# Synthesis and characterization of biomaterials for medical application using nanocellulose from orange peels with nanoparticles

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## INTRODUCTION

In the current agroindustry field, Brazil is the world's largest orange producer, generating large amounts of biomass waste. One particularly promising byproduct among whole vegetable discards is orange peel (OP), which have pectin as main biopolymer, and in minor extension, cellulose and hemicellulose. Pectin is already industrially produced by OP, however, the cellulose from this biomass has not been extensively explored. Thus, new ways to add value to cellulose from OP have been developed. Cellulose is widely employed in different fields, mainly in its nano-size form, such as nanofibers and nanowhiskers. Tissue engineering is one of the main emerging areas for cellulose application, with the increasing demand to replace synthetic polymers opening ways for the use of cellulose from orange biomass. These materials have properties such as protection, healing, and high biocompatibility. [1-2]

Nanocellulose consists of many microcrystalline domains of cellulose, with potential properties such as high surface area, optical transparency, and mechanical strength. This material, when combined with other biopolymers, like carrageenan, a polysaccharide from red algae with antioxidant and healing properties, forms a permeable porous, and strong network structure, useful for the effective delivery of therapeutic agents and film formation. Thus, in this study we aimed to develop a nanocellulose/carrageenan composite for wound healing. Nanocellulose can be produced by chemical treatment of raw cellulose from despectinated OP. It is the first time that such materials have been developed, which represents a great advance in terms of the application of natural materials.[3,4,5,6]

## METHODOLOGY

### 1. Orange peel pretreatment for pectin and other polysaccharides removal

100 g of orange peel (OP) was heated in distilled water at 90 °C under stirring for 30 min and filtered. Then 700 mL of 1.0 molL$^{-1}$ HCl solution was added to the filtrate and stirred for 60 min at 90 °C. The mixture was filtered and washed with distilled water once to remove the acid excess.

### 2. *Alkaline treatment for lignin removal*

To the filtrated pellet, 500 mL of 10% (w/v) NaOH solution was added, and the mixture was placed into an autoclave at 120 °C for 30 min. Then the residue was filtered for the lignin removal and washed with distilled water.

### 3. *Bleaching*

Residues and pigmentation were removed using a 10% (v/v) $H_2O_2$ solution at 90°C under stirring for 45 minutes. It was repeated twice. Then, the obtained cellulose was filtered and washed with hot distilled water until neutral pH.

### 4. *Cellulose hydrolysis - nanonization*

For the preparation of cellulose nanofibers (CNF) 5 g of cellulose from OP was heated at 50 °C in different concentrations of $H_2SO_4$ (10, 20, 30, and 45% v/v). These samples were named as AM10, AM20, AM30, and AM45, respectively. The samples were washed until neutral pH. The samples were characterized with ATR-FTIR, $^{13}$C-NMR (CP/MAS), and XDR.

### 5. *Composite Production*

The gel mixture was prepared with changing the cellulose/carrageenan rate in mind. The gel was made using 25% of cellulose and 75% of carrageenan. 0,39 g of carrageenan was heated in 8 mL of MilliQ water at 90° C under stirring until completely dissolved. Then 0,078 g (15% of total mass) of glycerol was added into the carrageenan solution and stirred. At the same time, 0,13 g of AM45 was added in 2 mL of MilliQ water and underwent ultrasound treatment for 15 min with 80% amplitude, 10 s off, and 2 s on pulse. Subsequently, nanocellulose was added into de carrageenan with 0,26 g of $Mg^+$ dissolved in 1 mL. The mixture was submitted to an ultrasound for 1 min for better homogenization. For the SEM analysis, the produced gel was freeze-dried for 48h.

### *Cellulose and composite characterization*

ATR-FTIR, $^{13}$C-NMR (CP/MAS) and XDR were used for cellulose and nanocellulose characterization. FTIR spectra were obtained in a CARY 630 spectrometer (Agilent, USA) at a resolution of 4 cm$^{-1}$ in the 400-400 cm$^{-1}$ with 128 scans. $^{13}$C-NMR spectra were obtained in a Bruker spectrometer in a field of 400 MHz, with cross-polarization and magic angle spinning (CP/MAS). A pulse of 90° was used for 1.5 ms and an 800 ms contact pulse sequence at a 10 kHz MAS rate. For crystallinity index measurement a XRD D2-Phaser (Bruker) with a voltage of 30 kV and electric current of 10 mA in continuous scanning mode with 0,3 s per step and 5-60° range was used for 2 hours each sample.

