

Chemical Composition And Structure Of Modified Agave Fiber As Related To The Rheology Of Gelatin Film-Forming Solutions With Montmorillonite And Fiber Addition

Perla Alanís-López¹, Paulo J. Amaral Sobral², Ana Monica Quinta Barbosa Bittante², Izabel Cristina Freitas Moraes², Emmanuel Flores-Huicochea¹, Isidra Guadalupe Ruiz-Martínez, Javier Solorza-Feria^{1*}

¹Center for Research on Biotic Products (CEPROBI-IPN), National Polytechnic Institute. Calle Ceprobi 8, Col. San Isidro, Yautepec, Morelos, ZC. 62731. MEXICO.

²Department of Food Engineering, FZEA, University of São Paulo, 13635-900, Pirassununga/SP, Brazil.

Corresponding Autor: J. Solorza-Feria

ABSTRACT: In order to reduce the use of synthetic polymers derived from petroleum, some researches have been done with starch and fiber to improve its film characteristics. Another important polymer is bovine gelatin, no effect on its thickness. Micrographies of the fibers, which has excellent film-forming properties. An alternative to increase macromolecules structure strength is to incorporate strengthening compounds to polymeric matrices, such as plant fibers and nanoclays. The aim of this study was to characterize some chemical, physical, and structural properties of Agave lignocellulose fiber, by chemical analysis, scanning electron microscopy, Fourier transform infrared spectroscopy and X-ray diffraction and to evaluate the rheological properties of bovine gelatin-based solutions, loaded with Agave fibers and montmorillonite. Hydrophilic groups of the agave fiber (hemicellulose and lignins) were removed by alkaline treatment, resulting in lower moisture content, but keeping all its components with showed for the control sample, aligned openings all along its structure, while the alkaline treated sample, showed a slightly closer structure. The FTIR spectra showed typical transmittance areas from cellulose and its derivatives, with similar crystallinity patterns irrespect of the fiber treatment as seen in the X-ray diffraction results. The gelatin filmogenic solutions showed viscoelastic behavior with predominance of the elastic over the viscous character, with transition temperatures Tgel-sol and Tsol-gel, without significant differences among the samples, the nanoclay incorporation in the filmogenic solution favored the formation of a more elastic gel.

KEYWORDS: lignocellulosic fiber, chemical analysis, gelatin solutions, rheology, characterization.

Date of Submission: 31-07-2018

Date of acceptance: 15-08-2018

I. INTRODUCTION

Due to its structural arrangement, the degradation of the conventional plastics from petroleum derivatives, is very slow (up to more than 100 years in some cases), and can generate by ignition highly polluting compounds. This has increased the interest in producing new plastic materials from renewable sources, named biodegradable plastics. These materials are degraded by the action of biological agents (e.g. microorganisms), causing its de-polymerization and demineralization, being thus treated as organic residues [1, 2]. Proteins are a good raw material for the biodegradable materials industry, with special attention to the bovine gelatin (a water soluble protein obtained by partial acid or alkaline enzymic hydrolysis of collagen), produced world-wide at a relatively low cost. At the present time, the food industry has increased the use of bovine gelatin as an emulsifier, foaming agent, stabilizer, encapsulator and in biodegradable packing materials [3]. The excellent film-forming capacity of gelatin is due to its ability to form physical gels. Gel-formation in aqueous solution of gelatin, implies a process where the structures tend to pass from a disordered state, to triple helices structures, characteristic of collagen in its native state. This structural re-arrangement is reflected in the physical properties of its gels.

The use of ligno-cellulosic fibers to reinforce protein films mechanical properties is becoming common. *Agave tequilana* Weber, considered one of the most important agricultural resources in Mexico, is a rich source of fiber, employed mainly in the production of "Tequila" (an alcoholic drink of Mexican origin) [4]. The ligno-cellulosic fiber presents long cellulose chains, having intra and intermolecular hydrogen bonds, conferring to the system a hydrophilic character, finding it in an extended pattern within the central cavity of the fiber. Besides, some chemical modification of these fibers might improve the interaction between fibers and biopolymers [5].

Nanoparticles are species whose use to strengthen materials structure is increasing, e.g., nanoclays or nanominerals, they consist of layers formed by two silicon atoms in tetrahedral arrangement, linked to an octahedron sheet with sheared borders of either aluminum or magnesium hydroxide. Several kinds of nanoparticles have been used as nanoscale additives. Among the nanoparticles, the sodium montmorillonite (MMT) is perhaps the specimen most used in studies on biodegradable films reinforcement. The MMT is a nanoclay that belongs to the smectite family or extensible minerals composed mainly by Al_2O_3 , 4SiO_2 and $4\text{H}_2\text{O}$. It is considered as a reinforcement material, chemically and thermally stable, and has been studied in nanocomposite films based on gelatin produced by casting [6, 7].

Some of the properties of the films could be related to the rheological properties of its film-forming solutions. Rheological studies of protein solutions report commonly a viscoelastic behavior. This term refers to the destruction of an incomplete tridimensional structure of the protein when a stress is applied, that recovers partially when this stress is removed. Thus, the more resistant the structure to the breakage, the more elastic the material will be and vice versa, the easier the structure to break, the more viscous predominance will be observed [8].

Considering that studies on biopolymers based film, added with fibers and nanoparticles are required in this domain, and that these specimens could affect the rheological properties of film-forming solutions, the objective of this work was to characterize some physical, chemical and structural properties of *Agave tequilana* lignocellulose fiber, and to evaluate the rheological properties of gelatin-based solutions, loaded with *Agave* fibers and montmorillonite.

II. MATERIALS AND METHODS

The materials used in the films manufacture were, the biopolymer bovine gelatin (240 Bloom, Gelita South America: Sao Paulo, Brazil), Glycerol (J.T. Backer, New Jersey, USA) as plasticizer; sodium montmorillonite (Sigma-Aldrich, USA), as a nanoscale additive; and a natural fiber from *agave* (*Agave tequilana*) supplied by the Center for Research on Biotic Products (CEPROBI), as a microscale load. These fibers were used in its native state (control), and also, after an alkaline chemical treatment.

Ligno-cellulosic fiber Treatment

Before the treatment, the fiber was washed with hot water at 75°C for 20 min, to deactivate lipolytic enzymes, eliminating possible dust and sugar as well. Subsequently, the fiber was dried in an air oven at 45°C during 24 h. Finally, the fiber was ground in a rotary mill (CFW 08, Inverter MA-090CFT), at 6559 rpm, to obtain a particle size of approximately 0.007 to 0.131 mm. The fiber obtained was chemically modified with a 10% (w/v) NaOH solution, placing 200 ml of solution for each 10 g of fiber at ambient temperature for 1 h. After this reaction time, the fiber sample was washed with distilled water until the supernatant was clear, then, it was verified that the pH was neutral (pH=7), before placing the fiber in aluminum plates to be dried in an oven at 45°C for 24 h, to be ready for use.

