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COMPARISON OF TWO METHOD FOR ISOLATION OF FIBRILLATED CELLULOSE FROM LIGNOCELLULOSIC BIOMASS

MONIKA STANKOVSKÁ, JURAJ KRIŠTA, ŠTEFAN BOHÁČEK,
ALBERT RUSS, ANDREJ PAŽITNÝ
PULP AND PAPER RESEARCH INSTITUTE

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ABSTRACT

Fibrillated cellulose from distillery refuse based on maize starch was prepared by two different procedures. The effect of sonification was evaluated atacid-alkali extractionas well as the type of used acid. The results from the alkali-acid procedure were compared with these obtained by method of steam explosion at different temperatures. The acid-alkali method brings a better result regarding degradation of hemicellulose and lignin as well as cellulose. Lignin/hemicellulose were only released from lignocellulose network using steam explosion at 120-180°C. At higher temperature, the results were comparable with those obtained by acid-alkali method. Similarly pore size distribution of filter paper decreased more significantly when fibrillated cellulose from acid-alkali treatment was applied. After steam explosion, higher extend of longer still fibres remains.

KEYWORDS:Nanofibrillated cellulose, steam explosion, acid/alkali treatment, infrared spectroscopy, fibre length distribution, lignin, hemicellulose.

INTRODUCTION

Steam explosion is a pre-treatment method that enhances the accessibility of biomass polymers for subsequent processes like hydrolysis and fermentation (Pažitný et al. 2019a,b, 2020, 2022). It involves treating biomasswith hot water under pressure, followed by an explosive decompression that ruptures the biomass fibres due to steam generation, thereby improving the recovery of sugars and other useful compounds from the biomass (Stelte 2013). Due to the high cellulosic fibre content, various straw and grasses are also suitable for steam explosion pre-treatment and subsequent hydrolysis with simple sugars release (Pažitný et al. 2013, 2019b, Warren-Walker et al. 2024).The severity of the treatment can vary, leading to different levels of fibre separation, from minor cracks to complete defibrillation (Tanahashi 1990).Wang et al. (2015) listed advantages of this process as limited use of chemical;

avoidance of excessive degradation of monosaccharides; considerably lower energy requirements; high susceptibility of steam-exploded biomass to the action of cellulases.

Fibrillated cellulose especially nanocellulose has a wide range of application in several areas such as biomedicine, nanocomposites, food industry, environmental applications paper and packaging(Randhawa et al. 2022, Trache et al. 2020). Bacterial nanocellulose does not contain any other lignocellulosic components such as hemicellulose, lignin, etc., it is very pure and is therefore often used for medical purposes(Phanthong et al. 2018,Trache et al. 2020).

The methods for isolating nanocellulose can generally be classified into mechanical and chemical treatment. Mechanical methods include homogenization (Bhattacharya et al. 2008), grinding (Abe and Yano 2009), microfluidization (Xiang et al.2016), high-speed blending (Uetaniand Yano 2011), intense ultrasonification (Wang et al.2016) and cryocrushing(Alemdar et Sain 2008). Chemical methods include acidic or alkaline treatment using of 2,2,6,6-tetramethylpiperidine-1-oxyl-TEMPO-mediated oxidation (Liu et al. 2016).

The combination of steam explosion and TEMPO-mediated oxidation was used (Khadraoui et al. 2022) as pretreatment to produce cellulose nanofibrils (CNF) from sea grass *Posidonia oceanica*. The steam explosion was also used for isolation of nanocellulose from pineapple leaf fibres (Cherian et al. 2010). Crystalline cellulose fibres were successfully extracted from palm leaves *Phoenix canariensis* (Pérez-Limiñana et al. 2022) by using of four-step method consisting of an alkaline pre-treatment, a steam explosion, oxidation treatment and bleaching. Stepwise method which included steam-explosion pretreatment, alkaline treatment, sodium hypochlorite bleaching, high-speed blending, and ultrasonic treatment was used to obtainCNFs from corncobs (Yang et al. 2017). The nanocrystalline cellulose from sisal fibres were extracted by steam explosion-assisted mild concentrated chemical treatments followed by mechanical grinding (Vishnoi et al. 2023).Zhang et al. (2020) used steam explosion for pretreatment of poplar wood. The next step was enzymatic hydrolysis with cellulase combined with sonication for the preparation of nanocellulose. The poplar wood as a source of nanocellulose was also used by Haddis et al. (2023). Isolation of nanocellulose from hardwoods such as *Eucalyptus tereticornis* and *Casuarinaequisetifolia L*was provided by Vishu et al. (2023) by using multiple steam explosion in acid medium under high pressure produced nanofibres of high crystallinity, thermal stability and chemical purity. The steam explosion and conventional soda cooking methods were compared by Nader at al. (2022).

