Comparative Characteristics of Hemp Nanocellulose Extracted by Different Methods

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Abstract: The study describes the extraction of nanocellulose from organosolv hemp pulp (OHP) by different methods: acid hydrolysis, oxidation in the medium of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and in deep eutectic solvent (DES). OHP was obtained from renewable plant material - hemp fibers by extraction with NaOH solution and cooking using a mixture of acetic acid and hydrogen peroxide. SEM and FTIR data confirmed the reduction of cellulosic fibers and the removal of non-cellulosic components from hemp samples during their sequential thermochemical treatment. The data of X-ray structural and thermogravimetric analyzes confirmed that with increasing crystallinity index and resistance of cellulose-containing hemp samples to the influence of temperature, the obtained nanocelluloses are arranged in the following order: OHP – NCD – NCT – NCH. The values of physical and mechanical parameters of hemp nanocelluloses obtained by different methods are compared. It was established that with approximately the same values of the transverse size of hemp nanoparticles, nanocellulose obtained in the process of acid hydrolysis (NCH) has higher values of physical and mechanical parameters than nanocellulose obtained in TEMPO-medium (NCT) and in the DES (NCD).

Keywords: Hemp fiber, Organosolv pulp, Nanocellulose, Hydrolysis, TEMPO oxidation, DES.

INTRODUCTION

In recent decades, there has been a growing interest in the development and use of nanomaterials, in particular nanocellulose, as a potential nanoreinforcing filler in biocomposites for industrial and biomedical applications. Nanocellulose (NCs) are sustainable and renewable material and an alternative to environmentally harmful polymers from fossil energy sources - oil, gas, coal. NCs is a relatively new material with unique properties: good mechanical properties and low density, high transparency and aspect ratio, low toxicity and thermal expansion. Nowadays, the cost of nanocelluloses is about 0.6 USD per dry kg, which is equal to the cost for the starting bleached wood pulps for papermaking [1] and with the development of more nanocellulose applications, this cost will be further reduced.

Therefore, NCs is widely used in industry in the production of biopolymers and biocomposites, packaging and flexible electronics, pharmaceutical science and medical applications, as well as hygiene and sanitation products [2-5]. It is used to increase the mechanical strength and improve the barrier properties of paper and cardboard, polymer and cement composites, electric batteries and sorbents [6-8].

The raw material for the production of nanocellulose is cellulose - the most common polymer on Earth. At the same time, mechanical, chemical and biotechnological methods are used for the extraction of NCs from cellulose-containing materials. The choice of NCs extraction method depends on the type of original cellulose-containing material, the previous stages of its processing and the desired final quality indicators of nanocellulose.

Mechanical methods for the production of nanocellulose use various types of mechanical processing: homogenization, grinding, microfluidization, ultrasonic treatment, ball mill, and cryogenesis, and they are characterized by significant energy consumption [9, 10] and lead, as a rule, to to obtain cellulose nanofibrils (CNF).

Biotechnological methods use a lot of time, complex equipment and expensive enzymatic treatment of lignocellulosic materials [11]. Therefore, chemical methods are more often used in the production of NCs using various reagents. For example, in the process of acid hydrolysis, mineral acids such as sulfuric, hydrochloric, nitric, hydrobromic and phosphoric acids, or their mixtures, are used [12]. To obtain NCs, other chemicals are used: organic acids, deep autectic solvent (DES), oxidizing agents: 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and phthalimide-N-oxyl (PINO) [9, 13-16]. The use of the above chemicals leads to the formation of so-called cellulose nanocrystals (CNC), which usually have smaller transverse size than CNF [1]. Theoretical aspects of nanocellulose extraction by hydrolysis, oxidation in TEMPO and DES medium are described in detail in works [17, 18] and [14], respectively.

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The main source of cellulose for the extraction of NCs remains pulp from wood. The global demand for wood is growing along with the growth of the world economy. This desire for high-quality wood has led to the non-renewable export of hardwood in many developing countries, which poses a serious threat to the environment [19]. In order to minimize the impact on the environment, many countries have passed legislation that prohibits commercial logging, which has led to a significant increase in the cost of natural wood products. For countries with limited forest resources, the use of non-wood plant materials is economically feasible, which will contribute to the preservation of wood stocks and the improvement of the environment. One of the representatives of non-wood plant raw materials is industrial hemp - an annual bast fiber plant of the Cannabis family, which is grown in many countries for obtaining fiber and seeds.

The demand for hemp fiber pulp in Europe and the United States already stands at over 6 million tons per year [20]. Hemp is used to make a variety of commercial and industrial products, including furniture, automotive, paper, construction, rope, textiles, clothing, shoes, food, bioplastics, insulation, biofuel [21]. Hemp fiber pulp can be used as a raw material for the production of special high-quality white paper (banknote paper, securities, ultra-thin paper, highly porous cigarette paper) and chemical processing [22], in particular for the production of hemp nanocellulose.

Although the production of NCs has been extensively investigated in articles from various sources of natural fibers, a comparison of the efficiency of different extraction methods of nanocellulose from hemp fibers as a source of natural fibers for the production of NCs has not yet been widely used.

Taking into account the fact that the market size for nanocellulose was close to USD 146.7 million in 2019 and will grow at a compound annual growth rate of 21.4% from 2020 to 2026 [23], many lignocellulosic materials, including hemp, can be considered as feedstock for its production.

