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Development of gels composed of pectin/microcellulose from mango and peg for biotechnological applications

M.H. Ferraresi[1*]; V.M.C. Medal[1]; B.D. Neto[1]; I.D. Coutinho[2]; L.A. Dutra[3]; A. Dametto[4]; W. R. Lustri[1]; R.M. Nascimento de Asuncion[5]; M.R. Costa Iemma[1]; M.C.A.Ferreira Rezende[6*]; M.A. Sabino[7]; H.S. Barud[1]; R.A. Rezende[1*]

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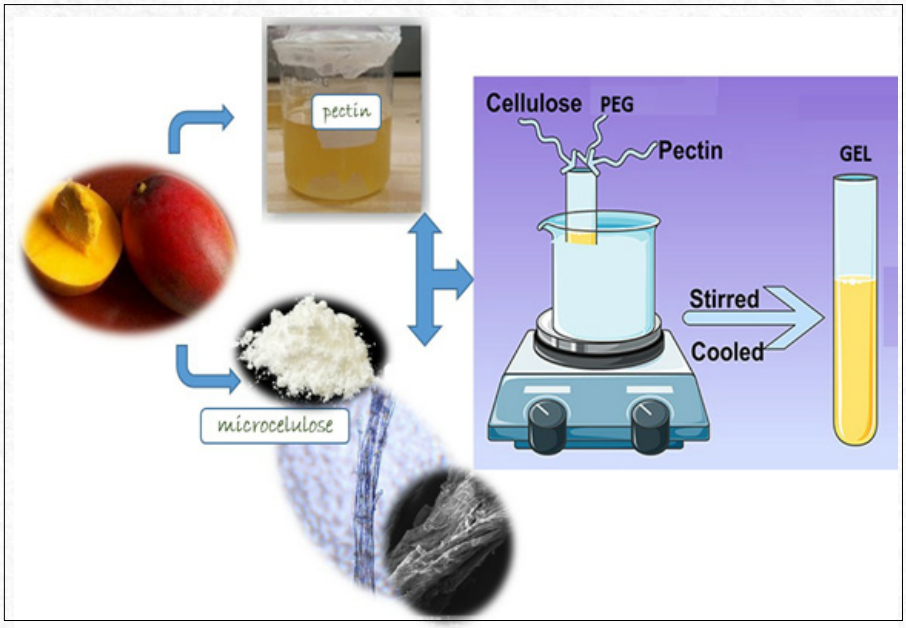
*Corresponding author: E-mail address: rarezende@uniara.edu.br



Abstract: Based on the concept of circular economy, waste from vegetable sources, mango between them, are reused and can be extracted some biomolecules as pectin and cellulose to be used in biotechnological applications, also for cosmetic industry, even use as biomaterials. In this context, the thermal, morphological, and rheological characterization of pure biopolymers and blends as gel was carried out to analyze their potential to produce membranes, scaffolds or bioprinted structures. FTIR analysis was performed demonstrating similar behaviors and groupings between the studied materials. The crystallinity index and amorphous areas obtained by XRD assay. Furthermore, other analysis performed was concerning the degree of polymerization according to the viscosity of the samples demonstrating the flow time of the polymers. After characterizing the polymers, formulations of gels combined mango pectin, mango microcellulose and vegetable microcellulose with wood sources were produced through calorimetry, scanning electron microscope (SEM), and rheology analyses. PEG was added with the purpose of improving the rheological properties and compatibility between the phases of these gels. We conclude through the characterization of these materials the viability in the production of structures for biotechnological applications as scaffolds in the medical areas between others.

Keywords: Pectin. Microcellulose. Gels. Rheology. Mango. Biotechnological Applications.

[1]Postgraduate Program in Biotechnology in Regenerative Medicine and Medicinal Chemistry, University of Araraquara (Uniara), Araraquara-SP, Brazil.
[2]Natcrom Sustainable Solutions, Araraquara-SP, Brazil.
[3]Institute of Chemistry of Araraquara, Araraquara-SP, Brazil.
[4]BioSmart Nanotechnology, Araraquara-SP, Brazil.
[5]Federal University of Uberlândia, Faculdades de Ciências Integradas do Pontal, Ituiutaba-MG, Brazil.
[6]3D BioEng, Avenida J. Fernandes Mattos, 311, 80 Distrito Industrial, 14808-162, Araraquara-SP, Brazil.
[7]Universidad Simón Bolívar, Caracas, Venezuela.

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Introduction

Mango is a tropical fruit highly valued worldwide for its flavor, aroma, and nutritional value. Pectin and cellulose are two compounds or by-products found in mango that have gelling properties. Pectin is a water-soluble fiber widely used to thicken and stabilize food and pharmaceutical agents. Cellulose, in turn, is a water-insoluble fiber that has the ability to absorb water and form a firm gel.[1]

Brazil's valorization of mango by-products to produce biotechnological products is still a little-explored area, but there are some studies and initiatives in this direction. Some examples include mango residue extracts (peel, seed, and residual pulp) with bioactive compounds; they can produce bioethanol through fermentation [2]; the enzymes present in mango residues can be used in industrial biomass hydrolysis processes[3]. Cellulose fibers can be used as raw material for paper production[4]; they can be used as a source of nutrients in organic fertilizers, etc.[5] It is still necessary to invest in research and development in this area to explore the full potential of these mango by-products.

But, in general, based on the scientific literature some research has been reported related to gels combining pectin and cellulose and derivatives. A case about improving the texture and consistency of food products, in addition to providing functional properties to the final product.[6] In addition, these gels can also be used in pharmaceutical products, such as controlled drug delivery systems.[7] Additionally, due to their biocompatible and bioactive properties, pectin and cellulose are considered promising materials for tissue engineering.[8]

In the case of hydrogels, which are materials with high water content and mechanical properties similar to those of biological tissues, mango pectin and microcellulose gels could be used in the production of hydrogels through various techniques, such as, for example, chemical gelation or physics, which can also lead to thinking about the fabrication of 3D scaffolds that are structures that provide mechanical support for cell growth and can be produced using these hydrogels..[9]

Based on the concept of circular economy, residues from vegetable sources, particularly from mangoes, can be explored, and pectin and cellulose microfibers can be extracted to be used as biomaterials.[10]

In this sense, this work aims at developing gels mixing pectin and mango microcellulose including a modifying phase (polyethylene glycol PEG) to improve the rheological behavior and proceed with their characterization.

Materials and Methods

Mango pectin (PECM) samples were provided by NATCROM P&D ("https://natcrom.com/") and sized using the Polymix® PX-IG 2000 Impact Grinder Kinematica ball mill available from BIOSMART Nanotechnology LTDA ("https://www.biosmartnano.com/") for 5 minutes at frequencies of 20 Hz, resulting in powdered (PECM) particles. Dissolutions and gels of (PECM) in distilled water were made using the ratio of PECM:distilled H_2O for dissolution of 15% m/v; remaining in agitation for 30 minutes for complete homogenization.

The mango microcellulose (MCM) samples were provided by the company NATCROM P&D ("https://www.natcrom.com/").[11] They were extracted through the hydrolysis of AHP (Alkaline Hydrogen Peroxide) to eliminate hemicellulose and samples lignin. After hydrolysis, the samples were ultrasonicated for 1, 2, 3 hours in a 1% H_2O_2 solution with a low frequency ultrasound processor (20 kHz).

Drops of H_2SO_4 were added to the suspension (pH 5.0) and vigorously mixed using vortex mixer for 2 min to avoid agglomeration of disintegrated fibers avoiding possible equipment corrosion – subsequently lyophilized.[12]

The plant microcellulose (MCV) samples were provided by the Biopolmat laboratory at the University of Araraquara (UNIARA); being this MCV obtained through renewable and sustainable bases with reuse of wood fibers and used as a comparison material in relation to the extracted MCM. This MCV material was produced and donated by the Brazilian company Suzano.[13].

Polyethylene glycol-400 (PEG) use as dispersant and rheological helper was from Sigma Aldrich.

Characterization of pectin and microcellulose phases

Fourier transform infrared spectroscopy (FTIR)

In order to verify the chemical structure of the neat polymers PECM, MCM, MCV to be used to obtain the gels, the analysis was performed using an Agilent Cary 630 FTIR-ATR benchtop spectrometer with 64 scans (4500 cm^{-1} to 500 cm^{-1}), resolution of 4 cm^{-1}, present in the LABQUIM laboratory located at the University of Araraquara – UNIARA.

Degree of polymerization (DP)

Given the characteristics of gels obtained by mixing polysaccharide phases, it is important to know the size of the molecules. The degree of polymerization of the microcelluloses and pectin was obtained according to TAPPI standard T 2300 m-94: Viscosity of pulp (capillary viscometer method, 2013)[14] using the transparent Cannon-Fenske viscometer n°150.14 500 mg of each sample separately and added to 50 ml of distilled water, followed by homogenization in magnetic stirring

at 400 rpm/min for 5-min at room temperature. After this time, 50 ml of Ethylenediamine Cupric solution were added with hydrogen bath and taken again to magnetic stirring for 2 hours at 400 rpm/min. After the stirring time, for each case, 7 ml of the mixture were placed in the viscometer and inserted in a water bath at a temperature of 25 °C for 5-min. They carried out measurements of the flow times in triplicate and the average obtained was used to calculate the degree of polymerization according to the calculations proposed by Andritsou et al.[15]

X-Ray Diffraction

It is important to have information about the morphology of the materials, especially when the properties of the gels can reflect their composition related to the phases in the biocomposite material. X-ray diffraction (XRD) was performed using a Shimadzu model XRD-6000 diffractometer, operating at a power of 40 kV with 40 mA of current and CuKα radiation (λ = 1.54148 Å), in the angular range of 2θ from 5 to 40°, sweep speed of 2°/min and angular step of 0.02°. The samples submitted to XRD were: PECM, MCM, and MCV. The experiment was carried out at the Institute of Exact and Natural Sciences at the Federal University of Uberlândia, Campus Pontal, Ituiutaba, MG-Brazil.

