



Biofilms from micro/nanocellulose of NaBH₄-modified kraft pulp

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Abstract. Industrial applications of microfibrillated cellulose (MFC) and nanofibrillated cellulose (NFC) have been in use for some time; however, there is a need to improve the production steps and at the same time to obtain better quality products. NFC and MFC were generated from NaBH₄-modified kraft pulp, produced from a red gum tree plant (*Eucalyptus camaldulensis*). The generated NFC and MFC were characterized by high-performance liquid chromatography, Fourier transform infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA) and ¹³C-nuclear magnetic resonance (NMR). Morphological and viscoelastic properties were investigated by scanning electron microscopy and rheometry, respectively. The storage moduli of biofilms produced from NFC and MFC were investigated by dynamic mechanical thermal analysis (DMTA). Both exhibited mostly identical FTIR spectra. When the spectra were compared with those of NaBH₄-modified kraft pulp, minor shifts were observed due to crystallinity. In NMR spectra, disordered cellulose structures were observed for both NFC and MFC, and these findings were also confirmed by differential scanning calorimetry. Rheology studies revealed that the lowest viscosity was observed with MFC. TGA results showed that NFC degraded earlier compared with NaBH₄-modified kraft pulp. DMTA exhibited that NFC films had about six times higher storage modulus compared with MFC.

Keywords. Biofilm; enzyme; MFC; NaBH₄; NFC.

1. Introduction

Cellulose, composed of anhydroglucose units linked by β 1-4 glycosidic bonds, is a linear and natural homopolymer and has free hydroxyl groups (C-2, C-3 and C-6 positions) with different reactivities forming intra/intermolecular bonds [1]. Crystal packing controls not only the physical properties of cellulose but also ordered/disordered cellulose structure as well as free hydroxyl groups [2].

The complex structure of lignocellulosic biomass is the main obstacle in fractionating cellulose, which is organized into fibrils in the structure. The fibrils are aligned parallel to each other and surrounded by a matrix of lignin and hemicelluloses. The lignin limits cell permeability and causes insignificant cell destruction. Consequently, to improve the cell permeability, lignin removal from the structure is essential. In addition, it should be mentioned that the hemicellulose removal has slight effect on the nanocellulose synthesis compared with lignin removal [3].

Nanocellulose, is of great interest owing to many applications, belongs to the nanofibrillar domain; the nanofibrils can be separated from each other when inter-fibrillar hydrogen bonds are broken down by harsh caustic chemicals, specific

enzymes and/or intense mechanical forces. Rod-like cellulose nanocrystals and longer cellulose nanofibrils are of interest due to their nanoscale dimensions, which improve the properties of elastic modulus [4], axial thermal expansion coefficient [5] and specific surface area [6].

Several methods have been applied to break down the hydrogen bonds to produce nanoscale cellulose. One approach, acid hydrolysis, breaks 1,4 glycosidic bonds in the cellulose. Sulphuric acid, hydrochloric acid, hydrobromic acid and mixed acetic and nitric acids are capable of hydrolysing the cellulose. The method consumes less energy and isolates rod-like cellulose nanocrystals effectively. These chemicals do not esterify the cellulose surfaces, resulting in higher yields [7]. It should be noted that chemical methods are not economically feasible and also environment friendly [8].

Mechanical treatments, high-pressure homogenization [9], grinding [10], ultrasonication [11], cryocrushing [12] and high-speed blending [13] have been studied to improve the yield up to 100% [14]. The treatments generate longer (several micrometers) but less uniform (5–100 nm wide) [15] and less crystalline [16] cellulose nanofibrils. On the other hand, the treatments consume high amount of energy [17].

In order to overcome the disadvantages, enzymatic pretreatments have been conducted to produce nanoscale cellulose [18]. Enzymatic applications increase the fibre swelling [19] and make the nanoscale cellulose production easier. Because of the environmental concerns, enzymatic hydrolysis is more advantageous compared to chemical and mechanical applications [20].

Consequently researchers have been modifying the nanocellulose production processes regarding economic and environmental concerns. Sulphite and dissolving pulps are of interest in nanoscale cellulose production. On the other hand, the disadvantage is their price [21]. Therefore, researchers have focused on alternative raw materials and cheaper kraft pulps to produce NFC. Modification is assumed to be advantageous for nanoscale cellulose production. To produce the nanoscale cellulose with a lower energy requirement, pulps may include higher hemicellulose contents. Therefore, it could be hypothesized that a chemical such as sodium borohydrate (NaBH_4), which is a powerful reducing agent, could be utilized in pretreatment step to effectively degrade the lignin. In addition, using NaBH_4 preserves the carbohydrates by converting the carbonyl group in the reducing end units to hydroxyl groups [22]. This could result in higher hemicellulose content in the structure, which could give higher production yield. Preserving hemicellulose might improve the swelling that could cause effective nanocellulose production by preventing homogenizer blocking.

The aim of this work was to produce microfibrillated cellulose (MFC) and nanofibrillated cellulose (NFC) from NaBH_4 -modified Eucalyptus kraft pulp, which was then treated with enzyme Pulpzyme HC 2500. Literature has very limited knowledge on the effect of this enzyme. The chemical, rheological and morphological as well as thermal properties of MFC and NFC were examined in this study. In addition, the produced films of MFC and NFC were examined regarding the dynamic mechanical thermal analysis (DMTA) and morphological properties.

2. Experimental

2.1 Materials

For this study, freshly cut logs of a 16-year-old river red gum tree (*Eucalyptus camaldulensis*) collected from Tarsus, Turkey, was used as the raw material. The bark and cambium were carefully removed from the logs, which were reduced to chips of suitable size for the subsequent kraft- NaBH_4 pulping operations. The chips were air-dried and screened to establish a uniform size throughout pulping. Pulpzyme HC 2500 (Novozymes, Bagsvaerd, Denmark) was employed to break down the cellulose and hemicellulose structures.

2.2 Methods

2.2a Pulping and bleaching: In this study, the kraft method was modified by adding 0.3% NaBH_4 (oven-dried (o.d.) chips). Pulp was produced using 500 g of chips (o.d.). The cooking was made in a 10 litre rotating digester (Uniterm Rotary Digester, Uniterm Lab.). The highest pulping temperature was 150°C. The highest temperature was reached in 30 min and cooking continued for 150 min after reaching the maximum temperature. The *H*-factor was 408. The cook was achieved at 18% active alkali and 28% sulphidity charges. The liquor-to-wood ratio (litre kg^{-1}) was 5:1. The produced pulp was disintegrated and washed with hot tap water, and then screened using a flat laboratory screen (Somerville Flat Screen, Techlab Systems) with a slot width of 0.15 mm (Tappi T275). The pulp yield (screened/unscreened) and rejects were determined according to Tappi T210 via gravimetric measurements in the laboratory environment.