The purpose of this work was the extraction of NCs from hemp fibers by methods of acid hydrolysis using H_2SO_4, oxidation in 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and in deep eutectic solvents (DES), comparison of the characteristics of these NCs: surface morphology and nanoparticle sizes, chemical purity and crystallinity index, mechanical properties and thermal stability. For this purpose, the hemp NCs obtained by various methods were characterized by the methods of SEM and FTIR, AFM, XRD and thermogravimetric analysis.

2. EXPERIMENTAL

2.1. Materials and Methods

To obtain pulp for extraction NCs, we used hemp fibers from the Sumy region of Ukraine after the 2021 harvest. The fibers were crushed to 4±1 mm and stored in a desiccator to maintain a constant moisture content and chemical composition. The chemical composition of hemp fibers was determined according to TAPPI standards [24]. The following chemicals: sodium hydroxide, glacial acetic acid, hydrogen peroxide, sulfuric acid, ethanol, 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), sodium bromide, and sodium hypochlorite; choline chloride, oxalic acid dihydrate and $FeCl_3•6H_2O$ were of analytical grade and were used without additional purification after receiving from Khimreaktiv LLC (Ukraine). Distilled water was used throughout the experiments.

2.2. Obtaining of Hemp Pulp

The production of pulp from hemp fibers was carried out according to the method described in previous publication [25, 26]. Briefly, the chopped hemp fibers were placed in a conical flask, where NaOH solution was added at a consumption of 5% of the raw material to remove most of the hemicelluloses and minerals and partially remove lignin from the plant material. Hemicelluloses have a lower degree of polymerization and lower mechanical properties compared to cellulose, and therefore it is desirable to remove them from vegetable raw materials in the same way as the amorphous part of cellulose and other extractive substances. The mixture was boiled under reflux on a hot plate with a water bath for 180 minutes. At the second stage, to remove residual lignin and extractives, organosolv cooking was carried out using a solution of glacial acetic acid and 35% hydrogen peroxide in a volume ratio of 70:30% at a temperature of 97 ± 2 °C for 180 min. After the end of the cooking time the pulp was washed several times with distilled water to ensure complete removal of residual lignin and non-cellulosic components. The obtained organosolv hemp pulp (OHP) was stored wet in an airtight bag to produce nanocellulose. Never-dried pulp is better because the dried cellulose fibers lose their ability to swell due to irreversible cornification.

2.3. Extraction of the Hemp NCs

2.3.1. Extraction of the NCs by Acid Hydrolysis (NCH)

The values of the relevant technological parameters, which were used in this work to obtain nanocellulose from hemp organosolv pulp, were taken on the basis of previous experimental studies conducted by the authors [25-27] and literature data [9]. Preparation of NCs by acid hydrolysis (NCH) was carried out using sulfuric acid followed by ultrasound treatment according to the method described in detail in the article [26]. Briefly, the hydrolysis of OHP was performed with solutions of sulfuric acid with concentrations of 50% at a temperature of 60 ^{o}C, duration from 90 minutes using mechanical stirring. At the end of the reaction time, hydrolysis was stopped by adding excess 10-fold chilled distilled water followed by centrifugation. The resulting nanocellulose was washed three times with distilled water in a laboratory centrifuge at 4000 rpm to remove acidic solution. The precipitate was collected, resuspended in distilled water, and dialyzed in distilled water until a neutral pH value was reached. The nanocellulose suspension was sonicated at 22 kHz using ultrasonic dispersant for one hour to disperse the particles. The NCH suspension was poured into Petri dishes and dried at room temperature to obtain the films that were analyzed, or the NCH suspension was stored at room temperature in closed containers for further studies.

2.3.2. Preparation of the NCs by TEMPO-mediated Oxidation (NCT)

The nanocellulose has been prepared by TEMPO-mediated oxidation using the TEMPO/NaBr/NaOCl system followed by ultrasonic treatment [27]. Briefly, to obtain nanocellulose, 1 g of OHP was transferred into a beaker, 100 ml of distilled water was added, and the resulting aqueous suspension of cellulose was mixed with 0.16 g of sodium bromide and the TEMPO at the rate of 1.6% by weight of OHP. To uniformly impregnate cellulose suspension with these reagents, we used sonication in the ultrasound disintegrator at 22 kHz for 5 min. A solution containing NaClO at 10 mmol/g OHP is added to the slurry and pH 10 to start the TEMPO-mediated oxidation, with continuous addition of 0.5 M NaOH to maintain pH 10. The duration of the TEMPO oxidation was 24 h. To stop the TEMPO-oxidation process, we added 100 ml ethanol. Then, the suspension was centrifuged from TEMPO, salts, and other compounds present in the oxidizing mixture three times with the addition of distilled water at 4000 rpm for 10 min each. Ultrasonic treatment of a suspension of nanocellulose oxidized by TEMPO was carried out for 30 min until a transparent nanocellulose gel was formed. The resulting suspension of NCT was poured into Petri dishes and dried in air at room temperature to obtain NC films or the NCT suspensions were stored at room temperature in closed containers for use in further research.

