

## Nanocomposites Based on Cassava Starch and Chitosan-Modified Clay: Physico-Mechanical Properties and Biodegradability in Simulated Compost Soil

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Organic-inorganic nanocomposites based on cassava starch, glycerol and chitosan-modified Veegum<sup>®</sup> HS clay mineral at two different low polymer-to-clay ratios (2.5 and 5.0 wt.%) were prepared by extrusion producing flexible, transparent and homogeneous plastics as potential candidates for agricultural purposes. X-ray diffraction and transmission electron microscopy images revealed the presence of both intercalated and exfoliated nanocomposites in all samples, in which exfoliation is the predominant type of microscopic structure. Statistically significant improvements of over 20% on the tensile strength and Young's modulus were observed for samples containing chitosan-modified clay in comparison to pristine thermoplastic starch. Chitosan deeply affects the conversion of polymer carbon to CO<sub>2</sub> through biodegradation. Mineralization values for the sample loaded with 5.0 wt.% of chitosan-modified clay in simulated compost soil showed a reduction of almost 40% in comparison to thermoplastic starch, benefiting applications where delay degradation is required.

**Keywords:** organic-inorganic hybrids, nanocomposites, extrusion, cassava starch, Veegum clay

### Introduction

Polymers produced from biodegradable resources have been intensely investigated over the past decades in an effort to decrease human reliance on fossil-based materials, especially over growing concerns about improper discard of these materials.<sup>1-3</sup> In particular, naturally occurring polymers or commonly known as biopolymers, macromolecules with large abundance across the globe, seem potential candidates to replace partially or totally oil-based plastics in many applications.<sup>1,3</sup> Among them, biopolymers made from or blends with starch appear in a growing number of reported studies as a potential material with a broad array of applications. In fact, starch possesses several properties desired in modern materials, such as large availability from different sources obtained in all

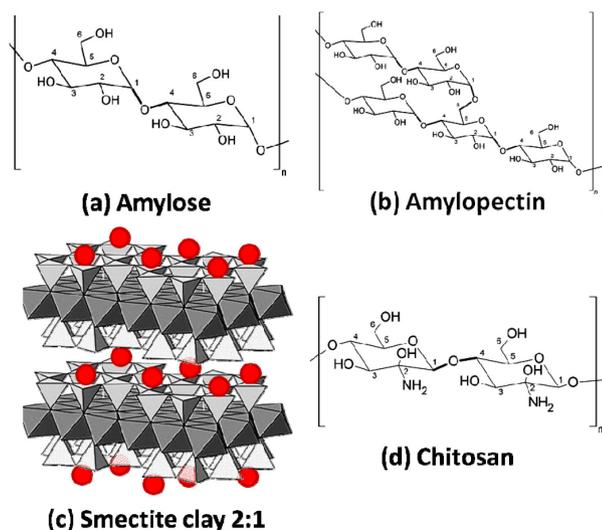
regions, low cost associated to both cultivation/harvesting and also processing to obtain the final product and full biodegradability under regular disposal conditions.<sup>4,5</sup>

Cassava starch is composed mainly of two types of polysaccharides, amylose and amylopectin.<sup>1,6</sup> Even though amylose to amylopectin ratio might not be constant depending on the cassava species, it is usually around 1:4, respectively.<sup>7</sup> While both polysaccharides are constituted by the same monomer unit called  $\alpha$ -D-glucopyranose, amylose possesses almost exclusively 1-4  $\alpha$  linkages, originating short linear chains (Figure 1a).<sup>1,8</sup> On the other hand, amylopectin features not only the presence of 1-4  $\alpha$  linkages, but also 1-6  $\alpha$  linkages, yielding much larger and coiled structures (Figure 1b).<sup>9</sup>

In order to transform cassava starch on a plastic material, it is necessary to disrupt its hierarchical coiled structure.<sup>10</sup> Addition of plasticizers such as glycerol or urea, among others, is often needed during the production

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of thermoplastic starch (TPS) to prevent recrystallization of amylopectin chains (a process called retrogradation).<sup>1,11</sup> However, TPS, the resulting material after processing starch with plasticizers using water/shear stress and temperature, shows considerably poorer mechanical properties in comparison to oil-based plastics.<sup>12</sup>



**Figure 1.** Structures of (a) amylose; (b) amylopectin; (c) smectite clay 2:1 (spheres represent the cations which neutralize the negative charged layers); (d) chitosan.