Fiber Proximate and Chemical Composition

The proximate and chemical analysis of control and treated fibers was carried out in triplicate, according to AOAC methods [9]. Moisture content was calculated by weight loss after drying at 105°C for 4 h (method 925.09). Crude protein was determined with a Keltec system (Tecator, Sweden) and by calculating protein content as nitrogen $\times 6.25$ (method 954.01). Crude fat was quantified after a 1 h extraction with hexane in a Soxhlet digestive system (Tecator, Sweden) (method 962.09). Fiber content was calculated after acid and alkaline digestion with a Soxhlet system (Tecator, Sweden) was done (method 962.09). Ash content was the remaining portion of samples after heating in a furnace at 550°C for 4 h (method 923.03). To evaluate the rough fiber, hemicellulose, Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF), Silva's methodology [10] was followed.

Scanning electron microscopy (SEM)

A Scanning Electron Microscope (JEOL JSMP 100, Japan) was used to take SEM photomicrographs from fiber samples, all with a magnification of 300 to 800x. The samples were fixed to stainless steel stubs, dehydrated with osmium tetroxide (OsO_4) and covered with a layer of colloidal gold of about 40-50 nm of

thickness in a JEOL ionizer of metals. They were immediately placed through the microscope's conduct under the influence of an electron beam; such a beam was passed over the surface, making a scan following a parallel line. Samples were observed with 5kV of voltage.

Fourier Transform Infrared Spectroscopy (FTIR)

Control and treated fiber samples were analyzed by Fourier transform spectroscopy, after 7 days of silica gel pre-conditioning (RH close to 0%), for water elimination in the material. Infrared spectra were analyzed using a Spectrum-One spectrophotometer (Perkin-Elmer). This device contained an UTAR (universal total attenuated reflectance) accessory, that allowed sample analysis by applying the material on the sample slide. Spectra were determined in transmittance mode, always in triplicate, between the wavenumbers 3800cm^{-1} and 400cm^{-1} , with a resolution of 2cm^{-1} , after 20 scanings.

X-Ray diffraction

X-ray diffraction is a technique that can reveal information about samples structure and physical appearance, based on the X-ray dispersion intensity observation affecting the sample, depending on the incident angle, the polarization and wave energy. X-ray diffractograms of fibers were determined by using an X-ray diffractometer (Rigaku Miniflex 600, Japan), which had a germanium detector, equipped with Scintag DMSNT Version 1.37 software. It operated at 40 kV and 15 mA (radiation by $\text{CuK}\beta$, $\lambda = 0.15405\text{nm}$). Spectra were obtained at 25°C , in a range $2\theta = 2^\circ$ to 70° , with a rate of $2^\circ/\text{min}$.

Film-forming solution preparation

The formulations and quantities of the different additives involved, as well as the symbology used to describe the film-forming solutions (FFS) are shown in Table 1. Firstly, a solution with 30 g of glycerol/100 g of gelatin, and a dispersion of 5 g of sodium montmorillonite (MMT)/100 g gelatin in water were prepared. To disperse MMT in water, this was hydrated for 1 h at 40°C together with glycerol. Afterwards, it was stirred for 10 min with an Ultraturrex equipment (2500 rpm), then introduced to a S-450D, Branson sonicator for 10 min in order to allow the creation of exfoliated and interspersed structures in the biopolymer moieties, as well as removing possible bubbles in the dispersion.

Besides, a gelatin solution (8 g macromolecule/100 g FFS) and fiber dispersion (15 g/100 g of gelatin) were prepared separately. The gelatin was firstly hydrated for 30 min, and then placed in water bath (Marconi, TE 184) at 70°C for 15 min to be dissolved. The glycerol solution and the MMT dispersion were then added very slowly (drop by drop) in the gelatin solution, under mechanical stirring for 2 min., being sonicated for 10 more min, for bubbles removal. Then, the fiber dispersion was added, also under mechanical agitation. This stirring was maintained until the FFS temperature reaching about 27°C .

Table 1. Terminology used for the different gelatin film forming solution formulations*

| FFS NAME | COMPONENTS (g/100g filmogenic solution) | | | |
|----------|---|-----------------------------|-------------------------------|----------------------------------|
| | (8%) ¹ Protein | (30%) ² Glycerol | (5%) ² Nanoclay | (15%) ² AgaveFiber |
| 8G0N | 8 | 2.4 | --- | --- |
| 8G5N | 8 | 2.4 | 0.4 | --- |
| 8G0N15FS | 8 | 2.4 | --- | 1.2(FS) |
| 8G0N15FT | 8 | 2.4 | --- | 1.2(FS) |
| 8G5N15FS | 8 | 2.4 | 0.4 | 1.2(FT) |
| 8G5N15FT | 8 | 2.4 | 0.4 | 1.2(FT) |

*1.- on the filmogenic solution basis, 2.- on the protein basis, FS.- fiber treated with 10% NaOH, FT.- control fiber.

Film-forming solution rheological tests

Rheological assays were carried out in a rheometer (AR2000 Advanced Rheometer, TA Instruments, New Castle, DE, USA) using a parallel-plate geometry (diameter= 60mm), with a $1700\text{ }\mu\text{m}$ gap, for both dynamic and steady shear experiments. The temperature was controlled by a Peltier system. The results were analyzed using the Rheological Advantage Data Analysis V.5.3.1 software (TA Instruments).

For dynamic tests, firstly, an amplitude strain test was carried out in order to identify the linear viscoelastic domain. Strain sweep (0-30%) essays were run at a constant frequency (1Hz), in both gel or solid state (5°C), and sol or liquid domain (35°C). Then, temperature sweep tests were performed within the linear viscoelastic region using constant values (frequency = 1Hz; strain = 3%). The cooling and heating ramps were undertaken between 10 and 45°C , at a heating rate of $1^\circ\text{C}/\text{min}$. The storage (G') and loss (G'') moduli were determined and the sol-gel ($T_{\text{sol-gel}}$) and gel-sol ($T_{\text{gel-sol}}$) transition temperatures (sol and gel indicate liquid and gel state respectively) were evaluated as the temperature where G' changed drastically as an inflexion point[11]. To avoid water loss by evaporation and dehydration, the system was covered with a solvent trap containing water. A Frequency sweep from 0.1 to 10 Hz with a 3% rate of strain at 10°C was also performed

for all formulations, letting the sample rest for 5 min before each test, to dissipate any previous strain and ensure the tested system homogeneous setting. The results were presented as G' and G'' in function of frequency. All data were analyzed with the measuring equipment integrated software.

Statistical analysis

Results were analyzed with the analysis of variance method (ANOVA) at a significance level of 5% ($\alpha=0.05$). The SigmaStat, version 2.03, program was applied to the data. When important differences were met, the Tukey's multiple comparisons test was applied, with the same mentioned significance level.

