Crystallinity and nanostructure of cellulose from different sources

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ABSTRACT

Atomic force microscopy (AFM) and Fourier transformation infrared (FT-IR) spectroscopy was used for assessment of structural differences of celluloses of various origins. Investigated celluloses were: bacterial celluloses cultured in presence of pectin and/or xyloglucan, as well as commercial celluloses and cellulose extracted from apple parenchyma. The crystallinity index ($X_C^{\text{RAMAN}}\%$) varied from -25% for apple cellulose to 53% for microcrystalline commercial cellulose. Significant differences in geometrical dimensions of cellulose microfibrils examined by means of atomic force microscope were observed. Roughness analysis of the AFM topographies profiles showed that the smallest dimensions of the microfibrils had cellulose from apple cell walls in comparison with cotton and bacterial cellulose. Differences between cotton and bacterial cellulose were slighter. Celluloses from studied materials had different elastic characteristics after hydration but almost no differences were found in dry state. Significant dependence between cellulose microfibrils geometry and degree of crystallinity was found. Materials with smaller microfibrils diameters were more amorphous than materials with thicker microfibrils. Nano-structural characterization of cellulose from different sources let to explain various behavior of these materials in macro-scale, e.g. different swelling capacity or tension strength. Quantitative description of the nanostructure properties of cellulose may give useful information for scientific and industrial postharvest technology groups.

Keywords: Raman spectroscopy; AFM; cellulose; crystallinity degree; microfibrils diameter.

INTRODUCTION

Cell walls are the main element of fruit and vegetables parenchyma architecture. From among different cell wall substituent cellulose is the biopolymer responsible for mechanical properties of the tissue which significantly influences the texture of plant foods. The texture of plant foods, especially such parameter as stiffness, crunchiness, crispness, decides on consumer’s acceptability and technological suitability [1 – 3]. Cellulose and other cell walls biopolymers play also important function in daily diet because cell walls are the main source of dietary fiber.

Mechanical properties of plant tissue are subordinated to the way the cellulose microfibrils are arranged. The biopolymer is considered as a two-phase system consisting of amorphous and crystalline domains [4, 5]. Except mechanical properties, also the physico-chemical behavior of cellulose is strongly related to its degree of crystallinity influencing directly the accessibility for chemical derivatization, swelling, water-binding. Therefore, the degree of crystallinity is an very important property which needs to be taken into account when considering the manufacturing and applications of cellulose and cellulosic materials. Crystalline regions are resistant to solvent penetration and due to the fact water affects as an plasticizer only amorphic regions.

Sequential chemical analysis and drying of potato parenchyma cell walls showed that cellulose microfibril diameter depends on presence of pectin and hemicellulose on the surface of the microfibriles. Microfibril diameter in hydrated state which were not subjected to chemical treatment was on average 26.2 and 11 nm after drying and extraction which caused removing of a soluble polysaccharides [6]. Davies and Harris (2003) applied the atomic force microscope (AFM) to measure of cellulose microfibrils in partially hydrated sampled of model dicotyledon Arabidopsis thaliana and monocotyledon – onion [7]. Diameter of cellulose microfibrils of A. thaliana were about 3.2 nm and of onion were 4.4 nm after extraction. According
to the AFM studies of Thimm et al. (2000), cellulose microfibrils dimensions of celery varied in the range from 6 to 25 nm [8].

The aim of this work was analysis of the nanostructure of cellulose from different sources: plant cellulose from apple, cotton and bacterial cellulose.

**MATERIALS & METHODS**

In the experiment different types of cellulose were used: bacterial cellulose, commercial cellulose and cellulose extracted from apple tissue. For the isolation of the cellulose from apple tissue (Malus domestica cv. Ligol) fruits were used stored in cold room for two months directly after harvest. Apple pectin with a methylation degree at about 30% was purchased from Herbstreith and Fox (Neuenburg, Germany). Xyloglucan from tamarind seeds (Tamarindus indica L.) was purchased from Megazyme (Bray, Ireland).

Additionally, commercial microcrystalline cellulosics were used: Avicel PH101 and PH302 (50 and 100 µm, alpha-cellulose obtained from wood pulp, FMC Biopolymer, Belgium), cellulose powder (20 µm, obtained from cotton linters, Sigma Aldrich) named further as Aldrich. All commercial cellulose were used without further purification.

