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Recovery of Nanocellulose from Agri-Food Residues through Chemical and Physical Processes

Annachiara Pirozzi, Giovanna Pappalardo, Franceso Donsì*

Department of Industrial Engineering, University of Salerno, via Giovanni Paolo II, 132, 84084 - Fisciano, Italy
fdonsi@unisa.it

This work proposes a biorefinery approach for the exploitation of agri-food by-products, such as tomato pomace (TP), through the combination of mild chemical hydrolysis and high-pressure homogenization (HPH) in water not only to promote the recovery of cellulose but also its defibrillation to obtain nanocellulose. In particular, the cellulose pulp was isolated from TP using different combinations of chemical and physical processes, by applying HPH treatment (i) directly on the raw material, (ii) after the acid hydrolysis, and (iii) after alkaline hydrolysis. Moreover, the isolated cellulose was deconstructed to obtain cellulose nanoparticles, also through the application of the HPH treatment, enhancing the polymer properties. The structural and physical features of cellulose nanoparticles from TP were analyzed through Fourier-transform infrared spectroscopy (FT-IR) analysis, ζ-potential measurement, and morphological analysis with SEM. The results clearly showed that the HPH treatment (80 MPa, 20 passes) at different stages of the process caused only a slight increase in the yield of cellulose recovery, but significantly contributed to obtaining defibrillated cellulose particles, characterized by smaller irregular domains containing elongated needle-like fibers.

1. Introduction

Food waste has been a global problem and food produced for the initial phases of harvesting, sorting, and washing, to processing and household consumption, valued at around 88 million tons, is wasted (EFSA, 2019), with unbearable environmental, social, and economic consequences. The exploitation of AFRs represents a great opportunity for a more sustainable approach to mitigate this issue by recovering biologically-active or techno-functional compounds, such as polyphenols, antioxidants, proteins, dietary fibers, sugars, and flavors, which can be profitably recovered and exploited for further applications. Among different food companies, the tomato processing industry is one of the most productive and thriving food sectors in Mediterranean countries. In all steps of tomato processing, a significant quantity of tomato pomace (TP) waste is produced, consisting mainly of peels, seeds, and fibrous residues. Actually, it is used mainly for animal feed or fertilizer, or only partially exploited as source for the organic extraction of lycopene, or as feedstock for anaerobic digestion to produce biogas. In the view of enhancement and integration of synergic solutions for waste management, the tomato industry faces a challenge to apply better approaches of valorization of its AFRs in accordance with the circular economy. In this scenario and due to its composition, TP could be considered a good source of the complex carbohydrates composing the lignocellulosic plant cell wall and therefore can be used as an environmentally-sustainable source of cellulose (Pirozzi et al., 2022). Lignocellulosic compounds are the most abundant and less recycled in agri-food residues (Verdini et al., 2021), because of the presence of lignin, cellulose, and hemicellulose held together to form a strong network in plants, giving structural support and protection. However, they can be recovered for different industrial applications. For example: (i) isolated lignin can be used for structural fortification in the polymeric matrix, as low-cost carbon fibers, thermoplastic elastomers, and in substitution of fuels and chemicals from petroleum (Ragauskas et al., 2014); (ii) hemicellulose can find use in cosmetic, pharmaceutical and food fields as a carrier, because of its hydrophilicity and the lower heating value than lignin (Qaseem et al., 2021); (iii) cellulose, the main component of natural fibers, representing about 30% of plant tissue, which is mainly used in the paper-making sector, can also be exploited in innovative applications, such as stabilizers in Pickering emulsions (Pirozzi et.al, 2021a) and reinforcement in composite

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materials (Pirozzi et al., 2021b). Cellulose, because of its good mechanical properties, semicrystalline structure, chirality, and ease of chemical modifications has gathered increasing interest, and its recovery is currently carried out through different methods addressed to break the compact lignocellulosic structure, which holds the cellulose inside.
In this scenario, this work proposes a biorefinery approach for the recovery of cellulose from TP agri-food residues, by combining acid and alkaline hydrolysis and physical high-pressure homogenization (HPH) treatments, to isolate cellulose with tailored morphological properties, along with the valorization of the value-added compounds still contained in the biomass. More specifically, an integrated process is developed to promote not only the isolation of cellulose but also to deconstruct it to obtain cellulose nanoparticles. The effect of the processing conditions is assessed through the characterization of chemical and structural features of nanostructured cellulose, through FT-IR, ζ-potential, and SEM.