## RESULTS AND DISCUSSION

ATR-FTIR, $^{13}$C NMR (CP/MAS), and XDR obtained for raw cellulose from OP after pectin and lignin removal showed success in the extraction of cellulose from this biomass. The ATR-FTIR spectrum shows the expected bands for cellulose structure such as the peak around 3340 cm$^{-1}$ that

corresponds to an intramolecular vibration from OH in $C_2$ and $C_6$, while the one close to 1420 cm$^{-1}$ corresponds to a $CH_2$ bonded in $C_6$ and the one around 1020 cm$^{-1}$ corresponds to β-1,4 glycosidic bond. $^{13}$C-NMR CP/MAS also shows the expected chemical shift for glucose carbons: $C_1$ at 105 ppm; $C_{2,3,5}$ between 70-80 ppm; $C_4$ as a dublet at ~84 ppm (disordered region) and ~88 ppm (ordered region); and $C_6$ between 60-70 ppm. Finally, the XRD pattern shows two peaks at ~15 and ~23 angles, as expected.
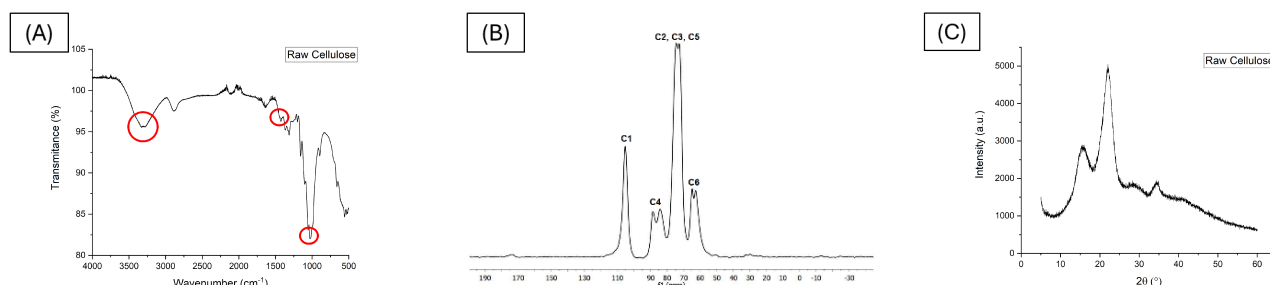


**Figure 1**: (A) ATR-FTIR raw cellulose spectrum. (B) $^{13}$C-NMR ssNMR (CP/MAS) raw cellulose spectrum. (C) XRD raw cellulose.

The best condition for CNF production from OP was evaluated by changing the parameters of acid concentration, and then, analyzed by $^{13}$C NMR (CP/MAS) and XDR to obtain the crystallinity index (CI), a parameter widely used for cellulose characterization. For CI measurement, two different types of methodology were used: the $C_4$ peak deconvolution from NMR, as described by Bernardinelli[7] et al., and the XRD which calculates the height of the 200 peaks around 22°, called $I_{200}$, and the minimum height between 200 and 101 peaks, around 18°, called $I_{AF}$ as indicated in equation 1.

$$CI = \frac{I_{200} - I_{AF}}{I_{200}} \text{ (Eq. 1)}$$
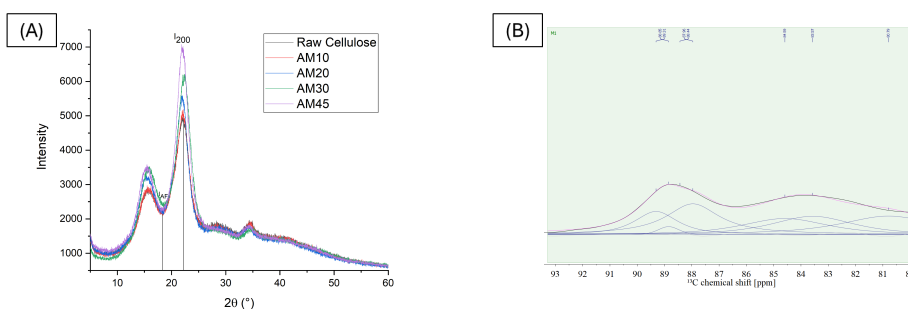


**Figure 2**: (A) XRD comparing different concentrations of $H_2SO_4$. (B) $^{13}$C-NMR (CP/MAS) deconvolution for $C_4$ peak.

The CI is an important parameter that shows what sample has the better propriety. Highly crystalline cellulose is more appropriate for application in composite materials since it provides desirable properties like thermal stability and high strength. The results shown in the table below show the best conditions for obtaining high crystalline cellulose from OP was $H_2SO_4$ at a concentration of 45% (v/v) in both methods. This sample was analyzed by AFM in terms of size and shape. It was observed that nanocellulose had the appearance of nanowhiskers with around 330 nm in size.

