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Extraction and characterization of nanofibrillated cellulose from yacon plant (*Smallanthus sonchifolius*) stem

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Abstract

This study aimed to evaluate the process of cellulose extraction from yacon stem using combined pulping and bleaching processes for produce nanofibrillated cellulose (NFC). First, chemical pulping process with NaOH was applied and, subsequently, the pulp obtained was bleached. From the chemical pulp (CP) bleached, NFC was obtained by the mechanical defibrillation in a colloidal grinder. Then, chemical composition, color, and infrared analysis of the pulps was performed. The pulping process showed a lower amount of extractives and lignin content, as a low yield and an excessively dark pulp. The CP bleached with $NaClO_2$ showed the best results increased whiteness of the pulp. A suspension of NFC with fibers of 5–60 nm in

diameter, high crystallinity index, and thermal stability was obtained. The results are promising and demonstrate the technical feasibility of obtaining NFC from yacon stems waste which is ideal to apply to other materials of the industry.

Keywords

lignocellulosic biomass; chemical process; cellulose fibers; bleaching; nanotechnology

Introduction

Yacon (*Smallanthus sonchifolius*) is a perennial plant native to the Andes that belongs to the Asteraceae family. The plant is adaptable to different altitudes, types of climatic conditions and soil because it is grown both at sea level (Brazil, Germany, Japan, New Zealand, Russia, and the United States) and in the Andean mountains, which reach up to 3200 m in altitude [1,2]. Also, presents a very branched root system underground, and stems, leaves, and flowers in the aerial part plant.

The stem represents the largest fraction of the aerial part of the yacon plant, about 74%, the other percentage is made up of leaves and flowers. According to Kamp et al. [3], the density of the yacon plantation can vary from 12500 to 30000 plants.ha^{-1}, as it depends on the propagation method. Also, each plant has 4 to 12 stems that can reach up to 3 m in height [1,4]. It is composition includes approximately 23.82% to 26.85% fiber, 9.73% to 11.37% protein, 9.60% to 10.23% ash and 1.98% to 2.26% lipids [5]. The researches of its stem are more insufficient than its leaves and roots, and its stem is actually discarded or used as animal feed [5,6]. Therefore, the yacon stem is one promising biomass to be used as raw material with use of the fiber, as represents a considerable fraction of chemical composition.

The materials derived from lignocellulosic biomass have received great attention because they have a high potential as substitutes for raw material of fossil origin, due to their abundance, availability and renewability and biodegradability [7]. As well, the stimulus is promoted by different aspects, as its policy, laws, and international treaties. Although the use of these materials may further advance to reduce environmental impacts, it will also require properties similar or superior to those seen in conventional materials [8].

The nanofibrillated cellulose (NFC) obtained from non-wood biomass has gained the attention of several industry sectors, and have been applied in food packaging, biosensors, drug delivery, because of its biocompatibility, biodegradability, renewability, availability, lower cost of raw material, lower weight, higher technical and mechanical strength [9-11]. The NFCs are a tangled of nanofibrils with a diameter within nanoscale dimensions – i.e. up to 100 nm – and with several length micrometers [12] However, the choice of cellulose source and the production process has a significant impact on the quality and characteristics of NFC. To obtain NFC, the lignocellulosic biomass is submitted to pre-treatment processes such as pulping and bleaching [13-16], followed by a refinement treatment [17].

The chemical pulping process is the most employed in the industry, where the alkaline chemical process with sodium hydroxide (NaOH) is the most widely used and known [18]. As advantages, alkaline treatments can efficiently remove lignin, in addition to reducing the solubilization of hemicelluloses and be applied in mild temperature conditions [19]. After the alkaline pulping, the cellulose pulp has dark-colored, requiring the subsequent application of bleaching processes. Bleaching occurs when chemical agents oxidize the non-cellulosic compounds presents in the pulp. Sodium hypochlorite (NaClO), hydrogen peroxide (H_2O_2) and sodium chlorite ($NaClO_2$) have been used to

bleach and, at the same time, promotes the delignification of cellulose pulp [18, 20-22].

The fibers obtained in these processes are long, so additional refinement processes can be used to obtain NFC [23]. NFC can be isolated through various processes, one of them being the defibrillation process performed in a colloidal grinder, which has been considered an appropriate method to produce NFC in a more economically viable way [24]. To obtain smaller fibers or to produce a more uniform product, the suspension can be treated by multiple turns through the defibrillator [15, 21].

