



Article Blends of Chitosan and Water Kefir Grain Biomass Incorporated with Nanosilica

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Abstract: The use of by-products from the food industry for the development of bioplastics represents an outstanding strategy in meeting current demands for material circularity. In this work, films based on chitosan (CH) and water kefir grain (WKG) biomass incorporated with different concentrations (3%-10% w/w) of nanosilica (SiO_2) were developed for the first time. The key properties required for food packaging were assessed. There are no structural changes (FT-IR) upon nanosilica incorporation. However, the XRD analysis indicates a tendency toward an increase in the amorphous character of the films. For the films with higher proportions of nanosilica, the SEM images indicate particle agglomeration points. The control film (CH/WKG) experienced a 5% mass loss at 68.3 °C, while the CH/WKG10%SiO₂ film showed the same mass reduction at 75.7 °C, indicating the nanoparticles increased the moisture-associated thermal stability of the films. The tensile strength was not significantly influenced by the incorporation of nanosilica, but there was an increase in elongation at break, from $25.01 \pm 3.67\%$ (CH/WKG) to $40.72 \pm 4.89\%$ (CH/WKG/3%SiO₂), followed by a drastic reduction to $9.10 \pm 1.99\%$ (CH/WKG/10%SiO₂). Overall, 3% of SiO₂ may be the most promising concentration for CH/WKG blends in future application as sustainable alternatives for food packaging, since it is possible to improve properties, such as ductility and thermal stability, at this concentration without marked losses in the tensile strength of the films.

Keywords: films; waste valorization; food packaging; coating; nano-SiO2

1. Introduction

The use of renewable resources, which includes the recovery of waste and industrial by-products, is an alternative to reduce costs and environmental impacts, aiming at the use of solid waste that has resulted from industrial processing [1,2]. In this context, to reconcile science, technology, and sustainability, applications that go beyond disposal in landfills have been studied, aiming at an alternative use of waste [2]. The use of waste and by-products not only solves environmental problems, but it also allows for their reuse as a sustainable biological material [3]. Thus, biopolymers obtained from waste and/or by-products have become a very promising application for the scientific and industrial community. As an example, the development of sustainable food packaging is a field of great prominence [4,5].

Among the biopolymers produced from industrial waste, chitosan (CH) is one of the most abundant sources of polysaccharides in nature [5], and it is obtained from the deacetylation of chitin, which is found mainly in the exoskeleton of crustaceans, mollusks, fungi, and insects [6]. The main sources of commercial CH are by-products of the seafood processing industry [7]. CH consists of two subunits, D-glucosamine (deacetylated unit) and N-acetyl-d-glucosamine (acetylated unit), which are linked linearly by 1,4-glycosidic



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). bonds [6,8–11]. Besides presenting low toxicity, biodegradability, and biocompatibility, CH also presents antimicrobial, antioxidant, and good film-forming properties, which makes it an attractive material in the production of packaging films [6,11]. However, although it is possible to obtain CH films with relatively good tensile strength, they are generally brittle, which limits their applications [6]. Moreover, CH still has a relatively high cost when compared to other sources of natural polymers, such as starch, pectin, and alginate, among others [8].

In this sense, the formation of CH blends with other biopolymers derived from waste and/or industrial by-products becomes a promising strategy to improve CH properties. Blends of CH with water kefir grain (WKG) biomass have been previously reported by our group, showing excellent potential for film production [8]. The biomass of WKG is the result of the process used in the production of fermented probiotic drinks [12], and WKG is mainly composed of dextran [13]. The kefir fermentation process is carried out with a sucrose solution added from fruits, usually figs and lemons, for up to three days when microorganisms form transparent cauliflower-shaped granules [13–15]. Only probiotic drinks are commercialized, and WKG is employed again in the next fermentation processes. However, as fermentation cycles occur, the amount of WKG biomass increases and the excess biomass is usually discarded [8,12]. Hence, an alternative for the valuing of this residual biomass is its incorporation in biopolymer-based matrices for sustainable film development.