III. RESULTS AND DISCUSSION

Fiber characterizations

The ligno-cellulosic fiber subjected to a chemical treatment with 10 % (w/v) NaOH solution, presented visual changes in color, apparently being darker, with a brown tone in comparison with the untreated fiber, such coloration was possibly caused by lignin-containing groups, such as hydroxyphenyl-propane, which degraded in alkaline medium causing a change in the coloration of the ligno-cellulosic materials. The main purpose of the chemical treatment was the lignin removal from the middle lamella as well as carbohydrates, especially hemicelluloses, which are soluble in alkaline medium; with the removal of these compounds, the adhesion with the polymer matrix where they are incorporated, is improved [12].

Thickness and diameter of control and treated fibers

The average thickness of Agave fibers was 0.101 ± 0.07 mm for the control, and 0.104 ± 0.05 mm for the treated ones, while the average diameter was 0.81 ± 0.49 mm and 0.89 ± 0.56 mm for the control and treated samples, respectively ($n=30$). However, both the fiber's thickness and diameter did not present significant differences ($\alpha=0.05$) (data not shown), and so it can be affirmed that the alkaline or basic treatment had no direct influence in such parameters.

Fiber proximate and chemical analysis

Table 2A, shows the proximate composition of the control and treated fibers as compared to blue Agave. Significant differences were found in all fiber components respect to the blue Agave, being especially notorious those of protein and carbohydrate contents. The alkaline treatment provoked a decrease in the moisture content but an increase in the carbohydrates. According to Sreekala and Thomas [13] (2003), the alkaline treatment makes the fiber's surface less hydrophilic, due to water molecules being mainly absorbed by the fiber's amorphous region. This difference could be related to the treated fiber's lower moisture content and to the chemical and physical changes during the modification.

Agave fibers were composed mainly by cellulose, hemicellulose and lignin as seen in the results obtained individually (Table 2B). The changes found in the composition were mainly moisture and hemicelluloses content. The moisture was higher in the untreated fiber and decreased in the treated fiber sample. With the alkaline treatment, hemicelluloses content decreased notoriously, this diminution could be related to the treated fiber's lower moisture content and to the chemical and physical changes during the modification.

González-García et al. [14] carried out a chemical characterization of the agave fiber grown in Jalisco (Mexico), and observed different results from those in this work, which could be attributed among other causes, to the region and soil where the agave was grown, the season of the year and plant's age.

Table 2A. Proximate composition (%) of control and treated *A.tequilana* ligno-cellulosic fiber

| Components | Control Fiber ¹ | Treated Fiber ¹ | Blue Agave* |
|---------------|----------------------------|----------------------------|--------------------|
| Moisture | 14.77 ^b | 5.27 ^c | 18.3 ^a |
| Protein | 2.8 ^b | 2.04 ^b | 0.53 ^a |
| Ether Extract | 0.49 ^a | 0.32 ^b | Nd |
| Ashes | 2.96 ^b | 2.85 ^b | 8.8 ^a |
| Carbohydrates | 78.98 ^b | 89.52 ^a | 72.37 ^c |
| By difference | | | |

¹Average of three measurements. The same letter in the same row indicates no significant statistical difference ($P > 0.05$). *before González-García et al. [14]. Nd= not determined.

Table 2B. Chemical composition (%) from control and treated *A.tequilana* ligno-cellulosic fiber (dry basis)

| Components | Control Fiber ¹ | Treated Fiber ¹ | Blue Agave* |
|---------------|----------------------------|----------------------------|-------------|
| Rough Fiber | 52.70 | 46.28 | 50.6 |
| ADF | 63.03 | 62.86 | 46.7 |
| NDF | 84.53 | 77.53 | 58.8 |
| Cellulose | 52.89 | 50.10 | 41.9 |
| Hemicellulose | 21.49 | 14.68 | 12.1 |
| Lignin | 4.89 | 4.03 | 7.2 |

¹Average values.*before González-García et al. [14]. Nd= not determined. ADF Acid detergent fiber that contains cellulose, lignin and silica, NDF Neutral detergent fiber, measures hemicelluloses, cellulose and lignin.

Fiber scanning electronic microscopy

Morphology and structural changes on the surface of control and alkali-treated fiber are shown in Fig. 1. In the control fiber (Fig.1a) a lot of aligned openings or gaps are seen, which might well be the result of carrying out the fibers milling, washing and drying processes, the structure remains on this stage, keeping in mind that the fibers were subjected to a juice obtaining process for the agave distillate production.

In the treated fiber's surface a somehow different structure is observed (Fig.1b) respect to the control, the above mentioned aligned openings or gaps are not seen in the same amount, but some small whitish pores in an elongated shape can be seen, along with some discontinuous areas or even wider gaps, probably brought about during the alkaline treatment. Sreekala and Thomas [13] studied the superficial differences in fiber, by modifying it with different chemical reagents, detecting that when performing alkaline treatment, the amorphous cuticle layer was leached out, creating some pores or gaps in the surface.

Geethamma et al. [15] also reported that alkaline treatment generates gaps, promoting a better mechanical anchorage between the fiber and a polymeric matrix, since with the fibers surface improvement, the water adsorption is reduced. Also, alkaline treatment caused hemicelluloses and lignin diminution, giving place to pores or gaps formation, thus increasing the superficial area in these fibers, allowing better interaction between the fiber and the gelatin matrix and improving water adsorption.

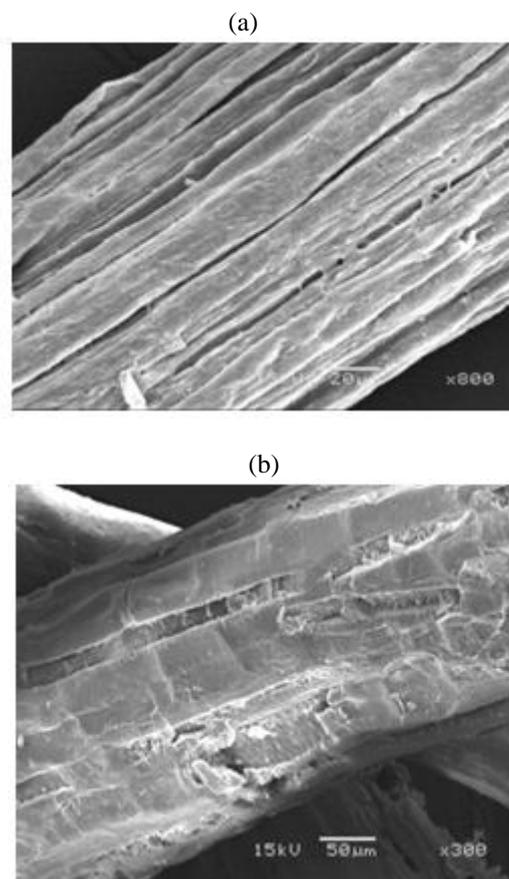


Fig. 1: Photomicrographs by SEM of fibers from *A. tequilana* fiber: (a) control and (b) sample treated with solution at 10% of NaOH.