The aim of this study was to compare two method which were used for isolation of fibrillated cellulose from distillery refuse in terms of lignin/hemicellulose presence as well as pore size and fibre length.

MATERIAL AND METHODS

Materials

The distillery refuses, as a by-product from the first-generation bioethanol production, were provided by Envirala.s. in Leopoldov (SK).A commercial HEPA filter (Eko-Šimkos.r.o., SK) and chemicalsHCl, H_3PO_4, NaOH (Slavus, s.r.o., SK) were provided as well. The reactor for steam explosion (Amar EquipmentsPvt. Ltd., India),ultrasonic cleaner Manufacturer expert,moisture analyzer IR 35 (Denver Instrument),laboratory digital pH meter OP-211/1 (RedelkisBudapest),Jokro mill Prym (Germany),mechanical homogenizer MLW ER 10 (Germany),centriguge IEC CL30 (Thermo Scientific), ADV-3 analyser of fibre length (SK) and UV-VIS spectrometer Helios β (ThermoSpectronic) were used.

Methods

Isolation of fibrillated cellulose by acid-base treatment

Fibrillated cellulose was obtained from the distillery refuse by extraction with acid combined with alkali. The dry matter of the distillery refuse was determined at 32.2%. The first step was washing-up with water with the use of centrifuge, then beating for 30 min by using Jokro mill.The solution was washed twice with the use of centrifugation. The extraction with HCl or H_3PO_4 which was performed by heating the suspension to 60°C followed by acidic treatment to adjust pH 3.1 and standing at this temperature for 1 h. The solution was processed in the same way with NaOH (pH13) and was neutralized after 1 h to neutral pH. The sample was divided into two parts, one part was subjected to sonication for 10 min. In the end, fibre solutions werewashed-up tentimes using the centrifuge, and the dry matter was determined.

Steam explosion treatment

The reactor for steam explosion was heated at different temperatures (120, 150, 180 and 205°C). One-step process of steam explosion was applied on raw material ofthe distillery refuse adjusted to 15% of dry matter. The retention time was 20 min, the initial pressure 10 bar provided with air from the compressor was increased by heating to 12.9 bar (at 120°C), 17.2 bar (at 150°C), 24.5 bar (at 180°C) and 30.9 bar (at 205°C). After treatment the samples were washed-up ten-times by using of centrifuge.

Application of fibrillated fibres on HEPA filter substrate

The suspensions of isolated fibrillated cellulose in aqueous solution were manually applied to the surface of commercial HEPA filter paper by three-times coating with a rod no. 19 (Gardco, Paul N. Gardner, Inc.).

Measurement of IR spectra

Spectra were measured in the near-infrared region using Nicolet iS10 laboratory FTIR-NIR/MIR spectrometer with Omnic 8 software (Thermo Scientific) by the middle-infrared (MIR) method. 128 scans with a resolution of 4 were applied using thediffuse reflection method in the range of wavenumbers 4000 to 700 cm^{-1}. The monitored wave number range of the spectra is 1274, 1700-1750 and 2890 cm^{-1}. Before measurement the samples of isolated fibrillated cellulose were dried in oven at 50°C for 24 h on Petri dish.

Sonification

Sonification was performed without heatingfor 10 min, the temperature of sample was spontaneously increased from room temperature up to 40°C. Ultrasonic frequency was 40 kHz and ultrasonic power was 80 W.

Pore size distribution measurement

The pore size distribution of coated filter papers was determined using Quantachrome 3G porometer (Anton Paar, Austria) using Porofil inert fluorinated liquid with a surface tension of 16,000 dyn/cm and a viscosity of 1 500 Pa.s to wet the paper sample. A distribution curve was obtained and the minimum, maximum and average pore sizes were measured.

RESULTS AND DISCUSSION

Infrared spectra

Steam explosion has proven to be a very effective method for making glucose available for e.g. enzymatic hydrolysis and in this study, this method for lignin/hemicellulose removal was tested. Analysis of the IR spectra of fibrillated cellulose obtained by two isolation procedures was performed (acid-alkali treatment and steam explosion). For the measurement, dried samples were used in tracking the positions of the spectra at 1274, 1700-1750 and 2890 cm^{-1}. The infrared spectra of untreated distillery refuse are illustrated in Fig.1. Infrared spectra of fibrillated cellulose obtained by beating connected with acid-alkali treatment are shown in Fig. 2. Similarly, the infrared spectra of isolated cellulose fibres obtained after steam explosion are shown in Fig.5. The infrared spectra of fibrillated cellulose obtained by both processing were compared.