2. Materials and methods

2.1 Raw materials

Fresh TP, mainly composed of skins and seeds, was kindly supplied from Salvati Mario & C. spa (Mercato San Severino, Italy), and dried in an oven at 50 °C for 48 h. The dried material was milled with a lab knife grinder and sieved to a final particle size of ≤ 2 mm. The milled pomace (sample TP) was packed in vacuum-sealed flexible pouches and stored at 5 °C, until use.
Commercial cellulose (CL) pulp Arbocel® BWW40 was kindly supplied by Rettenmaier Italia (Brescia, Italy), with a average length of 200 µm.

2.2 Chemicals

Sulfuric acid (H_2SO_4, 95.0 - 98.0 %, ACS GR, Fluka, Charlotte, NC, USA), sodium hydroxide (NaOH, beads, PanReac, Barcelona, Spain), hydrogen peroxide (H_2O_2 6 % solution, ACS GR, VWR Chemicals, Radnor, PA, USA), Folin-Ciocalteau's Reagent (1.8 - 2.2 mol/L, PanReac, Barcelona, Spain), sodium carbonate ($NaCO_3$, ACS GR, PanReac, Barcelona, Spain), TPTZ (2,4,6-Tris (2-pyridil)-s-triazine, ≥ 99.0 %, Sigma-Aldrich, St. Louis, MO, USA), chloridric acid (HCl, 36.5 - 38.0 %, ACS GR, PanReac, Barcelona, Spain), iron (III), chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, ≥ 98 %, Sigma-Aldrich, St. Louis, MO, USA), acetone ($(CH_3)_2CO$, ≥ 99.0 %, VWR Chemicals, Radnor, PA, USA), ethanol (C_2H_5OH, 99.9 %, VWR Chemicals, Radnor, PA, USA), methanol (CH_3OH, ≥ 95 %, Thermo Scientific, Waltham, MA, USA) were used as received without further purification. All water used throughout this work was purified by a Milli-Q water purification system (Barnstead™ Pacific TIIWater, Thermo Scientific, Waltham, MA, USA).

2.3 High-pressure homogenization (HPH) treatment on TP

The milled TP, suspended at 5 g/L of dry weight in distilled water, was pre-treated with high-shear mixing (HSM) at 20,000 rpm for 10 min using T-25 Ultra Turrax device (IKA®-Werke GmbH & Co. KG, Staufen, Germany) equipped with an S25-N18 G rotor in an ice bath. Subsequently, the suspension was homogenized at 80 MPa and 25 °C for up to 20 passes, using an in-house developed HPH system, with an orifice valve of 200 µm diameter (Tastan et al., 2016). At the end of the HPH treatment, the suspension was concentrated by using an R-200/205 Rotavapor (BÜCHI Labortechnik AG, Flawil, Switzerland) until a volume reduction of 70%, prior to being freeze-dried in a 25 L VirTis Genesis freeze-drier (SP Scientific Products, Stone Ridge, NY, USA) at 7 Pa for 72 h. The HPH freeze-dried extract (sample HPH-TP) was stored under at 4 °C before proceeding to the isolation of cellulose through the fractionation acid-alkaline hydrolysis process.

2.4 Cellulose isolation

The removal of lignin, hemicellulose and other minor compounds was obtained through acid and alkaline hydrolysis, followed by a bleaching step, both on TP and HPH-TP. The effect of HPH treatment was assessed after each chemical step, by applying TP after acid hydrolysis or after alkaline hydrolysis. The extraction consisted of three steps. Initially, (1) the samples were treated with a 4.7 % v/v H_2SO_4 solution (1:10 m_{sample}:$v_{solution}$) in a static autoclave at 121 °C for 45 min to hydrolyze polysaccharides, acid-soluble polyphenols, and part of hemicellulose; and, subsequently, rapidly cooled under running water. Then, (2) alkaline hydrolysis was performed carried out by using 4 N NaOH solution (1:10 m_{sample}:$v_{solution}$) at 25 °C for 24 h under continuous stirring (at 180 rpm) to dissolve the remaining hemicellulose, lignin, and other polysaccharides. Finally, (3) the solid was bleached with 4% H_2O_2 solution, with pH adjusted to 11.5 with NaOH (1:10 m_{sample}:$v_{solution}$) at 45 °C for 8 h under continuous stirring. At the end of each step, the solid residue was collected by vacuum filtration, washed with distilled water until the pH of the eluted water reached neutral values, and dried in an oven at 50 °C for 24 h.

