Nº PROYECTO: 3140036  DURACIÓN: 3 años  AÑO ETAPA: 2016
TÍTULO PROYECTO: FUNDAMENTAL SUTIDES OF BIOPOLYMER (STARCH, SALMON AND BOVINE GELATIN)-BACTERIAL CELLULOSE NANOFIBRIL INTERACTIONS FOR APPLICATIONS IN FOOD TECHNOLOGY AND TISSUE ENGINEERING.

DISCIPLINA PRINCIPAL: INGENIERIA QUIMICA
GRUPO DE ESTUDIO: INGENIERIA 3
INVESTIGADOR(A) RESPONSABLE: FRANCK JEAN CHRISTOPHE QUERO
DIRECCIÓN:
COMUNA: Santiago
CIUDAD: Santiago
REGIÓN: METROPOLITANA

FONDO NACIONAL DE DESARROLLO CIENTIFICO Y TECNOLOGICO (FONDECYT)
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**INFORME FINAL**

**PROYECTO FONDECYT POSTDOCTORADO**

**OBJETIVOS**

Cumplimiento de los Objetivos planteados en la etapa final, o pendientes de cumplir. Recuerde que en esta sección debe referirse a objetivos desarrollados, NO listar actividades desarrolladas.

<table>
<thead>
<tr>
<th>Nº</th>
<th>OBJETIVOS</th>
<th>CUMPLIMIENTO</th>
<th>FUNDAMENTO</th>
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<td>1</td>
<td>Production of nanofibrillated cellulose and its characterization by powder X-ray Diffraction (XRD) and Atomic Force Microscopy (AFM)</td>
<td>TOTAL</td>
<td>Nanofibrillated cellulose was successfully prepared, which was confirmed by AFM imaging. The typical crystalline structure of nanofibrillated cellulose was also confirmed by powder X-ray diffraction</td>
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<td>2</td>
<td>Production of starch-nanofibrillated cellulose composites</td>
<td>TOTAL</td>
<td>Starch-nanofibrillated cellulose composite films were successfully prepared by solvent casting</td>
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<td>3</td>
<td>Characterization of starch-nanofibrillated cellulose composite films by powder X-ray diffraction, Fourier-transform infrared spectroscopy, Raman spectroscopy and UV-visible spectrophotometry</td>
<td>TOTAL</td>
<td>Starch-nanofibrillated cellulose composite films were successfully characterized by powder X-ray diffraction, Fourier-transform infrared spectroscopy, Raman spectroscopy and UV-visible spectrophotometry.</td>
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<td>4</td>
<td>Study the micromechanical interaction between starch and nanofibrillated cellulose by Raman spectroscopy.</td>
<td>NO</td>
<td>It has been impossible carrying out this objective due to the fact that starch and cellulose are not spectroscopically different in the Raman shift range of 1050 to 1150 cm⁻¹. Due to the fact that starch also presents Raman band in that region following the shift of the 1095 cm⁻¹ Raman band from cellulose was not possible.</td>
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</table>

Otro(s) aspecto(s) que Ud. considere importante(s) en la evaluación del cumplimiento de objetivos planteados en la propuesta original o en las modificaciones autorizadas por los Consejos.
RESULTS OBTAINED:
For each specific goal, describe or summarize the results obtained. Relate each one to work already published and/or manuscripts submitted. In the Annex section include additional information deemed pertinent and relevant to the evaluation process.

The maximum length for this section is 5 pages. (Arial or Verdana, font size 10).

Main goal: This project aims to study the fundamental interaction between nanocellulose and biopolymers (starch and bovine and salmon gelatin) for applications in food technology and tissue engineering.

Organization for the 3-year work:
Year 1: Fabrication and characterization of bovine gelatin-cellulose composites (see details in transfer report year 2014).
Year 2: Fabrication and characterization of bovine and salmon gelatin-nanocellulose composites (see details in transfer report year 2015).

Year 3: Fabrication and characterization of starch-nanocellulose composites.