Nevertheless, the characteristics of NFCs depend mainly on the type of raw material and the processes that they were submitted, hence the investigation of their characteristics are very important. There are recent studies lignocellulosic biomass to obtain NFC [9, 25], but there are no reports in the literature about the use of yacon's agricultural waste (stem) for this purpose. Therefore, the objective of this study was to investigate the feasibility of using yacon stem to produce NFC from the bleached pulp. The processes' yields were compared, as well as the effects of treatments on the pulp and the resulting NFC characteristics. The results of the physical, thermal, and chemical properties of NFC from yacon stem were also evaluated.

Results and Discussion

Raw fiber and pulps characterization

Morphological analysis

Figure 1 shows the SEM micrographs of yacon stem (YS) and the fibers obtained by chemical process, as well as the bleached pulps. The structure of YS is like that of other plants in the Asteraceae family, which have vascular bundles that form a porous

network analogous to honeycombs with a variety of sizes in diameter [26]. The CP presented soft and clustered fibers with smooth connections, but trace residues of the material (YS) remained on the fibers. The morphological characteristics of the CP reflected the chemical composition of the pulp, indicating the efficiency of the chemical process. In general, bleaching processes promoted a superficial change in the pulp initially treated with NaOH, making them even smoother.

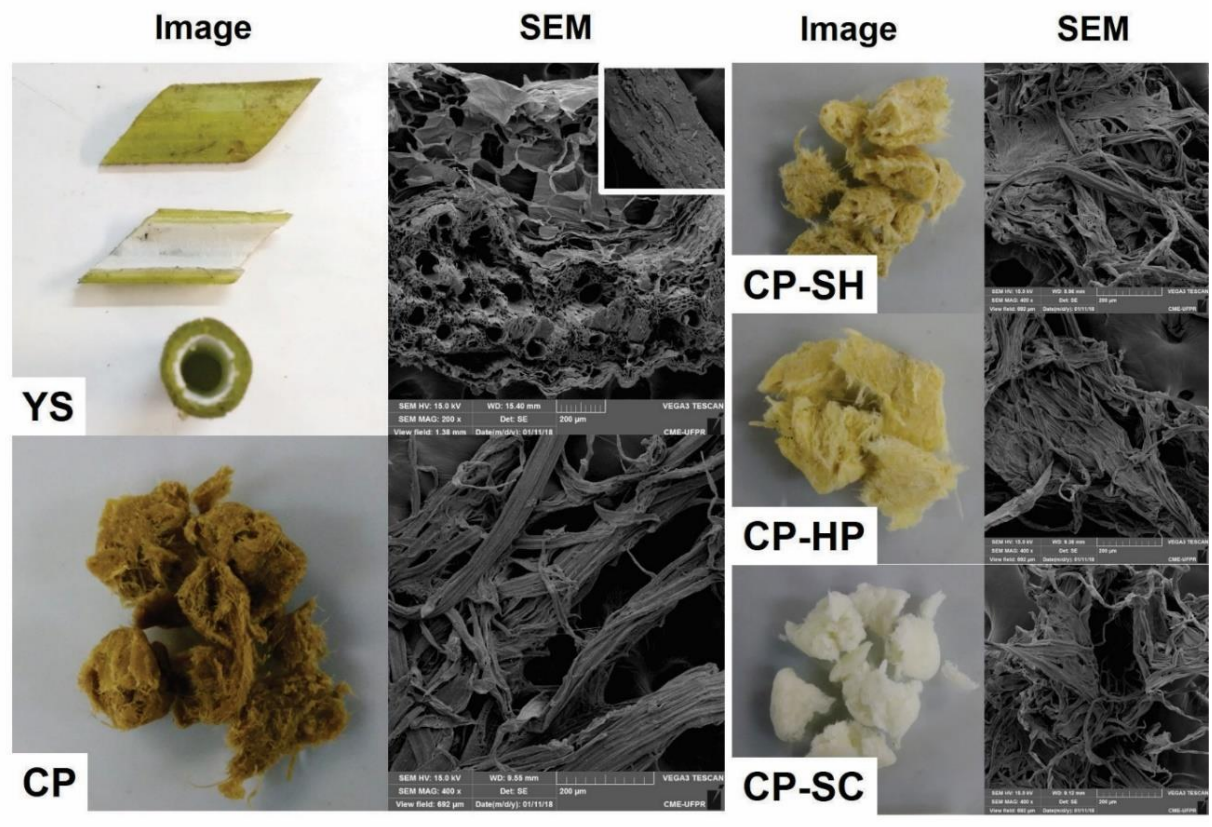

Figure 1: Image and SEM of the yacon stem (YS), CP=chemical pulp treated with NaOH and bleached pulp treated with NaClO (SH), H_2O_2 (HP) and $NaClO_2$ (SC).