As seen in Fig.1, in the region of 1700-1750 cm^{-1} is a larger peak when consideringuntreated corn distillery refuses, which corresponds to vibrations in the acetyl and ester groups from lignin or hemicelluloses (Lanxing et al., 2017).This peak, after beating of corn distillatory refusesconnected with acid-alkali procedure using both types of acids significantly decreased (Fig.4) which corresponds to the partial eliminationof lignin/hemicellulose during this type of treatment. In this procedure, the smallest peak is visible for fibrillated cellulose obtained with H_3PO_4 connected with sonification (blue line).The positive effect of sonification during isolation of nanofibrillated cellulose has been also proven by other studies (Alanazi, 2022, Mishra et al., 2011). Phosphoricacid hydrolysis is a good option because has low requirement to mechanical assistance while maintaining comparable or even lower hydrolysis temperature than the use of strong acid (Xuejuan ae al., 2013). H_3PO_4 has a strong ability to form hydrogen bonds with the hydroxyl group of cellulose and has been shown to be an effective swelling and dissolving agent for cellulose (Mahmud et al. 2019, Wang et al.2020). The synthesis of CNCs using the hydrolysis method with different types of acids suchas HCl, H_3PO_4 and H_2SO_4showed an effecton the CNCs' morphology (Kurniawan et al., 2023). The small presence of the band in the region of 1744 cm^{-1} showed that all ester linkages of the hemicelluloses were not cleaved by the acid-alkali treatment (Akinjokun et al.2021).The presence of peak at 1423 cm^{-1} is associated with the amorph of the cellulose crystal structure, which indicates that the amorph region cannot be removed entirely by the alkaline-acid hydrolysis process which is in accordance with the work of Sucyiati et al. (Sucyiati et al. 2021).

On the other hand, using of steam explosion did not decrease the peak in this region, only when highest temperature of 205°C was used, the peak for the acetyl and ester groups from lignin or hemicelluloses decreased (Fig.3, orangeline). It can be seen that when the temperatures 120-180°C were used, the peak in this region was even higher as in the case of untreated distillatory refuses (Fig.1 and Fig.3). This can be due to release of hemicellulose/lignin from lignocellulose network. The results show that hemicellulose and lignin are decomposed by the acid and base treatment, unlike when a steam explosion at lower temperature without additional treatments is used, which only results in their release. The same results were obtained in a region around 1274 cm^{-1} wavenumber which corresponds to the C-O stretching of the aryl group of lignin. The region around 2890 cm^{-1} indicates the stretching of O-H, C-O and C-H bonds, which corresponds to the presence of cellulose. Similar results were obtained as in case of hemicellulose and lignin. Due to beating and acid-alkali treatment the peak in this region decreased only slightly. On the other hand, the use of steam explosion treatment resulted in rapidly increase of peak except when the highest temperature was used (orange line) which correspondedto cellulose release but not its decomposition. When compared samples which were beated and acid-alkali treated, cellulose decomposition was mild in case of using of H_3PO_4 without sonification (Fig.2, red line), other treatment procedures lead to higher cellulose decomposition at the same level. The results regarding to steam explosion treatment itself are in accordance with many studies where the effective preparation of nanofibrillated cellulose with the use of steam explosion was reached only when combined with other pretreatments or post-treatments as was described in the part Introduction, mainly by alkaline pre-treatment which removes lignin and increases cellulose content and only after this procedure comes the steam explosion.

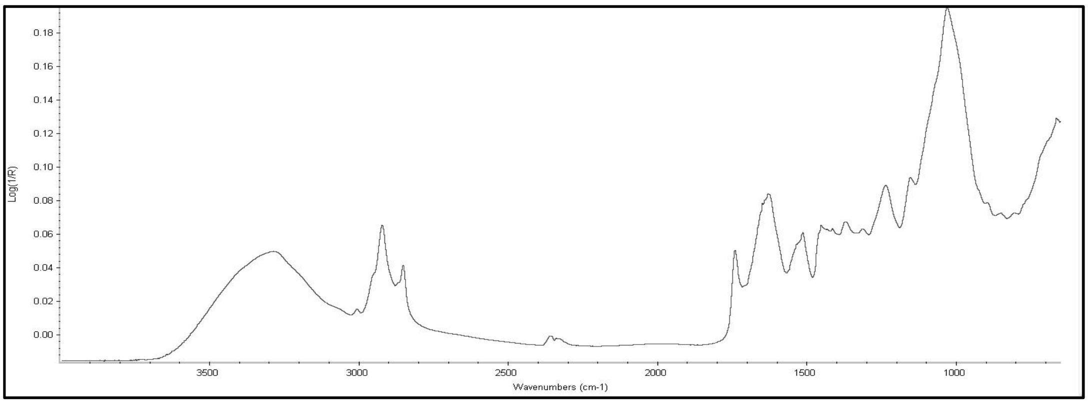

Fig. 1: Infrared spectra in MIR region of untreated distillery refuse.

