameter Size using SEM

The NFCs produced using different enzyme concentrations were observed by SEM. Representative SEM images are shown in Figure 4 for both NFCs.

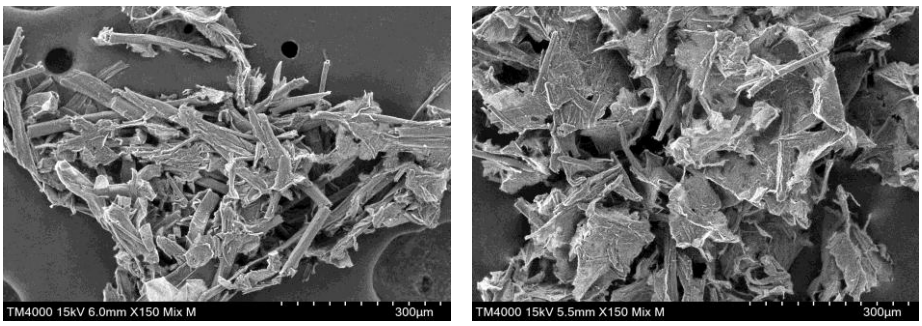

Fig. 4. (left) NFC A with 0.1% enzyme concentration; (right) NFC B with 1.0% enzyme concentration

The effect of enzyme concentration on NFC structure is clearly shown by SEM images. Due to the lower enzyme content of NFC A, the SEM image shows fibril structures that are large and unbalanced. This shows that fibrillation is not complete. Meanwhile NFC B, which is produced with a higher enzyme concentration of 1.0%, is significantly smaller and more uniform. Normally, the SEM image of NFC B would show a finer, more individualized network of nanofibrils with less aggregation. In order to optimize the performance of NFC in advanced material applications, it is necessary to increase enzyme concentration since this increases the fibrillation efficiency and produces smaller, more uniform fibrils with better dispersion.

Multiple studies have shown that the application of enzymes, especially cellulases, helps to reduce the amount of energy that is necessary for mechanical fibrillation. Additionally, the usage of enzymes results in the formation of nanofibrils that are finer and more uniform (Pääkkö et al., 2007). The scanning electron microscopy (SEM) images of NFC with low enzyme concentration often show bundles or clusters, but the SEM images of NFC with higher enzyme concentrations show fibrils that are well-dispersed and thinner (Iwamoto et al., 2005).

Effect of Enzyme Concentration on Polydispersity Index (PDI)

Polydispersity Index (PDI) are closely related, as PDI quantifies the broadness of the particle size distribution in a sample. The Polydispersity Index (PDI) of nano fibrillated cellulose (NFC) is a key indicator of particle size distribution, reflecting the uniformity of fibril sizes within a sample. PDI values range from 0 to 1, where values closer to 0 indicate a narrow size distribution with more uniform particles, while values closer to 1 signify a broader size distribution, suggesting greater variation in particle sizes. In NFC, a lower PDI typically indicates better uniformity, which can enhance material properties such as mechanical strength and transparency, making the NFC more suitable for applications like composites, films, or coatings (Wang et al. 2025). According to the results obtained in Table 1, the dispersion of the particles (PDI) for NFC A and NFC B shows some notable variations. NFC A has a relatively high polydispersity index (PDI) of 0.859, indicating a wide range of particle sizes. However,

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NFC B's lower PDI of 0.405 reflects more homogeneous particle dispersion, indicating better uniformity. NFC B emerges as the preferred option due to its lower PDI. While NFC A's higher PDI implies greater size variation, NFC B's lower PDI indicates higher homogeneity, making it more suitable for applications requiring stability and uniformity. Therefore, NFC B is likely the better choice, especially in applications demanding stable dispersion.

Table 2. Polydispersity Index (PDI) for (left) NFC A with 0.1% enzyme concentration; (right) NFC B with 1.0% enzyme concentration

NFC	Polydispersity index (PDI)
NFC A (0.1%)	0.859
NFC B (1.0%)	0.405

Cellulase hydrolysis, an enzymatic treatment, plays a significant role in altering the dimensions and morphology of cellulose nanofibrils (CNFs). The enzyme's ability to break down the cellulose structure can produce smaller fibrils with a more uniform size distribution. Additionally, this may lead to particle sedimentation, contributing to the observed size trends. The decreasing count rate and particle size in NFC A further support this, indicating potential sedimentation or dissolution. The PDI reflects the consistency of particle sizes in a sample. A high PDI, like 0.859 in NFC A, indicates a wide size range, suggesting uneven cellulose breakdown due to less controlled hydrolysis or variations in enzyme concentration and duration. In contrast, a low PDI, like 0.405 in NFC B, shows a more uniform size distribution, likely resulting from a more effective enzyme treatment that produced consistent particle sizes (Zhu et al., 2018). Higher enzyme concentrations could increase enzyme-cellulose interactions, reducing surface charge and promoting aggregation, potentially causing the decrease in particle number and size seen in NFC A (Baig., 2020; Mazega et al., 2023). According to Hansini et al., (2025), nanocelluloses produced using Trichoderma reesei enzyme have lower PDI. So that, by increasing the amount of enzyme will decrease the PDI of the NFC produced.

CONCLUSIONS

This study successfully demonstrated the production of nano-fibrillated cellulose (NFC) from oil palm trunk (OPT) fibers through a sequence of pulping, bleaching, enzymatic pre-treatment, and mechanical fibrillation processes. The results make it very clear how important enzyme content is in shaping the properties of the NFC that is made. The high total solid content of NFC A (0.1% enzyme) and NFC B (1.0% enzyme) indicates efficient moisture removal and the development of a thick cellulose network. Despite a slight TSC difference between the two samples, further investigations revealed that NFC's characteristics significantly improved when the enzyme concentration increased. Results from Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) verified that smaller, more homogeneous fibrils with lower diameters and lengths were formed by increased enzyme concentrations. In contrast to NFC A's bigger, more clumped structures, NFC B's nanofibrils were finer and more evenly distributed. Furthermore, the aspect ratio decreased as the enzyme concentration increased, suggesting that the cellulose fibres were broken down more thoroughly. Additionally, NFC B's polydispersity index (PDI) was lower than NFC A's, according to the dynamic light scattering (DLS) study, suggesting a more uniform particle size distribution. Applications requiring constant performance, such composites, films, and coatings, benefit from this increased uniformity. Overall, our findings demonstrate that the quality of nano-fibrillated cellulose may be considerably improved by increasing the cellulase enzyme concentration during pre-treatment. In addition to providing a sustainable method of producing NFC, using oil palm trunk waste as a raw source helps to increase the value of agricultural wastes. To make the enzymatic

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process even better in the future, we could try using different types of enzymes and higher amounts to make NFC qualities fit a wider range of commercial uses.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

AUTHORS' CONTRIBUTIONS

Siti Nur 'Arifah M. Anuar carried out the research, data synthesis, and manuscript drafting, and revised the article. Rafidah Jalil, Amizon Azizan, and Latifah Jasmani designed the research, supervised the research progress, reviewed the manuscript, and contributed to its finalization.

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