Specific goals for year 3 (final report 2016):
1. Production of nanofibrillated cellulose and its characterization by powder X-ray Diffraction (XRD) and Atomic Force Microscopy (AFM).
2. Fabrication of starch and starch-nanofibrillated cellulose composite films.

Results:

1. Production of nanofibrillated cellulose by high pressure homogeneization (specific goal 1).

   The fabrication of nanofibrillated cellulose (NFC) consisted in wetting 30 g of cellulose fibers with distilled water to obtain a mixture of 10 wt% solid. The sample was then passed through a PFI mill at 20 000 revolutions. The milled fibers were then diluted in distilled water to obtain a final have 2 wt% of solid substance. The 2 wt% suspension was then disintegrated for 5 minutes at 24 000 rpm with the Ultra-Turrax T-25. The final step in the fabrication of a highly viscous NFC gel was to submit the fiber suspension to 20 homogenization passes at 800 bar using a GEA Niro Soavi PANDA Plus 2000. This procedure allowed converting cellulose fibers into NFC.

2. Characterization of nanofibrillated cellulose nanofibers by XRD and AFM (specific goal 1).

   Figure 1 reports AFM images of the obtained NFC. The length of the fiber was impossible to measure due to the micron scale entanglement of the nanofiber network. It was, however, possible to assess the diameter of the cellulose nanofibers. In Figure 1, the distribution of the diameter size of NFC is reported (frequency as a function of width). The diameter of the nanofiber varies between approximately 30 nm and 200 nm. Most of the fibers have a diameter below 100 nm, so one can qualify this material as nanofibrillated cellulose.

   ![Figure 1 Atomic force microscopy images of nanofibrillated cellulose.](image)
Figure 2 reports typical X-ray diffraction for the generated nanofibrillated cellulose. The typical diffraction planes for nanofibrillated cellulose can be observed namely (110), (1\overline{1}0), (200) and (040). The crystallinity index associated with the proportion of crystalline and amorphous phases in the cellulose nanofibers, was found to be 59 ± 2 % using Segal’s method. This value is typical for this material and related to the number of passes into the high-pressure homogenizer, which when increasing, decreases the crystallinity index.

![Figure 2](image)

**Figure 2** A typical powder X-ray diffraction pattern obtained for nanofibrillated cellulose.

3. Production of starch-nanofibrillated cellulose nanocomposite films by solvent casting (specific goal 2).

The production of starch-NFC composite films required the preparation of 3 wt% waxy starch suspensions. It is very difficult, or even impossible, to dissolve a larger amount of starch in a volume of 150 mL of distilled water. That is why most studies involving the preparation of a starch suspension report the use of a concentration of 3 wt%. Then controlled amounts of the 2 wt% NFC gel were added to the 3 wt% starch suspension to obtain upon water evaporation composite films with 2, 6, 10 wt% NFC (in relation to the weight of starch). The mixture of starch suspension with or without glycerol was heated at 90°C for 30 minutes, with continuous stirring, to ensure gelatinization as reported in Figure 3a. Starch films were also produced similarly and used as control materials. The suspensions were then poured into teflon moulds as shown in Figure 3b.

![Figure 3](image)

**Figure 3** Preparation of starch-nanofibrillated cellulose composite films: (a) preparation of a starch-nanofibrillated cellulose suspension, (b) starch-nanofibrillated cellulose suspension before drying, from left to right: 0 wt%, 10wt%, 6wt% and 2wt%. and (c) starch-nanofibrillated cellulose
composite films after drying at various wt% content of nanofibrillated cellulose, from left to right: 0 wt%, 10wt%, 6wt% and 2wt%.

As previously mentioned, the NFC was integrated at different loadings, 2, 6 and 10 wt% in relation to the weight of starch. Relatively low rate of NFC were chosen because this material is more expensive than starch. It is important to assess whether at low NFC content, the structure and properties of starch can be sufficiently to produce robust food coatings with optimized optical transparency (the food coating is directly in contact with the product, it must be sufficiently transparent to allow the consumer to easily see the food product.). If at low NFC content, good results are obtained, it will help to lessen the cost of production of these materials.