The modified kraft pulp was then bleached using elemental-chlorine-free (ECF) processes of ODEP (oxygen-chlorine dioxide-alkaline-peroxide). Oxygen (O) bleaching was conducted in a digester (Uniterm Rotary Digester, Uniterm Lab.) using 2% NaOH (as Na_2O -o.d. pulp) and 0.5% MgSO_4 (as carbohydrate stabilizer-o.d. pulp) at a pressure of 6 kgf cm^{-2} (90°C for 60 min). The consistency was 10%. The chlorine dioxide (D) bleaching was performed in a plastic bag, which was placed in a water bath (GFL 1023 Water Bath, GFL Lab.). The process was accomplished at 60°C for 60 min. Each pulp sample (10 g, o.d.) was treated with 97 ml ClO_2 (1% w/v) and 3 ml H_2SO_4 (98% w/v) solution. The alkaline extraction (E) was performed in a water bath (GFL 1023 Water Bath, GFL Lab.) at 60°C for 60 min. Each pulp sample (10 g, o.d.) was treated with 100 ml NaOH solution (2% w/v). The hydrogen peroxide bleaching (P) was conducted at 10% pulp consistency using 4% H_2O_2 , 0.5% Na_2SiO_3 (as a hydrogen peroxide stabilizer), 0.1% MgSO_4 and 1.5% NaOH (o.d. pulp). The process was carried out at 105°C for 120 min in a digester (Uniterm Rotary Digester, Uniterm Lab.). After each bleaching operation, the pulp samples were washed with water, squeezed and crumbled. The kappa number (Tappi T236) and the viscosity (SCAN cm 15-62) of the pulps were determined after each bleaching stages.

2.2b Gel preparation: The cell wall delamination of the bleached modified kraft pulp was accomplished in four stages: mechanical refining, enzymatic pretreatments, second mechanical refining and homogenizing. The bleached kraft- NaBH_4 pulp was first mechanically refined (2% w/w) for 10 min using a Waring blender (NuBlend Commercial Blender, Waring Commercial) to reach 30°SR [23]. The power input was 1.9 A at 115 V. The process was paused for 5 min to allow the material to cool down to approximately room temperature. The freeness of the pulp was measured using a Schopper Riegler device (SR/P Schopper Riegler, Thwing-Albert Instrument Company) (ISO Standard method 5267-1).

Refined materials (50 g o.d. pulp) were enzymatically hydrolysed using Pulpzyme HC 2500 of concentrations 25, 100 and 250 AXU g⁻¹ at 2% solid loading in 2.5 litres of phosphate buffer at pH 7.0. The phosphate buffer used in the enzymatic pretreatments was prepared from 11 mM KH₂PO₄ and 9 mM Na₂HPO₄. The enzyme reactions were accomplished in an incubator (Incubator ES-20, Biosan Lab.) at 50°C for 2 h. The samples were mixed manually every 30 min. At the end of the treatment, the samples were washed with deionized water and put into boiling water for 30 min to stop the enzymatic activity. Then the samples were again washed with deionized water. The optimum enzyme concentration was determined according to the ratio of removed glucan to xylan. The sample with the highest amount of xylan was selected for further analysis.

The enzymatically treated sample (optimum) was then refined using a Waring blender (NuBlend Commercial Blender, Waring Commercial) [23] to reach 90° SR. To prevent a bacterial growth in the material, 0.4 μl ml⁻¹ of a microbicide (5-chloro-2-methyl-4-isothiazolin-3-one) was added to the slurry.

In the MFC and NFC production stage, the sample was passed through a high-pressure fluidizer (2% w/w) (Microfluidizer M-110Y, Microfluidics Corp.). For the MFC production, the sample was passed once through a Z-shaped chamber with a diameter of 200 μm (14,000 psi). For the NFC production, the sample was passed once through a Z-shaped chamber with a diameter of 200 μm (14,000 psi) and then passed five times through a chamber with a diameter of 100 μm (24,000 psi).

2.2c Film manufacturing procedure: For MFC or NFC film manufacturing, the MFC or NFC was stirred for 1 h at room temperature to ensure homogeneous consistency. The MFC or NFC suspension was poured onto a glass plate. The materials were placed overnight in a dryer to evaporate water at 60°C. The NFC or MFC films were peeled off from the plate and kept at room temperature for 24 h before the DMTA analysis. The produced NFC and MFC films were almost 100 μm thick.

2.2d Analytical methods:

High-performance liquid chromatography analysis The sugar and the lignin contents of the samples were determined by Laboratory Analytical Procedures (LAP) from the National Renewable Energy Laboratory (NREL) [24]. The sugar contents were analysed using high-performance liquid chromatography (HPLC, Agilent 1200 System, Agilent Tech.) equipped with a Shodex SP0810 column (mobile phase: HPLC grade water—0.2 μm filtered and degassed; injection volume: 20 μl; flow rate: 0.6 ml min⁻¹; column temperature: 80°C) and a refractive index detector. The acid-insoluble and acid-soluble lignin were determined, respectively, by weighing and by the adsorption at 320 nm against a deionized water blank.

The reduction in lignin was calculated based on the initial dry weight of the lignin in the chip/bleached modified kraft pulp (LU) and the dry weight of the lignin in the remaining solids after the pulping, bleaching, refining, enzymatic hydrolysis and homogenizing treatments (LP). The percentage of lignin reduction was calculated using the following equation:

$$\text{Percentage of lignin reduction} = \frac{\text{LU} - \text{LP}}{\text{LU}} \times 100.$$

The solubilization of xylan and glucan during the treatments was also calculated in the same manner.

The percentage of solids recovered was calculated on an oven-dry basis as follows: percentage of solids recovered = $W_2/W_1 \times 100$, where W_1 is the dry weight of the whole biomass before treatment (g) and W_2 is the dry weight of the treated material (g).

Fourier transform infrared spectroscopy The infrared (IR) spectra were taken via an attenuated total reflectance (ATR)-Fourier transform infrared (FTIR) device (Shimadzu IR Prestige-21, Shimadzu Corp.). Sample suspensions of 0.5 ml were prepared in a concentration of 2% (w/w). The samples were gently dropped in a diamond attachment using an automatic pipette (0.1–1 ml). In order to elucidate molecular vibration signals in the range of 4000–600 cm⁻¹, 20 scans with a resolution of 4 cm⁻¹ were taken.