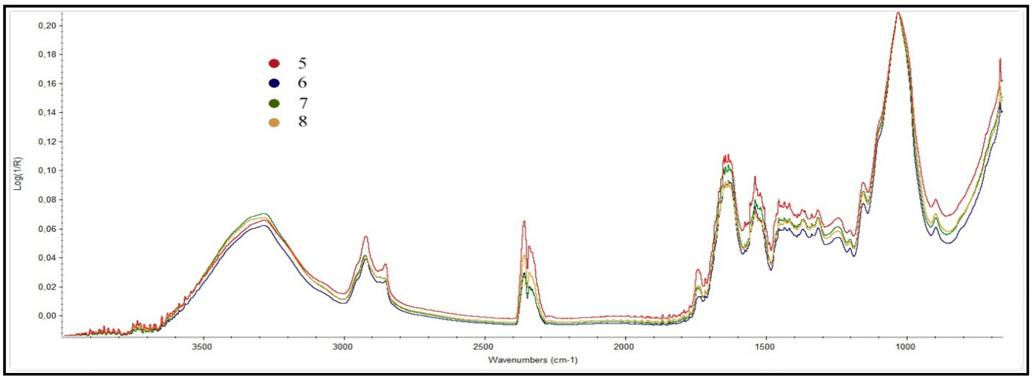

Fig.2: IR spectra in MIR region of fibrillated cellulose obtained with combined beating and acid-alkali treatment, when H_3PO_4 was used without (5)/ or with sonification (6) and when HCl was used without(7)/ or with sonification (8).

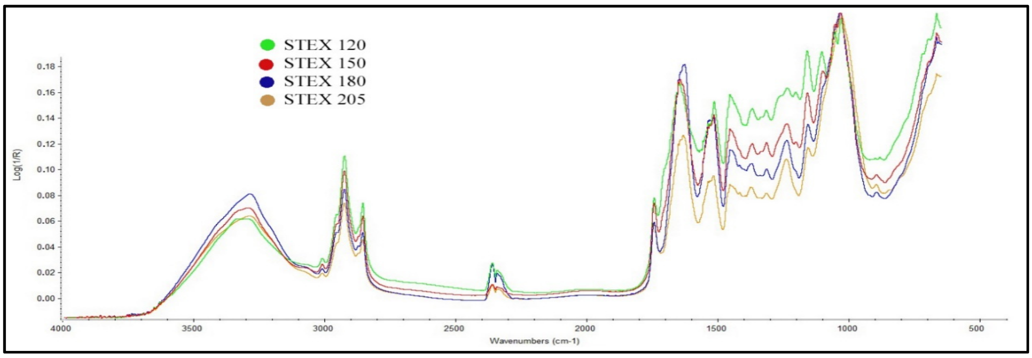

Fig.3: IR spectra in MIR region of fibrillated cellulose obtained by steam explosion at different temperatures.

Distribution of fibre length

Fig. 4 and 5 show the size distribution of the fibres obtained by procedure consists from beating and next acid-alkali treatment. The results showed that the use of phosphoric acid without subsequent sonification of sample was less effective in reducing fibres length compared to the procedure when sonication was used, in the first case, the weighted mean fibre length was higher (1.01 mm). When the sonification procedure was used, the weighted mean fibre length was 0.69 mm which is comparable to the use of hydrochloric acid (0.63 and 0.64 mm) regardless of whether sonication was also used. It is evident that the fibres longer than 2.0 mm were not presented in any of samples.

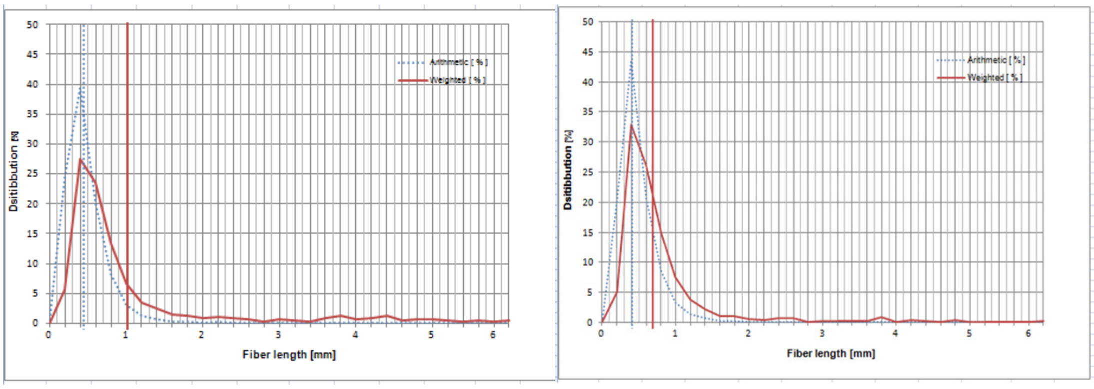

Fig.4: Fibres length after acid-alkali treatment when H_3PO_4 without (left)/ or with sonification (right) was used. Weighted average: 1.01mm (left) and 0,69 mm (right).