2.5 Structural carbohydrates and lignin content determination

After the bleaching step, a part of the solid was submitted to strong acid hydrolysis, with 72 % H_2SO_4 solution (0.2:1 m_{sample}:$v_{solution}$) at 30 °C in a water bath for 60 min, to hydrolyze the structural carbohydrate and lignin, and the composition was defined by analyzing the liquor. Subsequently, distilled water was added to obtain a final concentration in H_2SO_4 equal to 3 % v/v, and the mixture was autoclaved at 121 °C for 60 min. After cooling down at room temperature, the solid fraction was separated from the liquid. From the solid residue, the moisture was determined (105 °C in the oven for 24 h) together with ash content (muffler at 525 °C for 24 h). In the liquor, the acid-soluble lignin was determined by reading the absorbance at 320 nm in a V-650 UV-Vis spectrophotometer (Jasco Europe Srl, Cremella, Italy); cellulose content (expressed as glucan) was evaluated from the D-glucose concentration using the GOPOD-format kit assay (K-Glucose assay, Megazyme, Lansing, MI, USA) multiplied by the correction factor of 0.88; hemicellulose concentration was quantified from the xylose concentration (K-Xylose assay, Megazyme, Lansing, MI, USA) multiplied by the correction factor of 0.90.

2.6 Nanocellulose production

The bleached solids, and pure cellulose as a comparison, were suspended in 150 mL distilled water (5 g/L) and subjected to HSM (20,000 rpm, 10 min) in an ice bath. The suspensions were then treated by HPH (80 MPa, 5 °C, 20 passes) to defibrillate and reduce the size of cellulose to the nanoscale.

2.7 Nanocellulose characterization

The ζ-potential measurement was based on phase analysis light scattering using a Zetasizer Nano ZS, (Malvern Instruments Ltd., Malvern, UK) apparatus, by measuring the electrophoretic mobility at 25 °C.
Fourier-transform infrared (FT-IR) spectroscopy was used to obtain the spectra of the nanocellulose samples with an FT-IR-4100 series spectrophotometer (Jasco Europe Srl, Cremella, Italy) at ambient conditions using a single-reflectance horizontal ATR cell (ATR-PRO 470-H with a diamond prism, Jasco Europe Srl, Cremella, Italy). The infrared spectra were collected in absorbance mode from an accumulation of 128 scans over the wavenumber regions of 4000 - 400 cm^{-1} at a resolution of 4 cm^{-1}. Three repetitions from each sample were used for each spectra measurement. The resulting averaged spectra were smoothed with a five-point under adaptive-smoothing function to remove the eventual noises, and then baseline modification was applied.
Scanning electron microscopy (SEM) analysis was also performed on nanocellulose samples, using an FEI Quanta 200 FEG SEM using a secondary electron detector and an acceleration voltage of 10 - 30 kV. Before the SEM imaging, the samples were sputter-coated with a 10 nm thick gold/palladium layer.

2.8 Statistical analysis

Analyses were performed on three independently-prepared samples, unless differently specified, and the results were reported as means ± standard deviations. Differences among mean values were analyzed by one-way variance (ANOVA), performed with SPSS 20 (SPSS IBM., Chicago, USA) statistical package, and the Tukey test was performed to determine statistically significant differences ($p < 0.05$).

3. Results and discussions

3.1 Cellulose isolation

A possible strategy to improve cellulose extractability is the use of physical treatments, such as HPH, in different phased of the process (Figure 1). In general, the HPH treatment had a disruptive effect on the tomato structure, making cellulose isolation more efficient. With respect to tomato pomce an increase in cellulose isolation of about 10 % and 5 % has been achieved for HPH treatment applied on raw material and after alkaline hydrolysis, respectively. While only a slight increase in cellulose isolation (~ 3 %) has been obtained for HPH treatment applied after acid hydrolysis. These results can be attributed to the effect of the mechanical treatment, which could have disrupted some bonds of the lignocellulosic structure, therefore improving the separation from the cellulose of the lignin after alkaline hydrolysis, respectively.