Fourier Transform Infrared Spectroscopy (FTIR) of fibers

The fibers modification by alkaline hydrolysis and the changes in its structure was verified by FTIR (Fig. 2). The observed vibrations in O-H bonds in the 3322 to 2899 cm^{-1} area, are probably due to groups from the cellulose molecule [16, 17]. Both spectra (control and treated fiber) presented similar behavior during vibration in this area, indicating that the modification with NaOH had in a lesser extent, an influence on the cellulose structure.

Youn Oh et al. [18] conducted a study of cellulose modification with different concentrations of sodium hydroxide (5, 10, 15 and 20%) and carbon dioxide, assessing the best cellulose dissolution state through an organic solvent, pressure, reaction temperature and time. These authors described changes in the spectra with the different NaOH concentrations, being between 4000 to 2995 cm^{-1} , the spectrum commonly found for cellulose, with a significant reduction in 3352 to 3447 cm^{-1} area, in relation to the control sample. During this work, it was not possible to observe such changes, due to a sole (10%) NaOH concentration used; however, the results are consistent with those of such authors for modification under the same settings used

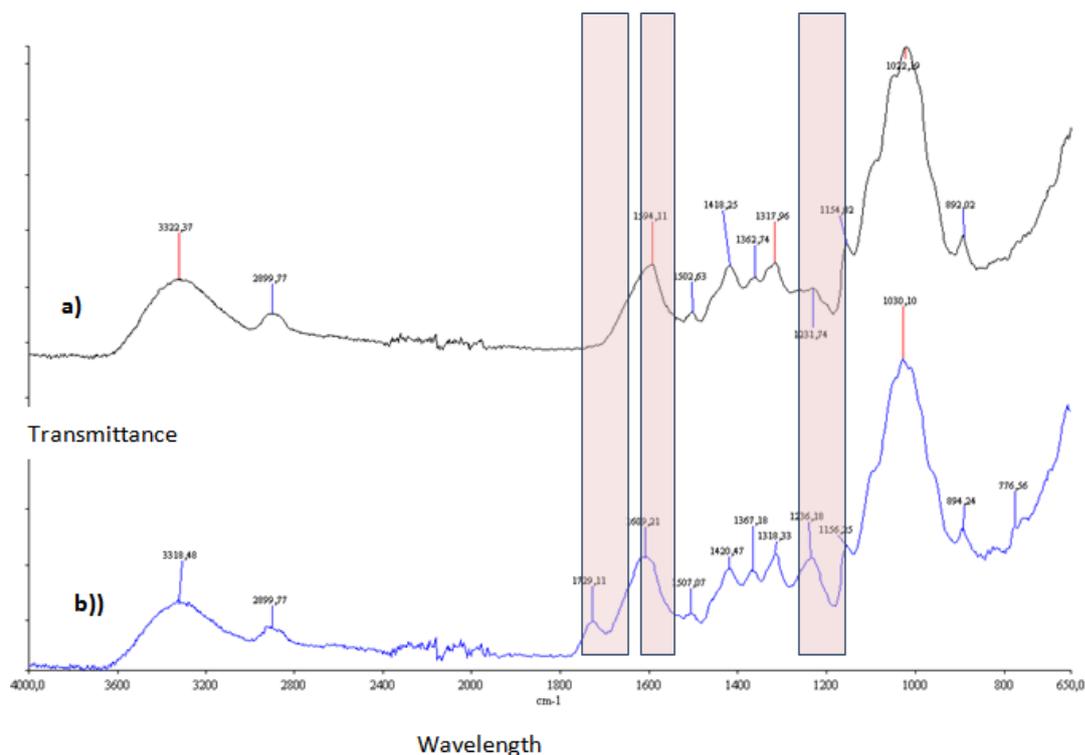


Figure 2. FTIR spectra from *A. tequilana* fiber: a) sample treated with 10 % NaOH and b) control sample.

Moreover, the control sample (Fig. 2b) showed an absorption signal in 1729 cm^{-1} , which could be due to the presence of hemicelluloses, that does not exist in the fiber treated with NaOH (Fig. 2a).

Several authors, [16, 17, 19] have indicated a typical vibration of carbonyl groups in a range from 1700 a 1800 cm^{-1} , which is consistent with the chemical analysis results (Table 2b), that showed a hemicellulose content reduction in the treated fiber, which could be related with the absorption absence due to hemicellulose degradation, which are soluble in alkaline aqueous solutions.

Valadez-Gonzalez et al. [20] found a peak at 1750 cm^{-1} band, for the fiber of *Agave sisalana*, treated with sodium hydroxide at 2% (w/v), attributed to hemicelluloses, similar to that observed in this work. An absorption decrease of the typical band of lignin from 1500 to 1600 cm^{-1} was also observed, presented by the compounds' structure of the C-C aromatic skeleton, and partially removed with the alkaline treatment [21]

Likewise, another signal in the infrared spectrum of untreated fiber was observed at 1235 cm^{-1} band (Fig. 2b), which corresponds to the C-H₂ bonds, typical vibration of sulfonic acid, that is observed as an elongation in the treated fiber spectrum. According to Dziuba et al. [22], this is due to the polysaccharides degradation in the fiber's cell wall, so that the surface area of the treated fiber is increased, these results could be correlated to the images from the pores presence in the fiber microographies.

X-ray diffraction of agave control and treated fiber.

Finding that the cellulose structure presents different levels of organization, ordered and stable conformations have been proposed, giving place to two sort of crystal structures: cellulose I and cellulose II, cellulose I being predominant in wood fibers and pulp [23]. The X-ray diffraction pattern of the control fiber and that caused by the alkaline treatment, are shown in Fig. 3. It can be observed that the proportion of crystalline cellulose was overall similar in the agave treated with alkali at 10%, to that in the control fiber, presenting pronounced peaks in $2\theta = 22^\circ$ and $2\theta = 15^\circ$, as well as small peaks in $2\theta = 72^\circ$, 60° , 58° , 46° , 48° , and 31° as it is shown. Authors as Astruc et al. [16] isolated nanospheres II from flax stems, stipulated that the peak between 14° and 16° belongs to amorphous materials. They obtained patterns of cellulose similar to those obtained in this work. The alkaline treatment dissolves smaller molecules such as lignins, hemicelluloses and possibly moieties from the less ordered cellulose [24], and part of the fiber amorphous material, affecting slightly but in this case not significantly, the shape of the X-ray pattern, which is overall, similar to that of the control.