¹³C Cross-polarization–magic angle spinning nuclear magnetic resonance spectroscopy The solid-state ¹³C cross-polarization (CP)–magic angle spinning (MAS) nuclear magnetic resonance (NMR) spectra of the samples were recorded using an Advance III 300-MHz NMR instrument (Bruker Corp.). The operating frequency was fixed at 75.385 MHz. A double air-bearing probe and a zirconium oxide rotor (4 mm) were used in the analysis. The MAS rate was 8500 Hz. A CP pulse was ramped at a contact pulse of 100 μs with the rotation of a 4 μs proton at 90° pulse (294.8 K). The delay between repetitions was 2.5 s. ¹³C data were processed offline using mestReNova processing software with a line broadening of 10 Hz. Cellulose crystallinity index (CrI) was calculated from the areas of the crystalline and amorphous C4 signals using the following formula [25]:

$$\text{CrI} = \frac{A_{86-92 \text{ ppm}}}{A_{79-86 \text{ ppm}} + A_{86-92 \text{ ppm}}}.$$

Rheological measurements In order to determine the rheological properties of the samples, a RST-CPS rheometer (Brookfield Corp.) was used. The measurements were made at the 37.5 mm diameter cone plate and the 25 mm diameter

parallel plate. The gap was fixed at 1 mm. Before the measurements, the shearing was applied to the materials at 20,000 rpm for 2 min (IKA T18 homogenizer, IKA Lab.) to disrupt any flocculated aggregates and the samples were then allowed to rest for 3 min.

Scanning electron microscopy The morphological properties of the samples were analysed by scanning electron microscopy (SEM, FEI Quanta FEG 250, FEI Corp.) images. The samples were first dried at 105°C overnight, and then coated up to 5 nm with a gold–palladium composite. Pictures were taken for all samples at 1–15 kV using a field emission gun equipped with a compacted secondary electron detector. Scales were selected as 100 µm for fibre materials and 1 and 100 µm for NFC and MFC, respectively. In addition, SEM analysis was carried out for MFC or NFC films.

Thermal analysis The thermal properties of the materials were examined using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). For the DSC, samples of approximately 3–5 mg of material were first squeezed, and then placed in an aluminium pan. In the device chamber the sample was heated from room temperature to 500°C at the rate of 10°C min⁻¹. All measurements were carried out in nitrogen flow (75 ml min⁻¹) using a Shimadzu DSC-60 Plus (Shimadzu Corp.) equipped with a thermal analysis data station. The heat flow was recorded as a function of the temperature. For the TGA, a Shimadzu DTG 60 (Shimadzu Corp.) equipped with a thermal analysis data station was utilized. The material samples were first dried at room temperature overnight. Approximately 5 mg of the material was placed in a platinum pan and heated from room temperature to 650°C at a rate of 20°C min⁻¹. Measurements were carried out in nitrogen flow (75 ml min⁻¹). The mass of the material was recorded as a function of the temperature.

Dynamic mechanical thermal analysis (DMTA) of nano-films and microfibrils DMTA tests were carried out to obtain thermo-mechanical characteristics (storage modulus) of the produced MFC and NFC films. The test was performed in tension mode at a controlled heating rate of 5°C min⁻¹. The temperature increased from 30 to 250°C at an oscillatory frequency of 1 Hz.

3. Results and discussion

3.1 Yield, kappa and viscosity analyses

The properties of unbleached modified (NaBH₄) kraft pulps are shown in table 1. Tozluoğlu *et al* [26] observed 45.7% (o.d. chip) of kraft pulp yield and the rejection was 0.03% (o.d. chip). Modified kraft pulp cooked under similar conditions gave almost similar pulp yield (45.3% o.d. chip) and rejection (0.04% o.d. chip). On the other hand, Istek and Gonteki [27]

and Gulsoy and Eroglu [28] observed slightly higher pulp yields when the kraft method was modified with NaBH₄.

The modified kraft pulp had lower kappa number (14.5) and viscosity (9.85 cP) compared with kraft pulp [26]. Modification diminished the kappa number by 21.2% (table 1). Istek and Ozkan [29] observed 19.5% reduction when the kraft method was modified with 3% NaBH₄ for poplar wood. On the other hand, much lower (8.02%) reduction was observed by Akgul *et al* [30] when the kraft method was modified with 2% NaBH₄ for brutia pine. The highest kappa reduction in this study could be due to the pulping conditions and raw material characteristics. The effect of modification on viscosity is a contentious issue. Akgul *et al* [30] observed higher viscosity for modified kraft pulp and explained this finding in terms of lower cellulose degradation. On the other hand, Copur and Tozluoğlu [22] found lower viscosity for modified pulp and explained the finding in terms of higher content of hemicelluloses having lower degree of polymerization (DP) compared with the cellulose.

The effect of bleaching sequences in kappa and viscosity was also studied and both properties, as expected, decreased when the pulp was bleached. Bleaching significantly diminished the kappa number (77.0%). A decrease in the kappa number and viscosity was observed after oxygen bleaching (O). On the other hand, a decrease in viscosity and kappa was observed when modified pulp was bleached with chlorine dioxide, and the lower viscosity was explained in terms of cellulose degradation [31]. In the next bleaching stage, the NaOH (E) extracted some lignin and low-molecular-weight materials and consequently diminished the yield. The pulp after E stage bleaching had higher viscosity (5.90 cP). This finding was also observed by Islam [32]. This finding could be due to the removal of some low-molecular-weight materials from the structure. The last stage, peroxide bleaching (P), also decreased both kappa and viscosity of the pulp.

3.2 Chemical properties

3.2a HPLC analyses: The effects of NaBH₄ modification on pulp's chemical composition were examined by HPLC. NaBH₄ utilized for kraft modification converts the carbonyl group in the reducing end units of carbohydrate chains to hydroxyl groups and therefore preserves the carbohydrates. On the other hand it delignifies the lignin more selectively [22]. Lignin, glucan and xylan solubilizations for NaBH₄-modified pulp were 95.7, 29.1 and 17.4% (o.d. chip), respectively.

The results obtained in this study showed that modification prevented carbohydrate reactions and thus preserved more glucan and xylan in the structure compared with the kraft pulp [26]. HPLC results indicated that modified pulp had 2.10% higher hemicellulose content. Therefore, this finding supports the information given earlier about lower viscosity of modified kraft pulp. The lignin, glucan and xylan solubilizations of the bleached pulps were found to be 96.7, 34.6 and 28.0% (o.d. chip), respectively. Bleached pulps had

Table 1. Yield, kappa and viscosity values of NaBH₄-modified kraft and bleached pulps.