TG curves

The SDT Q600 equipment from the company TA Instruments, present in the Biopolmat laboratory of the University of Araraquara – UNIARA, was used to carry out the thermogravimetric analysis (TG). Samples around 20 mg and 25 mg were heated at 10 °C/min into an alumina crucible from 30 °C to 700 °C, under a nitrogen atmosphere (30 mL/min flow rate).

Scanning Electron Microscopy – SEM

In the case of the PECM and MCM sample, it was also observed by optical microscopy (using a conventional four-lens binocular biological microscope by Olen) only with the aim of having evidence of the micrometric character before being observed by SEM.

In order to know the dimension or magnitude of the MCM and MCV microcellulose, the scanning electron microscopy (SEM) images were obtained using "Electronic microscope with field emission pistol - FEG-SEM Tescan model Mira 3 XMU with e-lithography beam and with EDS 60mm Bruker". QUANTAX EDS Bruker 60 mm with XFlash® energy dispersive X-ray detector and Espirit software. Located at the Center for Information Technology (CTI) Renato Archer, in Campinas, SP.

Cytotoxicity

Cell viability tests were carried out at Lecer laboratory of the University of Araraquara. The MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetryzoline bromide) assay was adopted. On the MTT assay, when there is the incubation with fibroblastic cells, the substrate is broken down by mitochondria, where the carbon dioxide emitted by the cells reacts with the MTT and it is transformed from a yellow compound into a dark blue compound (formazan) providing information on viable cells.[16]

For cytotoxicity evaluation, the culture medium of L929 cells grown in 24-well plate (1x10^4/well) was removed and a volume of 200 µl of conditioned medium was added. Cells were cultivated in the presence of different concentrations of conditioned media for each material (100, 50 and 25%) and the plate kept in an oven at 37° C with 5% CO_2 saturation for 24 hours. Subsequently, conditioned medium was removed and added 50 µl/well of the MTT solution (5mg/ml) in PBS1X. After incubating the plate for 4 hours in oven at 37° C with 5% CO_2 saturation, the MTT solution was removed and the formazan crystals were solubilized in DMSO for absorbance reading at a wavelength of 570nm, in a Spectra Max Gemini XS plate reader (Molecular Devices).

Preparation and characterization of gels

Gels were prepared by combining mango pectin PECM, mango microcellulose MCM, plant (wood) microcellulose MCV with specific concentrations of each material (see table 1). The samples were incorporated using a mechanical mixer present at the Biopolmat lab, for 5-min and 500-rpm. Prior to this mixture, the samples of MCM and MCV were subjected to oxidation with hydrogen peroxide (commercial) to improve solubility with water, time 1 hour with magnetic stirring.

Two gel samples were prepared with a percentage of polyethylene glycol (PEG), improving the compatibility between the phases and possibly improving the gels' rheological behavior.

Table 1 - Concentrations (m/v) of the phases used to prepare the gels.

Gel (PEC/MC)	% PECM(*)	% MCM	%MCV	% PEG
($PECM^{15\%}MCM^{1\%}$)	15	1	0	0
($PECM^{15\%}MCM^{2\%}$)	15	two	0	0
($PECM^{15\%}MCV^{1\%}$)	15	0	1	0
($PECM^{15\%}MCV^{2\%}$)	15	0	two	0
($PECM^{15\%}MCM^{1\%}PEG^{5\%}$)	15	1	0	5
($PECM^{15\%}MCV^{1\%}PEG^{5\%}$)	15	0	1	5

(*) 10, 15 and 20% m/v solutions were prepared, but the 10% solutions were too dilute, and the 20% solution was very viscous and lumpy, so 15% m/v was selected.

FTIR-ATR spectra

The components of the chemical group of the gels were identified by Fourier Transform Infrared Spectroscopy (FTIR) in Attenuated Total Reflectance mode (FTIR-ATR) located at the CTI Renato Archer Information Technology Center, in Campinas, SP. FTIR spectra were measured in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} on a spectrometer (PerkinElmer, Spectrum GX, USA).

Rheology Analysis

The rheology analyzes were carried out in the Biopolmat lab in the University of Araraquara – UNIARA, using the TA Instruments AR1500ex 8B3689 rheometer at room temperature 25°C, 10.0rad/s; 0.01 to 100%. Shear rate 0.01 to 100.0. The geometry used was a parallel plate of 40.0mm Peltier plate Sandblasted.

SEM

Scanning electron microscopy (SEM) were performed as described in point 2.1.5 above. But in the case of gels, after mixing, they were placed on a glass plate to form films by drying (solvent casting or slow evaporation of the solvent) and at room temperature, to then be finally metalized and observed by SEM.

Results and Discussion

Characterization of the base polysaccharides of the gels: PECM, MCM, MCV

Thesamples of PECM, MCM, and MCV submitted to FTIR-ATR analysis allow identifying the possible vibrations of the characteristic functional groups for each one of these polysaccharides (pectin and cellulose), thus these main bands can be observed in Figure 1. Particularly PECM shows its initial band at 1000 cm^{-1} corresponding to COC functional groups. Another band behavior between 2800 cm^{-1} and 3000 cm^{-1} corresponding to the CH group. Another band at 3300 cm^{-1} corresponding to the OH group, corroborating the structural formula of pectin. In the analysis of the MCM wavenumber was obtained at 1000 cm^{-1} corresponding to the COC group, and the band at 1300 cm^{-1} corresponding to the CH group, the band at 1500 cm^{-1} corresponding to the C=C group. Another band at 2800 cm^{-1} corresponding to the CH group and the band at 3300 cm^{-1} corresponding to intramolecular hydrogen bonds in cellulose OH. The MCV analysis demonstrated similar behavior with mango microcellulose which is fully expected, thus showing the 1750 cm^{-1} band approximately corresponding to the vibrations of the acetyl group and hemicellulose ester or carboxylic group.

Research carried out obtained similar cellulose functional groups and observed C=O groups in esters, CO in ethers, CH3 functional groups, and OH hydroxyl groups. Peaks around the 1505 cm^{-1} and 1512 cm^{-1} bands, attributed to the CC stretch, and the 1737 cm^{-1} band associated with the C=O stretch was also observed, the peaks in the 2900-2800 cm^{-1} region associated to CH stretching. The band in the region from 3900 cm^{-1} to 3300 cm^{-1} attributed to the OH group thus corroborating with the bands found in this research and compared with the cited bands.[17-19]

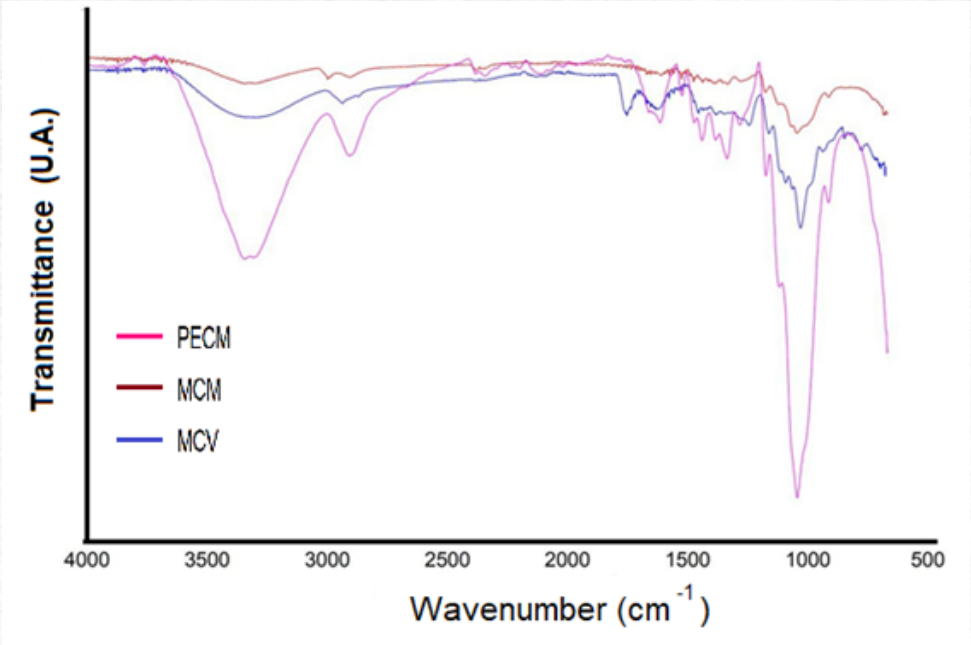
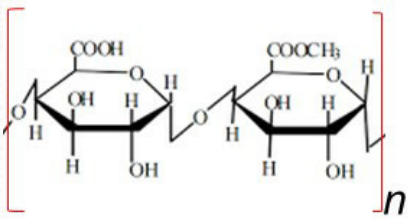
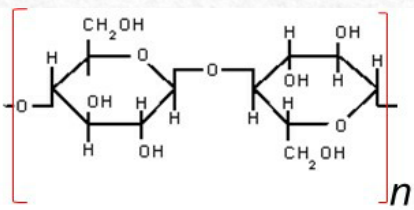


Figure 1 – FTIR of the analyzed samples PECM, MCM, and MCV.