Chen et al [17], produced highly crystalline nanocellulose, finding that all the diffractograms presented three main diffraction peaks; at $2\theta = 15.6^\circ$, 22.5° and 34.5° , which corresponded to the typical cellulose I β monoclinic lattice, meaning that the native crystalline structure of cellulose was also not damaged or disrupted during the nanocrystallization process.

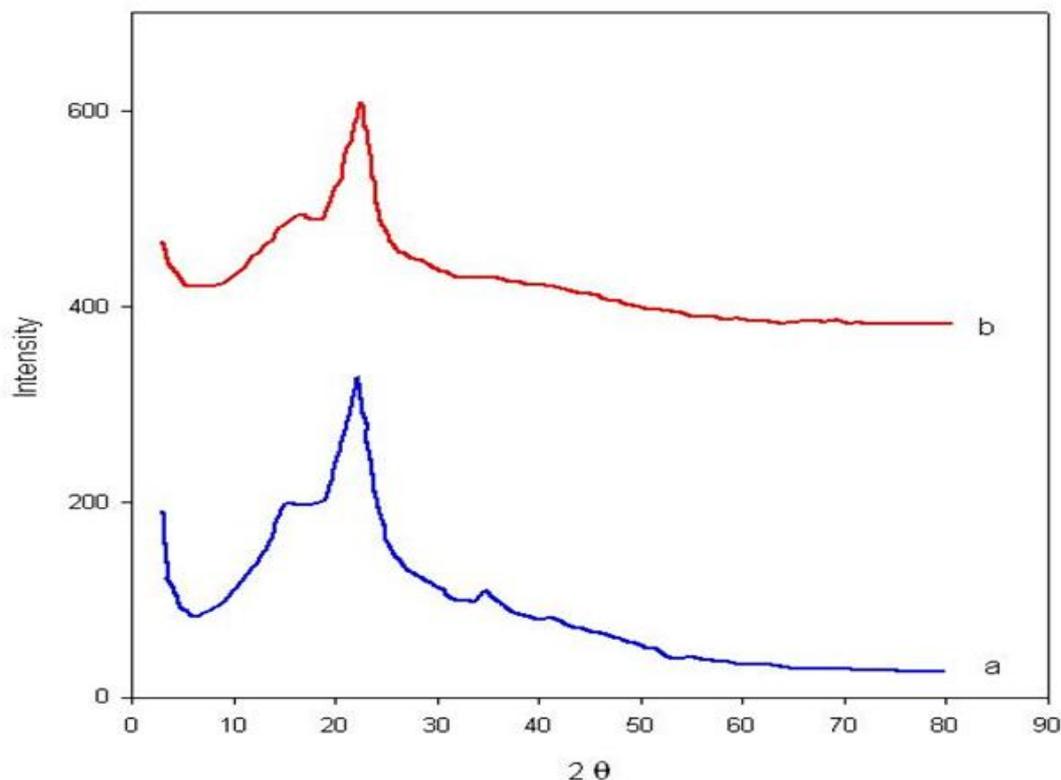


Fig. 3: X- ray diffraction pattern of Agave fiber: a)control sample, b)sample treated with 10% NaOH.

Dynamic tests: temperature sweeps

The gelatin's ability to create a thermoreversible gel is caused by the structural realignment of its amino acids in aqueous medium, wherein the protein tries to retake the initial triple-helix structure, going from a disorganized shape to a more organized one [25]. The rheological profiles of gelatin filmogenic solutions can be seen in Fig. 4. As expected, the storage (G') and loss (G'') moduli have higher values at low temperatures; among these moduli, it was possible to observe the typical inflection point indicating the change from solid to liquid phase and vice-versa. These results are typical of gelatin based systems without [11] or with a net charge [26]. Gelatin having physical gel behavior, shows two types of transitions: the one which goes from liquid to gel state or sol-gel (by cooling down, $T_{sol-gel}$) and the other way round or gel-sol (by heating up, $T_{gel-sol}$) [11, 7], such transitions were analyzed to determine the appropriate temperature.