Bleaching stages	Total yield (%)	Kappa	Viscosity (cP)
Kraft [26]	45.7 ± 0.46	18.4 ± 0.15	10.2 ± 0.38
Kraft–NaBH ₄	45.3 ± 0.61	14.5 ± 0.49	9.85 ± 0.38
Kraft–NaBH ₄ –O	43.6 ± 0.15	10.1 ± 0.32	8.57 ± 0.29
Kraft–NaBH ₄ –O–D	43.5 ± 0.23	3.79 ± 0.13	5.60 ± 0.26
Kraft–NaBH ₄ –O–D–E	41.4 ± 0.39	3.45 ± 0.00	5.90 ± 0.17
Kraft–NaBH ₄ –O–D–E–P	39.5 ± 0.18	3.34 ± 0.03	5.43 ± 0.45

Table 2. Chemical composition (%) of pulps after pulping and bleaching.

Chemical component	Chip	Kraft–NaBH ₄	Kraft–NaBH ₄ (ODEP)
Glucan	40.0 ± 1.53	62.6 ± 2.04	66.2 ± 0.81
Xylan	8.67 ± 0.19	15.8 ± 0.65	15.8 ± 0.85
Galactan	0.18 ± 0.14	0.10 ± 0.03	—
Mannan + arabinan	0.44 ± 0.20	0.33 ± 0.00	0.56 ± 0.20
Acid-insoluble lignin	27.6 ± 0.99	1.65 ± 0.69	1.28 ± 0.05
Acid-soluble lignin	0.74 ± 0.01	1.07 ± 0.03	1.08 ± 0.01
<i>Removed material (% o.d. chip)</i>			
Total material	—	54.7	60.5
Glucan	—	29.1	34.6
Xylan	—	17.4	28.0
Total lignin	—	95.7	96.7

Table 3. Changes in chemical composition (%) of kraft–NaBH₄ pulps after Pulpzyme HC 2500 treatment.

Chemical component	First refining	Enzymatic treatment concentrations (AXU g ⁻¹)			Optimum enzyme-treated sample (concentration of 100 AXU g ⁻¹)		
		25	100	250	Second refining	MFC	NFC
Glucan	65.9 ± 0.14	65.5 ± 0.47	66.1 ± 0.74	61.8 ± 0.08	64.9 ± 0.78	64.5 ± 0.22	64.3 ± 0.74
Xylan	15.7 ± 0.32	15.5 ± 0.65	15.6 ± 0.11	12.2 ± 0.15	15.3 ± 0.19	15.3 ± 0.41	15.3 ± 0.14
<i>Removed material (% o.d. chip)</i>							
Glucan	35.6	37.1	37.1	41.4	38.7	39.4	39.7
Xylan	29.2	31.3	31.5	46.6	33.3	33.7	33.9
<i>Removed material (% o.d. bleached modified kraft pulp)</i>							
Glucan	1.45	3.71	3.72	10.5	6.22	7.27	7.83
Xylan	1.63	4.53	4.79	25.9	7.37	7.84	8.11

higher glucan compared with modified pulp and this could be due to the selective delignification of lignin during bleaching (table 2).

The percentage of material loss and the glucan and the xylan content after each pretreatment step are shown in table 3. The first mechanical refining slightly diminished the glucan (1.45%, o.d. bleached pulp) and xylan (1.63%, o.d. bleached modified kraft pulp) contents. Chen *et al* [33] also observed similar results.

The refined sample was then enzymatically (Pulpzyme HC 2500) pretreated and the results showed that the enzyme effectively degraded xylan in the structure. On the other hand, some glucan degradation unexpectedly occurred and an increase in enzyme concentrations resulted in higher degradation. The enzyme removed up to 10.5% (o.d. bleached modified kraft pulp) of glucan and 25.9% (o.d. bleached modified kraft pulp) of xylan from the structure. Tozluoglu *et al* [26] observed 6.73% (o.d. bleached kraft pulp) of

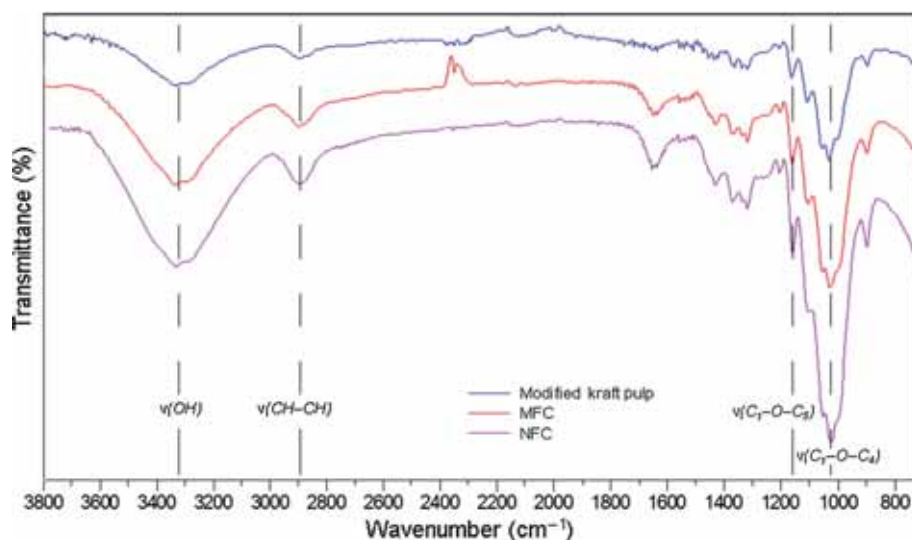


Figure 1. FTIR spectra of modified kraft pulp and MFC–NFC materials.

glucan and 8.65% (o.d. bleached kraft pulp) of xylan degradation for kraft pulp. The higher degradation in modified kraft pulp could be explained by the higher lignin delignification, resulting in more open structure for enzymatic activity.

The optimum enzyme condition regarding the ratio of removed glucan to xylan preserved more hemicelluloses in the structure, which is expected to diminish the cell wall cohesion and make the cell wall delamination easier [34]. Thus, it might prevent the blocking in the homogenizer [35]. Consequently, the Pulpzyme HC 2500 of 100 AXU g^{-1} condition, which removed only 4.79% (o.d. bleached modified kraft pulp) of xylan, was selected as the optimum for further analysis.