After the chemical structure of the components of the gels has been verified by spectroscopy, it is important to also verify the molecular size of these polysaccharides.[20] Other results of these calculations were based on the literature and are shown in Table 1.[1]

The results of the degree of polymerization (n) and the consideration of the molecular structure of the repetitive unit allow, through the molecular formula, to estimate a molar mass value of the polysaccharides studied in this research.

Table 1 – Degree of polymerization of pectin, microcellulose, mango cellulose microfibers and vegetal (wood) microcellulose samples.

Sample	Average Flow Time (s)	Degree of polymerization (n)	Molecular formula of the unit Monomeric	Molecular mass calculated (g/mol)*
PECM	175	436	 Pectin structure (FM= $C_{13}O_{12}H_{18}$)	≈ 1.6 x 10^5
MCM	79	194	 Cellulose structure (FM= $C_{12}O_{10}H_{20}$)	≈ 6.3 x 10^3
MCV	1501	2000		≈ 6.4 x 10^5

(*) KDa = 103 g/mol

In the case of pectin, there are reports in the scientific literature showing that the molar mass varies between 50-150 KDa, and more specifically there are reports that show values between (154-250) KDa for fruits such as grapefruit[21], and enter (164-247) KDa for orange[22], and for pectin extracted from mango peel there are reports that give approximate results between 20-22 KDa[23]. In the case of cellulose, regardless of its size on the micrometer scale, it will be the extraction process that will allow defining the molecular size. In this case, it was observed how the MCM has a molar mass almost 100 times less than that of the MCV, given that the latter would be extracted at an industrial level (given its commercial provider) and on the contrary in the case of the MCM that the extraction would be done at laboratory level, it is possible that the differences between these protocols lead to this structural difference between MCM and MCV. In general, the scientific literature reports results between 300-550 KDa for cellulose extracted from wood and other natural sources.[24]

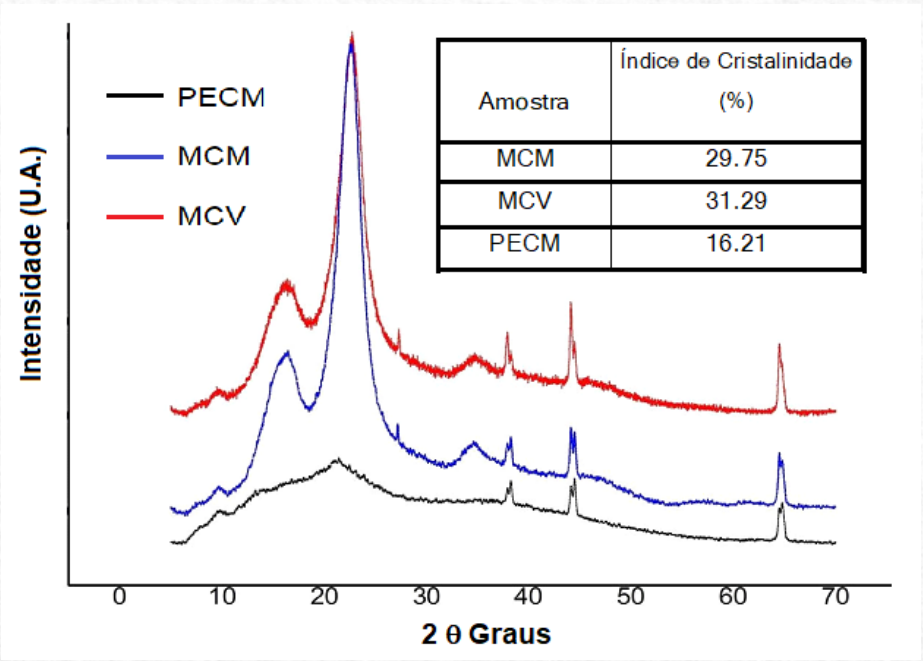
According to XRD results, there are several methods in the literature for calculating crystallinity indexes for natural and synthetic polymers [25]. Figure 2 shows the XRD-diffractogram of cellulose and pectin biopolymers with peaks of moderate intensity between 10 ° and 20 ° and peaks of greater intensity between 20° and 30° from mango cellulose showing characteristic behavior cellulose and low-intensity peaks for amorphous pectin demonstrating a typical region of non-crystallize pectin. [26] Peaks around 35° to 70° could be attribute to some partially hydrolyze cellulose chains. The calculation results of the crystalline and amorphous areas are also shown in the table into the Figure 2.

The literature has shown crystallinity indexes for corn husk cellulose microfibers, between 53% and 47%; microcellulose and cellulose microfibers derived from cassava are around 49%. The degree of crystallinity of commercial citrus pectin is equivalent to 17%. The values for MCM and MCV are a little below those reported in the literature. Still, it may be considering the different drying processes of each sample, which allows the reorganization of the chains, thus generating other crystallinity indices and a higher amorphous degree. In the case of pectin, the values found here coincide with those reported.[19,27,28]

TG/DTG curves, Figure 3, of PECM, MCM, and MCV allow us to observe the determining temperatures for the mass degradation of the materials submitted to analysis.

TG/DTG curves for PECM indicate that degradation temperature starts around 200 °C and shows the mass loss in three steps up to almost zero up to 700 °C. DTG presents degradation peaks at 268 and 302 °C. TG/DTG curves for MCM and MCV show similar thermal stability with degradation starting around 250 °C and a correspondent peak in DTG curves between 306 and 322 °C; the thermal decomposition process for celluloses is almost similar, except that the MCV shows a higher percentage of carbonaceous residue up to 700 °C. Notably, until the beginning of degradation, the materials present a loss of approximately 6-10% of moisture in the temperature range close to 100-120 °C.

The images obtained by optical microscopy and



Amostra	Índice de Cristalinidade (%)
MCM	29.75
MCV	31.29
PECM	16.21

Figure 2 – XRD of MCM and MCV microcelluloses and mango pectin. Calculated values of the crystallinity index of each phase to formulate the gels.

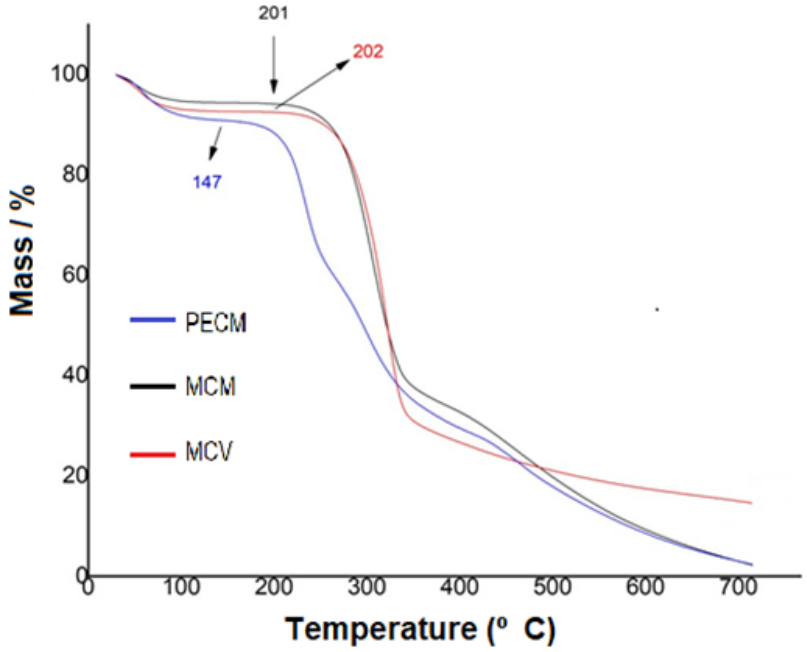


Figure 3 – TG/DTG curves for PECM, MCM, MCV.

SEM are summarized in Figure 4; demonstrating an irregular but homogeneous surface with a fibrillar character. The dimensions of the fiber diameter correspond to the micrometric scale, which is expected according to the extraction process. According to the scale of the images, it is possible to observe some fragmented irregularities of the cellulose, which could be attributed to oxidation caused by hydrogen peroxide. Actions of hydrogen peroxide on the cellulose of the green coconut shell were observed significant changes in the surface of the cellulose making it more irregular and fragmented than the cellulose without treatment with peroxide[29,30].