2.3.3. Extraction of the NCs by Deep Eutectic Solvent (NCD)

Preparation of nanocellulose from OHP was carried out in accordance with the methodology described in the article [9]. Briefly, the DES system was prepared by mixing oxalic acid dihydrate, choline chloride and $FeCl_3 \cdot 6H_2O$ in a mass ratio of 4:1:0.2 and heating this mixture in a spherical flask at 80 ^{o}C in an oil bath with constant stirring. After that, 1 g of OHP was added to the DES system and maintained at this temperature for 7 hours. After completion, the flask was immediately cooled to room temperature with tap water and the mixture was transferred to a beaker and then diluted with 100 mL of hot deionized water. The diluted mixture was centrifuged at 8000 rpm for 3 min to immediately remove DES. The separated precipitates were washed for another three times with distilled water at 4000 rpm to remove remaining DES. After centrifugation, the sediment was diluted with deionized water, and the resulting suspensions were dialyzed with deionized water using a dialysis membrane until neutral pH was reached. Ultrasonic treatment of a suspension of the NCD was carried out for 30 min until a transparent nanocellulose gel was formed. The obtained NCD suspension was stored at room temperature in closed containers for further treatments or for obtaining NC films.

2.4. Characterization Methods

The method of ***scanning electron microscopy (SEM)*** is used for morphological studies of hemp fibers, obtained OHP and NCs films. The research was carried out on a PEM-106I microscope (SELMI, Ukraine) at an accelerating voltage of 20 kV. The samples were sputter-coated with a layer of gold using the sputtering technique.

Fourier transform infrared spectroscopy (FTIR) spectra of the hemp fibers, OHP and NC films were recorded on Tensor 37 Fourier-transform infrared spectrometer with 4 cm^{-1} resolution in the 400–4000 cm^{-1} frequency range (BRUKER, Germany).

The X-ray diffraction (XRD) were measured for hemp fibers, OHP and NCs with an Ultima IV diffractometer (Rigaku, Japan) using Cu Kα radiation at 40 kV and 30 mA. Scattered radiation was detected in the range of 2θ = 5 – 40° at a scan rate of 5°/min.

The ***crystallinity index (CrI)*** was calculated from the heights of the peak of the crystalline phase 200 (I_{200}) and the minimum intensity between the peaks 200 and 110, which corresponds to the amorphous phase (I_{am},) using Segal's method [28]:

$$CrI = [(I_{200} – I_{am}) / I_{200}] \times 100\%,$$

where I_{200} is the intensity of the (200) reflection for the crystalline phase at 2θ = 22.5° and I_{am} is the intensity of the amorphous phase at 2θ = 18.5°.

Atomic force microscopy (AFM) was used to determine the topography and morphology of the NCs samples. Measurements were accomplished with Si cantilever, operating in a tapping mode on the device Solver Pro M (NT-MDT, Russia). The scanning speed and area were 0.6 line/s and 2×2 μm^2, respectively. The ***transparency*** of NCs films was determined by electronic absorption spectra, which were recorded at a wavelength of 600 nm on a double-beam spectrophotometer 4802 (UNICO, USA) with a resolution of 1 nm.

Thermogravimetric (TG) and derivative thermogravimetric (DTG) analyzes of samples of OHP and NCs films were carried out simultaneously by using a Q50 thermal analyzer (TA Instrument, USA) with a resolution of 0.02 μg as a function of temperature. The weight of samples was within 10 mg, the standard substance for calibrating the temperature scale is nickel, crucible material – platinum. Runs were carried out at heating rates 20°C min^{-1} from ambient temperature to 700°C under high purity nitrogen at a flow rate of 40 ml min^{-1}. Deviations of weight were registered and processed according to a program involving the use of computer technology.

The ***density*** of nanocellulose films were determined according to ISO 534:1988. The ***tensile strength*** of the nanocellulose films was measured at a controlled temperature (23 ± 1 °C) and humidity (50 ± 2%) according to ISO 527-1. Tension tests were performed at a crosshead speed of 0.5 mm/min on the TIRAtest-2151 (Germany) instrument equipment with a 2-N load stress. For testing, test strips with 10 ± 2 mm width and 25 ± 5 mm long were used. The tensile strength of the NC films was calculated on five test pieces, expressing the results as an average and standard deviation.

3. RESULTS AND DISCUSSION

3.1. Cooking Hemp Pulp

Hemp fibers contain 73.9% cellulose, which is significantly higher than the cellulose content in wood (41.0–47.8%). The content of lignin in hemp fibers (8.8%) is 2.7–3.5 times less than in wood (21.0% – 28.0%). At the same time, hemp fibers contain more minerals (1.6%) than softwood and hardwood (0.2% – 0.7%) [29]. Such values of the main components of hemp fibers a priori indicate the need for a lower consumption of chemical reagents, lower energy costs during their delignification and regeneration of spent cooking liquor compared to obtaining pulp from wood.

As a result of alkaline extraction and acetic boiling during 180 min of each treatment, the obtained OHP had a residual lignin content of 0.16% and an ash content of 0.08% and was used to obtain hemp nanocellulose. The obtained OHP has quality indicators close to those for organosolv pulps obtained earlier from other representatives of non-wood plant raw materials – wheat straw, flax, kenaf, miscanthus, reed [25-27] and has a lower residual lignin content than bleached coniferous cellulose, which was used to obtain nanocellulose in work [30] (lignin content 0.66%).