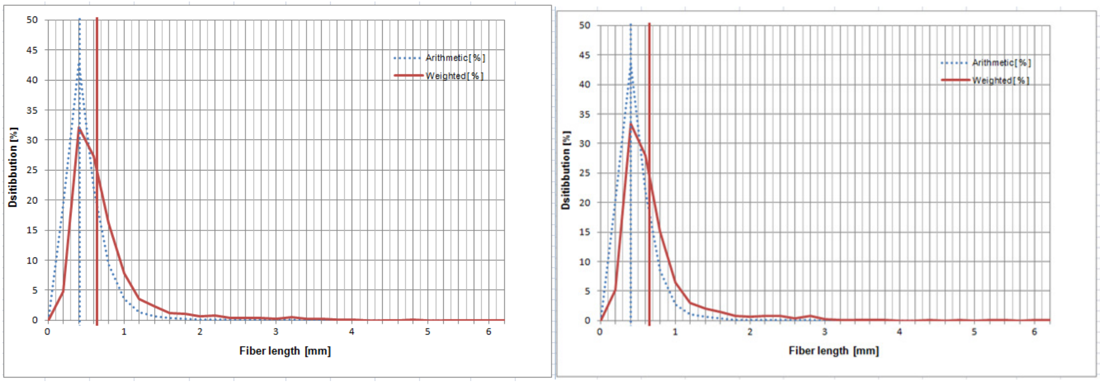

Fig.5: Fibres length after acid-alkali treatment when HCl without (left)/ or with sonification (right) was used. Weighted average: 0.63 mm (left) and 0.64mm (right).

When the acid-alkali procedure was compared with steam explosion, the results proved acid-alkali procedure to be more effective and even the use of high temperature of steam explosion failed to get the weighted mean of the fibre length comparable to the acid-alkali procedure. Within the steam explosion itself (Fig.6 and 7)a certain effect of increasing the temperature is visible, where at lower temperatures the weighted mean of the fibre length was 2.38 and 2.26 mm and by increasing the temperature, it decreased to 1.82 and 1.77 mm. All these results are consistent with the results from infrared spectra measurements where more lignin and hemicellulose destruction after acid-alkali treatment occurred and the use of sonification after treatment with H_3PO_4 improved it more. When comparing the measurement results for isolated nanofibers obtained by both methods, it is evident that after the steam explosion a certain proportion of fibres longer than 2.0 mm remains. On the other hand, after steam explosion, a smaller proportion of fibres shorter than 1.0 mm were found.

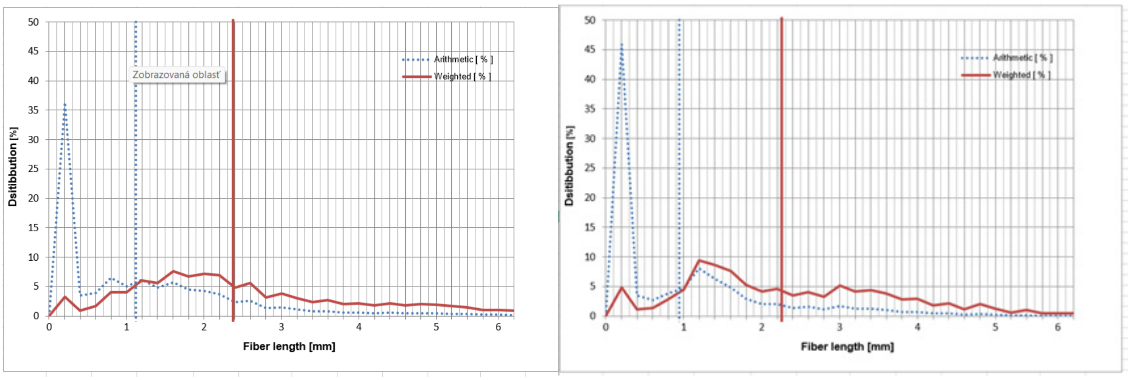

Fig.6: Distribution of fibres length of isolated fibrillated cellulose after steam explosion at 120°C (left) or 150°C (right). Weighted average: 2.38 mm (left) and 2.26 mm (right).