Chemical composition, surface color, and yields

The YS (24.4% extractives, 14.0% lignin, and 61.5% holocellulose) was submitted to chemical pulping process, and the chemical composition of these materials are presented in Table 1. The lignin content found is lower than in tobacco (23%), sunflower (26%), corn (19%), and bamboo (23–28%) biomass [25, 27,28]. This is an

advantage because it makes the process of fiber extraction less strict, demanding fewer chemical reagents and time. There are no reports in the literature on the composition of the yacon stem in terms of extractives, lignin, and holocellulose, highlighting the importance of this research.

The chemical process removed a significant ($p<0.05$) amount of the amorphous extractives of the fibers, which affected other properties, such as yield (low) and resistance to thermal degradation, further discussed.

The pulping process applied promoted a significant decrease in the extractives and lignin contents (Table 1). Alkaline pulping of the YS resulted in the removal of considerable amounts of extractives and lignin, approximately 94.6% and 85.7% respectively, showing a pulp with 96.8% holocellulose, but with 33.29% yield. Subjecting the plant matrix to treatment with alkaline solutions at high temperatures causes a disturbance in the cell wall structure due to cleavage of the ester and ether bonds between lignin and hemicellulose, resulting in its solubilization [29]. This explains the significant reduction of these constituents in the matrix, on the other hand, promotes an increase in the purity of cellulose fibers.

Table 1: Effect on chemical composition and yield of pulping and bleaching processes of pulps

Parameters	YS	CP	CP-SH	CP-HP	CP-SC
Extractives (%)	24.4 ± 0.7^{a}	1.3 ± 0.1^{b}	0.8 ± 0.1^{bc}	0.1 ± 0.0^{c}	0.1 ± 0.1^{c}
Lignin (%)	14.0 ± 0.1^{a}	2.0 ± 0.2^{b}	0.9 ± 0.1^{c}	1.0 ± 0.1^{c}	0.4 ± 0.1^{d}
HOLO (%)	61.5 ± 0.7^{d}	96.8 ± 0.4^{c}	98.3 ± 0.1^{b}	98.9 ± 0.1^{ab}	99.5 ± 0.1^{a}
Yield (%)	-	33.3 ± 1.4^{c}	94.9 ± 3.3^{a}	95.0 ± 2.5^{a}	85.9 ± 3.0^{b}

(-) not determined; (%) on dry basis; YS: yacon stem; CP: chemical pulp treated with NaOH; SH: NaClO-treated pulp; HP: H_2O_2-treated pulp; and SC: $NaClO_2$-treated pulp;

HOLO: Holocellulose. The mean ± standard deviations followed by different letters differ statistically at a significant difference (0.05) by the Tukey test.

Color analysis is a quality parameter that can be used in the industry to determine the efficiency of the pulp bleaching process. The effect of CP bleaching treatments can be observed by the color coordinates in Table 3 and Figure 1. Pulp bleaching with $NaClO_2$ (CP-SC) was more satisfactory, followed respectively by H_2O_2 (CP-HP) and NaClO (CP-SH), which is evidenced by analyzing the L* coordinate that represents the luminosity/whiteness. The bleaching of pulp also allows delignification, as it significantly reduced the lignin content in the pulp. The combined bleaching and hydrolysis helped with cellulose purification and isolation because of the removal of non-cellulosic components including lignin and hemicelluloses, besides facilitates mechanical defibrillation to obtain NFC [30]. The application of more bleaching stages can be used to obtain pulps with a higher level of whiteness. The bleaching of cellulose pulp in a single stage is an advantage as it reduces costs and process time.

Table 3: Effect on color coordinates (L*, a*, b*, ΔE) of pulping and bleaching processes of pulps

Parameters	CP	CP-SH	CP-HP	CP-SC
L*	40.46 ± 0.03^{d}	61.77 ± 0.04^{c}	69.39 ± 0.01^{b}	83.60 ± 0.04^{a}
a*	6.93 ± 0.03^{a}	3.15 ± 0.02^{b}	2.31 ± 0.04^{c}	-0.80 ± 0.02^{d}
b*	22.08 ± 0.01^{b}	22.07 ± 0.03^{b}	26.98 ± 0.06^{a}	13.15 ± 0.01^{c}
ΔE	0.00^{d}	21.64 ± 0.04^{c}	29.71 ± 0.02^{b}	44.73 ± 0.04^{a}

CP: chemical pulp treated with NaOH; SH: NaClO-treated pulp; HP: H_2O_2-treated pulp; and SC: $NaClO_2$-treated pulp; The mean ± standard deviations followed by different letters differ statistically at a significant difference (0.05) by the Tukey test.

