

Montmorillonite dispersion in water affects some physical properties of gelatin-based nanocomposites films

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ABSTRACT

The aim of this work was to study both the effect of montmorillonite (MMT) dispersion method in water and the nanoparticle concentration on some physical properties of gelatin based films produced with those dispersions. MMT was dispersed in water using two devices: Colloidal mill (CM) and ultrasonic processor (US). These dispersions were analyzed for determination of particle size and zeta potential. Films were produced by spreading and characterized for determination of mechanical properties, hydrophobicity, and microstructure, among others. It was obtained stable MMT dispersions with average particle size of 289 and 395 nm, for US and CM dispersions, respectively. In general, the films filled with MMT were up to 50% less glossy and rougher than control film. The tensile strength increased up to 50% in nanocomposites. The dispersion method used had a strong influence on the quality of dispersions, affecting the particle size and zeta potential, with evident effect on the properties of nanocomposite films.

Keywords: *particle size, mechanical properties, contact angle, roughness, natural polymer, biodegradable film.*

1. INTRODUCTION

The development of films based on biopolymers reinforced with nanoparticles is one of the possible alternatives to replace synthetic materials, and thus, to reduce environmental concerns in the area of food packaging. The main biopolymers used in the preparation of biodegradable films are the polysaccharides and proteins [1,2]. Because of its excellent film-forming property, gelatin has been widely used in studies on edible and/or biodegradable films [3,4].

In general, the plasticized gelatin films are characterized by it high sensitivity to environmental conditions, particularly the relative humidity, good mechanical resistance and high elasticity [5]. The formation of nanocomposites with nanoclays has shown evident improvement in the properties of several biopolymers with a low concentration of the filler, usually <10% (w/w) [6,7]. This improvement is strongly dependent on filler content and dispersion state; it is commonly recognized that exfoliated systems give better mechanical properties than intercalated ones [8].

Several researchers observed improvements for mechanical and barrier properties of films based on gelatin [9,10,11,12] and agar [6]. However, there exist some other works in which the improvements were not achieved, and even was observed a

decrease of the mechanical properties of those reinforced films [13,14,15,16,17]; thus, there is a lack of consensus in the results that may be related to differences in the biopolymer and nanoparticle types and concentrations, or incomplete dispersion of nanoparticle that prevents to obtain intercalated or exfoliated structures.

For films produced by casting/spreading, the improvements are still limited and probably it is due to dispersion problems of nanoparticles in water, the usual solvent for biopolymer-based films production, prior the formation of films. In a previous work [18], several dispersion methods of montmorillonite (MMT) in water were tested and one of those dispersions was chosen to prepare the nanocomposite films, based in particle size and zeta potential values. However, it was only considered the effect of concentration of MMT in the properties of gelatin films and it was not evaluated the effect of the various methods tested on the properties of films produced from these dispersions. In that context, the aim of this work was to study both the effect of MMT dispersion method in water and the nanoparticle concentration on some physical properties of gelatin based films produced with those dispersions.

2. EXPERIMENTAL SECTION

2.1. Material.

The biopolymer used in this study was a pigskin gelatin type A, with bloom 245 and molecular weight 52×10^3 Da (Gelita South America, São Paulo, Brazil). The films were plasticized with glycerol (Synth, Brazil). An unmodified commercial sodium montmorillonite (Nanomer® clay, PGV, Sigma) was used as filler.

2.2. Montmorillonite Dispersion.

The montmorillonite (MMT) particles were dispersed in distilled water, in a concentration of 1 g of MMT/100 g of water,

using either a colloidal mill CM (SPEX type) with 4 acrylic balls or an ultrasonic processor US (750W, Sonics) with a ½” probe for 30 min. After 18 hours of rest, the particle size and zeta potential of dispersions were analyzed at 25°C using a ZetaPlus equipment (Brookhaven Instruments Company, EUA) [19]. In order to get an initial value of those parameters, MMT was dispersed also with a magnetic stirrer for 30 min.