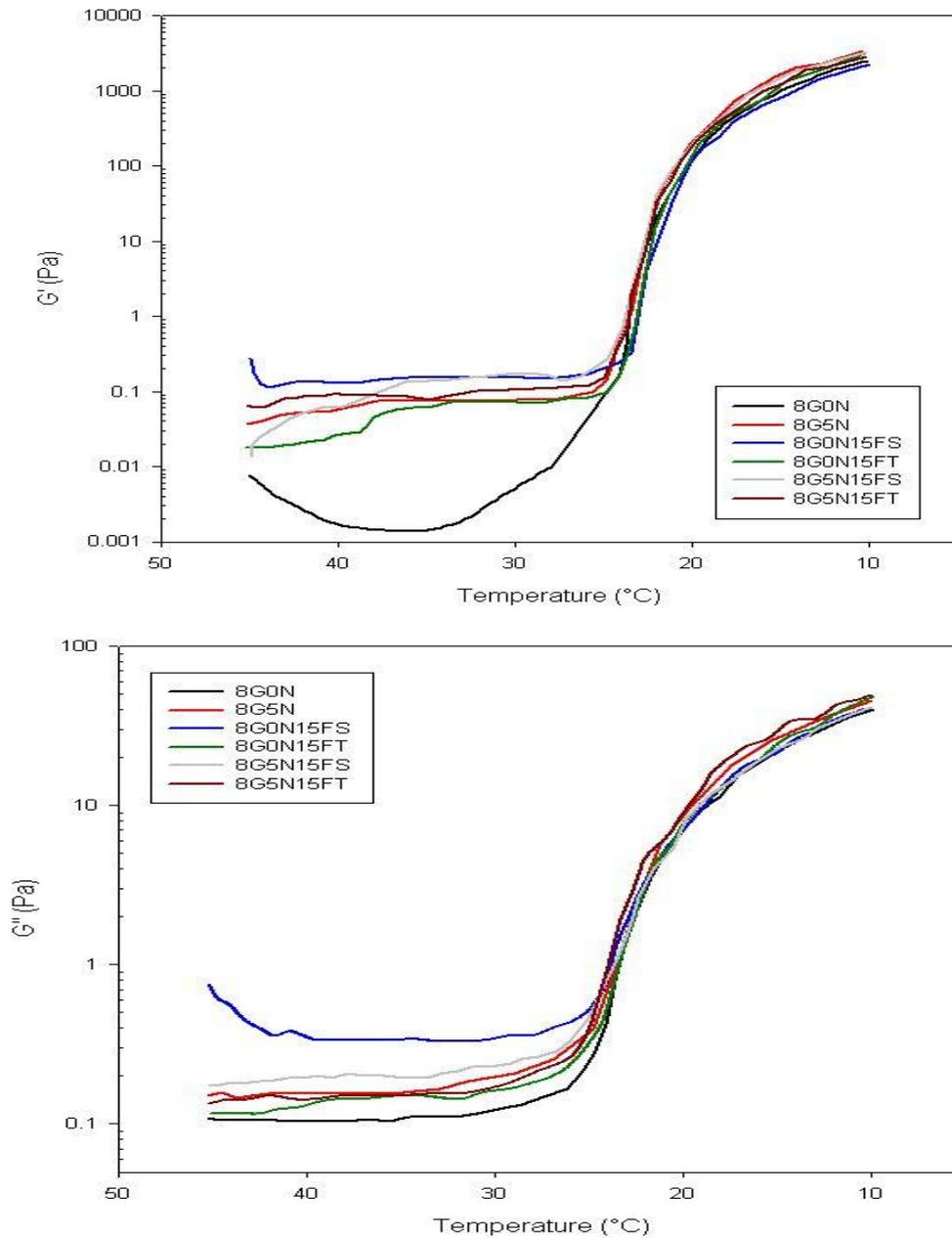


Fig. 4: The storage (G') and loss (G'') moduli, as a function of temperature of film-forming solutions from gelatin with montmorillonite and control and treated fiber. Rate of cooling temperature: $1^\circ\text{C}/\text{min}$.

When the temperature sweep was run from 45°C to 10°C (cooling), in all cases, as the temperature decreased, the elastic character (G') prevailed over the viscous character (G'') (Fig. 4).

In the case where the filmogenic solutions produced a rigid gel starting from 23°C , no statistically significant differences ($\alpha=0.05$) between treatments were found, suggesting that neither the additives (glycerol, nanoclay or control and treated fiber), nor the sonication step used to produce the filmogenic solutions, had an effect in the reorganization or creation of a triple-helix structure of the gelatin [27]. As increasing the temperature (heating), the control sample exerted no resistance on applying the strain (Fig. 5).

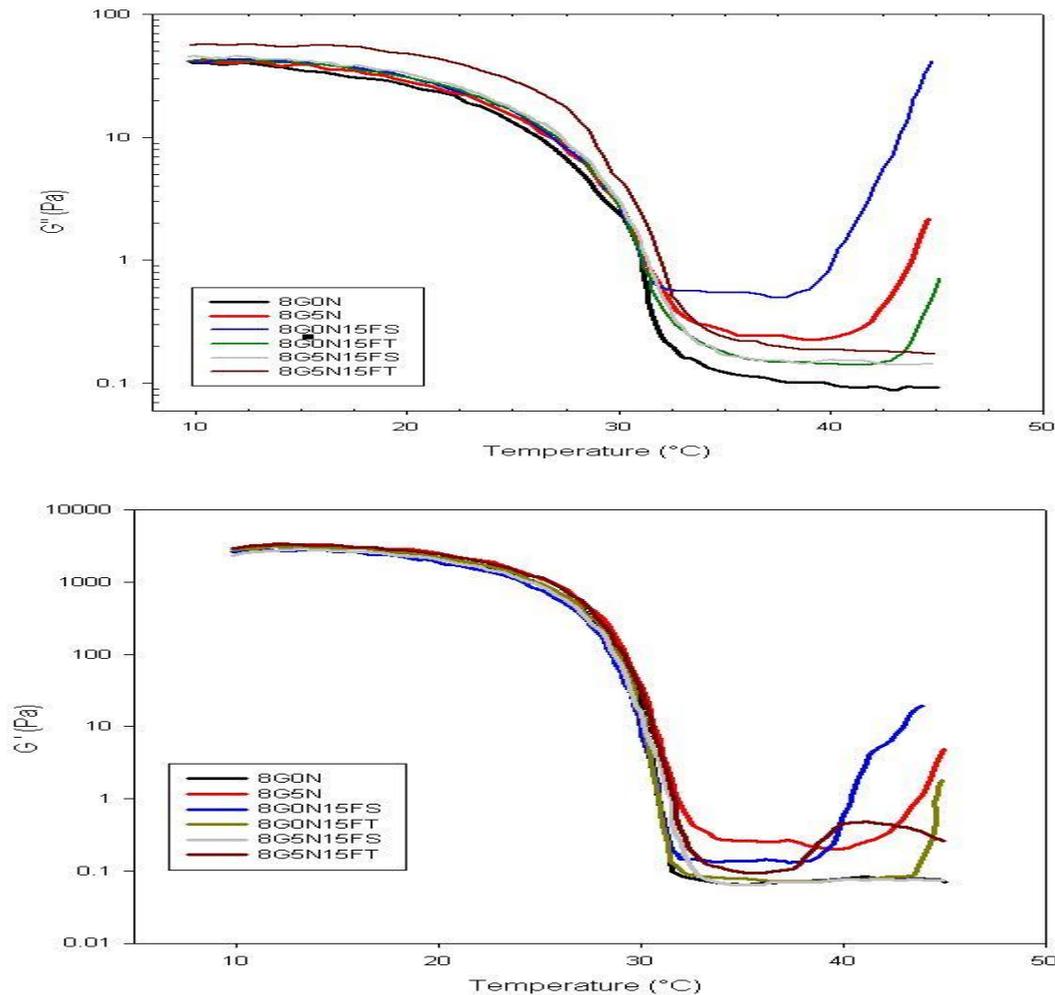


Fig. 5. The storage (G') and loss (G'') moduli, as a function of temperature of of film-forming solutions from gelatin with montmorillonite and control and treated fiber. Rate of heating temperature: $1^\circ\text{C}/\text{min}$.

The MMT improved the intermolecular interactions especially at high temperatures, where dissociation of the triple-helix structure dominated. It is worth mentioning that gelatin and MMT can form coacervates, because nanoclay platelets are electrostatically “cross-linked” by long gelatin molecules or vice-versa [26, 28]. Then, the effect of the MMT in the gelatin matrix could lead to higher viscoelastic moduli (G' and G''), compared to those obtained for the control, due to the great specific surface of the MMT, which gave place to interactions with the gelatin [26].

Similarly, the control and treated fiber has polar groups that could interact with protein amino acids, thereby improving stability and showing a trend to increase the storage modulus (G').

These results are different from those obtained by Cho et al. [27], who reported for pig gelatin dispersions with 6.7% solids, a transition temperature gel-sol of 36.5°C and a temperature sol-gel of 25.6°C . Such different values are probably due to the type and concentration of the gelatin used and to the additives incorporated in the film forming solutions. Transition temperatures sol-gel of the samples were in the ranges from 23.0 to 23.35°C and from 30 to 31°C for the transition gel-sol, without significant differences among the samples, both in derivative analysis and cross-linking analysis (Table 4). These results are different from those reported by Moraes et al. [11] for pig gelatin solutions, with a transition from sol-gel of 15.6°C , and gel-sol of 25°C , such differences might be related to the source and concentration of the gelatin used.