Figure 5 shows the morphological characteristics and dimensions of the fibers that make up the

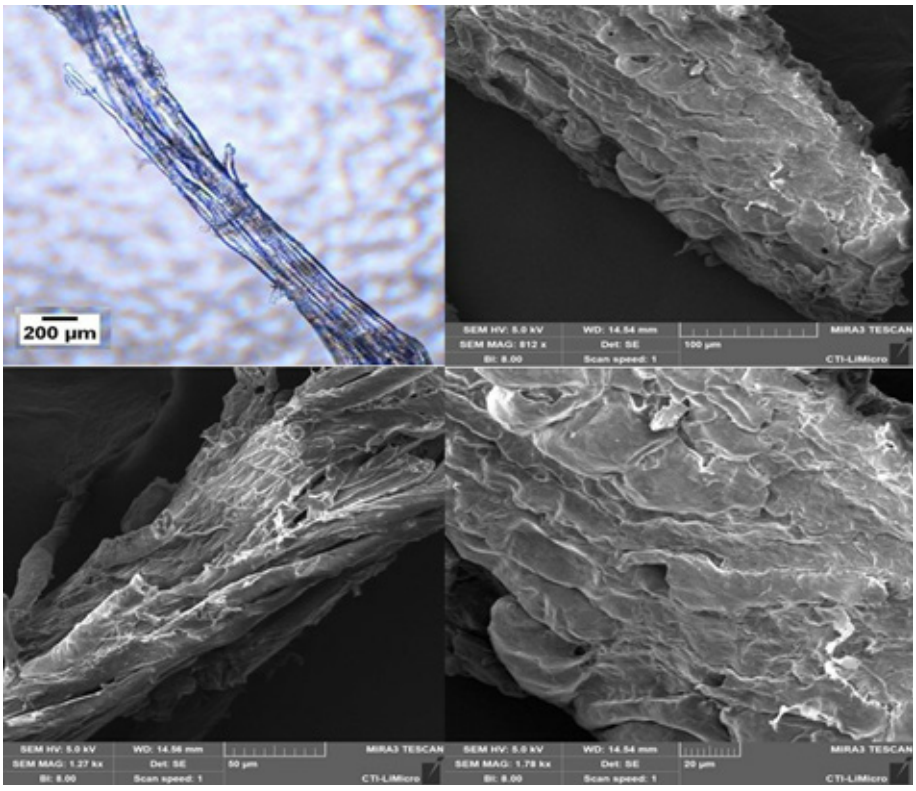


Figure 4 - SEM micrographs of the MCM sample that will be used in the preparation of pectin gels. There is evidence by optical microscopy that allows verifying the fibrillar character of the cellulose, which is characteristic of this material.

MCV. MCV shows characteristics similar to MCM, regardless of the extraction process and also its molar mass. MCV also has some surface and fragmented irregularities; this could be attributed to oxidation caused by hydrogen hydroxide.

Finally, pectin, given its character as a fluid and low-viscosity material, forms a film during the drying process so that it can be observed by SEM. The images show that the material owns certain surface irregularities. These irregularities and size of PECM particles can be viewed by the optical microscopy images (Figure 6).

For cytotoxicity, the method used was the reduction of the MTT*(3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide)*, which consists of analyzing the conversion of MTT (soluble) into formazan crystals (insoluble form) by the action of mitochondrial dehydrogenase enzymes. The absorbance results were represented as percentage of cell viability in relation to the control group (100%), treated and plotted in the GraphPad Prism software as can be viewed in Figure 7.

According to these results obtained, all materials showed non-cytotoxic behavior in 25%, 50% and 100% conditioned media. So these results can open a window to think about these gels as biomaterials for biomedical applications.

Similar results were found in other studies found in the literature analyzed the cytotoxic effect of cotton cellulose fibers on human dental pulp stem cells, obtaining cell proliferation and viability using the MTT assay in 96-well plates, at concentrations of (0.1; 1; 10; 50; 100 µg mL^{-1}) for 24 and 48 h. The authors point out that there was no cytotoxic behavior in any of the tested concentrations. Another study analyzed the cytotoxic effect of cellulose from sugarcane bagasse. Cytotoxicity assays were conducted with the exposure of 929 cells of the NCTC clone to the extract obtained from a sample of the membrane kept in contact for 24 hours in a culture medium DMEM; the authors obtained non-cytotoxic behavior in all analyzes[31,32].

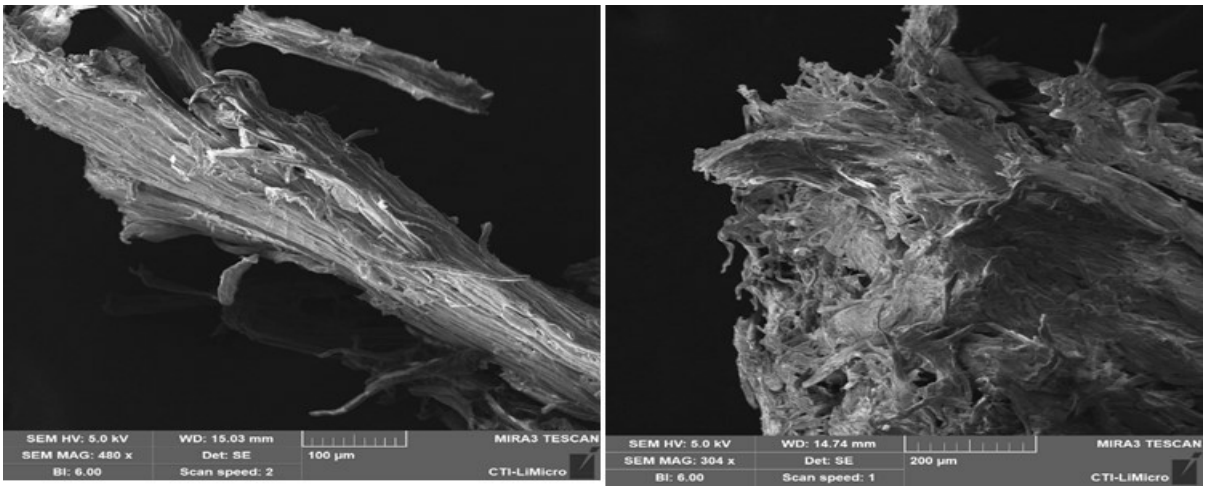


Figure 5 - SEM micrographs of the MCV sample that was use in the preparation of pectin gels.

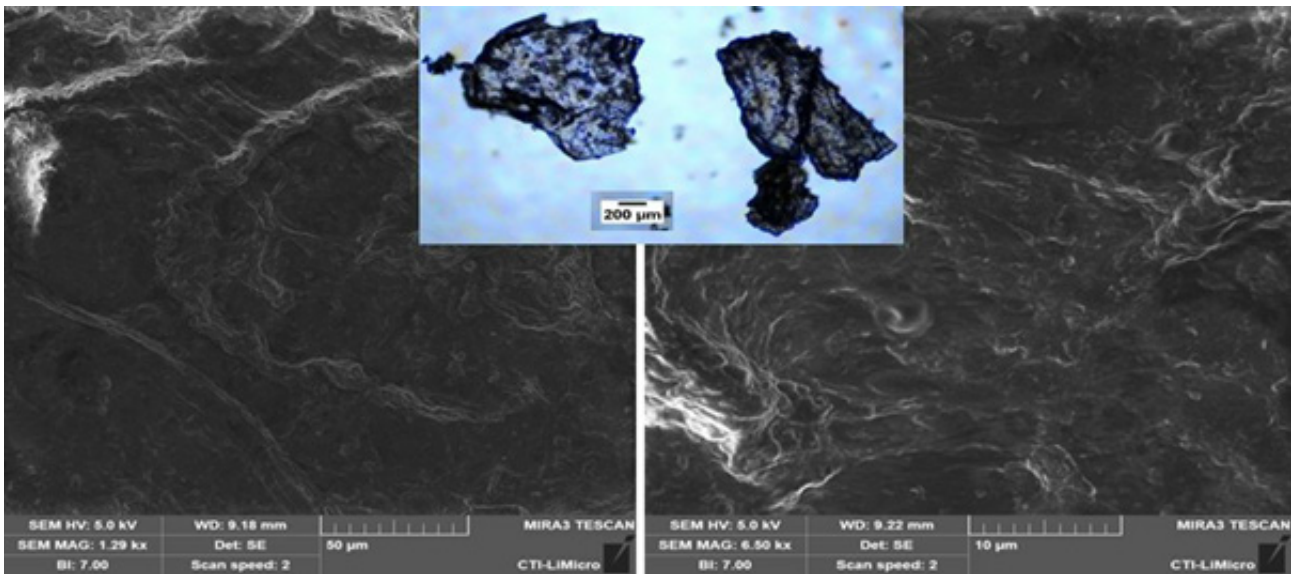


Figure 6 - Optical microscopy and SEM micrographs of the pectin sample.

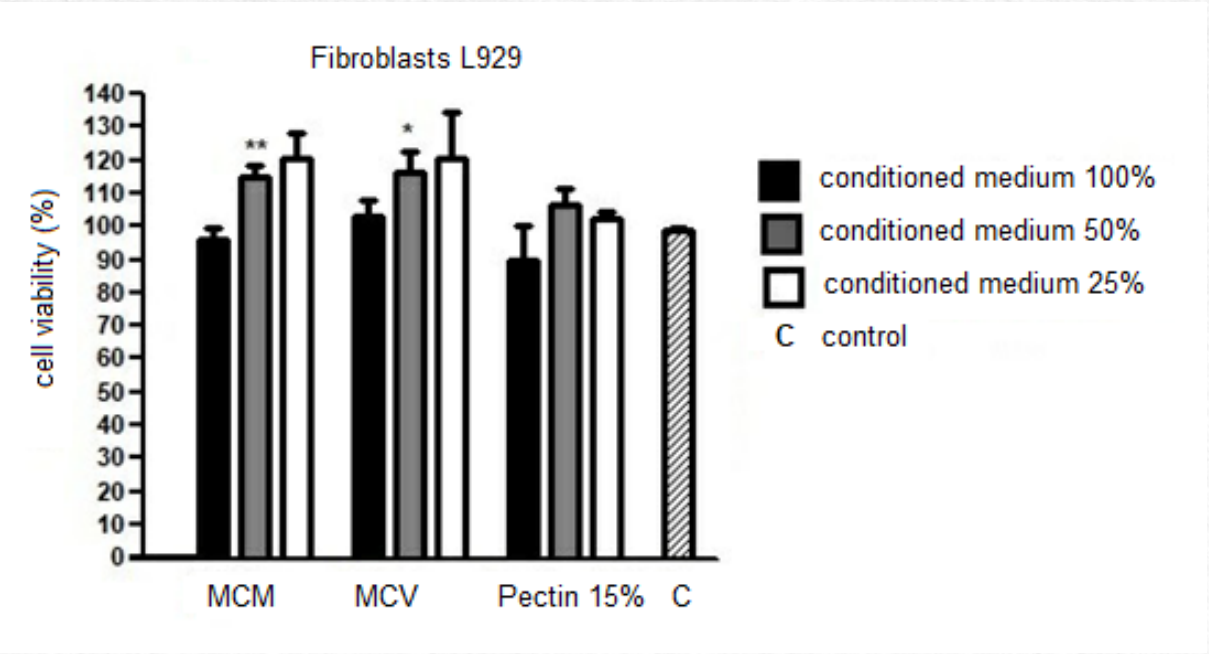


Figure 7 – Cytotoxicity analysis of MCM, MCV, PECM samples.