The a* and b* coordinates refer to chroma and the closer this value is to zero, the less it interferes with color. The color of the pulps ranged from reddish (+a*) to neutral (a* near 0). Bleached pulps are predominantly yellow (+b), although for pulps treated with SC, b* values are lower, which is visually perceived (Figure 1). The color of the pulps is associated with the chromophoric groups of the components, as cellulose and hemicelluloses are inherently white; as such, they do not absorb light in the visible spectrum and do not contribute to color. Organic substances composed of unsaturated bonds are generally the ones that color the pulp, especially those with type C=O and C=C bonds, as in the lignin structure [31]. With the global color variation (ΔE) it is possible to observe how much bleaching changed the general color aspect of the pulps used at the beginning of the process (CP). Bleaching mainly influenced the L* coordinate, where SH evidenced little change in the ΔE value of pulp CP-SH.

Fourier Transform Infrared Spectroscopy (FTIR)

The effects of pulping and bleaching processes on the chemical composition of YS fibers were assessed by infrared readings (Figure 2). The chemical pulping process with NaOH altered the chemical structure of the fibers as seen in the chemical characterization (Table 1). All infrared spectra of the samples have a high-intensity band around 3600 cm^{-1} attributed to the vibration of hydroxyl bonds (-OH), a functional group present in cellulose, hemicellulose, and lignins [32]. An elongation of this band is noticeable in the YS until about 3100 cm^{-1}, which may be related to the formation of hydrogen bonds from carboxylic and phenolic groups of the hemicellulose, lignins and extractives structures [33].

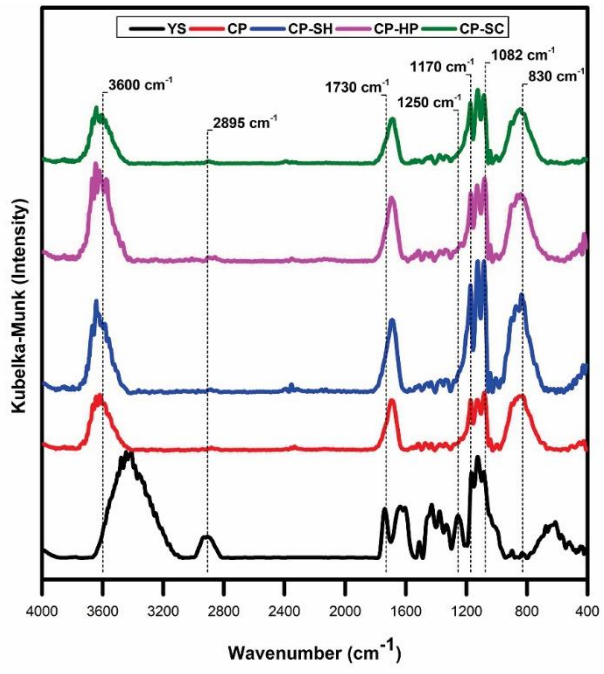

Figure 2: Infrared spectrum of the yacon stem fiber (YS), chemical pulp treated with NaOH (CP); and chemical pulp treated with NaClO (CP-SH), H_2O_2 (CP-HP) and $NaClO_2$ (CP-SC).

The band between 2920–2850 cm^{-1} represents the vibration of the C–H bond present in cellulose, hemicellulose, and lignin. The 1750 to 1720 cm^{-1} range reflects the vibration of C=O bonds, with an increase in intensity in this region (1730 cm^{-1}), possibly due to hydrolysis cleavage of hemiacetal bonds, which generate aldehyde groups [32, 34, 35].

The NaOH process, as well as the bleaching treatments, significantly reduced the lignin content of YS, observed in the 1500 to 1600 cm^{-1} range, where there are less peaks, which are attributed to the vibration of the aromatic structure [35]. The peak at 1250 cm^{-1} disappears after alkaline treatment on YS fibers due to the vibration of hemicellulose's C–O [36], corroborating the chemical composition found. Moreover, the peaks at 1170 and 1082 cm^{-1} are attributed to the vibration of the C–O–C group in the pyranose ring in polysaccharides [34]. A considerable inversion of the spectrum

signal occurs around 830 cm^{-1}, attributed to presence of carbohydrates, such as hemicelluloses [37]. Thus, among the bleached pulps and considering yields, color and residual lignin content in the fibers, the NaOH-treated pulp with $NaClO_2$ bleaching (CP-SC) offered a more satisfactory result to proceed with the extraction of NFC.