In general, films based on a single biopolymer, or blends of different biopolymers, have several limitations in their application as food packaging. Some of the main limitations include low mechanical strength and high sensitivity to water vapor [16]. An efficient strategy to overcome these hindrances is the incorporation of nanofillers, such as SiO₂ nanoparticles (nanosilica or nano-SiO₂). Nano-SiO₂ is an amorphous white powder with a three-dimensional structure [17–19], which is widely utilized in industry due to its high specific surface area, low toxicity, biocompatibility, optical transparency, and chemical and thermal stability [17,18]. Numerous studies have shown that nanosilica represents an interesting option to improve the mechanical, thermal, and optical properties of biopolymerbased films, including sodium alginate [20,21], alginate/pectin [22], alginate/hydrolyzed collagen [22,23], chitosan [24], potato starch [25], and carboxymethylcellulose [26].

To the best of our knowledge, there is no research on the incorporation of nanosilica in CH and WKG blends (CH/WKG). Therefore, the aim of this research was to develop CH/WKG-based films incorporated with different concentrations of nanosilica (3, 6, and 10% w/w) to understand how this nano-filler influence film properties, which could be helpful for future food packaging applications. Thus, this study covered structural and morphological characterization, as well as mechanical properties, thermal properties, and water sensitivity. A clear relationship between the structure and properties of CH/WKG blend films incorporated with nanosilica was established.

2. Material and Methods

2.1. Materials

Whole water kefir grains (WKG) without purification (Probióticos Brasil, São Paulo, Brazil); chitosan (CH) from shrimp shells, with a degree of deacetylation \geq 75% (product number C3646, CAS number: 9012-76-4, specification: PRD.2.ZQ5.1000008299, Sigma Aldrich, São Paulo, Brazil); glycerol PA; and glacial acetic acid (Ecibra, São Paulo, Brazil) were used as the materials.

2.2. Water Kefir Grain Biomass Preparation

Water kefir grains (WKG) were prepared as described in our previous work [8], which was adapted from Coma et al. [13]. In brief, in 2 L of water, at 25 °C, 100 g of WKG was supplemented with 100 g of brown sugar and 50 g of dried figs. Additionally, 1 lemon cut into four slices (0.5 cm) was added to the solution. Then, in a glass container, the solution

was covered with a porous tissue. Finally, the obtained WKG was dried in an oven at 105 °C. The WKG moisture content was 84.3%.

2.3. Film-Forming Solutions Preparation

The CH/WKG (control sample) film-forming solution was prepared as described previously [8]. Briefly, aqueous solutions, with 1.5% (w/v) CH, 0.3% (w/v) WKG, 2% (w/v) acetic acid, and 0.45% (w/v) glycerol, were prepared to obtain the samples without nanosilica. All these concentrations were defined as preliminary. Particularly, the choice of 0.3% WKG (20% in relation to the mass of CH) was the result of a detailed characterization of films prepared with different WKG concentrations [8]. After preparing the CH/WKG blends, other solutions with 3, 6, and 10% (based on the added CH dry mass) of nanosilica were obtained. The resulting film-forming solutions were stirred at 600 rpm and 50 °C for 1 h, followed by 15 min in an ultrasonic bath to eliminate bubbles. A total of 90 mL of the film-forming solutions was added to polystyrene Petri dishes (14 cm diameter), which remained in an oven at 45 °C for 48 h. The obtained film samples were labeled as CH/WKG (control), CH/WKG/3%SiO₂, CH/WKG/6%SiO₂, and CH/WKG/10%SiO₂, and they were stored at 25 °C and 75% relative humidity (RH) before characterizations.

2.4. Characterizations

2.4.1. Morphology

A Leo 440i/6070 Scanning Electron Microscope (SEM) (LEO Electron Microscopy Cambridge, England) was employed for this purpose. It was used with a 15 kV accelerating voltage and a 50 pA current. The magnifications of the surface were $50 \times$ and $1000 \times$, and the cross section was $1000 \times$.