The sample was then mechanically refined again and the results showed that the treatment led to almost no xylan or glucan degradation in the structure. The processes to obtain MFC and NFC removed slight amount of xylan and glucan from the structure [36].

3.2b FTIR analyses: FTIR spectra were investigated to elucidate molecular interactions for modified kraft pulp and MFC and NFC produced in this study (figure 1). Slightly broad O–H peaks were observed at 3328 cm^{-1} . These peaks were compared to those of the free OH groups on the cellulose molecules corresponding to intra- and intermolecular H-bonds (figure 1) [37,38]. Successive treatments to produce MFC and NFC increased the peaks intensity due to increasing of O–H concentrations [37,39–41]. This phenomenon could be explained by the cellulose disintegration, reduction of fibre dimensions and more specific surface area throughout the production of MFC and NFC from modified pulp.

In addition, O–H in-plane bending and O–H bending vibrations were additionally observed at 1336 and 1202 cm^{-1} , respectively. These observed peaks showed the same

behaviour with the reaction as that of the O–H 3328 cm^{-1} peak [42].

Bleached modified kraft pulp including MFC and NFC showed no lignin peaks of 1740 , 1509 and 1463 cm^{-1} . The bands represent carbonyl, aromatic C=C ring deformation and C–H deformation in methyl, methylene and methoxyl groups of lignin [37,43]. This indicated that bleaching treatments applied in this study significantly removed the lignin from the structure.

CH_2 asymmetric stretching vibration peaks at 2895 cm^{-1} gave some insight about crystallinity [44]. Successive treatments to produce MFC and NFC altered the peaks intensity [45]. The peaks observed at about 1200 and 1000 cm^{-1} represent C–O–C vibrations in the cellulose structure. Peaks at about 1163 cm^{-1} are related to $\text{C}_1\text{–O–C}_5$ asymmetric bridge stretching, showing the ether linkage in the pyranose rings [38]. The peaks observed at 1033 cm^{-1} show the glycosidic ($\text{C}_1\text{–O–C}_4$) deformation or pyranose ring skeletal vibration in the cellulose. On the other hand, NFC produced from modified pulp resulted in minor shifts in $\text{C}_1\text{–O–C}_4$. Similar results were observed when NFC was produced from kraft pulp [26].

3.2c ^{13}C CP–MAS NMR analyses: The chemical structures of the samples were analysed by CP–MAS ^{13}C -NMR (figure 2). Some minor chemical shifts observed could be attributed to the packing effect of the supramolecular structures due to different chemical reactions and physical processings [46]. C4 peaks give insights about crystalline and non-crystalline forms. They were obtained as doubled-collateral peaks at $62\text{–}74\text{ ppm}$ in this study. The former (left) peak displayed the crystalline character, whereas the latter (right) showed the amorphous character [35]. Cellulose crystallinity index (CrI) calculated from C4 peak signals was

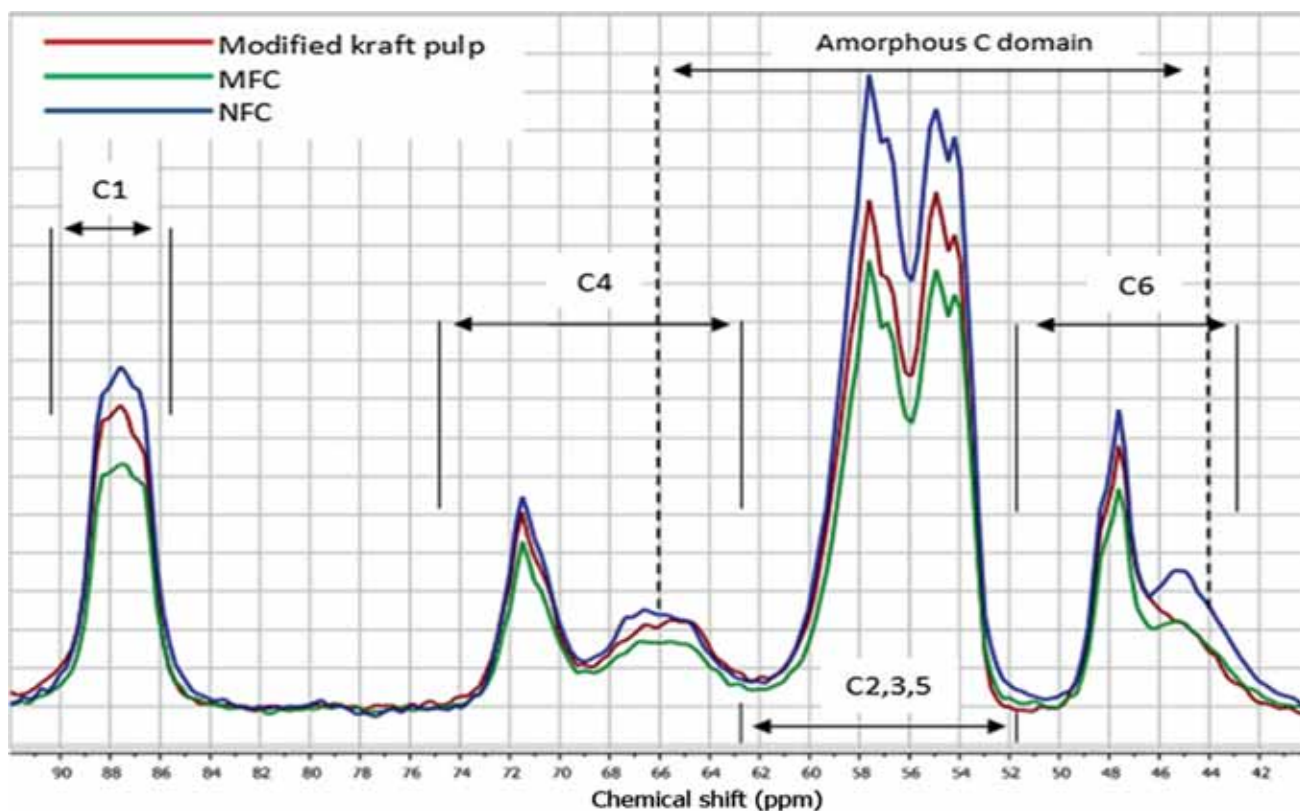


Figure 2. ^{13}C -NMR spectra of modified kraft pulp and MFC–NFC.

found to be 0.80, 0.57 and 0.49 for MFC, modified kraft pulp and NFC, respectively. A considerable decrease was observed for NFC compared with modified kraft pulp and MFC. CrI findings were also supported by DSC and TGA, in this study.