2.3. Gelatin nanocomposite films.

The film-forming solutions (FFS) were prepared based on the work of Jorge et al. [19], with 5 g of gelatin/100 g of FFS. The gelatin was hydrated in distilled water for 30 min at room temperature and then dissolved at 70°C for 30 min using a thermostatic bath (Marconi, model MA 179). After that, the glycerol (30 g of glycerol/100 g of gelatin) and the aqueous dispersions of MMT, as prepared in Section 2.2, were mixed conveniently with the gelatin solution. In order to eliminate any air bubbles formed, the FFS were treated in an ultrasound bath (Unique, model MaxiClean 1400) at 60°C for 10 min. The nanoparticles concentration in nanocomposite films was adjusted to 1, 3, 5 and 7 g of MMT/100g of gelatin. Films prepared with the MMT dispersed with the Ultrasonic Processor and with the Colloidal Mill were denoted as US films and CM films, respectively, and films without MMT were considered as control.

Films were produced by spreading 100 g of the FFS onto an acrylic plate (780 cm²) with an automatic film applicator (Speed II, TKB Erichsen). The spreading knife speed was set at 35 mm/s; the gap between the knife and the plate was 1.5 mm. The FFS on the plate was dried in oven at 30°C for 24h; then, conditioned in a relative humidity of 58% for 7 days [7].

2.4. Film Characterization.

The films were characterized for determination of thickness, solubility in water, mechanical properties, microstructure by scanning electron microscopy (SEM) and atomic force microscopy (AFM), gloss and water contact angle.

2.4.1. Thickness.

The thickness was measured with a digital micrometer (Mitutoyo, ± 0.001 mm) in 10 points of each film. The thickness was calculated as the average of 10 measurements.

2.4.2. Solubility in water.

Films were cut into 2 cm diameter disc-shape and immersed in distilled water and kept under agitation in an orbital shaker table (MA-141, Marconi) for 24 hours at 25°C. Subsequently, the non-solubilized sample was placed in an oven at 105°C for 24 hours to determine the moisture [20]. The solubility in water was calculated by dry mass weight difference.

2.4.3. Mechanical Properties.

Mechanical properties were determined by tensile test (samples cut into 15mmx100mm strips) with grip separation of 50mm and speed rate of 1 mm/s, according to ASTM D882 standard [21,22], and by puncture tests (samples diameter 53mm), with a 3mm probe and velocity of 1mm/s according to ASTM F1306 standard [23,20]. Both tests were carried out using a texture analyzer (TA.XT icon, SMS, Surrey, U.K.), at room temperature (~25°C).

2.4.4. Microstructure.

Films were analyzed using a scanning electron microscopy (SEM model TM30000, Hitachi) at the surface and the cross-section, at 15kV. The films were cryo-fractured after immersion in liquid nitrogen, and analyzed without further preparation. Films were fixed to aluminum stubs with double-sided tape. The images were taken at random positions of the films with 2000x magnification, accordingly with a previous work [18].

Atomic force microscopy (AFM model NTEGRA PRIMA, NT-MDT) was used to study the surface topography and roughness (as mean root square) of films using the tapping mode, as described by Martucci et al. [24]; analyses were performed in areas of 25µmX25µm at random positions of films.

2.4.5. Gloss.

Gloss measurement was performed using a glossmeter (Rhopoint NGL 20/60) with 60° angle in 10 points of the air drying film surface, according to ASTM D2457 standard [25,26].

2.4.6. Water contact angle.

The water contact angle was measured according to ASTM D7334 standard[27], using an optical tensiometer (Attension Theta lite, KSV Instrument, Finland). The films were attached to the equipment and a drop of distilled water (approx. 10 µL) was placed on the film with an automatic precision syringe and immediately photographed. The angle formed between the surface and the tangent to the drop was calculated with the software Attension Theta lite optical tensiometer. The measurements were performed in triplicate [7].

2.4.7. Statistical analysis.

All tests were executed in triplicate, and results submitted to ANOVA and Tukey tests ($\alpha=0.05$). Statistical analysis was performed with SAS software v9.0.

3. RESULTS SECTION

3.1. Dispersion of MMT in water.

The dispersion of the commercial MMT in water using a magnetic stirrer led to the breakdown of the agglomerated structures of the clay, producing particles with Zeta potential of -34.3 ± 3.1 mV and average size of 501.9 ± 27.6 nm. Then, these values were taken as the initial particle size and further reductions were attributed to the dispersion process used. It is known from literature that MMT particles presents a platelet shape with thickness of about 1 nm [28] and can be considered as nanoparticles according to ASTM E2456-06[29].