About the characterization of pectin/microcellulose gels

Pectin and cellulose gels have a wide potential for biotechnological applications, in areas such as food formulation, cosmetics and pharmaceuticals, bioremediation, packaging, and agronomy. These pectin and cellulose gels can exhibit physical properties that make them ideal to produce hydrogels and 3D scaffolds for tissue engineering. Their biocompatibility and ability to form emulsified systems also make them promising to produce materials with possibilities for use in additive manufacturing. Furthermore, studies have demonstrated that it is possible to modify the rheological properties of these gels through the addition of polymers such as PEG (polyethylene glycol), as previously mentioned what can further improve the rheological properties of these gels.

After production, the gels from mango pectin, mango cellulose and vegetal microcellulose were characterized using FTIR, TG, SEM and rheology analyses.

The results of the infrared spectroscopy of the PECM, MCM, MCV gels are presented in Figure 8, that all the curves presented similar behavior. Only seven bands are listed in the spectrometers of the gels, 1000, 1200, 1500, 1600, 1700, 2900 and 3300 cm^{-1}, which refers to the same bands of the materials when analyzed individually. The materials were incorporated and there was no negative interaction between them, since similar functional groups are found in each gel. In addition, opinions from some reports found in the literature the functional groups found are COC, CO, CH, C=O and OH. [18,19]

TG/DTG curves for gels, Figure 9, show similar thermal behaviors. The mass loss up to 100°C can be attribute to water evaporation, followed by the gel thermal degradation from 201°C with corresponding peaks DTG in 250 and 330-350°C. At 700°C is observe residues around 20-30%. According to the literature, the degradation of the chain polymeric pectin and the final degradation with the temperature at 330°C demonstrating the degradation of cellulose according to the literature. [6,17]

Pectin gels report pectin degradation temperature between 230°C and 250°C with approximate mass loss between 53%. Furthermore, pectin and chitosan gels show pectin degradation between 220°C and 280°C with mass loss between 50% and 65% corroborating with studies found in the literature.[33,34,35]

To characterize the surface of the gels developed based on pectin, mango microcellulose and vegetal microcellulose, scanning electron microscopy (SEM) analyzes were carried out at different magnifications to visualize the structures and morphology of the gels or blends. For this, a film was formed, which after drying, it was possible to be observed by SEM. The morphologies are shown in the micrographs of figures. [10-15]

In general, the SEM micrographs allow observing the homogenization of the gel, in addition to some fragments of granules of each type of microcellulose that were dispersed in the pectin (Figures 10-13). In these same images, at different magnifications, a smooth and compact uniform structure was observed between the dispersed phase and the matrix without major defects. Furthermore, the figures also show some elongated fibers that correspond to the microfibers of mango or vegetal cellulose, which are well disperse and made some

compatibility by the presence of PEG due to the addition of this polymer in the formulation of the gels (Figures 14 and 15). It is noteworthy that only the two gels with PEG in their composition showed this dispersion behavior, and it could be stated that the PEG phase substantially improves the dispersion of microcellulose fibers.[36]

Research with pectin hydrogels, starch and cellulose microfibers, had similar results in scanning electron microscopy, finding starch and pectin granules, which means that this dispersed phase within the matrix does not completely disaggregate due to the effect of the mixer.[34]

The homogeneity of hydrogels with PEG is a result of their power of miscibility, hydrophilicity, and formation of a better interphase with the polymeric chain through PEC/MC hydrogen bonds. Studies claim that the addition of PEG in hydrogels causes a relaxation of tensions arising from the long chain, making them more elastic and denser. [37,38] The latter can be proven by the results of the rheology of the gels, which are presented ahead.

So, the samples were submitted to rheology analysis to verify the viscoelastic capacity of the produced gels which means understanding the proportion as fluid and elastic solid behavior simultaneously. The elastic fraction of the deformation appears due to variations in the angle and the bonding distance between the atoms of the polymeric chain. The plastic fraction occurs due to the friction between the polymeric chains. [39]

In general, pectin/cellulose gels are hydrocolloid gels compound formed by pectin and cellulose fibers. In this case the dispersed phase is microcellulose fibers. The behavior of the storage modulus versus tension in these gels is affected by the interaction between the pectin and cellulose components and could also be affected by the size of the microfibers.[40]

In Figure 16, it can be observed that the gels present a pseudoplastic behavior at low shear rate, characteristic of gels that can be used in extrusion or bioextrusion processes. But after the increase in the shear rate, it seems that what predominates would be the majority phase of the PECM that generates a Newtonian behavior, however the microcellulose fibers, regardless of their origin, do not participate in the definition of the rheological behavior. For these gels, it is therefore important to consider only the shear values that allow them to have the required behavior for extrusion or bioextrusion processes.

Figure 17 shows the behavior of the storage modulus or elastic modulus versus the shear stress (within the range of pseudoplastic behavior in transition with Newtonian behavior). It is possible to see that at low strains, the gel storage modulus of pectin/cellulose behaves in a linear elastic manner, like that of pectin gels. However, the addition of cellulose fibers should cause the gel to exhibit a more pronounced non-linear viscoelastic behavior, expecting an increase in the storage modulus as strain increases. In this case it is observed that the presence of PEG produces (at low deformation) a slight increase in the value of the modulus G' and as the deformation continues to increase and appreciable a behavior with a tendency to decrease the elasticity of the material, that is, cellulose fibers can limit gel deformation, leading to a less pronounced plateau in the storage modulus. This can result in a gel that is more rigid and resistant to deformation than a pectin gel without cellulose fibers. The presence of PEG seems to help maintain the elasticity of the gels where this polymeric phase is present.

The loss modulus is a measure of the energy dissipated by the gel during deformation and is related to the viscous or damping behavior of the gel. In pectin/cellulose gels, the loss modulus normally increases as much stress increases, as the gel undergoes more deformation and energy is dissipated, as seen in figure 18. For strains below 5%, i.e. for at low strains, the modulus loss can be relatively low, indicating a relatively low degree of power dissipation. However, as the stress increases, the cellulose fibers start to interact with the pectin molecules, leading to a more pronounced non-linear viscoelastic behavior and an increase in the loss modulus, as happens here for strains between 5-10%.

Finally, in Figure 19, it seems that fiber size and molecular mass play an important role, given that gels formulated with MCV have a more pronounced behavior as a solid material than in the case of gels formulated with MCM. Again, it is appreciated that the presence of PEG really helps the gels perform more optimally, as expected. Here it seems that fiber size and molecular mass play an important role, given that gels formulated with MCV have a more pronounced behavior as a solid material than in the case of gels formulated with MCM. Again, it is appreciated that the presence of PEG really helps the gels perform more optimally, as expected.

A study published in 2019 evaluated the influence of pectin and cellulose concentration on the rheology of mango gels. The researchers observed that the addition of cellulose increased the viscosity of the gel, while the addition of pectin resulted in a weaker gel. Furthermore, the combination of pectin and cellulose led to a gel with a more uniform texture and less susceptible to syneresis during storage. [41]

Another study published in 2019 investigated

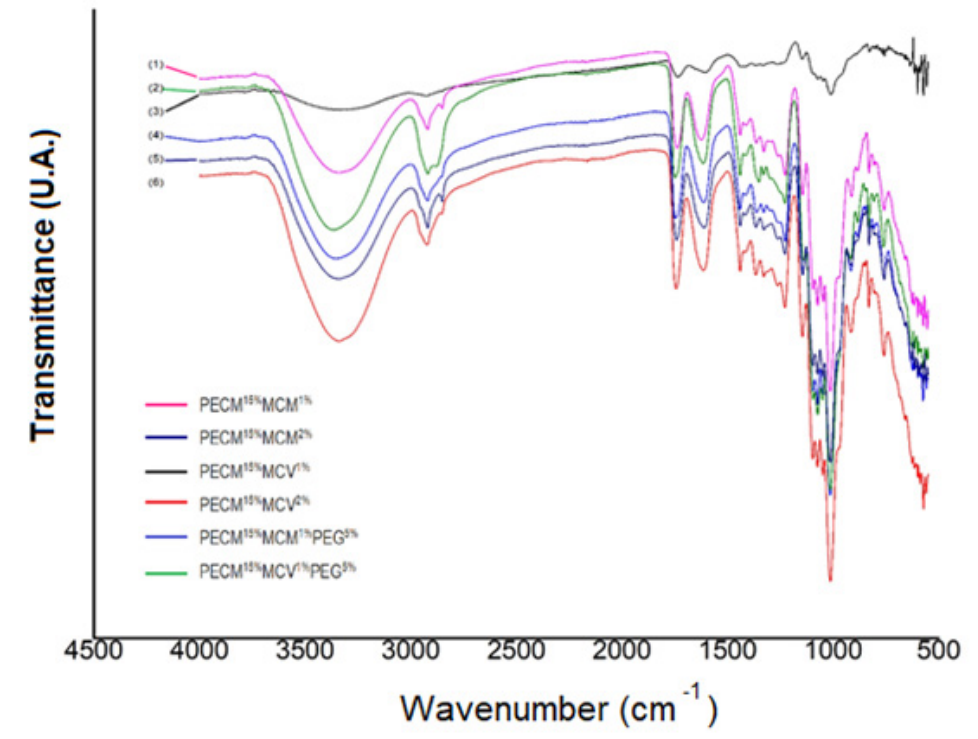


Figure 8 – FTIR spectra of PECM/MCM, PECM/MCV and PECM/MCM/PEG gels.