One strategy to improve physical properties of TPS is to combine it during starch gelatinization process with inorganic layered materials like clay mineral particles. In this case, the improvement of the physical properties can be related to the extent of the interaction that is established within the interface of the particles and the polymer matrix.<sup>13,14</sup> Some factors, which determine the range of improvement of such properties, are size and shape of the inorganic particles, its orientation within the polymer phase, and the type of interaction on the interface.<sup>15</sup> Usually, an increase in the hybrid material performance in comparison to the pristine polymer is noticed when mechanical properties are evaluated especially for inorganic particles with high anisotropy, like clay particles, since these 2-D particles possess high tensile strength in the direction of highest length.<sup>16</sup>

Veegum<sup>®</sup> HS is the commercial name for magnesium aluminum silicate, containing small amounts of other metal oxides, commonly used in cosmetics and pharmaceutical applications.<sup>17,18</sup> It is essentially a purified bentonite belonging to the smectite mineralogical group.<sup>19</sup> These mineral clays are different regarding their chemical composition, but possess identical crystalline structure comprised of two silica (SiO<sub>2</sub>) tetrahedral sheets organized in hexagonal rings and a central metal hydroxide octahedral sheet, known as 2:1 type clay (Figure 1c).<sup>1,20</sup> Veegum<sup>®</sup> HS has negative layers which are

charge balanced by the presence of cations between the layers. Due to the clay ability to form strong hydrogen bonds with hydrophilic polymers, display large surface area and platelets with high tensile strength, these inorganic particles are suitable candidates to produce organic-inorganic hybrid materials with superior properties in comparison to the pristine polymer matrix.<sup>1,21-23</sup> Additionally, the presence of exchangeable cations in the interlayer space allows chemical modifications of clays, admitting the presence of other species that contain functionalities not owned by neither polymer nor the clay phase. One interesting property that can be carried to nanocomposites is the capacity to restrict microbial growth. Several compounds exhibit such property and, among them, chitosan is one of the “green” options available. Chitosan is a linear polysaccharide biopolymer obtained from partial or total deacetylation of chitin (poly (β-(1→4)-N-acetyl-D-glucosamine)) (Figure 1d), commercially available from shrimps and other crustaceans.<sup>1</sup> It is recognized as a biodegradable, biocompatible and non-toxic material.<sup>24</sup> Chitosan presents several agricultural applications such as stimulation of plant growth, action against plant pathogens in the control of plant diseases, and time release of fertilizers and nutrients in soil.<sup>25,26</sup>

One remarkable property of chitosan is its antimicrobial activity (mainly yeasts and molds, followed by gram-positive and gram-negative bacteria).<sup>26</sup> One proposed mechanism of the antimicrobial action of chitosan involves the membrane damage. The interaction of this biopolymer with the outer membrane arises from the positive charge of the amino group attached to the C2 carbon monomer and the predominant anionic structures of the membrane such as lipopolysaccharides and proteins.<sup>27</sup> Protonation of the amino groups presented on chitosan is achieved during acidification of an aqueous solution to promote its solubilization.<sup>28</sup> Immobilization of chitosan in layered materials as clay minerals through ion exchange reaction can be considered a simple pathway to avoid polymer leaching, and maintaining its antimicrobial activity during a certain application. Considering that starch and chitosan have similar chemical structures, the modification of clay surface (bearing a negative electric charge) by intercalation of chitosan (in the protonated or cationic form) can improve the compatibility between the inorganic (clay) and organic (starch) phases. The presence of chitosan on different plastic formulations also decreases its overall hydrophilicity,<sup>29</sup> a problem that severely limits plastics applications containing highly hydrophilic polymers like starch.

This work describes the preparation of starch-based nanocomposites containing chitosan-modified clay particles and evaluates the impact of the mineral filler in the starch physicochemical and biodegradation properties through

the comparison to pristine TPS. Each component for nanocomposite preparation (starch, chitosan and Veegum<sup>®</sup> HS) was chosen considering sustainable principles such as availability, cost, biocompatibility, chemical compatibility, and origin (natural *vs.* synthetic).

## Experimental

### Materials

Erawan Marketing Company (Bangkok, Thailand) kindly supplied cassava starch. Glycerol was obtained from Sigma-Aldrich Chemical Company (Milwaukee, WI, USA). Chitosan (low molecular weight, degree of deacetylation from 75 to 85%) was also acquired from Sigma-Aldrich. Veegum<sup>®</sup> HS clay was provided from R. T. Vanderbilt Company (Norwalk, CT, USA). Glacial acetic acid was obtained from Acros Organic (Morris Plains, NJ, USA). Hydrochloric acid solution (37% by mass) and silver nitrate were gotten from Merck (Darmstadt, Germany). All materials were used as received without further purification.