The MMT dispersions prepared with Ultrasonic Processor showed particle size of 289 ± 3 nm. The reduction in relation to the value indicated above is consequence of the cavitation that

occurs on the system due to vibration of the water molecules that promotes the collision of the particles and destruction of agglomerates. Mekhamer [30] found that ultrasonication process for 30 min led to an increase of the surface area of bentonite of about 1.3 times, that was associated to the delamination of the larger particles, leading to a reduction of the size of the clay particles.

On another hand, using the high-energy mill to disperse the MMT in water favored the reduction of particle size to about 395 ± 4 nm, less than using US. This reduction was a result of high speed collisions generated by the equipment in which the movement "back-and-forth" is combined with lateral movement and oscillation, and the inertia of the balls causes them to impact

against the vial walls and bottom breaking the agglomerates. Similar particle size of 380 nm was obtained by Xia et al. [31], using a planetary ball mill during 2 h, obtaining mesoporous MMT.

The colloidal behavior of clay suspension can change with reduction in particle-size due to change of their surface charge. The surface charge implies in surface forces that, in many cases, affect the colloidal stability of the suspension [32]. In this sense, Zeta potential can be used to estimate the effect of the particle charge on such as aggregation. In this study, the zeta potential of MMT particles dispersed with US was -40 ± 11 mV, and using the CM was -47 ± 9 mV. Both dispersion could be considered as stable because the general established value for unstable suspensions is generally taken between $+30$ and -30 mV.

3.2. Nanocomposite films.

3.2.1. Film thickness and solubility in water.

The thickness of films of the CM treatment remained almost invariable, near to 0.066 mm, similar to control films. It was observed that the US treatment presented thicker films than those obtained with the CM treatment in most concentrations of MMT. The MMT addition resulted in a slight increase ($p < 0.05$) in film thickness as the MMT concentration was increased to 3%, this effect was observed only for US treatment (Figure 1), suggesting that the nanoparticles must have influenced the density of the nanocomposite. A similar result was obtained by Alboofetileh et al. [14] in films of alginate and MMT.

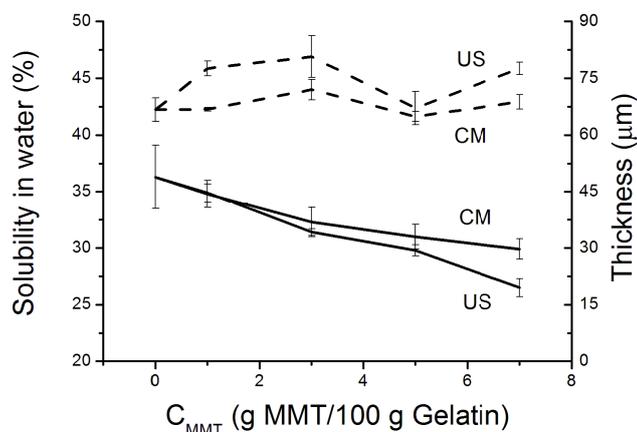


Figure 1. Thickness (dashed line) and Solubility in water (solid line) as a function of the montmorillonite (MMT) content in gelatin based nanocomposite films, for MMT dispersed by ultrasound (US) or colloidal mill (CM).

Nanocomposite films were up to 39% less soluble in water due to the presence of MMT. The film solubility in water decreased with the increasing concentration of nanoparticle, therefore, films with higher concentration of MMT were less soluble in water for both treatments used (Figure 1). No significant differences ($p > 0.05$) of film solubility were observed between the US and CM treatments, with the exception of the highest content of MMT (7%) in which, films of the US treatment were less soluble. This phenomenon of decrease in solubility in water with an increase in MMT content has been observed with different matrixes such as methylcellulose [33], corn starch [34] and sodium alginate [14]; and it is a consequence of some specific interactions between biopolymers and MMT, producing more organized networks that stabilize the film structure.

3.2.2. Mechanical properties.

The MMT caused an increase ($p < 0.05$) up to 50 % in both the tensile strength and the puncture force of the nanocomposite films, indicating that the MMT acted as reinforcement (Figures 2, 3), nevertheless, these behaviors were dependent of the dispersion process. The stiffness of the nanocomposites was also affected, since had a significant increase ($p < 0.05$) in the Young's modulus up to 80% due to the increasing content of MMT (data not shown). The main reason for the films mechanical property improvement was the strong interfacial interaction between the gelatin matrix and layered MMT due to the vast surface exposed of the clay layers [35].