Characterization of nanofibrillated cellulose (NFC)

Transmission electron microscopy (TEM)

Figure 3 gives an overview of the morphological characteristics of the NFC. In Figure 3 (a), the NFC suspension exhibited a gel-like viscous appearance where a non-phase separation has been verified during storage. Figure 3 (b) and (c) shows how the process of obtaining NFC allowed the individualization of the fibers. Mechanical defibrillation in a colloidal grinder yields a highly branched and interwoven structure with fibers diameters ranging from 5 to 60 nm very smaller than their lengths, which characterizes a nanomaterial. No reports were found in the literature about the production of NFC from the stem of the yacon plant. But, fibers of different lignocellulosic materials have a similar appearance and diameter to the fibers obtained [10, 11, 26, 38]. Therefore, it was possible to obtain NFC from the yacon stems, as wished.

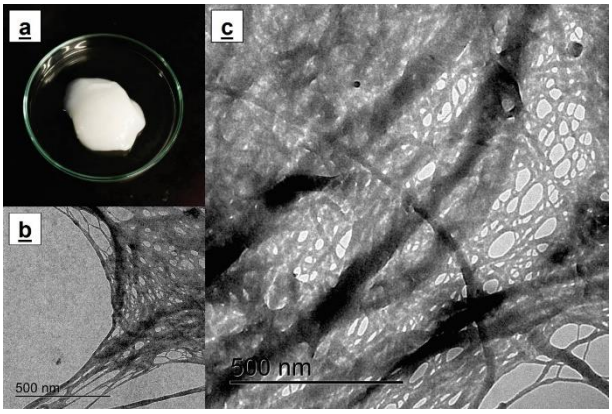

Figure 3: Image (a) and SEM (b) and (c) of nanofibrillated cellulose (NFC) with magnification of 15000×.

Thermogravimetric analysis (TGA/DTG)

The TGA/DTG curves of YS, CP, CP-SC, and NFC are shown in Figure 4. The constituents present in the analyzed materials exhibit three main stages of thermal degradation (Figure 4.a). The first stage starts at 30 ºC and extends to 110 ºC, which is mainly caused by the loss of water mass [12]. The second stage occurs between 150 ºC and 450 ºC possibly due to the depolymerization of cellulosic components (cellulose and hemicellulose) and due to the traces of lignin in the samples. There are considerable mass losses between 150 °C and 300 °C for YS in the second stage, which are not explicitly seen in pulps and NFC. It can be attributed to the thermal decomposition of extractive materials, such as low molecular weight polysaccharides - e.g. pectic substances [39].