### Modification of Veegum<sup>®</sup> HS with chitosan

Chitosan was previously dissolved in a 1 wt.% acetic acid solution in water to produce a 4.2 wt.% solution of polymer in acidic medium. During solubilization of chitosan, the pH of this solution was continuously checked with a digital pHmeter to assure a pH value of 4.5. Addition of small volumes of 0.1 mol L<sup>-1</sup> HCl solution was employed to decrease the pH to desired values. Later, in another flask, small amounts of Veegum<sup>®</sup> HS clay were slowly added under mechanical stirring in enough deionized water to produce a 3.5 wt.% clay suspension, which pH value was adjusted to 4.5 prior to its mixture with chitosan. Then, the chitosan solution was slowly added to the clay dispersion under mechanical stirring at room temperature. This mixture was stirred for 24 hours and then put on dialysis membranes and immersed in deionized water. Chitosan-modified clay was removed from dialysis when chloride test with AgNO<sub>3</sub> solution was negative. To remove the water content from the clay-chitosan material, it was freeze-dried until a pearl colored solid was reminiscent. The resulting material from this procedure was labeled as Vee-Chit.

### Preparation of starch-based nanocomposites through extrusion

#### Preparation of TPS

Cassava starch and glycerol was added to a Zip-Lock bag at the 70:30 ratio (wt.%). After sealed, the plastic bag

was hand mixed for 10 minutes, until a homogeneous and uniform mass was obtained. Later, the mixture was allowed to rest for 10 hours at 25 ± 2 °C before further processing. After resting, the mixture was processed using a vertical co-rotating twin-screw micro-compounder (DSM Research, Geleen, The Netherlands) attached to an injection molder. The extruder has 150 mm long screws with L/D ratio of 18 and barrel volume of 15 cm<sup>3</sup>. The equipment was set to screw rotation of 100 rpm and 130 °C of all three heating zones and the molten end closed prior to the start. Then, the machine was fed with single batches of 15 g of starch-glycerol mixture at a time. After insertion, the mixture was kept on the extruder for 5 minutes. Afterwards, part of the extruded material was transferred to a collecting tube of an injection molder, kept at 140 °C and transferred to the interior of a mold kept at 50 °C using a high-pressure pneumatic cylinder. The molds containing the injected materials were kept under pressure for 20 seconds before removal from the injection molder and then the TPS samples were removed from molds using a compressed air flow. Different types of molded TPS were obtained (discs, rectangles and dumbbells) and used as needed. The other part of the TPS extruded was allowed to flow freely from the equipment and was collected in a shape of spaghetti. Prior to the characterization, all the obtained TPS from the extrusion process was allowed to equilibrate on a controlled room with temperature set at 25 ± 2 °C and room humidity (RH) of 50 ± 3% for 4 days. The resulting material from this procedure was labeled as TPS.

### Preparation of starch-based nanocomposites containing chitosan-modified clay

Hybrid materials containing chitosan-modified clay were prepared similarly to the procedure described for the TPS. Initially, a solid mass comprising 70 g of cassava starch plus Vee-Chit (2.5 or 5.0 wt.% of modified-clay to starch) and 30 g (23.8 mL) of glycerol was mixed. Then, in different Zip-Lock bags, the mix of all previously measured materials were added, sealed and hand mixed for 10 minutes, until a homogeneous and uniform mass was obtained. Prior to extrusion, the mixture was allowed to rest for 10 hours at 25 ± 2 °C. After resting, the extruder was set to a screw rotation of 100 rpm and 130 °C of all three heating zones and the molten end closed prior to the start. Then, single batches of 15 g of starch-glycerol mixture were feed to the extruder at a time and processed as described above for the TPS samples. Samples were collected as discs, rectangles, dumbbells, and in the form of spaghetti. Prior to the characterization, all the obtained composite samples from the extrusion process were allowed to equilibrate on a controlled room with temperature set at 25 ± 2 °C and RH

of  $50 \pm 3\%$  for 4 days. The resulting material containing 2.5 or 5.0 wt.% of Vee-Chit in relation to starch was labeled as Star/Vee-Chit 2.5% or Star/Vee-Chit 5.0%, respectively.

#### Physicochemical characterization

X-ray diffraction (XRD) patterns were collected on a Bruker D8 Advance diffractometer (Karlsruhe, Germany) using Cu K $\alpha$  radiation ( $\lambda = 1.5451 \text{ \AA}$ ) operating at 40 kV and 40 mA. A step size of  $0.02^\circ \text{ s}^{-1}$  was used and the angular domain analyzed was comprised between  $(2\theta) 2.0$  and  $70^\circ$ .