Specifically, the tensile strength was significantly affected by the dispersion method used. Tensile strength presented a maximum as a function of nanoparticles concentration at 3 and 5% of MMT, for US and CM treatments, respectively (Figure 2). These behaviors were observed by several authors working with MMT in biopolymers films. Tunc et al. [36] observed an increase in tensile strength and Young modulus due to the increasing concentration of MMT in wheat gluten films with a concentration of MMT up to 5%. The same improvement behavior was observed for potato starch films [35] and fish gelatin films [10] reinforced with MMT. The reinforcement function of MMT in several polymeric matrixes is a well-known matter, and in the present work, the differences between the maximum mechanical properties achieved for the incorporation of MMT in gelatin films are due to the dispersion method used.

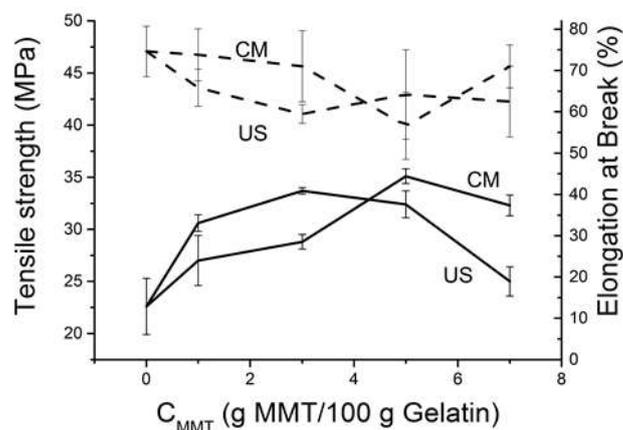


Figure 2. Tensile strength (solid line) and Elongation at Break (dashed line) as a function of the montmorillonite (MMT) content in gelatin based nanocomposite films, for MMT dispersed by ultrasound (US) or colloidal mill (CM).

The elongation at break was also affected by the dispersion method, films of the CM treatment presented similar values of the control film with a slight reduction at a MMT concentration of 5%, and increased at 7% of MMT (Figure 2). For films of the US treatment, the elongation at break was lower than control films and remain with almost the same values for all concentrations of MMT. The results observed from this study are in agreement with other studies of biopolymer films incorporated with MMT, this behavior could be attributed to the restricted mobility of gelatin molecules due to the incorporation of MMT [12].

On the other hand, a maximum in puncture force was observed only for films prepared using US methods, at 1% of MMT (Figure 3). The puncture force for films prepared using CM

methods increased monotonically as a function of MMT concentration. In overall, nanocomposite films of the US treatment had higher values of puncture force for all levels of MMT concentration tested, a similar result was obtained in a previous work [18]. The puncture deformation presented different behavior depending on the dispersion method used, for films of CM treatment it was observed a lower value of puncture deformation at MMT content of 5% and a slightly increased deformation at 3 and 7% of MMT; In films of the US treatment an increment of puncture deformation was observed only at 7% of MMT (Figure 3).

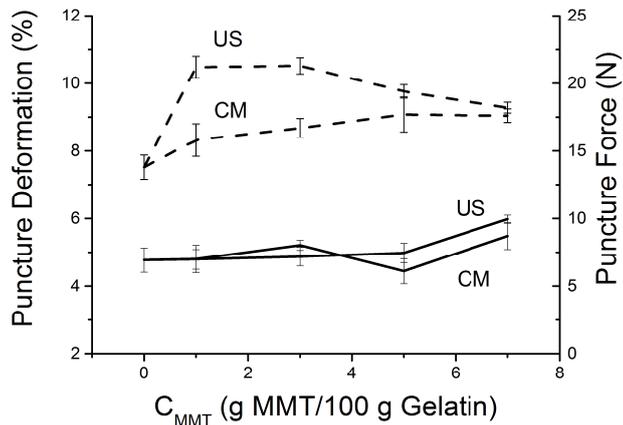


Figure 3. Puncture force (dashed line) and Puncture deformation (solid line) as a function of the montmorillonite (MMT) content in gelatin based nanocomposite films, for MMT dispersed by ultrasound (US) or colloidal mill (CM).