NC, obtained by extraction of organosolv hemp pulp by different methods, had different values of yield from the mass of OHP. Thus, the yield of hemp NC, extracted by the hydrolysis, was 45%; by the TEMPO-oxidation method – 73% and by the DES method – 70%. The slightly higher yield of NC TEMPO is explained by the lower destruction of cellulose under the action of the relatively mild oxidizing agent TEMPO in comparison with the action of oxalic acid in the DES solution and the even stronger action of sulfuric acid on cellulose. Such values of NC output correlate with the data of other authors. For example, the yield of the cellulose microfibers from kenaf was 26.3% (after action of 30% H_2SO_4 at 80°C with mechanical stirring for four hours) [31]. The yield of NC from wheat straw waste after p-toluenesulfonic acid hydrolysis was 45-55% [13], and the yield of NC obtained from eucalyptus cellulose by TEMPO oxidation varied between 40-95% [32], depending on the conditions of extraction. The yield of NC, obtained by processing coniferous bleached eucalyptus kraft pulp with DES solution, was in the range of 71-88% [9].

3.2. Characterization of the Morphology of the OHP and NCs

The change in the morphology of the hemp fibers, the OHP and NCs was studied by SEM method. As can be seen from Figure **1a**, the original plant material consists of several interwoven layers of fibrils with a transverse size in the range of 25–140 μm. In the process of alkaline treatment, hemp fibers swell and partially separate into fibers due to the removal of the main part of hemicelluloses and minerals and partial removal of lignin (Figure **1b**). In this case, there is a separation of layers of fibrils and an increase in the part of individual fibers with a diameter of several microns.

Carrying out further peracetic cooking of pulp leads to remove residual lignin and extractives, bleaching and shortening of fibers. As seen from Figure **1c**, this results in almost complete separation into cellulosic fibers with a transverse size of 5-30 μm.

The action of sulfuric acid or TEMPO or DES on OHP leads to the transformation of the hydroxyl group at the 6th carbon atom of the glucopyranose ring into an aldehyde and carboxyl group, and then to the formation of sulfuric or oxalic acid esters and to a decrease in the degree of polymerization, the breaking of 1–4 glycosidic bonds between the pyranose links of cellulose macromolecules, to the dissolution of the amorphous part of the cellulose [12, 14, 15] and a significant reduction in the size of the hemp fibers to nanoparticles (Figure **1 d**, **e**, **f**).

The change in the chemical composition of hemp fiber during its thermochemical processing was confirmed by infrared spectroscopy data. Figure **2** shows the Fourier IR spectra of hemp fibers, pulps

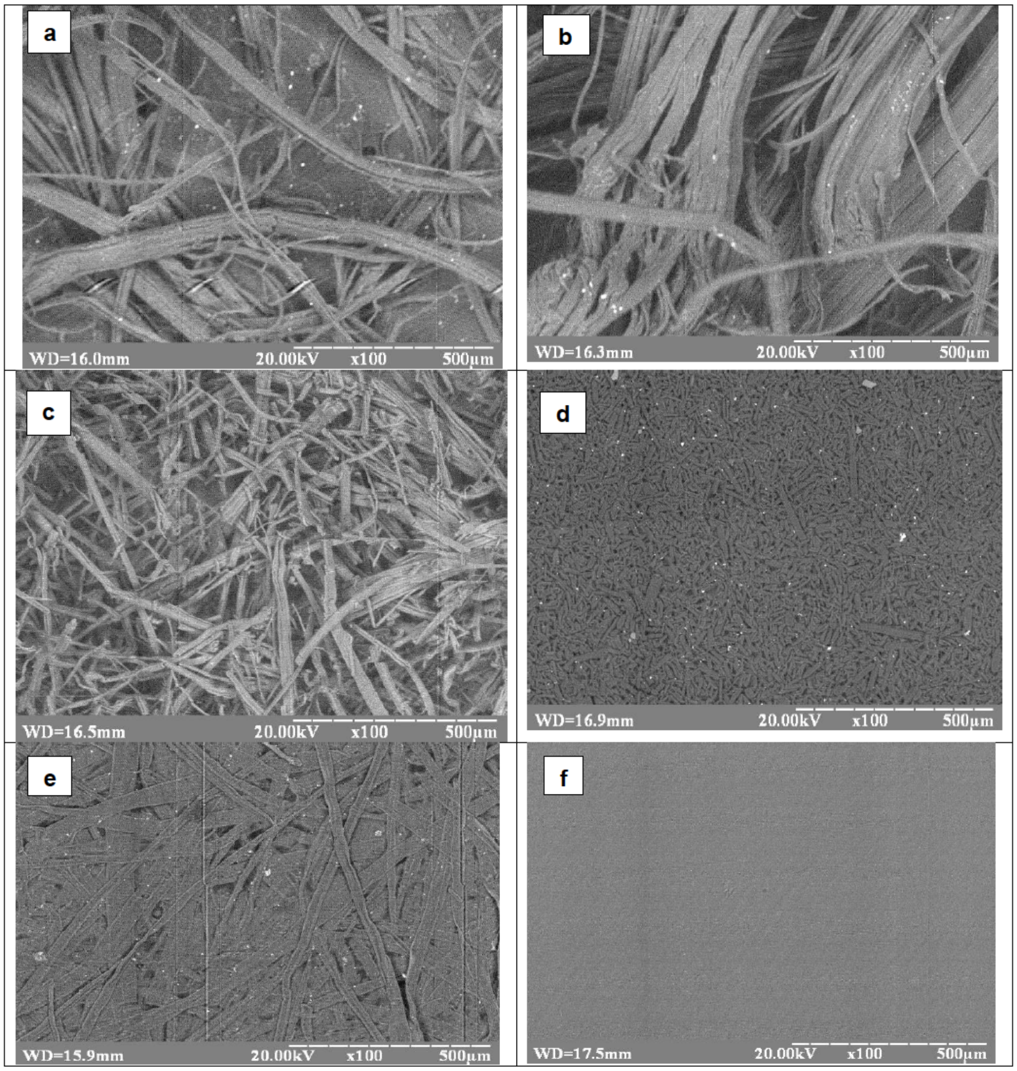

Figure 1: SEM images: (**a**) hemp fibers; (**b**) pulp after alkaline extraction; (**c**) OHP; (**d**) NCH; (**e**) NCT and (**f**) NCD.