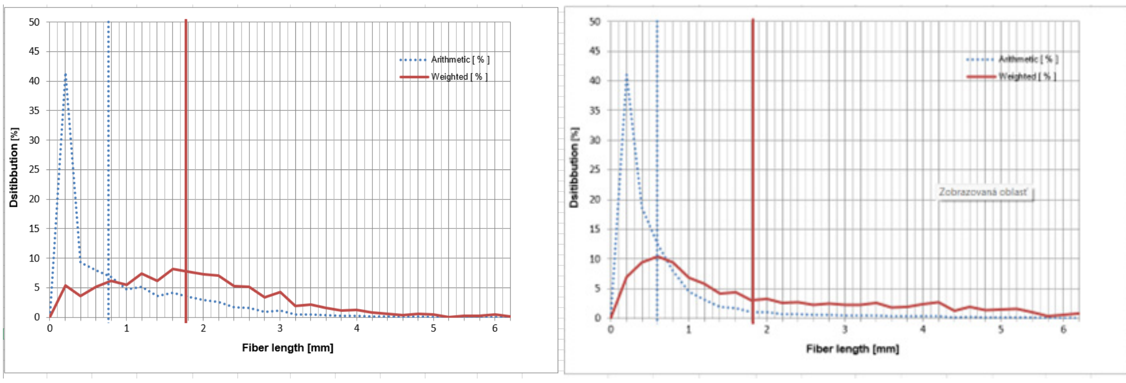

Fig.7: Distribution of fibres length of isolated fibrillated cellulose after steam explosion at 180°C (left) or 205°C (right). Weighted average: 1.82 mm (left) and 1.77 mm (right).

Pore size distribution

The porosity of isolated fibrillated cellulose applied on commercial filter paper was compared (Tab. 1). As a base, commercial filter paper was used and the average pore 10.18 µm was measured. The pore size was significantly reduced by applying all types of fibrillated cellulose fibres. The application of fibrillated cellulose obtained by acid-alkali treatment caused more significant decrease of the average pore size by 2.3-2.5 times. The use of sonification seems to be more effective when compared both types of acid, the use of hypochlorous acid results in lower pore size. After steam explosion of corn distillery refuses, the decrease of maximum pores was moderate while decrease of minimum and average pores was higher but average pores were significantly higher (6.75-6.90 µm) compared to results from acid-alkali treatment (4.05-4.48 µm). The pore size distribution of filter paper coated with fibrillated cellulose which was obtained by steam explosion at the highest temperature 205°C could not be measured due to clogging of the pores.

Tab. 1: Pore size distribution of filter paper with surface application of fibrillated cellulose (beating with acid-alkali treatment when H_3PO_4 was used without (1)/ or with sonification (2); and when HCl was used without (3)/ or with sonification (4)) or with treatment by steam explosion at different temperatures (STEX120-180).

Sample	Maximum pore (µm)	Minimum pore (µm)	Average pore (µm)
without treatment	13.42	7.38	10.18
5	11.36	4.23	4.28
6	10.78	3.96	4.05
7	11.93	4.31	4.48
8	11.34	4.01	4.26
STEX 120	12.16	5.40	6.90
STEX 150	12.08	5.40	6.82
STEX 180	12.02	5.34	6.75

CONCLUSIONS

Fibrillated cellulose from distillery refuse based on maize starch was prepared by two different procedures. One of the procedures involved acid-alkali extraction where the effect of sonification as well as the type of acid used was also evaluated. The results from the alkali-acid procedure were compared with these obtained by the method of steam explosion with the use of different temperatures. The use of acid-alkali method caused partial elimination of lignin/hemicellulose where the best results were obtained by using of H_3PO_4 connected with sonification. By using of steam explosion at temperatures of 120-180°C, lignin/hemicellulose were only released from lignocellulose network. The hemicellulose and lignin were partially decomposed by the acid-alkali treatment as well as by steam explosion with the use of high temperature 205°C. Similarly, cellulose was partially decomposed when alkali-acid procedure was used or steam explosion of high temperature. Steam explosion at lower temperatures only released cellulose without its decomposition. On the other hand, the use of steam explosion failed to get smaller weighted mean of the fibre length when compared to the acid-alkali procedure and negative effect of using of higher temperature was found.The steam explosion treatment load to decreasing of pore size in comparison to untreated sample but not such significantly as when the acid-alkali method of isolation was used.

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REFERENCES

1. Abe, K., Yano, H., 2009: Comparison of the characteristics of cellulose microfibril aggregates of wood, rice straw and potato tuber. Cellulose 16(6): 1017-1023.
2. Akinjokun, A.I., Petrik, L.F., Ogunfowokan, A.O., Ajao, J., Ojumu, T.V., 2021: Isolation and characterization of nanocrystalline cellulose from cocoa pod husk (CPH) biomass wastes. Helyion 7(4): e06680.
3. Alemdar, A., Sain, M., 2008: Isolation and characterization of nanofibers from agricultural residues – Wheat straw and soy hulls. Bioresource Technology 99(6): 1664-1671.
4. Alanazi, A.K., 2022: Polymers 14(10):1930,2022.
5. Bhattacharya, D., Germinario, L. T., & Winter, W. T., 2008: Isolation, preparation and characterization of cellulose microfibers obtained from bagasse. Carbohydrate Polymers 73(3): 371-377.
6. Cherian, B. M., Leão, A. L., de Souza, S. F., Thomas, S., Pothan, L. A., &Kottaisamy, M., 2010: Isolation of nanocellulose from pineapple leaf fibres by steam explosion. Carbohydrate Polymers 81(3): 720-725.
7. Dubois, M., Gilles, K.A, Hamuilton, J.K., Rebers, P.A., Smith, F., 1956: Colorimetric Method for Determination of Sugars nd Related Substances. Analytical Chemistry 28: 350-356.