3.2.3. Microstructure.

Plasticized gelatin films usually present regular, homogeneous and smooth surfaces [18]. But, it can be observed in Figure 3, that the MMT modified the surface and cross-section appearance of the nanocomposite films, being that higher the concentration, the greater the surface irregularity. Similar behavior has been observed by other researchers in films based on alginate [14], tilapia skin gelatin [16] and pigskin gelatin [7].

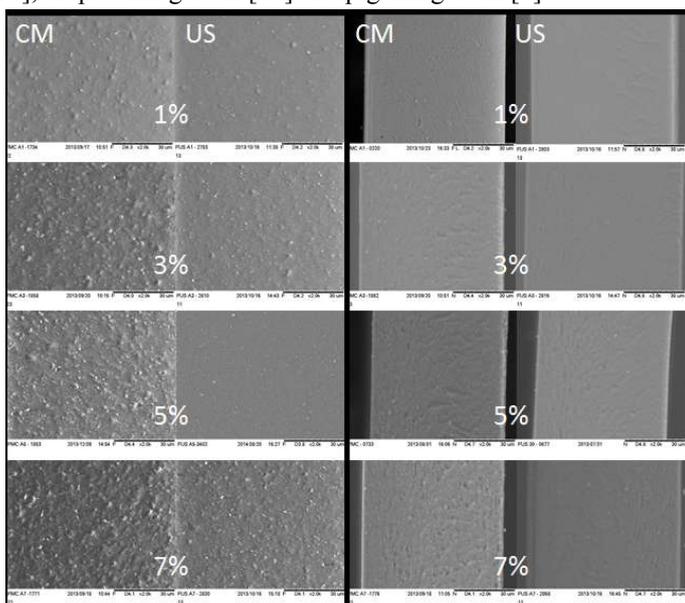


Figure 4. Scanning electron micrographs of gelatin nanocomposite films surface (left) and cross-section (right) for different montmorillonite (MMT) contents, and for MMT dispersed by ultrasound (US) or colloidal mill (CM).

Regarding the effect of the MMT dispersion method, the nanocomposite films of the US treatment appeared to have a clean surface and smoothed cross section, with small, highly dispersed particles and little agglomeration at the surface; while films of the CM treatment presented a highly irregular surface and cross section (Figure 4).

This effect of the MMT and dispersion methods on the microstructure of the films can be better observed analyzing the results of atomic force microscopy (AFM) (Figure 5). The appearance of a large number of peaks with the increasing content of the MMT was evident. Also, it can be noted a remarkable difference between the films of the both treatments tested, being that films of the US treatment have smaller particles with few and smaller peaks, whereas films of the CM treatment presented highest intensity peaks.

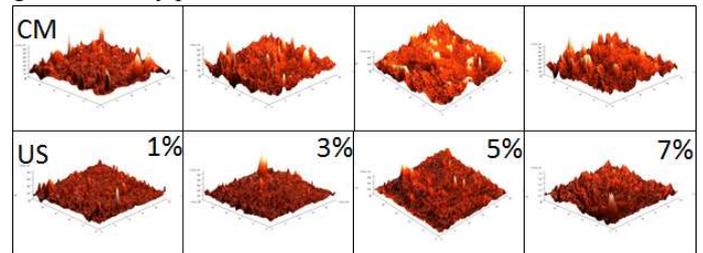


Figure 5. Atomic force micrographs of the gelatin nanocomposite films with different montmorillonite (MMT) contents, and for MMT dispersed by ultrasound (US) or colloidal mill (CM).

From these micrographs (Figure 5) the roughness values were calculated as a function of MMT concentration (Figure 6). Surface roughness increased almost linearly as a function of the content of MMT, being higher for films prepared using CM method. These results coincide with the reported by Rhim [6] using agar as matrix and a modified MMT. This change in the surface texture may be due to the formation of agglomerates by own interactions of the film components, or also because of the drying process that would displace the particles to the surface.