The sharpest and the most intense peaks were related to C2, C3 and C5 positions, which were observed at around 58–53 ppm. Results showed no significant shifts between the samples. C6 peaks were observed at around 48–44 ppm. The new observed peaks were obviously seen at about 45 ppm in MFC and NFC; this could be another indication related to CrI [47].

3.3 Rheological properties

The viscosity is plotted as a function of the shear rate for modified kraft pulp, MFC and NFC in figure 3. Increase in shear rate decreased the viscosity of all suspensions. This shows the shear-thinning behaviour and the materials tested in this study showed pseudoplastic behaviour [48]. MFC and NFC materials had lower viscosity compared with the modified kraft pulp. The decrease could be explained by lower floc size of fibrils, higher and narrower size distribution and gradual breaking of 3D network as well as higher Einstein coefficient—length to diameter ratio [49]. Consequently, this may cause higher specific surface

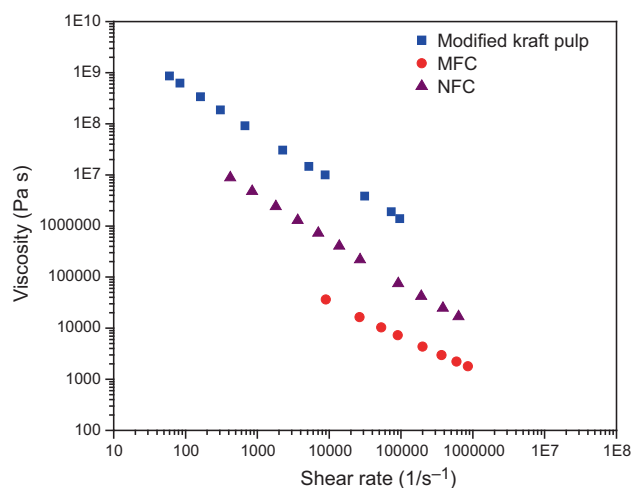


Figure 3. Viscosity as a function of the shear rate for modified kraft pulp and MFC–NFC materials.

area and thus increase the cellulose surface reactivity. A similar finding was observed when MFC and NFC were produced from kraft pulp [26]. On the other hand, modified kraft pulp has higher viscosity compared with kraft pulp, indicating the cellulose-preserving effect of NaBH_4 [22].

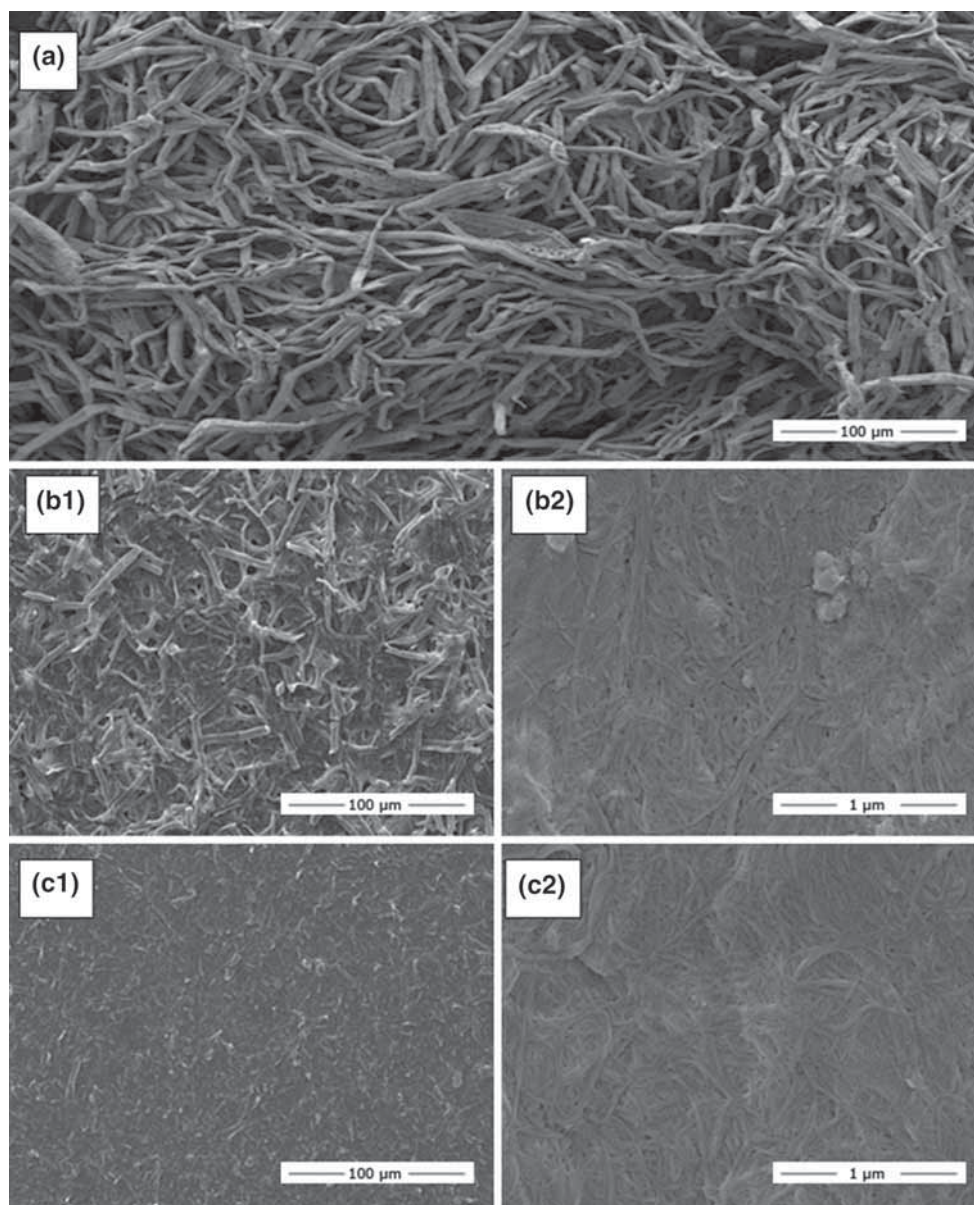


Figure 4. SEM images: (a) modified kraft pulp, (b1, b2) MFC and (c1, c2) NFC materials.

3.4 Morphological (structural) properties

Figure 4a–c shows the SEM images of the modified kraft pulp and MFC and NFC samples. Mechanical refining swells the fibres and creates damaged zones, which enhances the enzymatic activity [35]. Enzymatic treatments improve the fibrillation and result in efficient homogenization.