8. Haddis, D.Z., Chae, M., Asomaning, J., Bressler, D.C., 2024: Evaluation of steam explosion pretreatment on the cellulose nanocrystals (CNCs) yield from poplar wood. Carbohydrate Polymers 323: 121460.
9. Khadraoui, M., Khiari, R., Brosse, N., Bergaoui, L., &Mauret, E., 2022: Combination of steam explosion and TEMPO-mediated oxidation as pretreatments to produce nanofibril of cellulose from *Posidonia oceanica* bleached fibres. BioResources 17(2): 2933-2958.
10. Kurniawan, T.W., Sulistyarti, H., Rumhayati, B., Sabarudin, A., 2023: Cellulose Nanocrystals (CNCs) and Cellulose Nanofibers (CNFs) as Adsorbents of Heavy Metal Ions. Journal of Chemistry, 23 pages.
11. Mishra S.P., Manent A.S., Chabot B., Daneault C., 2011: Production of nanocellulose from native cellulose – Various options utilizing ultrasound. BioResearch 7(1):422–36.
12. Lanxing, D., Jinwu, W., Yang, Z., Chusheng., Q., Wolcott, M.P., Zhiming, Y.,2017: Preparation and Characterization of Cellulose Nanocrystals from the Bio-ethanol Residuals. Nanomaterials 7(3):51.
13. Liu, C., Li, B., Du, H., L, D., Zhang, Y., Yu, G., Mu, X., Peng, H., 2016: Properties of nanocellulose isolated from corncob residue using sulfuric acid, formic acid, oxidative and mechanical methods. Carbohydrate Polymers 151:716-724.
14. Mahmud, M.M.; Perveen, A.; Jahan, R.A.; Matin, M.A.; Wong, S.Y.; Li, X.; Arafat, M.T. Preparation of different polymorphs of cellulose from different acid hydrolysis medium. International Journal of Biological Macromolecules 2019, 130, 969–976.
15. Nader, S., Brosse, N., Daas, T., Mauret , E., 2022: Lignin containing micro and nano-fibrillated cellulose obtained by steam explosion: Comparative study between different processes. Carbohydrate Polymers 290: 11946-119970.
16. Pažitný, A., Halaj, M., Russ, A., Boháček, Š., Ihnát, V., Skotnicová, I., Šutý, Š., 2022: Steam explosion and steam extrusion pretreatment as auxiliary methods for concentration enhancement of monosaccharides from hydrolysates based on the selected lignocellulosic materials. Monatshefte für Chemie - Chemical Monthly 153 (11): 1077-1085.
17. Pažitný, A., Russ, A., Boháček, Š., Bottová, V., Černá, K., 2013: Utilization of energetic grass fibre for modification of recovered fibre properties. Wood Research 58 (2): 181-190.
18. Pažitný, A., Russ, A., Boháček, Š., Stankovská, M., Ihnát, V., Šutý, Š., 2019a: Various lignocellulosic raw materials pretreatment processes utilizable for increasing holocellulose accessibility for hydrolytic enzymes. Part II. Effect of steam explosion temperature on beech enzymatic hydrolysis. Wood Research 64(3): 437-448.
19. Pažitný, A., Russ, A., Boháček, Š., Stankovská, M., Ihnát, V., Šutý, Š., 2020: Effect of steam explosion on enzymatic hydrolysis of various parts of poplar tree. Wood Research 65 (4): 579-590.
20. Pažitný, A., Russ, A., Boháček, Š., Stankovská, M., Šutý, Š., 2019b: Various lignocellulosic raw materials pretreatment processes utilizable for increasing holocellulose accessibility for hydrolytic enzymes. Part I. Evaluation of wheat straw pretreatment processes. Wood Research 64 (1): 13-24.