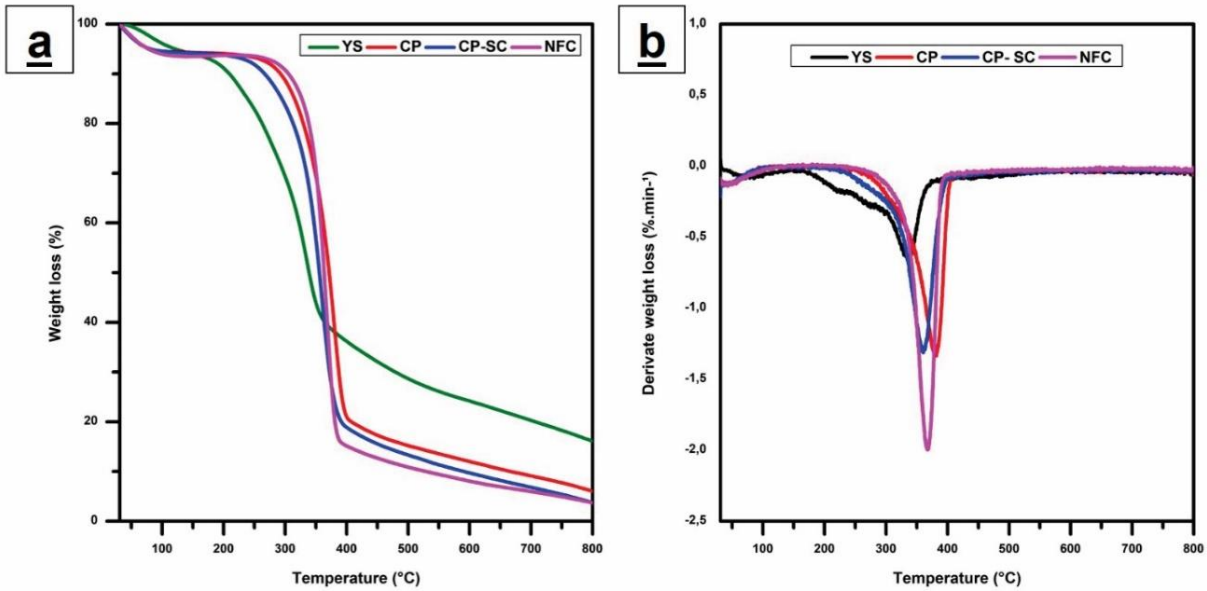

Figure 4: TGA (a) and DTG (b) of yacon stem fiber (YS), the chemical pulp (CP), $NaClO_2$- bleached chemical pulp treated with NaOH (CP-SC) and nanofibrilated cellulose (NFC) as a function of weight loss.

In the third stage, there is a small mass loss at 450 ºC, where the complete degradation of residual lignin mainly occurs [40]. The maximum thermal resistance temperature (Tmax) around 360 °C is attributed to cellulose, as hemicelluloses, as well as the other components, are considered amorphous and have a low degree of polymerization [26]. This characteristic is attractive to NFC, whose purpose is to be applied to materials in which the processing temperature is high, such as to biocomposites that may exceed 200 °C [38]. NFC obtained of source non-wood have been used as the base or auxiliary material to produce paper and board, coatings, packaging, adhesives, sensors, filters, biomedical, among others [41].

In Figure 4.b and 4.c, Tmax increases as YS (335 °C) undergoes alkaline pulping (360 °C) and $NaClO_2$ (380 °C) bleaching treatments, but Tmax lowers to 368 °C with ultrafine fibrillation. This lower resistance to thermal degradation of NFC may be related to the defibrillation to which CP-SC was submitted, as this process may cause changes in the crystalline regions of cellulose [42]. This effect can be noted by the XRD analysis.

X-ray diffraction analysis (XRD)

The processes' effect on crystallinity of samples can be visualized by XRD analysis (Figure 5). Similar intensity peaks were identified in all samples analyzed (YS, CP, CP-SC, and NFC) via XRD profiles, located at diffraction angles (2θ) near 17º and 22°. Another low-intensity peak is visible in the 34º angle, more evident in the pulps and the NFC. The samples have a typical diffraction curve of cellulose I, similar to other lignocellulosic materials [42,43]. The crystallinity index (CrI), that relate the crystalline phase to the amorphous phase of the material, was calculated according to Eq. 4, obtaining 52.21%, 65.15%, 71.28%, and 70.60% for YS, CP, CP-SC, and NFC, respectively, a clear increase from before to after the bleaching treatment. The chemical pulping process and pulp bleaching increased crystallinity by more 24.78%

and 36.52%, respectively, in relation to the matrix (YS). Such an increase in crystallinity is related to the removal of pulp amorphous components such as extractives and lignin [40], which can be confirmed by Table 1. In addition, a high value of crystallinity means greater rigidity of the fibers and this characteristic can be beneficial for the application as reinforcement for biocomposites [26]. It was also noted that the CrI of the NFC had a slight decrease when compared to CP-SC, which may be related to the effect of the mechanical defibrillation in a colloidal grinder and may have affected the crystal structure [42]. Although the process of obtaining NFC reduces the index, the crystallinity remains high (above 70%).

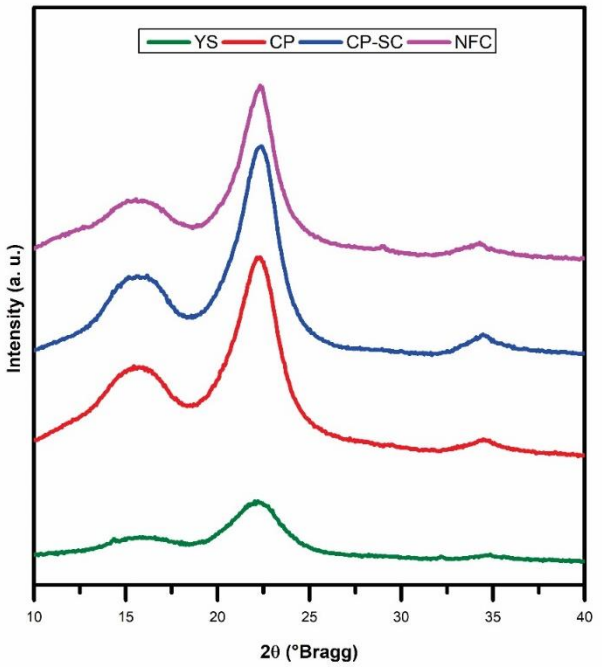

Figure 5: XRD of yacon stem fiber (YS), the chemical pulp treated with NaOH (CP), $NaClO_2$- bleached chemical pulp (CP-SC) and nanofibrillated cellulose (NFC).