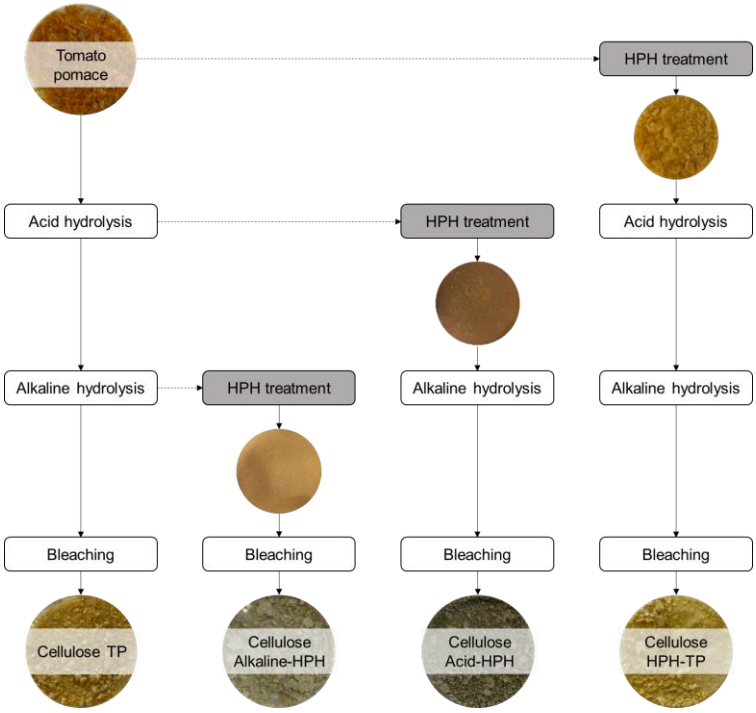

Figure 1: Flow sheet and visual observation of cellulose isolation process.

The content of soluble and insoluble lignin, hemicellulose, and ash, removed at the end of the process was evaluated, through the analysis of structural carbohydrates, with the results shown as the percentage reduction of each compound with respect to its initial total content in the raw material (Table 1).

Table 1: Lignocellulosic materials removed as a function of the combination of chemical and mechanical treatments, estimated as the percentage with respect to its initial total content in the raw material.

Component	Cellulose TP	Cellulose Alkaline-HPH	Cellulose Acid-HPH	Cellulose HPH-TP
Hemicellulose	91 %	83 %	88 %	97 %
Soluble lignin	88 %	91 %	77 %	77 %
Insoluble lignin	70 %	74 %	79 %	42 %
Ash	76 %	89 %	80 %	85 %

Interestingly, the reduction of ash content was higher for all HPH-treated samples than for the untreated one (TP). Conversely, the hemicellulose reduction was more relevant when HPH was applied to the TP before any hydrolysis, as shown by the highest concentration of xylose in the liquor after the bleaching. This high hemicellulose removal can be attributed to the physical pre-treatment applied before chemical hydrolysis wich is able to partially or completely degrade hemicellulsoe (Lu et al., 2021).

3.2 Characterization of the nanocellulose obtained through HPH treatment

After HPH treatment, the nanocellulose obtained was characterized to define the change in the chemical structure through FT-IR, the physical characteristic through ζ-potential analysis, and the morphological features through SEM. The HPH treatment was applied, at the same operating conditions and suspension concentration, on commercial pure cellulose (identified as PC), and used as a standard sample to compare the results.
The analysis of the chemical structure is an important characterization for the assessment of the HPH treatment because it can show the occurrence of structural modifications, the formation of new bonds, and the presence of residual lignin and hemicellulose. Figure 2 shows the spectrum range, where the most relevant peaks were detected. All samples exhibited peaks at the same wavelength, related to the presence of C=C and C-O stretching vibration groups, which are the linkages in glycosidic units (Pirozzi et al., 2022). The peaks observed at 1017, 1033, and 1053 cm^{-1} are attributed to C–O–C pyranose ring stretching vibrations of cellulose (Aguayo et al., 2018).

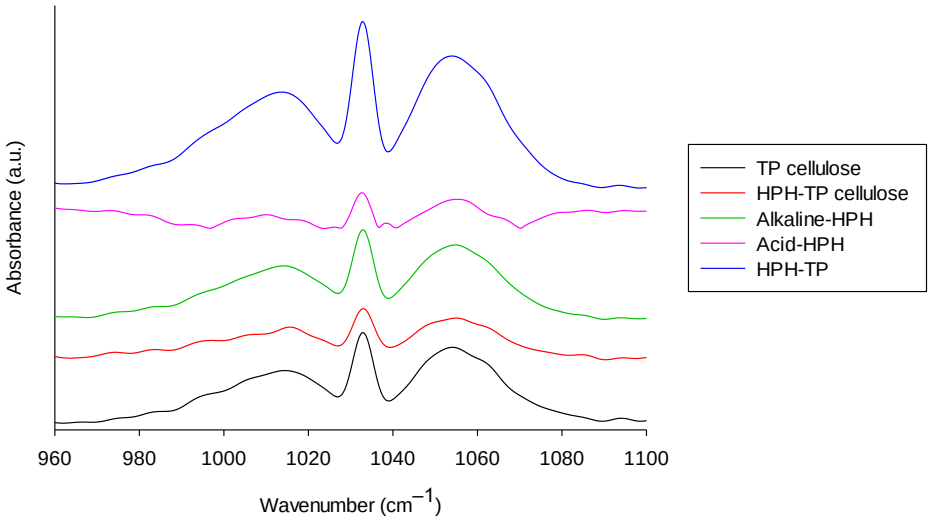

Figure 2: FT-IR spectra of nanocellulose isolated from CL, TP, Alkaline-HPH, Acid-HPH, and HPH-TP.