**Table 1** – Cellulose samples – CI (%)

| | *XRD* | *¹³C NMR* |
|---|---|---|
| *RAW* | 54.84 | 37.07 |
| *AM10* | 56.92 | 38.70 |
| *AM20* | 60.21 | 41.51 |
| *AM30* | 60.64 | 42.24 |
| *AM45* | 67.18 | 46.45 |
| *α-CEL* | 66.01 | 50.00 |
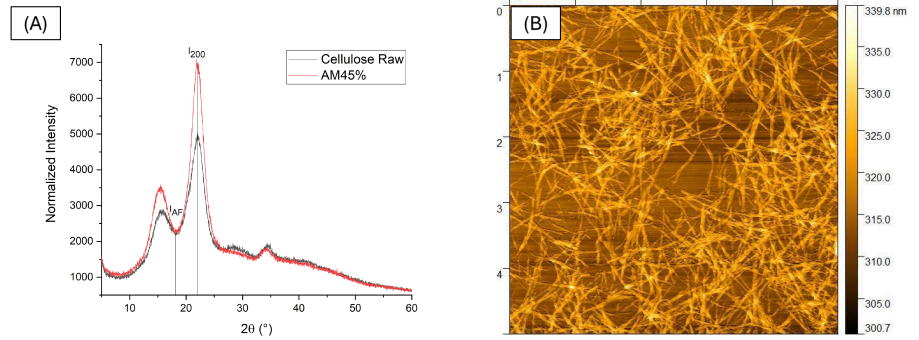


**Figure 3**: (A) XRD comparing raw cellulose and AM45. (B) AFM from AM45.

AM45 was used to synthesize a gel composite with carrageenan, a polysaccharide from red algae with antioxidant and healing properties. The composite was analyzed with SEM (Scanning Electron Microscope) to compare the influence of cellulose in the structure. For these, one sample of pure carrageenan was prepared following the same procedure but without cellulose. It is observed that the freeze-dried influences in porous formation and the organization of the structure, showing that the cellulose gave structure for carrageenan without losing its pores, suggesting that the crosslink between the two composts was effective.
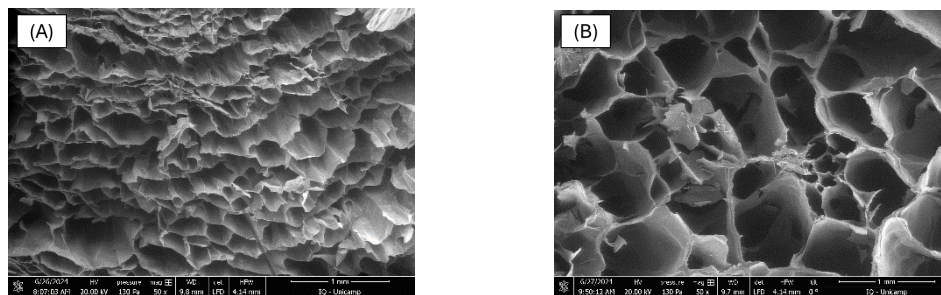


**Figure 4:** (A) Pores from pure carrageenan gel and (B) Pores from carrageenan with cellulose gel.
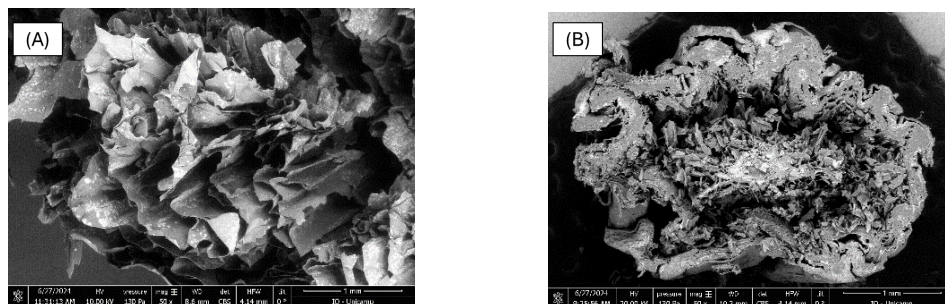


**Figure 5**: (A) Layers structure from pure carrageenan gel and (B) Compact structure from carrageenan with cellulose gel.

## CONCLUSION

The results show that nanocellulose was successfully obtained from OP. The interference of $H_2SO_4$ concentration is clearly observed in the crystallinity so it is possible to select what CI is better depending on the application. The interaction between carrageenan and cellulose was studied using SEM that shows the gel structure is formed with a great combination and it is a good signal for the next steps. Therefore, a new material with high potential in application as wound healing was produced by the combination of nanocellulose from OP, and carrageenan.

For the next steps it is expected to change the rates between carrageenan and cellulose to obtain different kinds of materials as well with characterization of these materials using NMR and SEM. Also is expected the synthesis of anti-biogenic nanoparticles for applying in the composite.

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