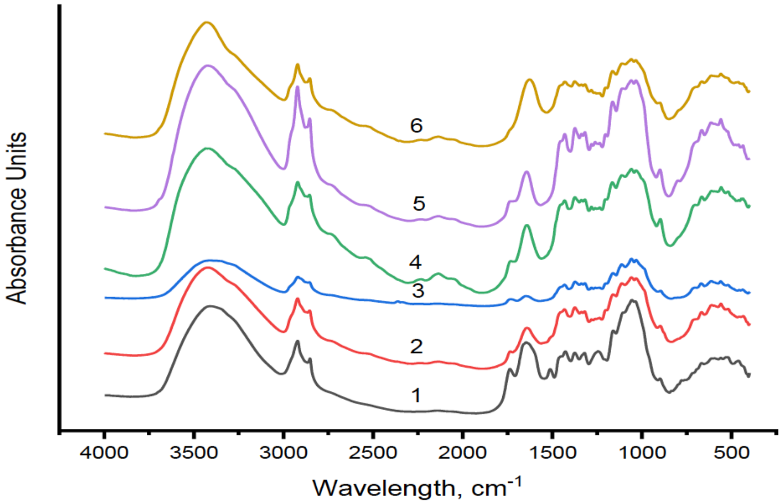

Figure 2: FTIR spectra of samples: (**1**) hemp fiber; (**2**) pulp after alkaline extraction; (**3**) OHP; (**4**) NCD; (**5**) NCH and (**6**) NCT.

after alkaline extraction and peracetic acid cooking and hemp NCs.

All spectra have the same typical peaks that characterize stretching vibrations of hydroxyl groups included in intramolecular and intermolecular hydrogen bonds (3000 – 3750 cm^{-1}), valence asymmetric (2920 cm^{-1}) and symmetric (2853 cm^{-1}) vibrations of the methyl and methylene groups, deformation vibrations of the bonds –CH_2 and –O–H in –CH_2OH groups (1236–1433 cm^{-1}), the valence vibrations of the C–O bonds and C–O–C bridge of the glucopyranose ring (1160 cm^{-1}, 1112 cm^{-1} and 1058 cm^{-1}) and deformation vibrations of C-H bonds (520 – 667 cm^{-1}) of cellulose [33]. The band in the spectrum near 1736 cm^{-1} is assigned mainly to the C=O stretching vibration of the carbonyl and acetyl groups in hemicelluloses and in lignin and the ester groups of cellulose in nanocellulose [34]. Alkaline extraction removes carbonyl groups from hemicellulose (spectrum ***2***), but subsequent organosolv cooking increases the amount of carbonyl groups due to oxidation of cellulose with hydrogen peroxide (spectrum ***3***). The absence of bands at 1512 cm^{-1} and 1244 cm^{-1} in the spectra ***2-6*** in Figure **2** testifies to the removal of lignin from the hemp pulp. Spectral bands in the region of 1645 cm^{-1} are associated with the presence of adsorbed water and characterize the degree of sample moisture [10].

The action of sulfuric acid or TEMPO or DES on the OHP leads to the transformation of the hydroxyl group at the 6th carbon atom of the glucopyranose ring of cellulose molecule into an aldehyde and carboxyl group, and then to the formation of sulfuric or oxalic acid esters in NCs [12, 14, 15] (spectra ***5, 4 and 6,*** respectively).

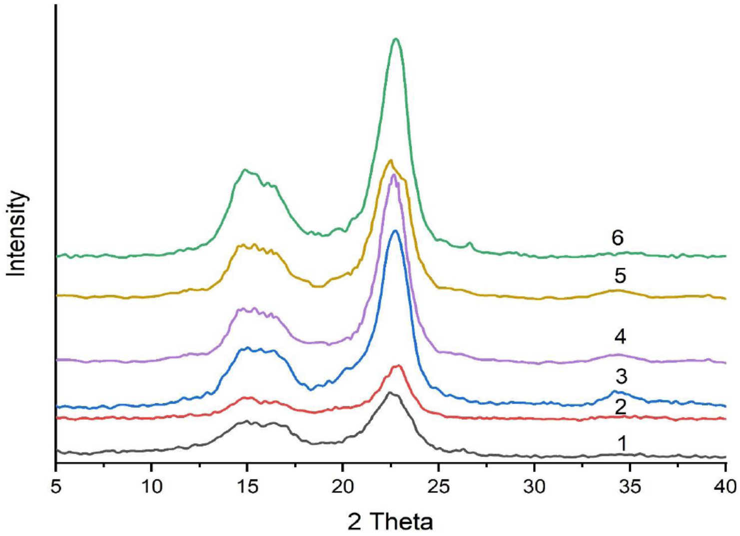

Figure 3: X-ray diffraction patterns of: (**1**) hemp fiber; (**2**) pulp after alkaline extraction; (**3**) OHP; (**4**) NCD; (**5**) NCH and (**6**) NCT.