2.4.2. Structural Evaluation

The FT-IR spectra were recorded in a Bruker Vertex 80 device, Bruker (Coventry, UK), ranging from 4500 cm⁻¹ to 500 cm⁻¹. It used a 4 cm⁻¹ resolution, 16 scans, and 16 s scan time. The accessories used were a 4225/Q Platinum ATR, Diamond Multiple Crystals CRY, and a KBr Beamsplitter. A X'Pert-MPD analytical X-Ray diffraction (XRD) device, (Philips, Almelo, Netherlands) was employed with Cu K α radiation ($\lambda = 1.54$ Å), ranging from 2 $\theta = 5$ to 35° at 0.0333°/s. The degree of crystallinity was determined from the ratio between the crystalline fraction and the sum of the crystalline and amorphous fractions in each XRD profile. Transmittance (FT-IR) and intensity (XRD) were reported as arbitrary unities (a.u.).

2.4.3. Interactions of the Films with Water

The initial mass (W_0) of a piece of film (2 cm × 2 cm) was determined. Then, the sample was dried in an oven, at 105 °C for 24 h, to ensure total moisture removal. After that, the dry mass (W_d) was determined. The same sample was then immersed in 20 mL of deionized water for 24 h at 25 °C. Finally, the sample was dried again at 105 °C for 24 h, followed by a determination of the final mass (W_f). The moisture content (MC) was estimated using Equation (1), and the water solubility (WS) was estimated using Equation (2).

MC (%) =
$$\frac{W_0 - W_d}{W_0} \times 100$$
 (1)

WS (%) =
$$\frac{W_d - W_f}{W_d} \times 100$$
 (2)

2.4.4. Thermal Stability

The thermogravimetric analysis (TGA) was carried out in a Hitachi STA 7300 device, with a N₂ atmosphere at 50 mL min⁻¹, a heating rate equal to 10 °C min⁻¹, and temperatures ranging from 25 to 525 °C.

2.4.5. Thickness and Mechanical Properties

The mean thickness of each sample was determined at 10 random points using a digital micrometer (Mitutoyo South America, Suzano, Brazil). Then, a RDS-100-C high-precision cutting device (ChemInstruments, Fairfield, OH, USA) was employed to obtain 15 mm wide strip samples, which were stored for 48 h ($25 \pm 2 \,^{\circ}$ C and $75 \pm 5\%$ RH) before analysis. The tensile strength (MPa) and elongation at break (%) were determined according to the ASTM-D882 (2018) [27], using an Instron 5966-E2 universal testing machine (Norwood, MA, USA). The tests were performed with a 1000 N load cell, with initial grip separation of 50 mm and 12 mm min⁻¹. Five replicates were performed.

2.5. Statistical Analysis

The quantitative data were assessed with analysis of variance (ANOVA) and Tukey's test using Minitab[®] 21.1.0 (Minitab LLC, State College, PN, USA). The differences between the means were considered statistically significant when p < 0.05.

3. Results and Discussion

3.1. Morphology

Figure 1 presents the SEM images of the film surfaces (at magnifications of $50 \times$ and $1000 \times$) and the cross sections for each of the samples. From the lower magnification surface micrographs [Figure 1a–d], it can be observed that, as the nanosilica concentration increases, the films become rougher. Particularly, a higher surface roughness is observed for the CH/WKG/10%SiO₂ sample. Additionally, it is noted the presence of whitish spots on the surfaces [Figure 1g,h], and also in the cross sections [Figure 1k,l], at higher proportions of nanosilica, suggesting an incomplete dispersion of nanoparticles along the polymer matrix. Similarly, previous studies have also demonstrated SiO₂ agglomeration through its incorporation in chitosan [28] and in alginate/collagen-based films [20,22]. These research studies reported a proportion of nanosilica at around 10% is considered a determining factor for the appearance of agglomeration and relevant microstructural defects.



Figure 1. Surface and cross-sectional scanning electron microscopic images at different magnifications of the CH/WKG (**a**,**e**,**i**), CH/WKG/3%SiO₂ (**b**,**f**,**j**), CH/WKG/6%SiO₂ (**c**,**g**,**k**), and CH/WKG/10%SiO₂ samples (**d**,**h**,**l**). The red arrows indicate defective points in the microstructure of the films.