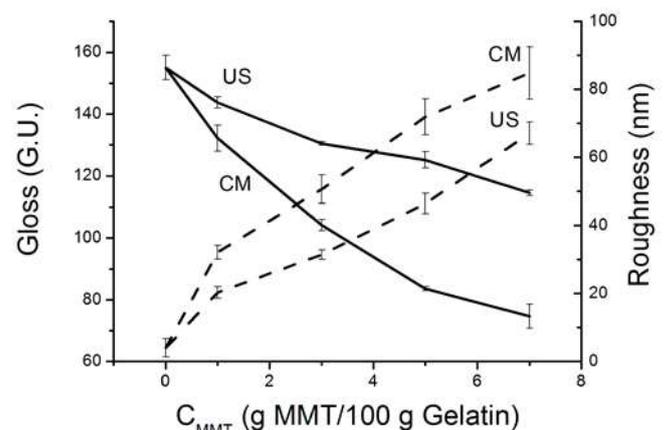


Figure 6. Gloss (solid line) and Roughness (dashed line) as a function of the montmorillonite (MMT) content in gelatin based nanocomposite films, for MMT dispersed by ultrasound (US) or colloidal mill (CM).

3.2.4. Gloss.

The gloss of a material is a surface property and therefore it is dependent of the smoothness or roughness of the surface, which influences the light reflection. Then, contrarily to the behavior of the films roughness, the films gloss decreased almost linearly as a function of MMT concentration, being that films of the US treatment presented remarkable higher values of surface gloss than

films of the CM treatment for all concentrations of MMT used (Figure 6). This reduction of surface gloss was 25% and 50% for films of the US and CM treatments, respectively, at the highest concentration of MMT. This behavior has been reported previously in different matrixes. Sothornvit et al. [13], working with whey protein isolate film and increasing content of a modified MMT, observed a reduction of about 34% when the concentration of the nanoclay was up to 10%. The result observed in this work was probably a consequence of the smaller particle size of the clay obtained with the ultrasonic processor that caused less superficial modifications to the microstructure of the film.

3.2.5. Water contact angle.

The surface hydrophobicity, measured as the water contact angle formed at the intersection of the liquid and solid phases, is one of the most important surface properties of biopolymeric films which is generally used to estimate the resistance of the film against liquid water [37]. The water contact angle of the nanocomposite films reduced as a function of MMT concentration (Figure 7), probably due to changes in surface texture as evidenced in the microstructure analysis (Figures 4, 5). Nanocomposite films of the US treatment presented slightly lower ($p < 0.05$) values of contact angle than films of the CM treatment (Fig. 7).

It is known that contact angles below 65° are typical of hydrophilic surfaces, while contact angles above 65° are characteristic of hydrophobic surfaces [38]. Then, all films

produced in this work could be considered as hydrophobic one ($75^\circ - 95^\circ$). As could be expected, the inclusion of MMT in the gelatin films increased the film resistance to water. These results disagreed with those obtained by Tunc et al. [36], whom observed that increasing concentration of nanoclay in wheat gluten become more hydrophilic the surfaces of the films.

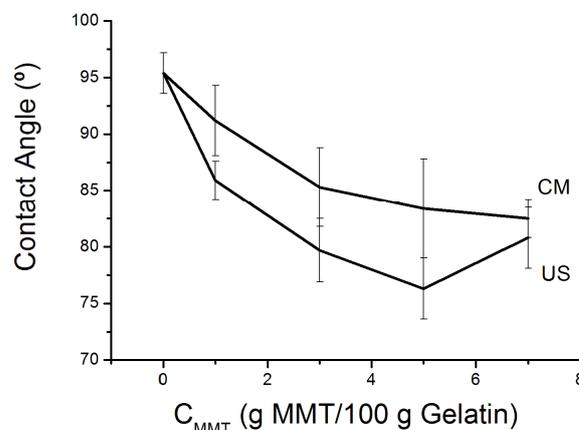


Figure 7. Contact angle with water as a function of the montmorillonite (MMT) content in gelatin based nanocomposite films, for MMT dispersed by ultrasound (US) or colloidal mill (CM).

4. CONCLUSIONS

The method used for montmorillonite dispersion had a strong influence on the quality of the nanoparticles dispersions, affecting the particle size and zeta potential, with evident effect on some properties of nanocomposites. Mechanical properties were improved, being that at low concentrations of MMT the films of the US treatment were more resistant and, at higher

concentrations, best results were obtained for films of the CM treatment. The main effect of the addition of MMT and the dispersion method used was observed in the microstructure and surface of nanocomposites. Smaller particles obtained with US treatment had a better distribution within the gelatin matrix with less impact on the films surface properties.

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