The mixtures of starch suspension and NFC placed in the Teflon molds, were finally dried in an oven for 48 hours at 60 °C, to let the water evaporate. At the end of the drying process, films as reported in Figure 3c were obtained. The thickness of the composite films was measured and found to be 0.13 ± 0.02 mm with no significant difference in thicknesses between all the films (important when assessing the visible transparency using UV-Visible spectrophotometry).

4. Characterization by powder X-ray diffraction, Fourier-Transform Infrared spectroscopy (FTIR), Raman spectroscopy, UV-visible spectrophotometry and differential scanning calorimetry (specific goal 3).

4.1 Powder X-ray diffraction

Figure 4 reports typical X-ray diffraction patterns for starch-NFC composite films at various NFC wt%. Figure 4a shows typical powder X-ray diffraction patterns for starch and NFC. The typical diffractions planes for starch and NFC are reported.

![Figure 4: Typical powder X-Ray diffraction pattern of starch and NFC](image)

Figure 4b reports typical powder X-ray diffraction patterns for starch-NFC composite films as well as NFC. For all the composite films, one can observe, three diffraction peaks at 17°, 19.6° and 21°. The intensity of these three peaks remains relatively unchanged upon the addition of NFC suggesting that these diffraction peaks would correspond to starch crystalline phases. The intensity of the peak located at ~21°, however, increases upon increase of the NFC content. This suggests that this diffraction peak arises due to the presence of NFC in the composite film materials. The analysis of the crystallinity for the nanocomposite materials cannot be obtained by X-ray diffraction, since the discrimination of the crystalline structure of starch and NFC is not clear. Indeed the peak at located at ~17° and ~21° present in the powder X-ray diffraction pattern of NFC, are also present in the powder X-ray diffraction pattern of starch as reported in Figure 4a. These results also suggest that the preparation of the starch-NFC composite films preserves the crystalline structure of both NFC and starch.
4.2 Fourier-Transform Infrared Spectroscopy

Figure 5a shows typical ATR-FTIR spectra for starch-NFC composite films at various NFC contents. One can observe that for all the materials, a peak located in the wavenumber range of 3500-3000 cm\(^{-1}\) is present and is related to the vibrational motions of OH groups. Peaks located at wavenumbers of \(\sim 858\) cm\(^{-1}\) and \(\sim 762\) cm\(^{-1}\) present for starch and starch-NFC composite films are absent in the spectra corresponding to NFC. Therefore these peaks can be attributed to the starch molecular structure. These peaks correspond respectively to the C-H, CH2 deformation and the C-C stretching.

The presence of noise in the 2500-2000 cm\(^{-1}\) region is due to the crystal diamond used for to perform the FTIR experiment in ATR mode. Peaks in the 1200-900 cm\(^{-1}\) region, are present in all spectra of NFC, starch and NC, therefore it is impossible to discriminate between NFC and starch in that wavenumber range, due to the very close molecular structure of starch and cellulose.

The peak located at \(\sim 1670\) cm\(^{-1}\) may be due to the bending mode of the absorbed water in the amorphous phase and some contributions from carboxylate group. The peak located at \(\sim 997\) cm\(^{-1}\) corresponds to the crystalline phase in starch. These peaks are used to determine the crystallinity of the starch phase in the composite materials.

Table 1 reports the calculated crystallinity index corresponding to starch and starch present in the starch-NFC composite films. The results reveal that the presence of NFC decreases the crystallinity of the starch fraction by 35-38% compared to the crystallinity of starch without the presence of NFC. Adding more NFC to the starch matrix does not additionally affect the crystallinity index of starch which remains at \(\sim 56\%\).

Figure 5b shows typical Raman spectra for starch, starch-NFC composite films and NFC. Raman spectroscopy is typically used to observe vibrational modes that are of weak intensity in FTIR spectroscopy since the Raman spectroscopy technique is complementary to IR spectroscopy. Meanwhile, Raman spectroscopy has a weak signal for some vibrational modes that are more easily observable using IR spectroscopy. However, water has a weak Raman signal, making the technique ideally suited for some areas of the food industry.