Dynamic tests: frequency sweep

The behavior of film-forming solutions was determined at 10°C as shown in Fig. 6. In all frequency profiles, the storage modulus (G') was greater than the loss modulus (G''); this behavior is typical of viscoelastic materials [8], and is related to the restructuration of gelatin molecules, which results in a system with predomination of the elastic behavior. Samples with MMT, control and treated fiber, displayed a tendency to increase the G' , suggesting that the interspersed structures of nanoclays, favors the formation of a more rigid gel, resilient and reversible, depending on frequency [11].

Such increase may be due to the macromolecular interactions between nanoclays and subsequently with fibers, since non-nanoclay and fiber solutions have a different behavior. The results of G'' showed the same behavior as G' , shown in Fig. 6, where it can be seen the effect of the addition of nanoclays to the control and treated fibers, which caused an increase in the values of G'' . Samples without nanoclays and ligno-cellulosic fibers (8G0N15FS) showed lower values of G'' respect to samples where the treated fiber (8G0N15FT) was incorporated, however, gel formation improved significantly in comparison to the control filmogenic solution (8G0N).

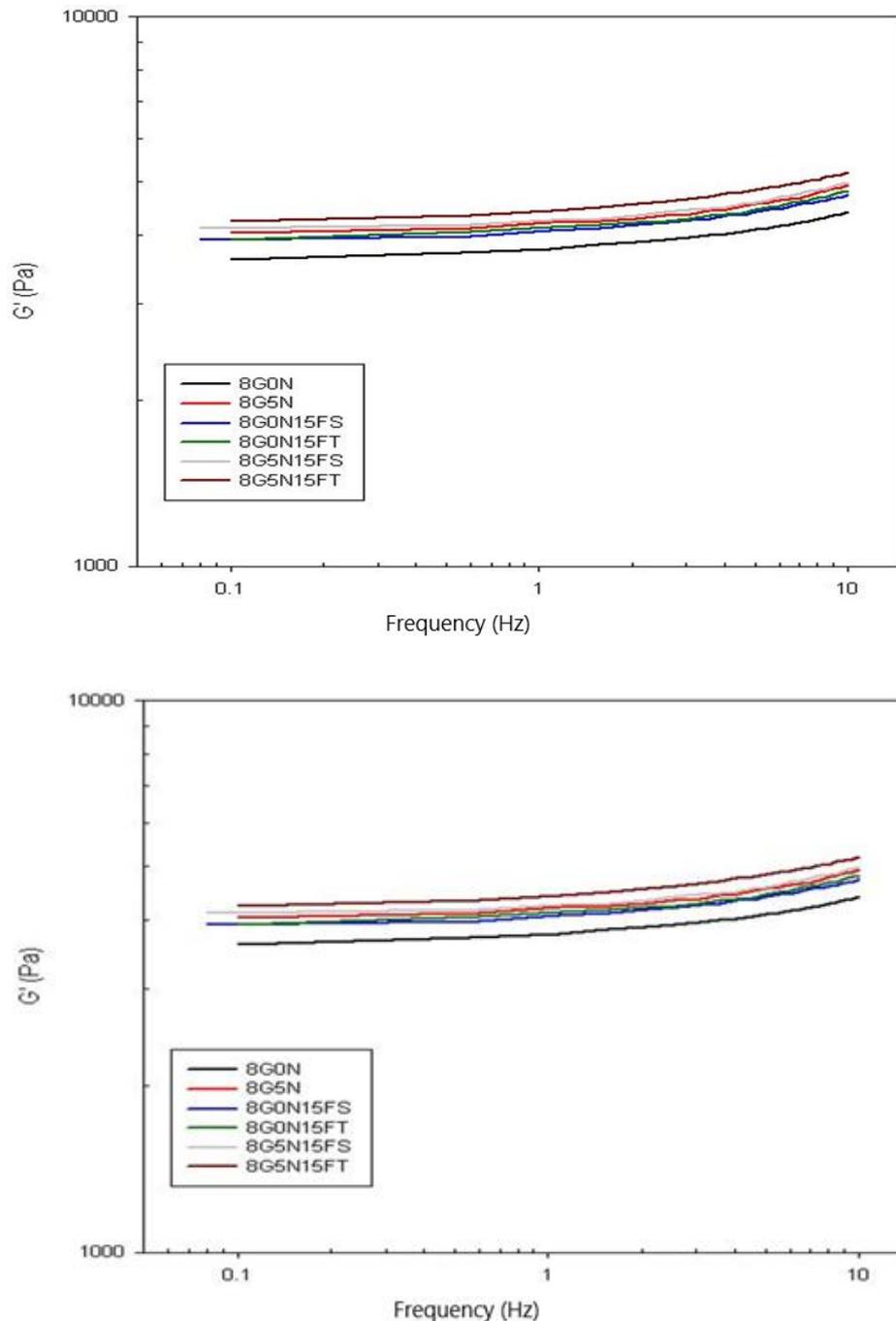


Fig. 6. The storage (G') and loss (G'') moduli, as a function frequency of film-forming solutions from gelatin with montmorillonite and control and treated fiber at 10°C .

IV. CONCLUSION

1. The hydrophilic groups of the agave fiber (hemicellulose and lignins) were removed by alkaline treatment; showing lower moisture content, but containing acid and neutral fiber with its corresponding components and no effect on its thickness.
2. The micrographs of the fibers, showed for the control sample, aligned openings all along its structure, while the alkaline treated sample, showed a slightly closer structure.
3. The FTIR spectra showed typical absorption areas from cellulose, with similar crystallinity patterns irrespect of the fiber treatment.
4. The gelatin filmogenic solutions showed viscoelastic behavior with predominance of the elastic over the viscous character, with transition temperatures Tgel-sol and Tsol-gel, without significant differences among the samples, the nanoclay(montmorillonite) incorporation in the filmogenic solution favored the formation of a more elastic gel.

ACKNOWLEDGMENTS

Thanks are due to CEPROBI-Instituto Politecnico Nacional in Mexico (project 20180187) and to São Paulo Research Foundation (FAPESP), for the Grants (09/53992-5, 10/50643-7).