The SEM image of MFC displayed nanofibrils and some micron-wide fibres. On the other hand, SEM image of NFC did not display micron-wide fibres and the collected dispersion was fairly homogeneous. Cellulosic nanofibres in this study had an average length of 2823 ± 241 nm and a width of 22 ± 5 nm. The average aspect ratio of generated NFC was 130 ± 34 . Moon *et al* [50] produced NFC with an aspect

ratio > 100 , and the diameter and length of NFC were 4–10 nm and several micrometres, respectively. Results showed that NFC produced from modified kraft pulp resulted in smaller size nanofibrils compared with kraft pulp (35 ± 12 nm) [26]. This finding could be due to the higher hemicellulose content of modified kraft pulp. Higher hemicellulose may decrease the cell wall cohesion and make cell wall delamination easier [34,35]. NFCs of about 10–100 nm, produced by different techniques, are reported in the literature [9,51,52].

3.5 Thermal characterization

3.5a DSC analyses: Thermograms for modified kraft pulp, MFC and NFC are shown in figure 5. Two considerable

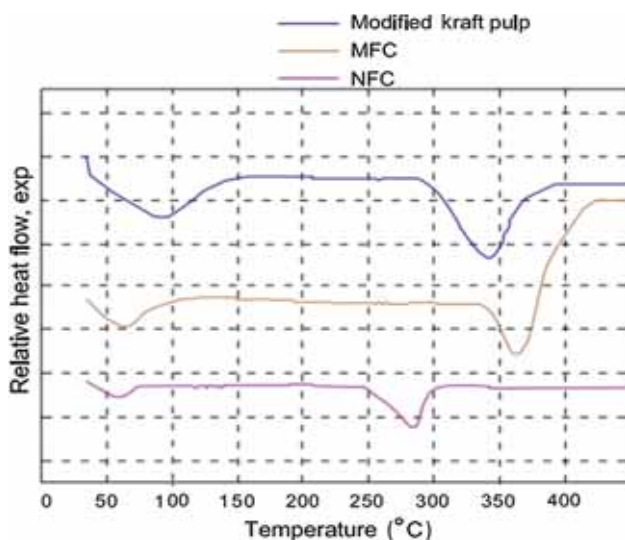


Figure 5. DSC curves registered over a range of room temperature to 600°C for modified kraft pulp and MFC–NFC materials.

endothermic peaks at around 25–150 and 350–380°C are observed. The earlier peaks could be attributed to the loss of water in the samples. Results showed that from modified kraft pulp to MFC and then to NFC, loss of water at lower temperatures was obvious. Results indicated that mechanic/enzymatic (Pulzyme HC 2500) pretreatments decreased the size and resulted in larger surface area, causing more water adsorption [39,53].

The later peaks observed at around 350–380°C indicate the degree of cellulose decomposition. The increasing of degradation temperature was associated with higher thermal stability, and this behaviour could be attributed to the high degree of material crystallinity [54]. Mechanical/enzymatical pretreatments to produce MFC and NFC lowered the degradation temperature. This finding could be due to the degradation in glycosidic bonds of cellulose, which changed the crystalline/amorphous ratio in the structure [55].

3.5b TGA analyses: The TGA curves of the modified kraft pulp, MFC and NFC are given in figure 6. Results showed that samples started decomposing at different temperatures depending on the chemical characteristics and the degree of crystallinity. The decrease in sample weight observed in the first range (25–150°C) for modified kraft pulp and MFC could be attributed to the losses of humidity and some low-molecular-weight compounds [56]. On the other hand, a constant weight loss up to 375°C was observed for NFC. The significant weight loss observed at about 375°C could be due to the cellulose and hemicellulose decomposition [57]. Up to 650°C, additional decomposition of hemicellulose and lignin was observed [58].

The NFC showed lower thermal decomposition and thermal stability (298°C) compared with MFC (342°C). The lower decomposition temperature could be attributed to the

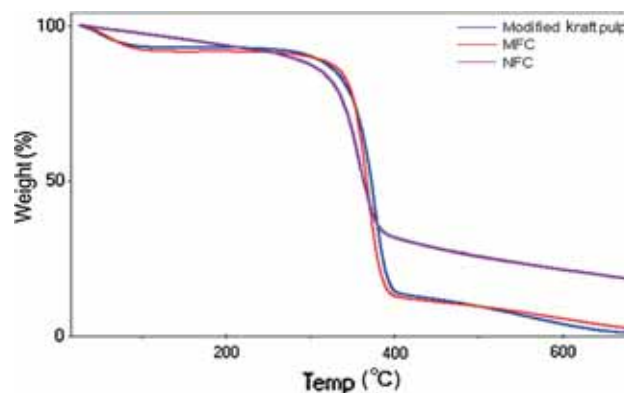


Figure 6. TGA curve of modified kraft pulp and MFC–NFC materials.

higher surface area and lower crystallinity of the NFC. NFC with higher surface area caused faster heat transfer and diminished the thermostability [59–61]. The decomposition temperature of kraft pulp [26] was lower compared with modified kraft pulp produced in this work. This finding could be due to the lower viscosity and crystallinity of kraft pulp. It should also be noted that higher solid residue for the NFC may be due to the presence of more stable cellulose I in the structure [43].

3.6 Dynamic mechanical thermal analysis (DMTA)

Figure 7 shows photographs of the (a) NFC and (b) MFC films. The clearly seen background through the NFC film indicated that the produced NFC film was transparent. On the other hand, MFC film showed opaque property. The obtained properties could be attributed to the cellulose dimensions of the films. The SEM images of NFC and MFC (figure 4) support the finding.

Figure 8 shows the (a1, b1) surface and (a2, b2) cross-sectional SEM images of fractured films. The NFC film had a smoother surface and more homogeneous cross-section compared with the MFC film. It should also be mentioned that the NFC film had a more uniform dispersion than that of the MFC film.

Figure 9 shows the DMTA results of the NFC and MFC films. The mechanical behaviour of films at elevated temperatures is critical for various industrial applications. The results indicated that the storage modulus of the NFC and MFC films increased with increasing temperature from 30 to 106 and 30 to 89°C, respectively. This finding could be attributed to easier water evaporation from the structure as well as the voids in the micro-structure. The MFC film removed water at lower temperatures compared with the NFC film, which had a more compact structure. After reaching the highest value, the storage modulus of the NFC or MFC films decreased with increasing the temperature up to 250°C.