As observed in the previous section using ATR-FTIR spectroscopy, all the Raman peaks, are present in all spectra of NFC, starch and NC, therefore discrimination between NFC and starch is delicate. Besides some Raman bands can be observed in the 1050 to 1150 cm\(^{-1}\) Raman shift range, which make impossible carrying out a micromechanical study using the 1095 cm\(^{-1}\) Raman band belonging to cellulose.

The Raman band associated with starch crystallinity that was visible in the starch and starch-NFC composite films. This peak is located at \(\sim 480\) cm\(^{-1}\) corresponding to the vibrational motions of C-H moieties, a band associated with "skeletal mode" vibrations. The peak at \(\sim 859\) cm\(^{-1}\)
corresponds to the amorphous phase. These bands were used to study possible changes in the crystallinity of the starch fraction in starch and in the starch-NFC composite films as function of NFC content. These results are reported in Table 1. The results are compared with the results obtained by FTIR spectroscopy.

The study of the crystallinity in starch and in the starch fraction in the starch-NFC composite films by Raman spectrometry, as reported previously by FTIR spectrometry, reveals that the presence of NFC decreases the crystallinity of the starch fraction in the starch-NFC composite films compared to starch. The crystallinity in the starch fraction in the starch-NFC composite films, however, does not change significantly when more NFC is added (from 2 to 10 wt%).

**Table 1** Crystallinity of starch-nanofibrillated cellulose composites determined by FTIR and Raman spectroscopy

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<tr>
<th>Xc (%)</th>
<th>Starch</th>
<th>2% of NFC</th>
<th>6% of NFC</th>
<th>10% of NFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTIR</td>
<td>94%</td>
<td>56%</td>
<td>55%</td>
<td>56%</td>
</tr>
<tr>
<td>Raman</td>
<td>76%</td>
<td>59%</td>
<td>57%</td>
<td>54%</td>
</tr>
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</table>

### 2.4 UV-Visible spectrophotometry

Figure 6 shows typical UV-Visible spectra for starch and starch-NFC composite films at different NFC contents. From the UV-Visible spectra, the level of transparency of the starch and starch-NFC composite films can be evaluated. The higher the transmittance is, the higher the visible transparency of films (in the 400-700 nm visible light range). As expected the presence of NFC decreases the transmittance of the composite films. A starch film has a transmittance of 80-85%. The addition of 2 wt% NFC decreases transmittance down to 70%. The addition of 6 and 10 wt% NFC decreases the transmittance down to ~65% and ~60% respectively. The increase in the NFC content, increases the opacity of the composite films as expected. This is due to some light scattering from cellulose nanofibers and possible aggregates. However, there is no evidence of the level of dispersion of NFC in the starch matrix. Also an increase in film roughness may be responsible for light scattering and so for the decrease in light transmittance, but the level of film roughness has not been measured in the present study. The transparency of the composite also depends on the difference in refractive index of starch and cellulose nanofibers.

![Figure 6](image_url)
During the third year of this postdoctoral project Dr. Quero was invited to contribute to a book chapter in the book entitled “Nanocellulose and Sustainability: Production, Properties, Application, and Case Studies”. This book is edited by Dr. Koon-Yang Lee and will be published in 2017. The book chapter entitled “Applications of Nanocellulose as Optically Transparent Papers and Composites”, has been submitted at the end of July 2016.