REFERENCES

- [1]. Uribe, V.M., Mehrenberger, P.Y. (1996). Los polímeros "síntesis, caracterización, degradación y reología". Instituto Politecnico Nacional. Primera Edición. México. pp. 239-263.
- [2]. Andrade-Pizarro, R. D., Skurtys, O., Osorio-Lira, F. (2015). Effect of cellulose nanofibers concentration on mechanical, optical, and barrier properties of gelatin-based edible films. *DYNA*, 82 (191): 219-226.
- [3]. Aguilar-Méndez M.A., Martín-Martínez E.S., Ortega-Arroyo L., Cruz-Orea A. (2010). Application of differential scanning calorimetry to evaluate thermal properties and study of microstructure of biodegradable films. *International Journal of Thermophysics*. 31: 595-600.
- [4]. Villanueva-Rodriguez, S., Escalona-Buendia, H. (2012). Tequila and mezcal: sensory attributes and sensory evaluation. In: John Piggott (editor). *Alcoholic Beverages. Sensory Evaluation and Consumer Research*. A volume in Woodhead Publishing Series in Food Science, Technology and Nutrition. Elsevier. London. pp. 359-378.
- [5]. Moreira, Alfonso, A.P. (2009). Caracterização de filmes biodegradáveis a base de blendas de gelatina e PVA reticulados com glutaraldeído. Relatório. Universidad de Sao Paulo. Facultad de Zootecnia e Engenharia de Alimentos.
- [6]. Bae H.J., Park, H.J., Hong, S.I., Byun, Y.J., Darby, D.O., Kimmel, R.M., Whiteside, W.S. (2009). Effect of clay content, homogenization RPM, pH, and ultrasonication on mechanical and barrier properties of fish gelatin/montmorillonite nanocomposite films. *LWT - Food Science Technology*, 42: 1179-1186.
- [7]. Vanin, F.M., Hirano, H. M., Carvalho, A. R., Moraes, F. I.C., Bittante, B.Q. A.M., Sobral, A.P.J. (2014). Development of active gelatin-based nanocomposite films produced in an automatic spreader. *Food Research International* 63: 16–24.
- [8]. Ferry J.R. (1980). *Viscoelastic properties of polymers*. 3rd Ed. John Wiley and sons. New York.
- [9]. AOAC. 1997. *Official Methods of Analysis*, 17th Ed (W. Horwitz, ed), Association of Official Analytical Chemists, Washington, DC.
- [10]. Silva, D.J. (2002). *Análise de Alimentos: Métodos Químicos e Biológicos*, editora UFV, Viçosa.
- [11]. Moraes, I.C.F., Carvalho R.A., Bittante A.M., Solorza- Feria, J. and Sobral P.J.A. (2009). Film forming solutions based on gelatin and poly(vinyl alcohol) Blends: thermal and rheological characterizations. *Journal of Food Engineering*. 95: 588-596.
- [12]. Andrade- Mahecha, M. (2011). *Uso de biopolímeros e nanofibras obtidos a partir de rizomas de biri (canna indica L.) para a elaboração de filmes biodegradáveis e coberturas comestíveis*. Tesis de Doutorado. UNICAMP. Departamento de Engenharia de Alimentos.
- [13]. Sreekala, M.S., Thomas, S. (2003). Effect of fibre surface modification on water-sorption characteristics of oil palm fibres. *Composites Science and Technology*. 63: 861-869.
- [14]. González-García, Y., Gonzalez-Reynoso, O., Nungaray-Arellano, J. (2005). Potencial del bagazo de agave tequilero para la producción de biopolímeros y carbohidrasas por bacterias celulolíticas y para la obtención de compuestos fenólicos. *E-Gnosis*. Universidad de Guadalajara, Mexico.
- [15]. Geethamma VG, Kalaprasad G, Groeninckx Gabriel., Sabu, T. (2005). Dynamic mechanical behaviour of short coil fiber reinforced natural rubber composites. *Composite: Part A*. 36:1499–1506.
- [16]. Astruc, J., Nagalakshmaiah, M., Laroche, G., Grandbois, M., Elkoun, S., Robert, M. (2017). Isolation of cellulose-II nanospheres from flax stems and their physical and morphological properties. *Carbohydrate Polymers*. 178: 352–359.
- [17]. Chen, W.Y., Lee, V.H., Hamid, A.B.S. (2017). Investigation of optimal conditions for production of highly crystalline nanocellulose with increased yield via novel Cr(III)-catalyzed hydrolysis: Response surface methodology. *Carbohydrate Polymers*. 178: 57–68.
- [18]. Youn Oh, S., Dong Il Yoo; Younsok Shin, Gon Seo. (2005). FTIR analysis of cellulose treated with sodium hydroxide and carbon dioxide. *Carbohydrate Research*. 340: 417–428.
- [19]. Cazaurang M. N; Peraza S; and Cruz-Ramos C. A. (1990). Dissolving-grade pulps from henequen fibers. *Cellulose Chemistry and Technology*. 24: 629–638.
- [20]. Valadez-Gonzalez A., Cervantes-Uca, J.M., Olayob, R., Herrera-Franco, P.J. (1999). Effect of fiber surface treatment on the fiber-matrix bond strength of natural fiber reinforced composites. *Composites: Part B*. v, 30: 309–320.
- [21]. Qing -Shen, A. B., Tao- Zhang, B., Mei-Fang Z. A.B. (2008). A comparison of the surface properties of lignin and sulfonated lignins by FTIR spectroscopy and wicking technique. *Colloids and Surfaces A: Physicochemical and Engineering Aspect*. 320: 57–60.
- [22]. Dziuba, B., Andrzej Babuchowski., Dorota Nalecz. and Marta Niklewicz. (2007). Identification of lactic acid bacteria using FTIR spectroscopy and cluster analysis. *International Dairy Journal*. 17(3): 183-189.
- [23]. Uhlin, K.L., Atalla, R.H., Thompson, N.S. (1995). Influence of hemicelluloses on the aggregation patterns of bacterial cellulose. *Cellulose*. 2(2): 129-144.

- [24]. Evans, R., Newman, R.H., Roick, U.C., Suckling, I.D. and Wallis, A.F.A. (1995). Changes in cellulose crystallinity during craft pulping. Comparison of infrared, X-ray diffraction and solid state NMR results. *Holzforschung*. 49 (6): 498-504.
- [25]. Arvanitoyannis I.S. (2002) formation and properties of collagen and gelatin films and coatings. In: Gennadios A. (editor). *Protein-Based films and coatings*. Boca Raton: CRC Press. pp. 275-300.
- [26]. Jorge, C.M.F., Flaker, C.C.H., Nassa ,F.S., Moraes, F. I.C., Bittante, B.Q. A.M., Sobral, A.P.J. (2014). Viscoelastic and rheological properties of nanocomposite-forming solutions based on gelatin and montmorillonite. *Journal of Food Engineering* 120: 81–87.
- [27]. Cho, S.M., Gu, Y.S. and Kim, S.B., (2005). Extracting optimization and physical properties of yellowfin tuna (*Thunnusalbacares*) skin gelatin compared to mammalian gelatins. *Food Hydrocolloids*. 19: 221–229.
- [28]. Qazvini, N.T., Bolisetty, S., Adamcik, J., Mezzenga, M. (2012). Self-healing fish gelatin/sodium montmorillonite biohybridcoacervates: structural and rheological characterization. *Biomacromolecules*. 13:2136-2147.

J. Solorza-Feria "Chemical Composition And Structure Of Modified Agave Fiber As Related To The Rheology Of Gelatin Film-Forming Solutions With Montmorillonite And Fiber Addition." *American Journal of Engineering Research (AJER)*, vol. 7, no. 08, 2018, pp. 165-176