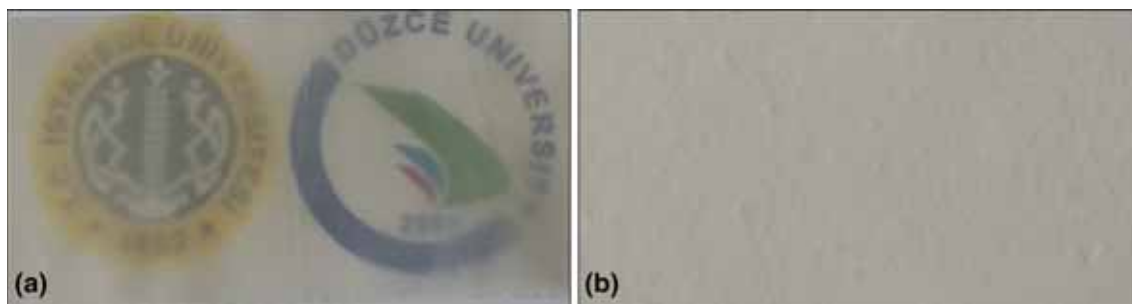


Figure 7. Photographs of the (a) NFC and (b) MFC films placed on a background.

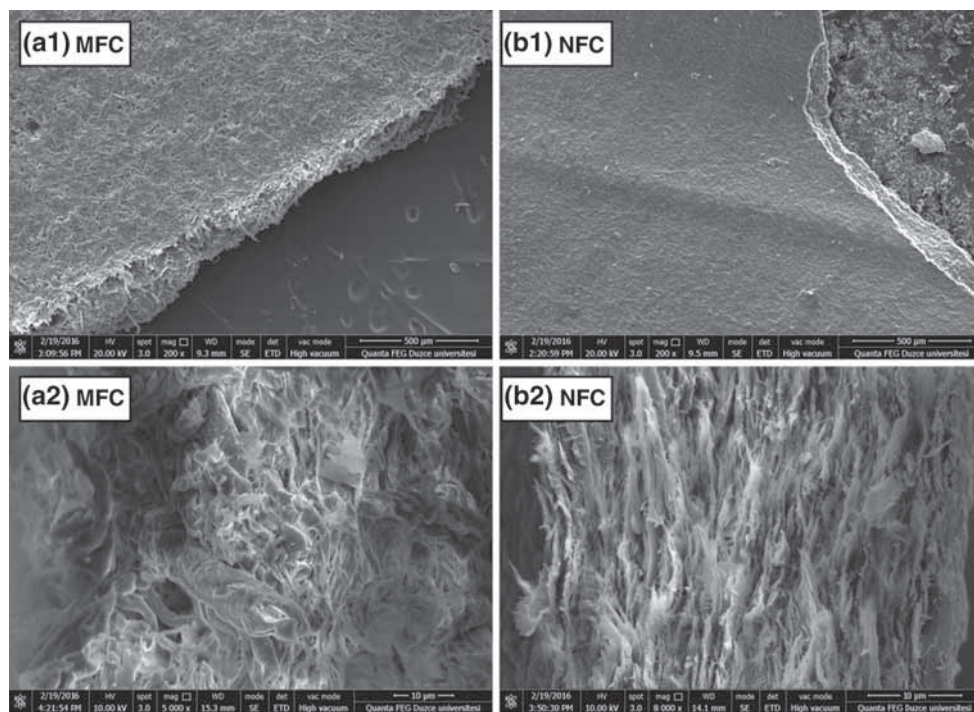


Figure 8. Scanning electron microscopy (SEM) images of (a1, b1) surface and (a2, b2) cross-section of the NFC or MFC films.

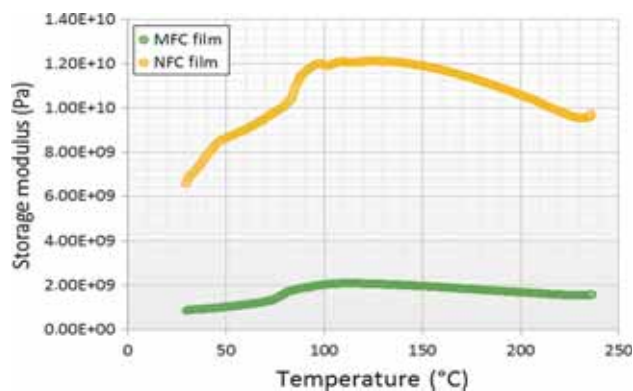


Figure 9. Storage modulus curves of the NFC or MFC films.

The average initial storage moduli of the MFC and NFC films were found to be 0.87 and 6.59 GPa, respectively. Honarato *et al* [62] examined mechanical properties of the TEMPO oxidized NFC films produced from Eucalyptus fibres, and the storage modulus of nanofilms was found to be 7.20 GPa. In addition, nanofilm produced from TEMPO oxidized bleached birch kraft pulp showed almost similar results [63]. On the other hand, Shankar and Rhim [64] produced a composite film using agar (90%) and nanocellulose (10%), which resulted in 1.15 GPa of storage modulus. Films of NFC with 1% PVA added showed storage modulus of 0.05 GPa [65].

The maximum storage modulus value of the MFC film was 2.10 GPa and the maximum storage modulus value of the NFC film was 12.10 GPa. When MFC and NFC were compared, the films produced from NFC improved the initial storage modulus by 657% and maximum storage modulus

by 476%. The results obtained in this study are consistent with the finding of Arjmandi *et al* [66]. They reported higher performances of nano-fillers, cellulose nanowhiskers, due to the enhanced stress transfer. Thus the modulus of the composite is higher than that of the microcellulose fibres.

4. Conclusions

Producing biomaterial-based nanoproducts is of great interest for industrial applications. Biomaterials consisting of a high amount of cellulose are a source to produce NFC. In this study, molecular structures of MFC and NFC exhibited minor differences compared to modified kraft pulp. Successive treatments decreased the crystallinity index of NFC, while the crystallinity of MFC was higher. In addition, significantly different thermal stabilities and decomposition behaviours were observed for NFC and MFC. Applied shear stress decreased the viscosity. The NFC films produced were transparent and had higher storage value compared with MFC. Using Pulpzyme HC 2500 in NFC and MFC production as well as modifying the kraft method with NaBH₄ prevented the aggregate formation, which made dispersion easier and caused uniform size distribution. Results revealed that the MFC and NFC could be utilized as reinforcement in polymer applications, additive in cosmetics, dye applications, etc.

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