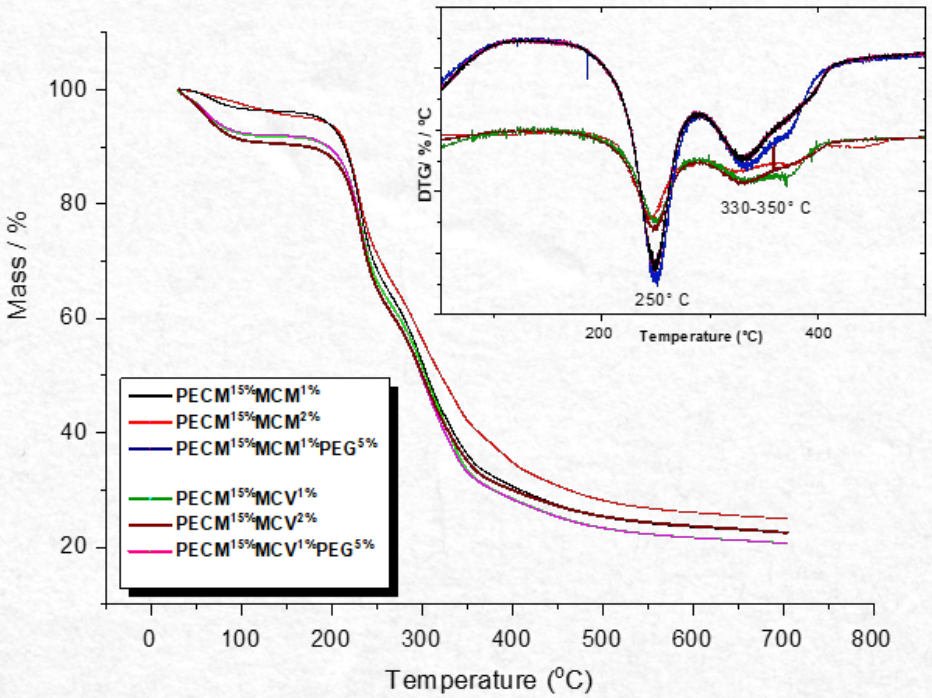


Figure 9 – TG/DTG curves for gels formulations.

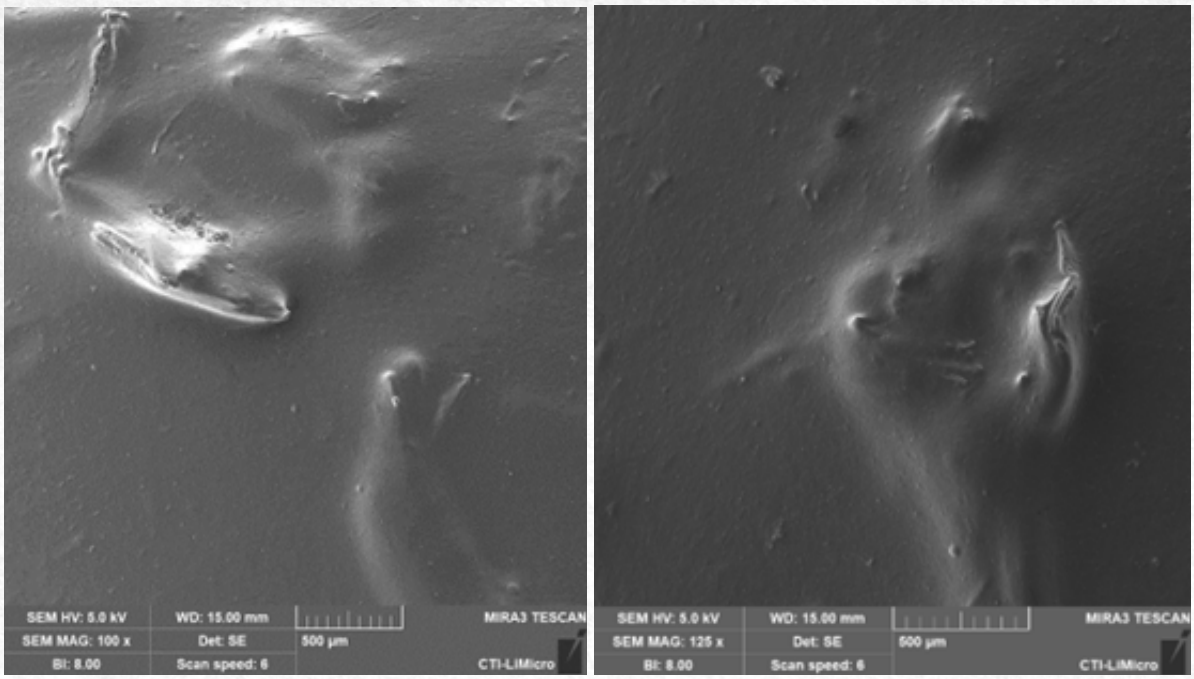


Figure 10 – SEM micrographs of the 1% PECT/MCM gel.

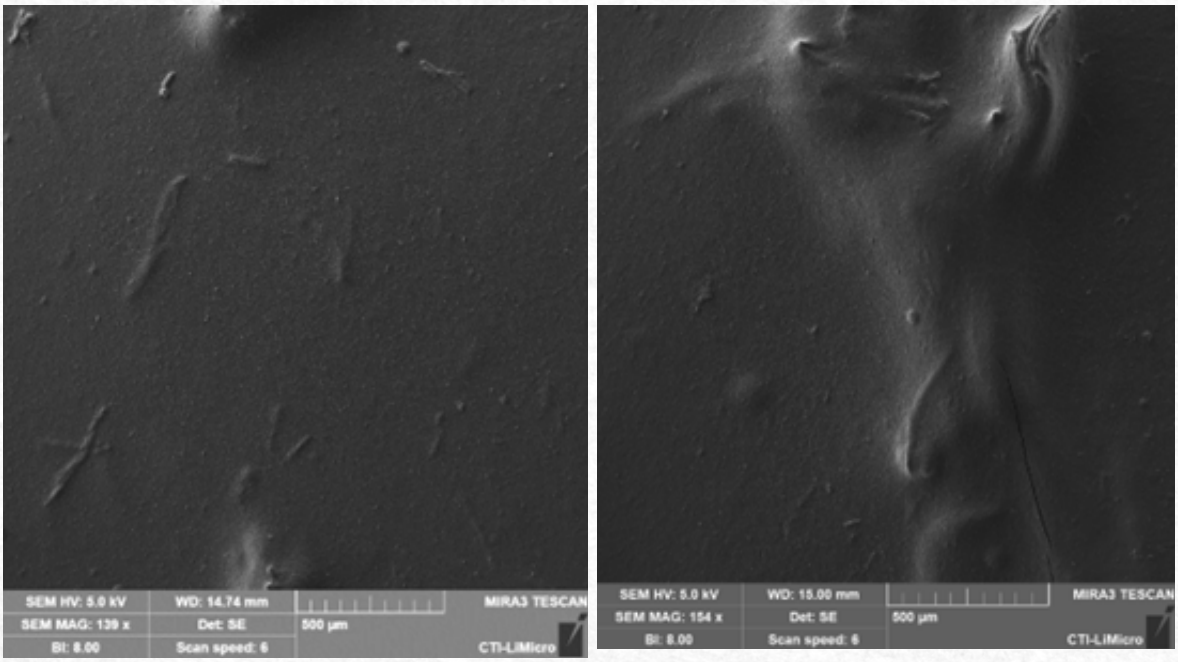


Figure 11 – SEM micrographs of the 2% PECT/MCM gel.