Table 1: The Value of the Intensity of the Lines on the Diffractograms and the Crystallinity Index of Cellulose-Containing Hemp Samples

№ of Sample	Cellulose Samples	Intensity at 2 theta		Crystallinity Index (CrI), %
		18,5°	22.5°	
1	Hemp fibers	104.5	374.0	72.0
2	Pulp after alkaline extraction	64.6	321.8	79.9
3	OHP	159.0	1041.0	84.7
4	NCH	160.5	1254.0	87.2
5	NCT	106.6	796.0	86.6
6	NCD	153.7	1075.0	85.7

3.3. Crystallinity Index of Cellulose-containing Hemp Samples

The X-ray diffractograms of initial hemp fibers, OHP and NCs are depicted in Figure **3**. Analysis of the X-ray diffractograms shows that the peak with greater intensity at 22.2º–22.9º 2θ reflection belongs to the (200) crystallographic plane of cellulose I. The peak with maximum reflection in the range 14.4º–14.9º corresponds to the crystallographic plane (1–10), the peak in the range 15.5–16.2º 2θ reflection assigned to the crystallographic plane (110) and the peak around 34.6º belongs to the crystallographic plane (004) cellulose I [10].

Presence of these peaks in all samples indicates that crystalline structure of hemp cellulose had not changed during the thermochemical treatments of sulfuric acid, DES or TEMPO solutions and belongs to the typical structure of cellulose I. Based on the analysis of diffraction patterns and changes in the ratio of the amorphous and crystalline parts of the samples under study, their crystallinity index (CrI) was calculated (Table **1**).

As can be seen from obtained data, crystallinity index of hemp samples increases in the following order: initial plant raw material – pulp after alkaline extraction – OHP – NCD – NCT – NCH. This is due to the fact that during thermochemical treatments, CrI increases due to the removal of non-cellulose components from the plant raw material and the removal of amorphous regions of cellulose under the action of sulfuric acid or TEMPO medium or DES. An increase in the CrI values of nanocellulose in comparison with the CrI of the initial pulp was also found for other representatives of plant raw materials [25, 35].

3.4. Properties of Hemp NCs

Topographic characteristics of hemp NCs were determinate by AFM. As can be seen from Figure **4**, hemp NCs particles form a multilayer structure between nanoparticles due to the action of hydrogen bonding and van der Waals forces [36].

Analysis of AFM images (Figure **4**) showed that suspensions of hemp NCs extracted by sulfuric acid, TEMPO oxidation and deep eutectic solvent methods consist of nanoparticles in the form of needles with a transverse size in the range of 6–35 nm (Figure **4**, right), and a length of several micrometers. Such values of the transverse dimensions of hemp NCs are confirmed by the data for nanocellulose extracted by acid hydrolysis of organosolv pulps from other representatives of non-wood plant raw materials: wheat straw (10–45 nm), flax (15–65 nm), kenaf (10–28 nm), miscanthus (10–20 nm) [25].

Table **2** shows some comparative values of the physical and mechanical parameters of hemp NCs obtained by different methods of OHP extraction.

Table 2: Parameters of the Obtained Hemp Nanocellulose

Nanocellulose	Transverse size according to AFM data, nm	Density, g/cm^3	Tensile strength, MPa	Transparency, %
NCH	8 – 35	1.54	66.7	85.5
NCT	7.5 – 34	1.45	62.0	79.8
NCD	6 – 33	1.25	51.2	70.3

A comparison of the data in Table **2** shows that with approximately the same values of the transverse dimensions of hemp nanocellulose nanoparticles, the nanocellulose obtained in the process of acid hydrolysis (NCH) has somewhat higher values of physical and mechanical parameters than NCs obtained by TEMPO-mediated oxidation (NCT) and deep eutectic solvent (NCD).

3.5. Thermal Analysis

The effect of temperature on the stability of OHP and NCs was investigated by thermogravimetric (TG) and derivative thermogravimetric (DTG) analyzes (Figure **5**).

TG curves show changes in the weight of the samples during heating, and its derivative (DTG) shows the temperature peaks at which significant weight loss occurs. As can be seen from the data in Figure **5**, when the temperature rises from 30 ^{o}C to 100 ^{o}C, the evaporation of surface-bound moisture from OHP and NCs is observed, and with a further increase in temperature, the evaporation of water, which is bound between molecules by hydrogen bonds. Table **3** shows the temperature values at which up to 5% of the mass