Conclusion

This study was the first to characterize and use yacon plant stem biomass for nanofibrillated cellulose production. The best result obtained in terms of yield, color and lignin content was the use of alkaline pulping process with NaOH followed by

bleaching with $NaClO_2$. The yacon NFC obtained show high crystallinity index and thermal resistance, which demonstrate the potential application in other materials, for example in biocomposites and packaging, as well as assisting in future research in this area.

Experimental

Materials

The yacon plant stems were supplied by a farmer in São José dos Pinhais, Paraná, Brazil (coordinates: 25 ° 37′8.37 ″ S 49 ° 07′15.72 ″ W; at 882 m altitude). The reagents used in the chemical characterization of the plant and the NFC were: Absolute ethyl alcohol 99.8% (Neon©, Brazil), toluene 99.5% (Anidrol©, Brazil), sulfuric acid 98% (Sigma-Aldrich©, Brazil), sodium hydroxide 97% (Neon©, Brazil), sodium hypochlorite in 10–12% solution (Neon©, Brazil), hydrogen peroxide 35% (Neon©, Brazil), sodium chlorite 78% (Neon©, Brazil), glacial acetic acid 99.7% (Dynamics©, Brazil).

Sample preparation

The stems were washed in running water and then manually cut into pieces of approximately 5 cm in length. About 12 kg of samples with 85.96% moisture were dried in a forced-air circulation oven at 40 °C for 48 hours, to reach a moisture of 6.79%. The moisture levels were determined according to the T264 method of the Technical Association of the Pulp and Paper Industry, in an oven at 105 °C for 24 hours [44]. Subsequently, the dried yacon stems (YS) were packed in polyethylene bags, sealed, and stored in a dry and ventilated environment until the experiment was performed. The preparation of the biomass for the chemical composition analysis of the YS followed the procedures described in T 257-cm02 [45].

Pulping process of cellulosic pulp

The process to obtain the chemical pulp (CP) was performed with NaOH, following the operational conditions described by [26] with some adaptations. The YS samples were placed in containers on a dry basis proportion of 1:10 (m:v) in NaOH solution 5%, and submitted to heat treatment in a laboratory autoclave (Phoenix, Brazil) at 120 ºC under pressure (98 kPa) for 1 hour, with automatic time and temperature control. Subsequently, the CP was washed and disintegrated in the disc refiner with water at room temperature, in low consistency (1:20, m:v) for 5 minutes. Finally, the CP was purified in a Brecht-Holl fiber classifier (Regmed®, model BH-6/12, Brazil) and then centrifuged (3000 rpm for 5 minutes) and stored in polyethylene bags under refrigeration (8 °C).

Bleaching processes of cellulosic pulp

The CP were bleached as the experimental conditions described in Table 3. The bleaching treatments were applied in a single stage with diluted solution of the NaClO (SH), H_2O_2 (HP), and $NaClO_2$ (SC). A standard consistency was adopted in the proportion of 1:10 (m:v) pulp on a dry basis:solution. Briefly, the pulp was placed in a glass becker, followed by the bleaching solution and the mixture was submerged in a thermostatic bath. After treatment, the bleached pulp (CP-SH; CP-HP; CP-SC) were washed in running water, in order to eliminate possible reagent residues, then centrifuged (3000 rpm for 5 minutes) and stored in polyethylene bags under refrigeration (8 °C).

Table 3: Parameters of bleaching treatments of the chemical pulp (CP).

Parameters	Treatments

	NaClO [a]	H_2O_2 [b]	$NaClO_2$ [c]
Treatment encoding	SH	HP	SC
Solution concentration	5%	1%	1.7%
Solution pH	11	11.5	4.5
Adjustment of solution pH	NaOH 4M	NaOH 4M	$C_2H_4O_2$
Temperature	70 °C	80 °C	80 °C
Time	60 min	45 min	120 min

Source: Adapted from (a) Balea et al. [20], (b) Berglund et al. [21] and (c) Cara et al. [22]. A standard consistency of 1:10 (m:v) dry weight biomass:solution was set. NaClO: Sodium hypochlorite; H_2O_2: Hydrogen peroxide; $NaClO_2$: Sodium chlorite; NaOH: Sodium hydroxide; and $C_2H_4O_2$: Acetic acid.