3.2. Structural Evaluation

Figure 2a illustrates the FTIR spectra obtained for the chitosan and water kefir grain blend (CH/WKG) and the samples incorporated with nanosilica (CH/WKG/3%–10%SiO₂). The bands around 3264 cm⁻¹ are associated with the vibration of hydroxyl groups (–OH) present in CH [7], and they are also found in the constituents of WKG (particularly dextran, proteins, and residual sugars) [8,12,13,29]. At approximately 2925 cm⁻¹, it is possible to observe signals related to the symmetric and asymmetric vibrations of C-H. Additionally, the C=O peak associated with amide groups appears at 1636 cm⁻¹, and the amide II band appears at around 1550 cm⁻¹. The transmittance peaks at 1217 and 1151 cm⁻¹ can be assigned to the C-O-C asymmetric stretching, and the peaks at 1066 and 1033 cm⁻¹ are due to the C-O stretching [7]. Typical peaks of nanosilica are expected to appear at 790 cm⁻¹ [21]. However, regardless of the proportion used, these peaks are not observed for all samples incorporated with SiO₂, possibly due to the low concentration used.



Figure 2. (a) FTIR spectra, (b) DRX patterns of the CH/WKG film and the samples incorporated with different proportions of nanosilica, and (c) simplified scheme of the possible effect of macromolecular clearance from the incorporation of nanosilica. In this scheme, we consider only the CH chains as an example, although the polymeric matrix used in this study is a blend of CH and WKG.

In previous reports, the X-ray diffraction (XRD) profile of pure nanosilica indicates an amorphous halo with a maximum intensity appearing around $2\theta = 23^{\circ}$ [21,29]. For a pure CH-based film, the characteristic peaks at around $2\theta = 10^{\circ}$ and 20° can be associated with the anhydrous crystalline phase of this biopolymer [7,8]. Figure 2b presents the XRD profiles of the CH/WKG blends which, as reported in our previous research [8], form a semicrystalline structure, with a broad peak at around $2\theta = 21^{\circ}$. The estimated degree of crystallinity for this sample is 13.1%, suggesting a mostly amorphous structure. The addition of nanosilica apparently did not change the XRD profiles of the films. However, it contributed to a reduction in the degree of crystallinity to 11% (CH/WKG/3%SiO₂), 11.5% (CH/WKG/6%SiO₂), and 9.7% (CH/WKG/10%SiO₂), suggesting that the nano-filler contributed to the amorphization of the films. Similar results have been reported in the literature [21,30,31].

Overall, the incorporation of nanosilica in CH/WKG films did not cause the appearance of new bands, indicating the non-occurrence of covalent chemical bonds between the materials involved. In this sense, it suggests that there are only intermolecular interactions between the biopolymers and the nanoparticles. Figure 2c schematizes the occurrence of hydrogen bonds between chitosan macromolecules. After the incorporation of nanosilica, part of these chitosan–chitosan and/or chitosan–dextran bonds (and/or other WKG constituents' interactions) are replaced by secondary bonds between the macromolecules and SiO₂ nanoparticles. The incorporation of nanosilica may have pulled away the polymeric chains, contributing to a greater tendency for structural amorphization at higher concentrations of the additive, as shown in the XRD profiles [Figure 2b].

3.3. Interactions of the Films with Water

Table 1 shows the estimated water solubility (WS) and moisture content (MC) values of the CH/WKG-based films with nanosilica. In all the proportions used, the incorporation of nanosilica causes a significant increase in MC in relation to the control film. Nanosilica is known to be quite hydrophilic, and although there is considerable dispersion of particles along the polymeric matrix, the SEM images (Figure 1) indicate the presence of agglomeration points, especially at higher nanosilica loads. This factor may have contributed to the greater moisture retention at these points. In addition, the possible macromolecular displacement that results from the incorporation of nanosilica [Figure 2c] may have been another contributing factor. The existence of more free volume may enable the accommodation of more water molecules [23], resulting in an increase in MC.