Thermogravimetric analysis (TGA) were conducted on a TA Instruments Q500 equipment (New Castle, DE, USA) using 10 mg of a given sample on an aluminum pan, under a synthetic air flow ( $50 \text{ mL min}^{-1}$ ) and a heating rate program of  $10^\circ \text{ C min}^{-1}$  from 30 to  $500^\circ \text{ C}$ .

Transmission electron microscopy (TEM) images were registered using a JEOL 100-CX equipment (Tokyo, Japan) operating at 100 kV. Prior to the analysis, all analyzed samples were cryo-sectioned using a RMC Boeckeler Instruments CR-X ultramicrotome (Tucson, AZ, USA) operating at  $-55^\circ \text{ C}$ , employing a  $45^\circ$  angle diamond-cutting knife and using isopropanol as collection medium. After that, samples were deposited onto a carbon-coated Cu microgrid.

Mechanical properties were investigated using an United Testing Systems SFM-20 equipment (Huntington Beach, CA, USA). All tests were performed at  $23^\circ \text{ C}$  and RH of 50%, according to ASTM D790-07.<sup>30</sup> Initial grip distance was set for 5.0 cm and a loading cell of 9.075 kg (20 lb) was employed. A grip separation rate was set to  $70 \text{ mm s}^{-1}$ , and the results were reported as the mean values of three samples.

Carbon content of the analyzed samples was determined through elemental analysis carried out on a PerkinElmer 2400 CHN analyzer (Branchburg, NJ, USA), using 2.0 mg for each sample at Central Analítica of Instituto de Química (Universidade de São Paulo (USP), São Paulo, Brazil).

#### Biodegradability assay

Biodegradation tests under composting conditions were performed according to Kijchavengkul *et al.*<sup>31</sup> In summary, a direct measurement respirometer (DMR) system analyses the  $\text{CO}_2$  released by an array of bioreactors connected to a  $\text{CO}_2$  infrared analyzer. Each bioreactor, comprised of a glass jar contained 300 g (wet basis) of a mixture of organic fertilizer previously screened using a 1 cm sieve opening and later heated at  $60^\circ \text{ C}$  for 24 hours, vermiculite and deionized water at the proportion of 4:1:2.5 (wt.%) called compost. Three sets of jar were used in this experiment. The first set was comprised of 300 g (wet basis) of compost and was used as a control sample (blank). The second set contained

300 g of compost (wet basis) and 10 g of powdered cassava starch was used as a positive control. The remaining set was comprised of 300 g (wet basis) of compost and 10 g of sample cut in small pieces with diameter lower than 2 mm. Each experiment was run on triplicate. Prior to the analysis, the content of the jars were mixed using a disposable spoon. Bioreactors were allocated in an environmental chamber with temperature set at  $58 \pm 2^\circ \text{ C}$  and continuously purged with decarbonated airflow with RH  $55 \pm 5\%$  during 60 days. The obtained data were converted to percent of mineralization (% Mineralization). The total carbon from the analyzed sample was converted to  $\text{CO}_2$  through microbiological decomposition after a certain period and expressed according to equation 1:<sup>31</sup>

$$\% \text{ Mineralization} = \frac{(s\text{CO}_2 - \text{CO}_{2b})}{W \times \frac{\%C}{100} \times \frac{44}{12}} \quad (1)$$

where  $s\text{CO}_2$  is the total carbon mass released by the sample,  $\text{CO}_{2b}$  is the total of carbon mass released by blank bioreactor, W is the mass of sample used, %C is the carbon content percentage of the sample (determined through carbon elemental analysis).

The term  $s\text{CO}_2$  is a converted parameter from concentration values detected through mass detection according to equation 2:<sup>31</sup>

$$s\text{CO}_2 = \frac{C \times F \times t \times 44}{22414 \times 10^6} \quad (2)$$

where C is the determined  $\text{CO}_2$  and corrected concentration obtained through a calibration curve (values in ppm), F is the gas flow ( $\text{cm}^3 \text{ min}^{-1}$ ), and t is the waiting time between two respective measurements.