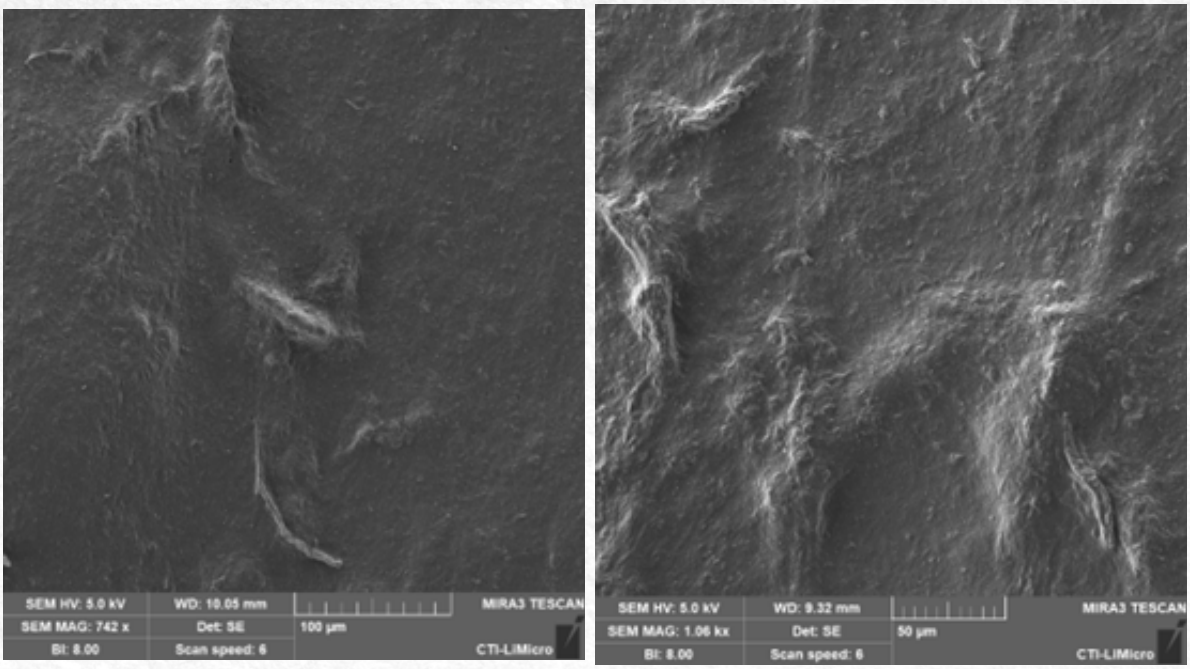


Figure 12 – SEM micrographs of the 1% PECT/MCV gel.

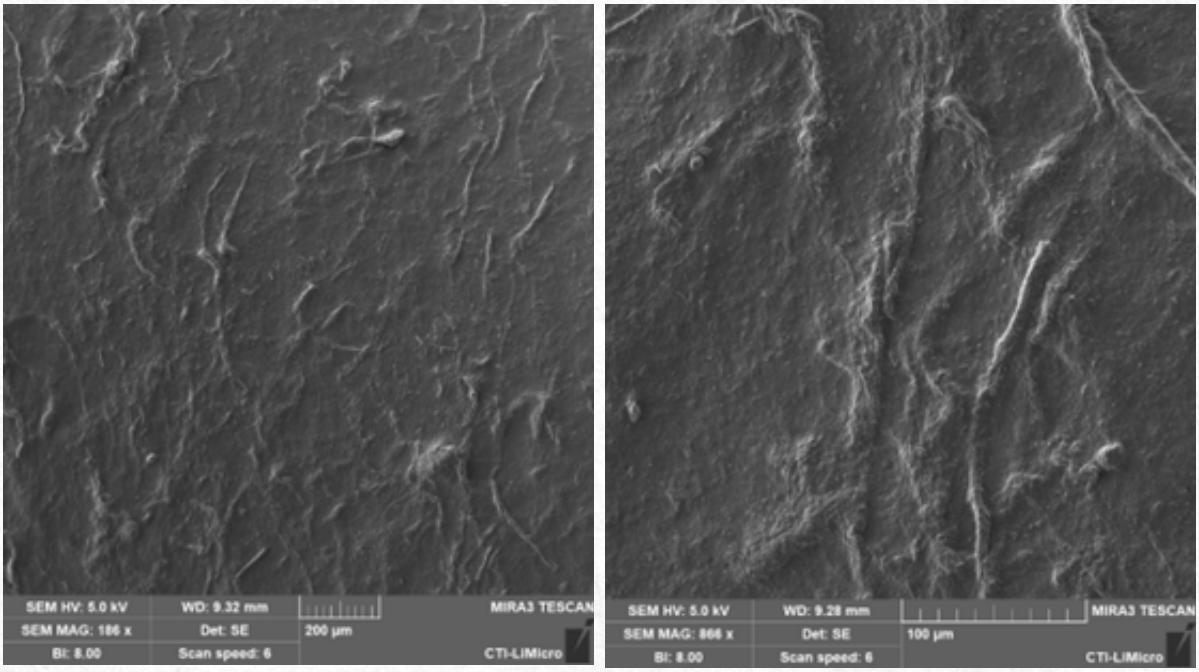


Figure 13 – SEM micrographs of the 2% PECT/MCV gel.

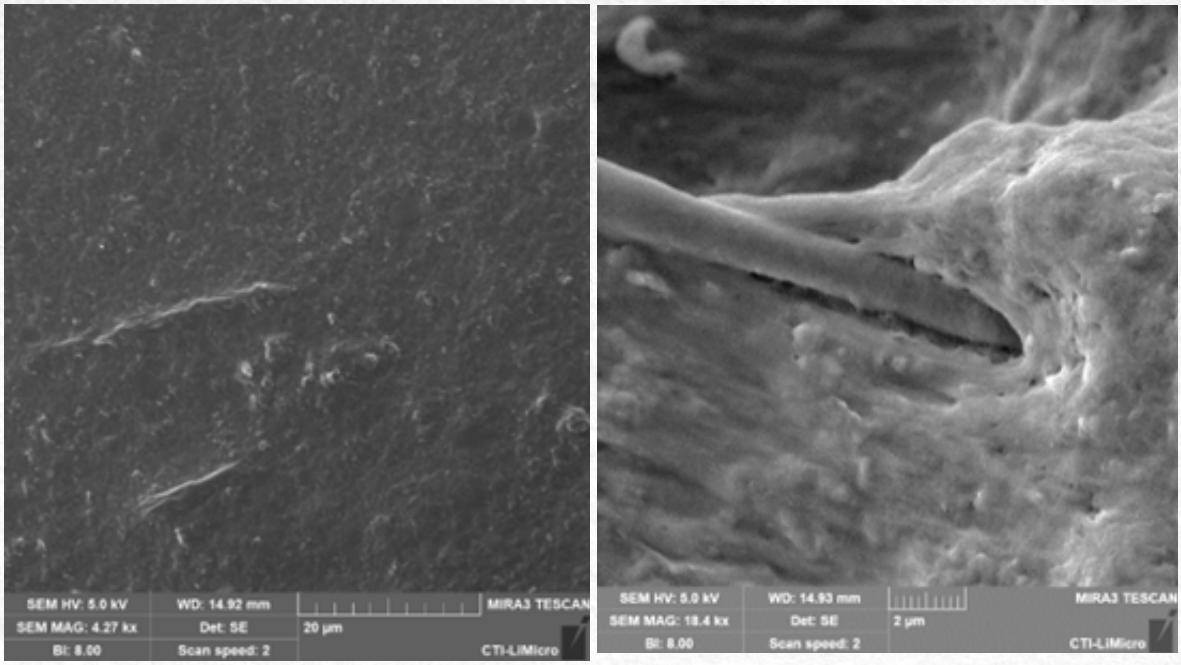


Figure 14 – SEM micrographs of the PECT/MCM(1%)/PEG(5%) gel.

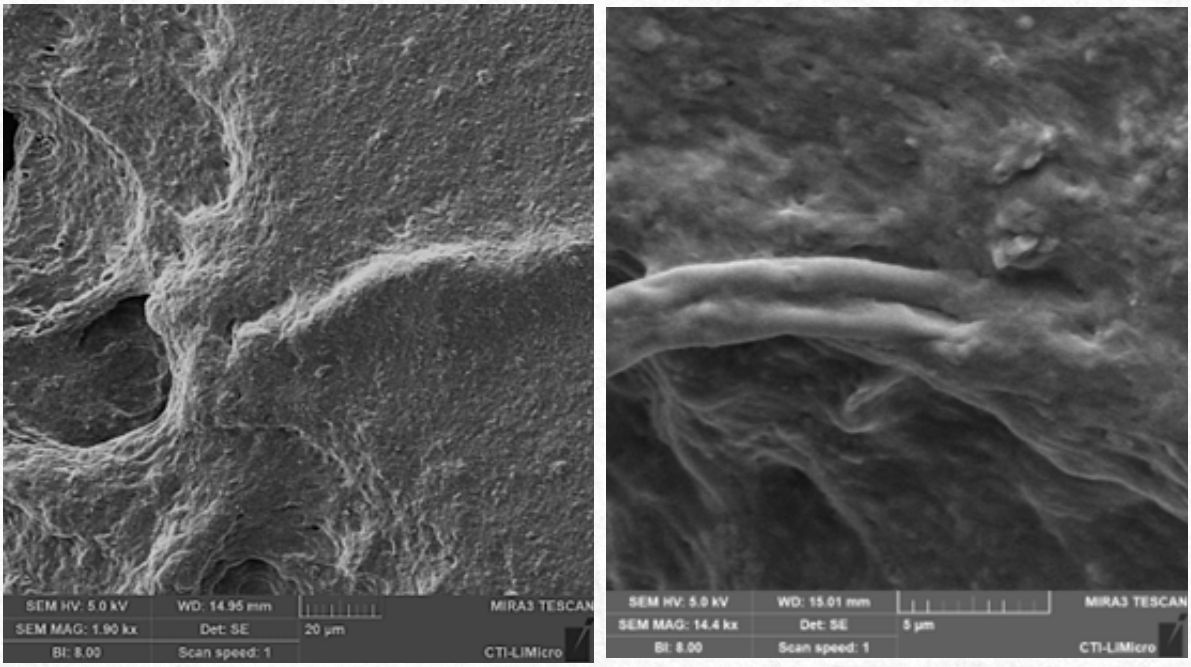


Figure 15 – SEM micrographs of the PECT/MCV(1%)/PEG(5%) gel.