The characterization of starch-nanofibrillated cellulose was performed during the third year of this project. This work was performed at Universidad de Chile in the framework of a Master’s student (Miss Geraldine Desjoyeaux) university placement at Universidad de Chile (supervised by Dr. Quero) from the middle of March 2016 to until the end of June 2016. During her placement, Miss Geraldine Desjoyeaux was trained to produce starch-nanofibrillated cellulose composite films and characterize the generated materials. She determined the molecular structure of the materials by FTIR and Raman spectroscopies and determined the optical properties by UV-visible spectrophotometry of the composite materials. A scientific report has been written by her and has been presented by Geraldine Desjoyeaux at the University of South Brittany (France) to obtain her Master’s degree. This internship was a great opportunity in order to establish a long-term internship exchange program with the University of South Brittany to carry out internships in the frame of other research grants to be funded by Conicyt. Another study from the same university, Miss Audrey Tiercin, performed her internship under the supervision of Dr. Quero. Another student, Mr. Aymeric Fechotte, will start his internship in March 2017.

During this step of the project, Dr. Quero was, this time, unable to travel to Exeter to carry out Raman spectroscopy experiments due to teaching commitment. Dr. Quero has been hired in April 2016 by the Facultad de Ciencias Físicas y Matemáticas of Universidad de Chile as Assistant Professor.

With respect to the presentations to seminars and conferences, Dr. Quero participated to the following events during the last step of this postdoctoral project:

- Dr. Quero presented a poster entitled “Structural, Morphological and Micromechanical Characterization of Gelatin-Bacterial Cellulose Composites” at the Jornada Científica de Salud at the Universidad de los Andes, Santiago in November 2015.

- From the 5th to the 7th of September 2016, Dr. Quero participated to IV Congreso Nacional de Nanotecnología where he gave a poster presentation entitled “Stress Transfer and Physical Properties of Gelatin-Nanofibrillated Cellulose Composites”.

- On the 1st of September 2016, Dr. Quero presented in the Ciclo de Seminarios 2016 at the Universidad Técnica Federico Santa María, Department of Physics, Valparaiso, Chile the presentation entitled “Micromechanical Interfacial Interactions and Deformation Mechanisms in Cellulose-Containing Composites”. Dr. Quero was invited by Dr. Cristian Acevedo. Data obtained from this research project were presented.

- Dr. Quero participated, from the 23rd to the 28th of October 2016, to the XV Simposio Latinoamericano de Polímeros (SLAP2016) and XIII Congreso Iberoamericano de Polímeros where he gave an oral presentation entitled “Stress Transfer Quantification in Gelatin-Nanofibrillated Cellulose Composites by Raman Spectroscopy”. An abstract was published in the frame of this conference as a conference proceeding.