Bacterial cellulose was obtained by purification of bacterial artificial cell walls produced in various medium. Bacterial artificial cell walls materials were produced using protocols described by Cybulska et al. [9, 10]. Depending on the medium composition different artificial cell walls were obtained:

- BC - bacterial cellulose,
- BCX – bacterial cellulose embedded in xyloglucan, obtained by adding xyloglucan from tamarind seeds to the medium up to 5 g l⁻¹,
- BCP - bacterial cellulose embedded in pectin, obtained by adding apple pectin to the medium up to 5 g l⁻¹ and 12.5 mM calcium chloride,
- BCPX- bacterial cellulose with pectin and xyloglucan created by adding the apple pectin (2.5 g l⁻¹), xyloglucan (2.5 g l⁻¹) and 6mM calcium chloride to the culture medium.

In order to test the influence of matrix polysaccharides during production of cellulose microfibrils in culture medium on cellulose crystallinity microfibrils were isolated from artificial cell walls. In order to remove culture medium and bacterial cells which might be trapped in material and caused the fluorescence during obtaining Raman spectra artificial cell walls (BC, BCP, BCX, BCPX) were washed three times in 0.1M HCl at 85 °C and three times in 1M NaOH at 80 °C for 30 minutes each bath and then washed several times in boiling deionised water (100 °C).

Cellulose from apple tissue was obtained during sequential extraction. Briefly, apple cell wall material was isolated using modified phenol-buffer method proposed by Renard [11]. The sample was vacuum dried. Further, obtained cell walls were purified by method of sequential extraction with modifications proposed by Redgwell et al. [12].

The cellulosic powders and bacterial cellulose films (BC, BCX, BCP, BCPX) were applied on microscope glass. Raman spectra were collected on DXR Raman Microscope (ThermoScientific, Waltham, MA, USA) with laser 532 nm and maximum power 10 mW. The spectra were recorded over the range 3500 – 150 cm⁻¹ using an operating spectral resolution 1.9285 cm⁻¹ of Raman shift. Spectra were taken with exposure 2s and 10mW laser power output. For each material, 15 samples under the same conditions were examined. For each sample, 64 scans were averaged. Then for a given material, final average spectrum was calculated. These spectra were normalized to 1.0 at 2900 cm⁻¹.

All the cell wall materials (bacterial celluloses, alpha cellulose and commercial cellulosics) were subjected to observations by means of the Atomic Force Microscope (NanoScope III, Digital Instruments, Veeco Metrology Group, Dourdon, France). Prior to the microscopic observations the dried specimen were stored in airtight containers to prevent rehydration. Dried sampels were attached to microscope glass and subjected to AFM observations.

**RESULTS & DISCUSSION**

Raman spectra can be divided into two regions. The region below 1600 cm⁻¹ is most sensitive to the conformation of the cellulose backbone (especially below 700 cm⁻¹) whereas the region above 2700 cm⁻¹ is more sensitive to hydrogen bonding [13, 14]. Figure 1 presents Raman spectra of cellulose I for spectral range 150 – 1650 cm⁻¹. The spectra are for microcrystalline cellulosics (cellulose pulver, Aldrich) and for cellulosics from apple parenchyma as an example of different proportions of highly crystalline and more amorphous cellulose, respectively. Comparing these spectra, broadening and loss of resolution of apple
cellulose could be observed. This would suggest that microcrystalline cellulose contains higher percentage of crystalline cellulose than apple cellulose.

Schenzel et al. has shown that intensity of peaks at 1462 and 1481 cm\(^{-1}\) which correspond CH\(_2\) bending, relates to crystalline/amorphous proportions in cellulosic sample [15]. The higher peak at 1481 cm\(^{-1}\), cellulose crystallinity degree is higher either. In the case of the amorphous cellulose predominate crystalline one, therefore is only evidence of broad peak at 1462 cm\(^{-1}\) and deconvolution function must be made. On this basis one can estimate crystalline index by counting a relative percentage amount of crystalline fraction in a cellulosic sample. Thus, degree of crystallinity could be calculate by Eq.1 [15]:

\[
\%X_C^{\text{RAMAN}} = \frac{I_{1481}}{I_{1481} + I_{1462}},
\]  

here \(I_{1462}\) and \(I_{1481}\) represent the Raman intensities of particular bands at 1462 and 1481 cm\(^{-1}\), respectively.