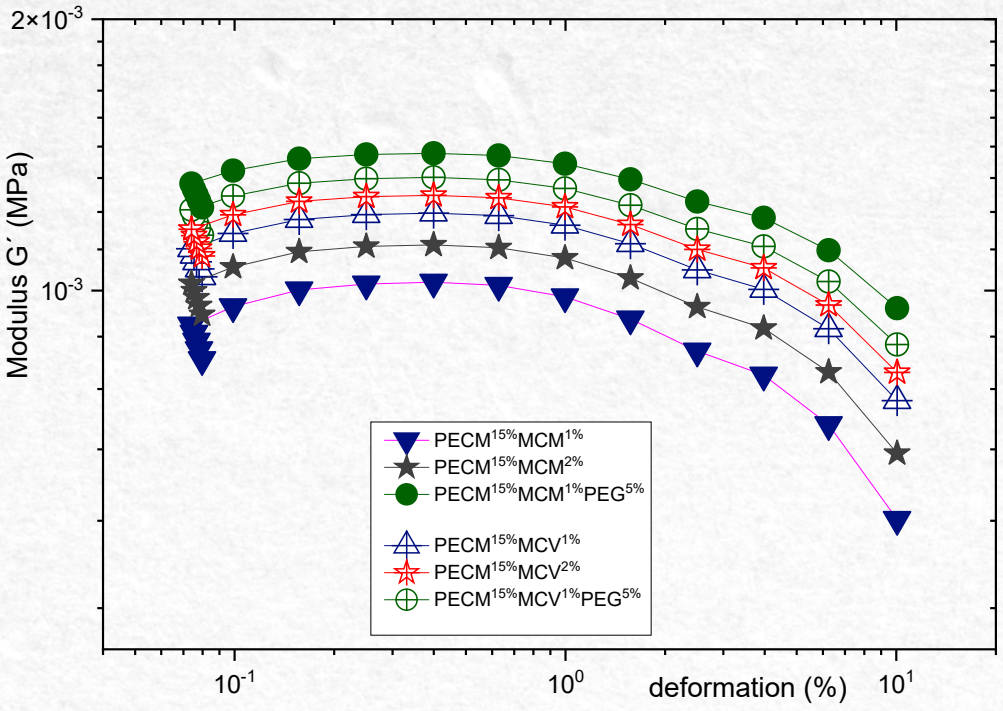


Figure 17 - Variations of G´ as a function of pectin and microcellulose gels deformation, also with the PEG phase.

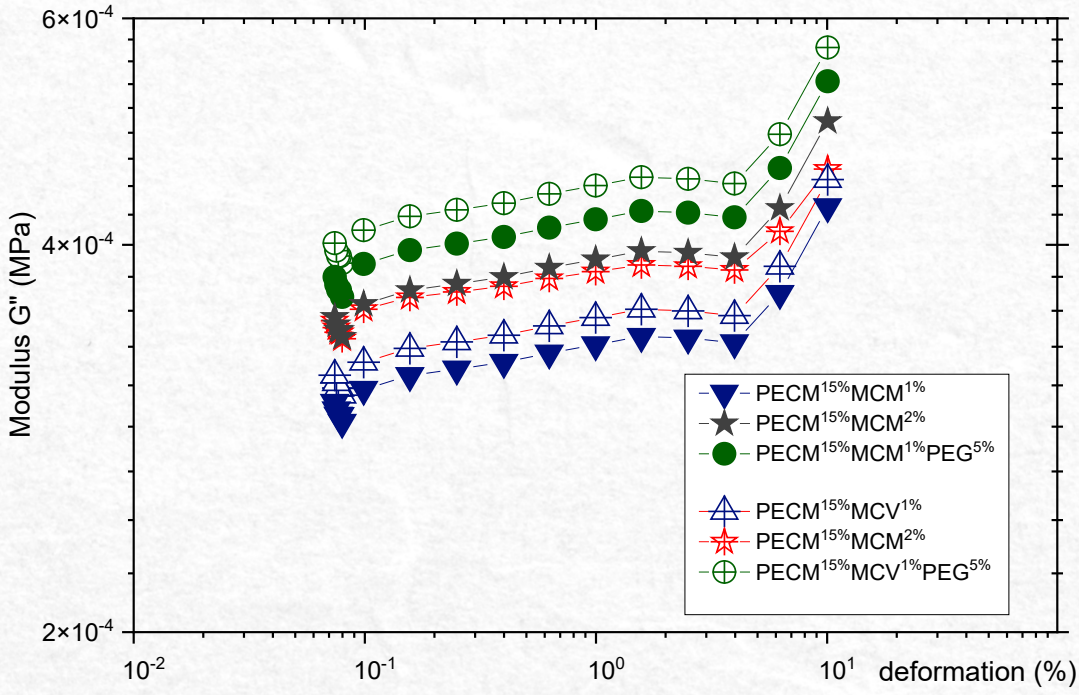


Figure 18 - Variations of G" as a function of strain for pectin and microcellulose gels, also with the PEG phase.

the rheology of mango pectin and cellulose gels. The researchers observed that adding cellulose to pectin resulted in an increase in gel viscosity and strength. Furthermore, the addition of cellulose also led to a decrease in syneresis (liquid release) during gel storage. It was concluded that blend pectin and cellulose can be an interesting option for the development of fruit gels with improved rheological characteristics. [42]

A third study, published in 2018 in the International Journal of Biological Macromolecules, investigated the rheology of pectin and cellulose gels derived from mangoes and other fruits. The researchers observed that the addition of cellulose increased the viscosity of the gel, while the addition of pectin resulted in a weaker, less elastic gel. Furthermore, the study reported that the combination of pectin and cellulose can result in a gel with a more uniform texture and less susceptible to syneresis during storage. [43]

Finally, a study published in 2020 in the International Journal of Biological Macromolecules investigated the effect of adding PEG on the rheology of pectin and cellulose gels derived from mango. The researchers observed that the addition of PEG resulted in an increase in elasticity and a decrease in gel rigidity, without significantly affecting viscosity. Furthermore, the addition of PEG also led to a decrease in syneresis during gel storage. [44]

Another research published in 2019 in the journal Carbohydrate Polymers investigated the influence of adding PEG on the rheology of apple-derived pectin and cellulose gels. The researchers observed that the addition of PEG resulted in an increase in gel viscosity, without significantly affecting elasticity and rigidity. Furthermore, the combination of PEG with other rheology modifying agents resulted in gels with even further improved rheological properties. [45]

Conclusion

Therefore, the use of gels composed of pectin and cellulose from mango can be a promising option for the development of new products for the biotechnological area which includes food, pharmaceutical and biomedical sector, given their functional properties and possible health benefits and even for the clothing and footwear industries.

Pectin and cellulose gels have unique rheological properties that are influenced by the interaction between the two phases and the incorporation of PEG in some formulations. The addition of cellulose fibers can improve the mechanical properties of pectin gels, leading to more pronounced nonlinear viscoelastic behavior with a more rapid increase in storage modulus as strain increases. It could be thought that cellulose fibers can act as physical crosslinks within the gel network, increasing its stiffness and strength, as demonstrated by rheological analyses.

In this sense, the storage modulus of pectin/microcellulose gels is a key rheological property that characterizes their stiffness and strength. The addition of MCM and MCV fibers increases the storage modulus of pectin gels, leading to a more pronounced non-linear viscoelastic behavior with a faster increase in the storage modulus as strain increases, and exhibiting a pseudoplastic behavior when and analyzed the viscosity. In this case, the behavior of the modulus loss in these gels increases with increasing stress, indicating a greater degree of energy dissipation.

The inclusion of Polyethylene glycol (PEG) further improves the properties of the gels. PEG is a polymer soluble in water and in the pectin solution and can act as a plasticizer, reducing the rigidity and increasing the deformability of the gel. The addition of PEG can also improve the water holding capacity of the gel and increase its stability over time.

Pectin/microcellulose gels can have numerous biotechnological applications due to their unique rheological properties analyzed in this research, in addition to their biocompatibility. They could be used as thickeners, stabilizers, and gelling agents. Furthermore, they could be used in pharmaceuticals, cosmetics and biotechnology for drug delivery, wound healing and possibly in tissue engineering applications.

As prospects for this type of gels can focus on the development of new formulations and methods to produce these pectin/microcellulose gels with specific properties to meet particular requirements of these different applications. For example, the concentration and orientation of cellulose fibers can be optimized to achieve specific mechanical and rheological properties. Furthermore, the addition of other natural or synthetic polymers, such as chitosan or polyvinyl alcohol, can be investigated to further modify the properties of the gels.

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Our sincere tribute and gratitude to scientist and friend Dr. Jorge Vicente Lopes da Silva for his pioneering work and contribution to the areas of additive manufacturing and biofabrication.

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