21. Pérez-Limiñana, M.A., Pérez-Aguilar, H., Ruzafa-Silvestre, C., Orgilés-Calpena, E. Arán-Ais, F., 2022: Effect of processing time of steam-explosion for the extraction of cellulose fibers from *Phoenix canariensis* palm leaves as potential renewable feedstock for materials. Polymers 14 (23): 5206.
22. Phanthong, P., Reubroycharoen, P., Hao, X., Xu, G., Abudula, A., & Guan, G., 2018: Nanocellulose: Extraction and application. Carbon Resources Conversion 1(1): 32-43.
23. Randhawa, A., Dutta, S. D., Ganguly, K., Patil, T. V., Patel, D. K., & Lim, K.-T., 2022: A review of properties of nanocellulose, its synthesis, and potential in biomedical applications. Applied Sciences 12(14): 7090.
24. Stelte, W., 2013: Steam explosion for biomass pre-treatment.Taastrup: Danish technological institute.
25. Suciyati, S.W., Manurung, P., 2021: Comparative study of Cladophora sp. cellulose by using FTIR and XRD. Journal of Physics: Conference Series 1751: 012075.
26. Tanahashi, M., 1990: Characterization and degradation mechanisms of wood components by steam explosion and utilization of exploded wood. Wood Research 77: 49-117.
27. Trache, D., Tarchoun, A. F., Derradji, M., Hamidon, T. S., Masruchin, N., Brosse, N., Hussin, M. H., 2020: Nanocellulose: From fundamentals to advanced applications. Frontiers in Chemistry 8, 392.
28. Uetani, K., & Yano, H., 2011: Nanofibrillation of wood pulp using a high-speed blender. Biomacromolecules 12(2): 348-353.
29. Vishnoi, V., Trivedi, A.K., Gupta, Singh, H., MavinkereRangappa, S., Siengchin, S., 2023: Extraction of nano-crystalline cellulose for development of aerogel: Structural, morphological and antibacterial analysis. Heliyon 10, e23846.
30. Vishnu R., Revathi, R., 1, Kizhaeral , S.S., Kalappan, T.P., 1, Kalichamy , Ch., Elaveetil, V.A., Cintil, J.Ch., 2023:Isolation and characterization of nanocellulose from selected hardwoods, viz., *Eucalyptus tereticornis* Sm. and *Casuarina equisetifolia* L.by steam explosion method. Scientific Reports 13: 1199.
31. Wang, H., Zhang, X., Jiang, Z., Yu, Z., & Yu, Y., 2016: Isolating nanocellulose fibrills from bamboo parenchymal cells with high intensity ultrasonication. Holzforschung 70(5): 1-9.
32. Wang, K., Chen, J., Sun, S.-N., & Sun, R.-C., 2015: Steam Explosion. In Pretreatment of Biomass, Eds: A. Pandey, S. Negi, P. Binod, Ch.Larroche, Elsevier B.V., pp. 75-104.
33. Wang J, Wang Q, Wu Y, Bai F, Wang H, Si S, Lu Y, Li X, Wang S., 2020: Preparation of Cellulose Nanofibers from Bagasse by Phosphoric Acid and Hydrogen Peroxide Enables Fibrillation via a Swelling, Hydrolysis, and Oxidation Cooperative Mechanism. Nanomaterials (Basel) 10(11), 2227.
34. Warren-Walker, D., Ravella, S.R., Gallagher, J., Winters, A., Charlton, A., Bryant, D.N., 2024: Optimising parameters for pilot scale steam explosion and continuous pressurised disc refining of miscanthus and sugarcane bagasse for xylose and *xylo*-oligosaccharide release. Bioresource Technology 405: 130932.
35. Yang, W., Cheng, T., Feng, Y., Qu, J., He, H., & Yu, X., 2017: Isolating cellulose nanofibres from steam-explosion pretreated corncobs using mild mechanochemical treatments. BioResources 12(4): 9183-9197.

36. Xiang, Z., Gao, W., Chen, L., Lan, W., Zhu, J. Y., & Runge, T., 2016: A comparison of cellulose nanofibrils produced from *Cladophora glomerata* algae and bleached eucalyptus pulp. Cellulose 23(1): 493-503.
37. Xuejuan, J., Yingwen Ch., Chong, S., Yangfan, Y., Peng, W., Xiaoxiong, Z., Tao, W., 2013: Preparation and Characterization of Cellulose Regenerated from Phosphoric Acid. Journal of Agricultural Food Chemistry 61: 12405−12414.
38. Zhang, Y., Chen, Zhang, L., Zhan, Liu, N., Wu, Z., 2020: Preparation of nanocellulose from steam exploded poplar wood by enzymolysis assisted sonication. Material Research Express 7: 035010.

MONIKA STANKOVSKÁ*, JURAJ KRIŠTA, ŠTEFAN BOHÁČEK,
ALBERT RUSS, ANDREJ PAŽITNÝ
PULP AND PAPER RESEARCH INSTITUTE
DÚBRAVSKÁ CESTA 14, 841 04 BRATISLAVA
SLOVAK REPUBLIC
*Corresponding author: stankovska@vupc.sk