Extraction of nanofibrillated cellulose (NFC)

The NFC extraction was performed from the bleached pulp that presented the best results in terms of yield, surface color, and Klason lignin content, following the defibrillation process proposed by Iwamoto et al. [15], with some modifications. The bleached pulp was dispersed and homogenized in distilled water to a consistency of 1% in dry weight, using a food processor with 450 W of power. The pulp suspension went through the mechanical defibrillation in a colloidal grinder (Masuko Sangyo®, model MKCA6-2J, Japan) four times, at 1500 rpm, with a 0.1 mm space between the grinding stones. The NFC suspension was placed in polyethylene bottles and refrigerated (8 °C).

Raw fiber and pulp characterization

Scanning electron microscopy (SEM)

The morphology was visualized through a scanning electron microscope (TESCAN®, VEGA3 LMU model). The samples were fixed on metal support (stub) covered with copper conductive tape and metalized with a gold thin layer, and the images were captured with an acceleration voltage of 15 kV.

Chemical composition

The chemical composition was performed in triplicate. The total extractives content was determined by standard method T 204-om97 [46] and Klason lignin by T 222-om02 [47]. The holocellulose content (HOLO), which represents the amount of cellulose and hemicellulose, was determined by difference according to the following equation:

$$HOLO\ (\%) = 100 - (Extractives + Klason\ Lignin) \quad (1)$$

Yield

The gravimetric yields of the pulps were calculated considering the dry weight of the recovered sample (P_f) and the dry weight of the sample used in the process (P_i) according to the following equation:

$$Yield\ (\%) = (Pf\ /\ Pi) \times 100 \quad (2)$$

Surface color

Pulp color measurement was determined using a previously calibrated colorimeter of reflectance MiniScan XE Plus (Hunter Associates Laboratory Inc., Naperville, USA) operating in the CIELab system (L*: 0=black and 100=white; a*: positive = red, negative = green; b*: positive = yellow, negative = blue). The angle of observation used was 10° under D65 illumination. The global color variation (ΔE) was obtained by

difference (Δ) between the sample and the standard according to the following equation (Standard: the chemical pulp used in bleaching):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (3)$$

Fourier transform infrared spectroscopy (FTIR)

The functional groups found in the samples were identified by FTIR spectrometer (Bruker, Vertex 70 model, USA), in diffuse reflectance mode (DRIFT), and, for each sample, 512 scans were performed in the 4000 to 400 cm^{-1} range and with a resolution of 4 cm^{-1}. The spectra were manipulated in Kubelka-Munk units, correcting the baseline using the concave rubber band correction method.

Characterization of nanofibrillated cellulose (NFC)

Transmission electron microscopy (TEM)

A transmission electron microscope (JEOL©, JEM 1200EX-II model), with accelerating voltage of 60 kV, was used to visualize the structure of the NFC. The NFC was dispersed in water solution (1:1000, v:v), and a drop of this mixture was placed on a copper grid, layered with Parlodion film. The ImageJ® program determined the diameter range of cellulose fibers [43].

Thermogravimetric analysis (TGA/DTG)

The TGA/DTG study was performed on a thermogravimetric analyzer (PerkinElmer©, model 4000, USA) with adapting the conditions of Xie et al. [40]. The assays were carried out under a dynamic nitrogen atmosphere of 20 $mL.min^{-1}$ and heat flow of 10 $°C.min^{-1}$, in a temperature range of 30 °C to 800 °C.

X-ray Diffraction (XRD)

The crystallinity index (CrI) was obtained by XRD using a diffractometer (Bruker©, D8 Venture model). The diffraction curves were obtained by Cu-Kα radiation (λ = 1.54 Å)

at 40 kV and 20 mA and with diffraction intensities in a 2θ angular range (Bragg angles) from 10° to 40°. The CrI was calculated by Eq. 4, where I_{002} and I_{am} represent the peak intensities near 2θ = 22° and the minimum near 18°, respectively [48].

$$CrI(\%) = \left[\left(I_{002} - I_{am} \right) / I_{002} \right] \times 100 \quad (4)$$

Statistical analysis

The results of the experiments were subjected to variance analysis ANOVA and the means compared with the Tukey test at 5% significance level with the support of the StatSoft®, version 13.0 (USA) Statistica software. FTIR, TGA/DTG, and XRD curves were analyzed with OriginPro 8.6 (OriginLab®, Northampton, MA, USA), using the Savitzky-Golay method at 15%-point cut, which reduces possible noises coming from the equipment.

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