- After the end of this project, Dr. Quero will participate to an outreach activity at “Maison de France” in Santiago. Dr. Quero has been invited by Dr. Patricio Jorquera (FCFM, UChile) to give a general presentation about biocomposites and cellulose. This project will be mentioned during the presentation. This activity is scheduled on the 7th December 2016.
El trabajo desarrollado el Dr. Franck Quero (Franck) en BIOPREL UANDES permitió generar nueva información sobre el efecto de la adición de fibras de celulosa en la micromecánica de compósitos de gelatina. Este trabajo desarrollado en conjunto con investigadores de UBristol y UExeter permitió publicar el siguiente trabajo: F. Quero, A. Coveney, A. E. Lewandowska, R. M. Richardson, P. Díaz<Calderón, K. <-Y. Lee, S. J. Eichhorn, M. A. Alam, and J. Enrione. Stress Transfer Quantification in Gelatin-Matrix Natural Composites with Tunable Optical Properties, Biomacromolecules, 2015, 16, 1784<1793. (Impact Factor: 5.750). Franck también supervisó adecuadamente el trabajo de magister de una estudiante de la Universidad AgroSup Dijon (Francia), quien desarrolló su trabajo en BIOPREL. Este trabajo se focalizó en la preparación de nanocristales de celulosa para I nuevos nanocompósitos de gelatina. Este material se caracterizó térmica (DSC), mecánica (tensión) y ópticamente (spectrofotometría). Actualmente se está preparando el siguiente nuevo manuscrito para ser enviado a ACS Applied Materials and Interfaces: Another article is currently being prepared and will be entitled: F. Quero, A. E. Lewandowska, P. Díaz<Calderón, C. Saez, J. Luengo, A. Berg, L. Caballero, F. Melo, S. J. Eichhorn, and J. Enrione. Stress Transfer and Mechanical Properties of Gelatin<Nanofibrillated Cellulose Natural Composites: Comparing the Effect of pH and Gelatin Origin, ACS Applied Materials and Interfaces, in preparation. (Impact Factor: 6.723). Durante este período, Franck también participó activamente en diferentes instancias en el proyecto redes NewtonCPicarte (PCI Nº 140144) que actualmente dirijo. Por otro lado, durante este año Franck no pudo continuar con su trabajo experimental en BIOPREL UANDES dado que en Abril 2016 fue incorporado como profesor asistente en la Facultad de Ciencias Físicas y Matemáticas de la Universidad de Chile. Durante esta última etapa de su posdoctorado, junto con sus nuevas responsabilidades académicas, participó en la elaboración de un capítulo de libro titulado “Applications of Nanocellulose as Optically Transparent Papers and Composites” en el libro titulado “Nanocellulose and Sustainability: Production, Properties, Application, and Case Studies”. Este libro es editado por Dr. Koon-Yang Lee y será publicado en 2017. Franck también ha apoyado la colaboración que actualmente mantengo con el profesor Cristian Acevedo del Departamento de Física de la Universidad Técnica Federico Santa María. En este sentido, actualmente se están realizando las últimas correcciones para la aceptación final del manuscrito en Journal of Applied Polymer Science titulado "Synergistic effects of crosslinking and chitosan molecular weight on the microstructure, molecular mobility, thermal and sorption properties of porous chitosan/gelatin/hyaluronic acid scaffolds" Sumado a las publicaciones indicadas anteriormente, Franck ha presentado su trabajo en el Congreso Chileno de Ingeniería Química, Universidad de Concepción, 15th, 16th and 17th October 2014) y en 20th International Conference on Composite Materials (ICCM 20) (19th to the 24th of July 2015). Más recientemente Franck ha presentado su trabajo en los siguientes congresos: Jornada Científica de Salud at the Universidad de los Andes, Santiago (November 2015), IV Congreso Nacional de Nanotecnología (5 a 7 de Septiembre 2016), Ciclo de Seminarios 2016 en la Universidad Técnica Federico Santa María, Departamento de Física, Valparaíso, Chile (1 Septiembre 2016), XV Simposio Latinoamericano de Polímeros (SLAP2016) and XIII Congreso Iberoamericano de Polímeros (23 al 28 de Octubre 2016).Puede afirmar con seguridad que Franck ha desarrollado adecuadamente su trabajo con independencia y dedicación, logrando para cada uno de años las actividades necesarias para el cumplimiento de gran parte de los objetivos principales de su proyecto. Lo anterior a pesar de haber sido incorporado como académico con jornada completa en la Universidad de Chile. Se espera que nuestra colaboración se mantenga en el futuro, como se manifiesta en mi reciente posulación Fondecyt Regular “Effect of cellulose nanofibers on thermomechanical and shape memory properties of salmon gelatine based composites”, en donde Franck participa como Co-investigador.

Javier Enrione.
Profesor Asociado
Universidad de los Andes
PRODUCTOS

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Nº : 1
Autor (a)(es/as) : Quero, F.; Coveney, A., Lewandowska AE.; Richardson, RM.; Díaz-Calderón, P.; Lee KY.; Eichhorn, SJ.; Alam, MA.; Enrione, J.
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OTRAS PUBLICACIONES / PRODUCTOS

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**CONGRESOS**

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<td>Structural, Morphological and Micromechanical Characterization of Gelatin-Bacterial Cellulose Composites</td>
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<tr>
<td>Nombre del Congreso</td>
<td>Jornadas Científicas en Salud - Universidad de los Andes</td>
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Ciudad : Santiago
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Ciudad : Olmue
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Nombre del Congreso : XV Simposio Latinoamericano de Polímeros and XIII Congreso Iberoamericano de Polímeros
País : MEXICO
Ciudad : Cancún
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