The ζ-potential measurement defines the stability of nanocellulose particles in suspension and also their tendency to agglomerate. Many studies reported that good stability is reached when the potential is higher, in absolute value than 30 mV (Mohaiyiddi et al., 2016; Prathapan et al., 2016) making the sample suitable, for example, as a stabilizer in emulsions (Fujisawa et al., 2017). The results show that all samples exhibited a negative potential at neutral pH because the applied pressure partially destroyed the cellulose crystalline region, causing a higher exposition of carboxyl groups (Ni et al. 2020).

Table 2: ζ-potential values of nanocellulose suspensions.

	ζ-potential (mV)
Pure cellulose	-1.20 ± 0.08
Cellulose TP	-11.37 ± 0.42
Cellulose Alkaline-HPH	-3.92 ± 0.32
Cellulose Acid-HPH	-3.62 ± 0.34
Cellulose HPH-TP	-1.72 ± 0.30

The morphology of the cellulose nanoparticles was observed using SEM (Figure 3). The nanocellulose from pure cellulose appeared in the form of fibers of irregular morphology, where the original cell structure could be detected. The cellulose isolated from tomato pomace through different combinations of chemical and mechanical treatments were, instead, completely different, with smaller agglomerates of irregular shape, including long needle-like debris. The significant variation in size and shape could be attributed to the fluid-mechanical stresses exerted by the HPH treatment, which improved the cellulose defibrillation and contributed to trimming down the length of the fibers. These observed structures, with a high defibrillation degree, are likely to ensure, through their larger specific surface area than pure nanocellulose, improved techno-functional properties, which can be exploited for example for the stabilization of Pickering emulsions (Pirozzi et al., 2021a).

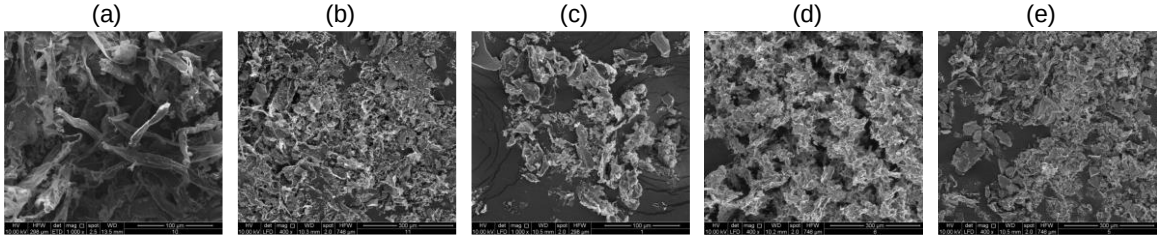

Figure 3: SEM images of nanostructured cellulose obtained from (a) PC, (b) TP, (c) Alkaline-HPH, (d) Acid-HPH, and (e) HPH-TP solid residues.

4. Conclusions

High-pressure homogenization treatment, in combination with mild chemical treatments, was applied to tomato pomace agri-food residues, to recover cellulose and then isolate cellulose nanoparticles applying an innovative and greener approach. For the cellulose recovery, the HPH treatment was applied to raw material and samples after the acid and alkaline hydrolysis, to evaluate the effects on untreated biomass and at different levels of destructuring. Results showed that, in general, the application of this physical treatment caused changes in the structure, resulting in higher removal of lignin and hemicellulose, so a higher residual amount of cellulose was obtained. Moreover, when HPH was applied to the cellulose pulp, stretching and defibrillation phenomena of cellulose chains were observed, resulting in nanocellulose fibrils with different lengths, as revealed by the morphological analysis. Finally, the ζ-potential analysis suggested that the isolated nanocellulose exhibited high stability in water dispersion at neutral pH, turning out to be suitable for innovative applications, such as Pickering emulsion stabilization. In conclusion, the use of HPH on lignocellulosic biomass is a valid technology to obtain valuable products. It represents a suitable alternative process to the traditional chemical cellulose isolation methods, enabling to reduce the concentration of chemicals reducing the pollution load, and also a promising technology for comminution purposes.

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