Table 1. Moisture content (MC) and water solubility (WS) of the films. The averages of the results followed by the same letter (^a, ^b) in the same column do not differ from each other (p < 0.05).

| Film Sample | MC (%) | WS (%) |
|----------------------------|------------------------------|-------------------------------|
| CH/WKG | 17.54 ± 0.97 ^a | 15.19 ± 0.32 ^a |
| CH/WKG/3%SiO ₂ | 19.57 ± 0.55 ^b | 16.95 ± 0.30 ^b |
| CH/WKG/6%SiO ₂ | 19.24 ± 0.48 ^b | $16.05\pm0.42~^{ m ab}$ |
| CH/WKG/10%SiO ₂ | $20.69\pm0.36~^{\mathrm{b}}$ | 15.31 ± 0.32 $^{\rm a}$ |

The dissolution of hydrophilic biopolymers, such as CH/WKG, involves the penetration of water to the matrix and swelling. The hydrogen bonds between macromolecules become dissociated due to the competition with water molecules, which results in deformation and dissolution of the film [32,33]. Chemical degradation, which generates oligomers and monomers, can also occur via hydrolysis or enzyme-catalyzed hydrolysis [34]. Regarding WS, Table 1 indicates that only the CH/WKG3%SiO₂ sample displays a significant increase. Although statistically significant, it is noted that the incorporation of nanosilica has not caused a marked increase in the solubility of the films. The values range from 15.31 ± 0.32 to $16.95 \pm 0.30\%$, which can be considered low. These results suggest that, although there is macromolecular displacement, the nanosilica might not have interrupted the intermolecular association of the CH and WKG component chains in the films. Furthermore, this may be a strong indication of the intermolecular interactions existing between the polymeric matrix and the SiO₂ nanoparticles.

3.4. Thermal Stability

Figure 3 highlights the two main thermal events observed for the CH/WKG blends incorporated with nanosilica. The first one is associated with the moisture loss of the films, which MC varies between 17 and 20% (Table 1). The second thermal event, occurs between 260 and 330 °C, is related to the degradation of the biopolymers that constitute the film matrices. The stability test was carried out proportionately up to 525 °C because the aim was to verify the impact of the nanosilica on the first two main thermal events. Notably, the incorporation of the nanosilica provides an increase in the thermal stability of the films, according to the displacement of the curves in Figure 3. A quantitative assessment of how much the nanofiller has influenced the thermal stability can be seen in Table 2.



Figure 3. Thermogravimetric profile (**a**) of the films and its first derivative, DTG (**b**), with indication for the two main thermal events.

| Film Sample | т (°С) | 1st Thermal Event | | 2nd Thermal Event | | | |
|--------------|----------------------|-------------------------|-----------------------|--------------------------|-------------------------|-----------------------|--------------------------|
| | 1 _{5%} (C) | T _{onset} (°C) | T _{max} (°C) | T _{offset} (°C) | T _{onset} (°C) | T _{max} (°C) | T _{offset} (°C) |
| CH/WKG | 68.3 | 47.4 | 80.4 | 114.8 | 257.3 | 289.2 | 329.0 |
| CH/WKG/3%SiG | D ₂ 73.1 | 46.0 | 93.0 | 123.0 | 259.0 | 291.0 | 333.0 |
| CH/WKG/6%SiG | D ₂ 67.4 | 48.0 | 79.0 | 113.0 | 258.0 | 292.0 | 334.0 |
| CH/WKG/10%Si | O ₂ 75.7 | 68.0 | 99.0 | 129.0 | 260.0 | 292.0 | 332.0 |

Table 2. Temperatures of 5% mass loss and temperatures related to the two main thermal events.

The second column of Table 2 indicates the temperature at which 5% of mass loss occurred in each sample. The control film (CH/WKG) shows this mass loss at 68.3 °C, while the CH/WKG10%SiO₂ sample shows a 5% mass reduction at 75.7 °C. At the same time, it is verified that the T_{onset} of the first thermal event also increases from the incorporation of the nanosilica. However, the T_{onset} of the second thermal event apparently has no significant change from the incorporation of the nanosilica. Therefore, it is suggested that nanoparticles increase the moisture-associated stability of the films. These findings indicate that the nanosilica enables moisture retention in a more pronounced way. In addition to films with higher MC values, the water retained in the agglomerated nanoparticles binds more intensely with this material, requiring a greater amount of energy to remove it from the films.

3.5. Thickness and Mechanical Properties

The thickness and mechanical properties of the films are presented in Table 3. Based on the statistical analysis, it can be observed that there is no significant change in the thickness of the films with the addition of the nanosilica. The same happens with the tensile strength. However, interestingly, the elongation at break increases for the CH/WKG/3%SiO₂ and CH/WKG/6%SiO₂ films, and then it decreases drastically for the CH/WKG/10%SiO₂ film.