The highest calculated degree of crystallinity was obtained for microcrystalline cellulose is 53 %. For apple estimation of cellulose crystallinity is difficult due to low intensity of peak at 1481 cm\(^{-1}\) however rough estimation suggests value about 25 % only. This confirms that apple cellulose is mostly amorphous [16]. The intensity of peaks was estimated after applying a deconvolution function and summary of results are presented in Table 1.

Table 1. Calculated values of cellulose crystallinity, percentage content of cellulose \(I_p\), FWHM (full width at half maximum) and intensity of Raman peak around 913 cm\(^{-1}\) for bacterial, microcrystalline and apple celluloses. In parenthesis standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>(%X_C^{\text{RAMAN}})</th>
</tr>
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<tbody>
<tr>
<td>BC</td>
<td>49.5 (3.2)</td>
</tr>
<tr>
<td>BCP</td>
<td>44.1 (5.1)</td>
</tr>
<tr>
<td>BCX</td>
<td>47.6 (2.7)</td>
</tr>
<tr>
<td>BCPX</td>
<td>50.2 (1.4)</td>
</tr>
<tr>
<td>PH101</td>
<td>53.8 (1.4)</td>
</tr>
<tr>
<td>PH302</td>
<td>51.8 (2.4)</td>
</tr>
<tr>
<td>Aldrich</td>
<td>53.8 (1.4)</td>
</tr>
<tr>
<td>Apple</td>
<td>25.3 (1.8)</td>
</tr>
</tbody>
</table>

Model bacterial cell walls in dry state were also subjected to AFM observations (Fig. 1). All the pictures presented are in the same scale in order to simplify their qualitative comparison. The materials had different chemical composition and this difference was reflected in the microstructure. Pure bacterial cellulose (BC) characterized the most porosity structure in comparison with the other materials. Any matrix was not visible but only chaotically located network of branched microfibrils. Cellulose microfibrils were arranged mainly randomly but there are also some bundles of several microfibrils. Diameter of cellulose microfibrils was different for studied materials. Diameter of bacterial cellulose microfibril in BC material amounted approximately 37 nm. Addition of pectin to the culture medium (BCP) caused thickening of bacterial cellulose microfibril to about 46 nm. However, xyloglucan supplementation (BCPX) had the most significant influence on microfibril dimensions which caused increase of diameter to about 75 nm.
Fig. 1. Topography of AFM images of bacterial model polysaccharide networks, height mode: a) bacterial cellulose BC, b) bacterial cellulose with pectin BCP, c) bacterial cellulose with pectin and xyloglucan BCPX, 5x5 µm.
Fig 2 shows the nanostructure of native cellulosics originated from apple parenchyma tissue and cotton. For apple cell wall materials, diameter of cellulose microfibril amounts about 13 nm for cellulose from apple parenchyma. Cotton cellulose microfibrils dimensions are significantly larger than apple cellulose, cotton cellulose diameter amounts approximately 60nm.

![AFM images](image)

Fig.2 Topography of AFM images, height mode: a) reconstituted apple cell wall material, 1x1 \( \mu \text{m} \) b) cellulose microcrystalline, 3x3 \( \mu \text{m} \).

CONCLUSION

Significant differences in geometrical dimensions of cellulose microfibrils examined by means of atomic force microscope were observed. Roughness analysis of the AFM topographies profiles showed that the smallest dimensions of the microfibrils had cellulose from apple cell walls in comparison with cotton and bacterial cellulose. Differences between cotton and bacterial cellulose were slighter. The most crystalline form is found in commercial specimens of cellulose microcrystalline obtained from cotton. Cellulose obtained from apple parenchyma probably consists of more amorphous than crystalline regions. Celluloses from studied materials had different elastic characteristic after hydration but almost no differences were found in dry state. Significant dependence between cellulose microfibrils geometry and degree of crystallinity was found. Materials with smaller microfibrils diameters were more amorphous than materials with thicker microfibrils. Nano-structural characterization of cellulose from different sources let to explain various behavior of these materials in macro-scale, e.g. different swelling capacity or tension strength. Quantitative description of the nanostructure properties of cellulose may give useful information for scientific and industrial postharvest technology groups.

REFERENCES