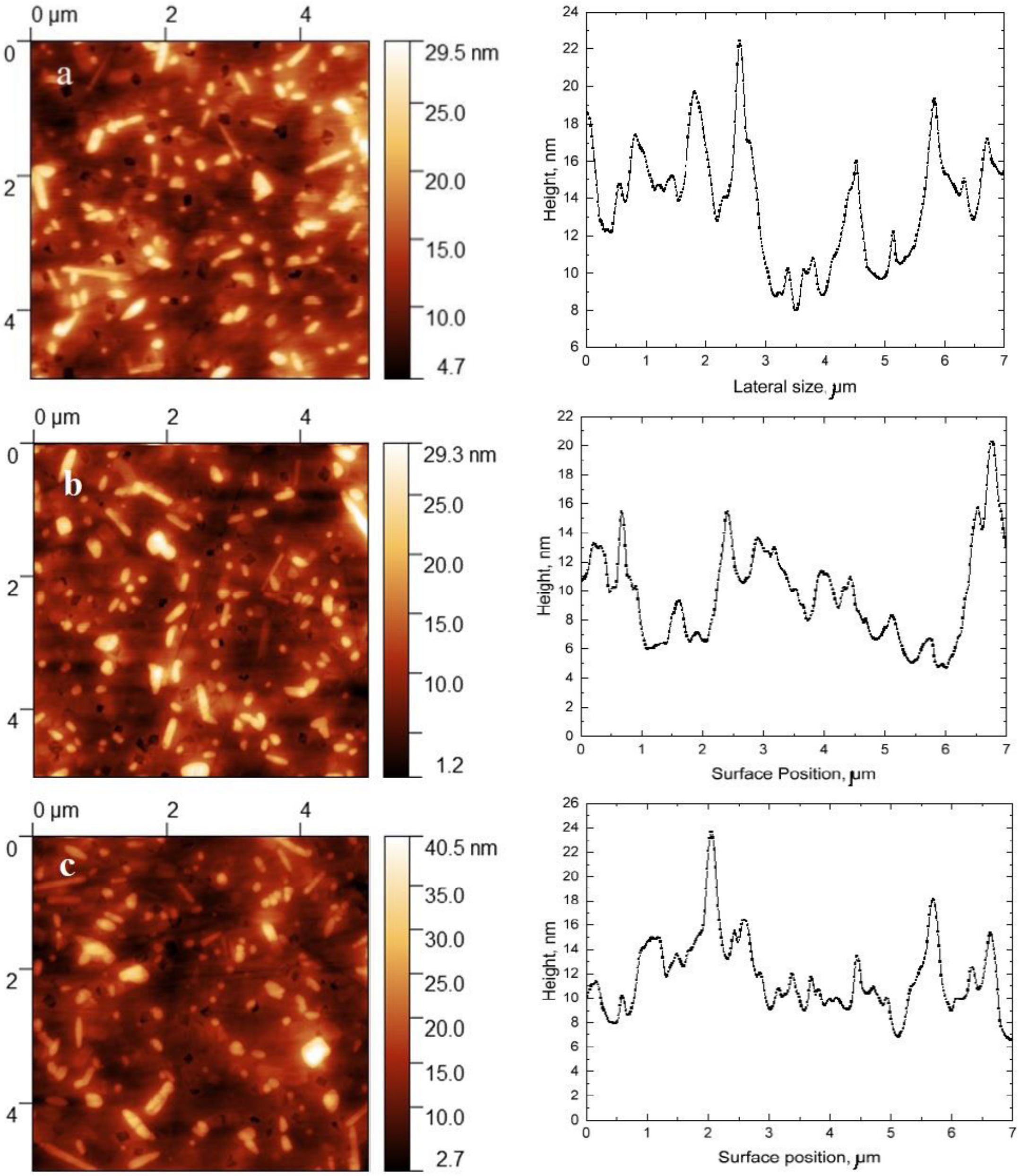

Figure 4: AFM images of a nanocellulose films in height (left) and surface position (right) of hemp NC particles extracted by (**a**) acid hydrolysis (NCH), (**b**) TEMPO oxidation (NCT) and (**c**) DES (NCD) methods.

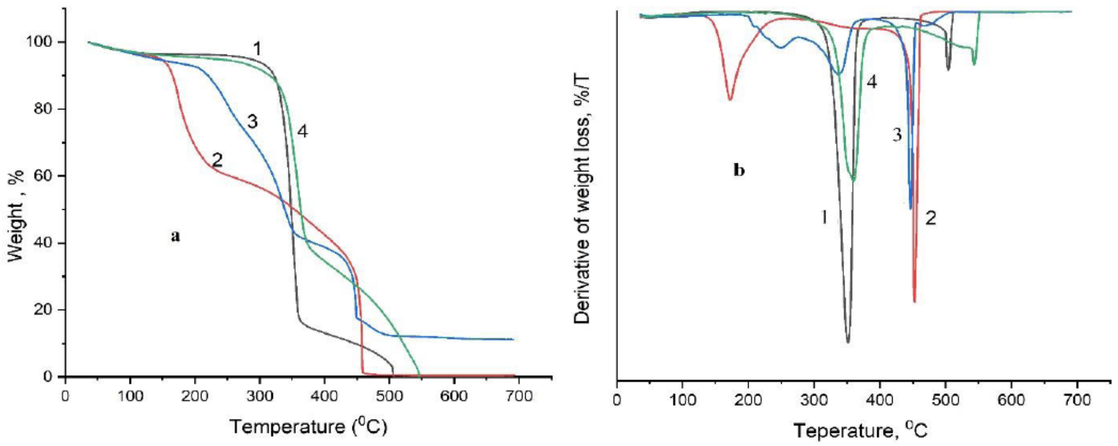

Figure 5: TG (**a**) and DTG (**b**) curves of thermal analysis: OHP (**1**) and NC films, extracted by acid hydrolysis (**2**), TEMPO-oxidation (**3**) and DES (**4**) methods.

of bound water is lost. After the loss of bound water, the degradation of the cellulosic material of OHP and NCs begins. Such degradation is characterized by different intensities, and the rapid degradation of the material reaches its peak at the temperatures given in Table **3** and is observed on the DTG curves.