Table 3. Thickness, tensile strength, and elongation at break of the films. The averages of the results followed by the same letter $(^{a, b})$ in the same column do not differ from each other (p < 0.05).

| Film Sample | Thickness (µm) | Tensile Strength (MPa) | Elongation at Break (%) |
|---------------------------|-----------------------------|--------------------------------|-----------------------------|
| CH/WKG | 151.54 ± 10.05 $^{\rm a}$ | 16.28 ± 2.55 $^{\mathrm{a}}$ | 25.01 ± 3.67 $^{\rm a}$ |
| CH/WKG/3%SiO ₂ | $161.24\pm4.74~^{\rm a}$ | 14.18 ± 1.57 ^a | 40.72 ± 4.89 ^b |
| CH/WKG/6%SiO ₂ | 167.40 ± 10.17 $^{\rm a}$ | 16.02 ± 0.83 $^{\mathrm{a}}$ | 37.21 ± 4.21 ^b |
| CH/WKG/10%SiO2 | 166.24 ± 16.11 $^{\rm a}$ | 16.04 ± 0.94 a | 9.10 ± 1.99 c |

The increase in elongation at break (film ductility) at the nanosilica concentrations equal to 3 and 6% suggests that this additive acts in the same manner as a "plasticizing agent", possibly locating itself between macromolecules and, consequently, increasing the free volume, which implies an increase in macromolecular mobility. This result again corroborates with the scheme proposed in Figure 2c. Nanosilica is rich in hydroxyl groups (OH), which could interact with biopolymers' matrix through hydrogen bonding [35] as this additive is located between macromolecules. In addition, particle agglomeration may also have influenced this result, which may have acted as an internal lubricant by reducing the frictional forces in the polymeric matrix [19,22]. When the results for the highest concentration of nanosilica (CH/WKG/10%SiO₂) are evaluated, a drastic reduction in elongation at break is observed, indicating that concentrations in this range can be considered the thresholds for this additive. In this concentration range, the incidence of microstructural agglomeration points (defects) can act as stress concentration points in the material, justifying such observed fragility.

No significant changes were observed in the tensile strength of the films at any concentrations evaluated, unlike the reduction expected based on the theory that the nanoparticles would act as "plasticizing agents". This indicates that it is possible to improve the ductility of the films without a loss of tensile strength if concentrations of 3 and 6% of nanosilica are used. Unlike what was observed in the present study, the incorporation of 10% SiO₂ in sodium alginate films containing a propolis extract provided a significant increase in tensile strength [20]. The same behavior was reported for blends of sodium alginate and pectin incorporated with 10% SiO₂ [22]. It is clear that the biopolymer matrices used in these mentioned studies are quite different from the major matrix (CH) used in the present study. While the former matrices are anionic, the latter is cationic. Therefore, these differences in mechanical behavioral trends may also be associated with these molecular factors.

4. Conclusions

This study aimed to develop films based on chitosan (CH) and water kefir grains (WKG) incorporated with nanosilica (SiO₂), targeting future applications such as food packaging. Overall, the incorporation of nanosilica provided the films with a higher moisture content, which is related to its hydrophilic nature, but there was no significant difference between the evaluated nano-filler proportions.

The incorporation of nanoparticles increased the moisture-associated thermal stability of the films. Additionally, the mechanical properties suffered a significant influence on only the elongation at break of the films, suggesting the additive acted in the same manner as a "plasticizing agent" at low concentrations. Particle agglomeration might have influenced this result, which might have acted as an internal lubricant by reducing the frictional forces in the polymeric matrix. Considering the set of evaluated properties, it is concluded that 3% of SiO₂ is the most promising proportion for the incorporation of this filler into CH/WKG blends. At this proportion, films with uniform morphology are obtained. Furthermore, it is possible to increase the thermal stability of the material, with a significant increase in ductility and without impairing their tensile strength. In this manner, such properties are more suitable for forthcoming studies as possible sustainable substitutes for food packaging applications.

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