The TG curve of the OHP indicates the beginning of its degradation at a temperature of 228.8 °C (Figure **5a**) and a sharp decrease in weight at a temperature of 351 °C due to the depolymerization of glycosidic bonds of OHP. In the temperature range of 351 °C – 500 °C, a gradual decrease in the mass of the OHP is observed due to its pyrolysis, and at 500 °C – a sharp oxidation and splitting of charred residues of the OHP (Figure **5b**).

TGA data show a relatively higher moisture content in NCs than in the OHP. During the treatment of OHP with sulfuric acid solutions or TEMPO or DES leads to the fact that some hydroxyl functional groups, in particular at the 6th carbon atom on the surface of nanocellulose particles, become sulfated or carbonated. These amorphous regions of cellulose nanofibers increase the interchain spaces that can capture water molecules due to the formation of new hydrogen bonds [37]. A further rise in temperature affects the destruction of OHP and NCs in different ways. On the DTG curve of hemp NCH (Figure **5a**), there are two sharp bends at 171 °C and at 452 °C. The first bend is observed at a lower decomposition temperature than for the OHP, and corresponds to the process of dehydration of chemically bound water and destruction of easily accessible sulfate ester groups of nanocellulose [34]. The second sharp bend in the DTG curve at 452°C corresponds to the final oxidation and decomposition of charred NC residues. Between these temperatures, a gradual decrease in the mass of hemp NC is observed, which corresponds to the processes of depolymerization and degradation of the main part of the amorphous regions of the NC and the breakdown

Table 3: Characteristics of Thermal Degradation of OHP and NC

Pulp and NCs	T of 5% wt% lost, °C	T of the beginning / end of rapid degradation, °C	T of maximum degradation, °C	T, at which 40% of the mass remained, °C
OHP	228.8	276 / 363 480 / 514	351 504	350
NCH	145.3	154 / 225 425 / 460	170 452	410
NCT	144.8	204 / 275 290 / 362 400 / 455	250 338 445	390
NCD	217.4	265 / 375 432 / 552	360 544	370

of glycosidic bonds under the action of sulfuric acid [38].

DTG curves (Figure **5b**) show that the decomposition of the NCH films begins at a temperature of 171 °C and the maximum rate of their decomposition is observed at 452 °C, while for OHP, the onset of decomposition began at 228.8 °C and the maximum rate of its decomposition is observed at 351 °C. The resulting NCs decomposes at a relatively lower temperature than OHP due to the presence of ester sulfate groups on the cellulose surface and a greater number of the free ends of the chains of NC which decompose at a lower temperature [10]. The presence of ester sulfate groups reduces the heat resistance of hemp nanoparticles, since less energy is required to remove sulfuric acid residues from the hydroxyl group at the 6th carbon atom in the pyranose ring of cellulose than to destroy the glucopyranose ring of the OHP. DTG curve of the NCT shows decomposition in three process of pyrolysis with beginning temperature of rapid degradation at 218 ^{o}C, 305 ^{o}C and 442 ^{o}C and maximum temperature peaks at 250 ^{o}C, 337 ^{o}C and 445 ^{o}C, respectively.

OHP and NCD have endothermic peaks on DTG dependences at temperatures in the range of 330-350 ^{o}C, which is significantly lower than for NCs extracted by hydrolysis and TEMPO-oxidation methods, for which endothermic peaks of the destruction of the main mass of nanocellulose are observed in the temperature range of 430-450 ^{o}C. Perhaps this is explained by the presence of a higher content of hemicellulose and lignin in them, which have a lower thermal stability than pure cellulose. At the same time, OHP and NCD have endothermic peaks at temperatures of 500 ^{o}C and 550 ^{o}C, respectively, which is explained by their higher content of residual mineral substances. By increasing the temperature at which hemp samples have 40% of the residual mass, which characterizes their thermal stability, hemp cellulose derivatives are arranged in the following sequence: OHP – NCD – NCT – NCH. Thus, it can be concluded that hemp nanocellulose obtained in the process of acid hydrolysis (NCH) has slightly higher values of physico-mechanical parameters than NC obtained by the method TEMPO-mediated oxidation (NCT) and deep eutectic solvent (NCD) with approximately the same values of the transverse size of NCs nanoparticles in the range of 6-35 nm.

4. CONCLUSION

Hemp fibers have been used as a renewable and sustainable source of cellulose to extract nanocellulose. Pulp from hemp fibers is obtained by an environmentally friendly organosolv method using a solution of peracetic acid. Extraction of nanocellulose from organosolv hemp pulp (OHP) is carried out by various methods: acid hydrolysis, oxidation in 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) medium and deep eutectic solvent (DES). SEM, FTIR, XRD, and AFM methods were used to investigate the structural and chemical changes of hemp fibers during thermochemical treatments using different methods of nanocellulose extraction. SEM and FTIR data confirmed the reduction of cellulosic fibers and the removal of non-cellulosic components from hemp samples during their sequential thermochemical treatment. The data of XRD and thermogravimetric analyzes confirmed that with increasing crystallinity index and stability of cellulose-containing hemp samples to the effect of temperature, the obtained nanocelluloses are arranged in the following order: OHP – NCD – NCT – NCH. The values of physical and mechanical parameters of hemp nanocellulose obtained by different methods are compared. It was established that hemp nanocellulose extracted in the process of acid hydrolysis has better quality indicators than nanocellulose obtained by TEMPO-mediated oxidation and in the deep eutectic solvent environment. Studies have shown that the considered chemical methods can be effective for the extraction of nanocellulose from hemp fibers.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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