#### Statistical analysis

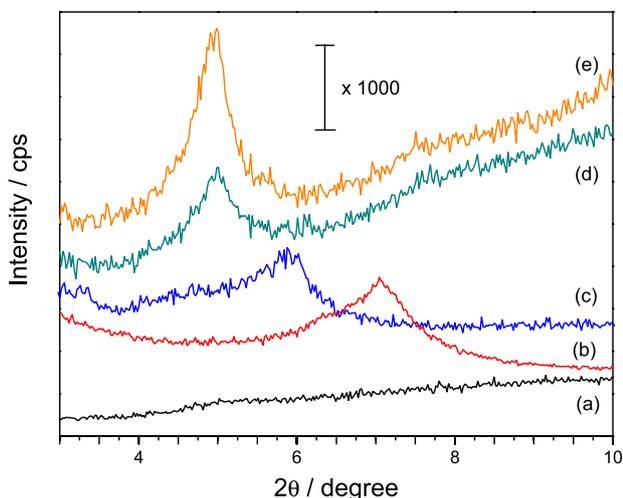
To evaluate statistical differences for distinct sets of values, Tukey's honest significant test was employed, using a confidence interval of 95%. All the raw values obtained from the tests were input in the Origin Pro 8 software, from Origin Lab Corp. (Northampton, MA, USA), and the results analyzed.

## Results and Discussion

#### X-ray diffraction (XRD)

The original cations in the interlayer structure of smectite clays can be replaced by ion exchange process

when keeping a clay suspension in contact with the replacing cations. This process in smectite clays is favorable due to their low layer charge density and the initial presence of hydrated cations, such as  $\text{Na}^+$  and  $\text{K}^+$ , disrupting the stacking structure.<sup>32</sup> Figure 2 shows the XRD patterns of the prepared materials. Pristine Veegum<sup>®</sup> HS clay is originally intercalated with hydrated  $\text{Na}^+$  cations,<sup>33</sup> evidenced by the presence of a diffraction peak centered ( $2\theta$ ) at  $7.05^\circ$  ( $d$ -spacing =  $12.5 \text{ \AA}$ ).



**Figure 2.** XRD patterns of (a) TPS; (b) pristine Veegum<sup>®</sup> HS clay; (c) chitosan-modified Veegum clay; (d) Star/Vee-Chit 2.5%; (e) Star/Vee-Chit 5.0%.

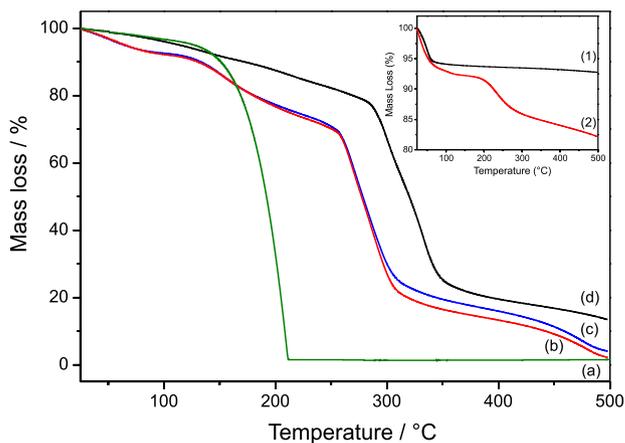
After immobilization of chitosan in clay by ion exchange reaction, chitosan-modified clay sample shows a peak at lower angle in comparison to pristine Veegum<sup>®</sup> HS, as observed in other publications using MMTs.<sup>34,35</sup> The reflection at ( $2\theta$ )  $6.0^\circ$  ( $d$ -spacing =  $14.7 \text{ \AA}$ ) can be attributed to the presence of a single chain of chitosan intercalated between two adjacent layers of clay, as reported by Darder *et al.*<sup>36</sup> XRD profile of pristine starch exhibits several peaks between the range of ( $2\theta$ ) 10 and  $25^\circ$ , which are closely related to semicrystalline pattern of arrangement of the polymer chains in three main groups, known as type-A, type-B or type-C.<sup>20</sup> However, when fully gelatinized, only a broad signal is observed between the same range, related to short-distance level of organization in thermoplastic starch. Therefore, no relevant information appears at higher angles than the showed in Figure 2 ( $2\theta = 10^\circ$ ). After processing chitosan-modified clay with starch and glycerol through extrusion, a diffraction peak centered at ( $2\theta$ )  $4.97^\circ$  ( $d$ -spacing =  $17.8 \text{ \AA}$ ) appears. When the chitosan-modified clay content on the extruded sample is increased from 2.5 to 5.0%, no variation of diffraction peak position is observed. However, the intensity of this signal is magnified almost three times, indicating the increase of

chitosan-modified clay content and that intercalation occurs in a larger extension on the sample containing the highest clay to starch content (i.e., Star/Vee-Chit 5.0% sample).

Since each nanocomposite sample contains a mixture of starch, glycerol and chitosan-modified clay, a careful analysis is needed to understand which of the cited species are contributing to the presence of the intercalation peak on Star/Vee-Chit composites. Glycerol is commonly used on the identification of smectite clays due to the characteristic peak observed with  $d$ -spacing of  $17.0 \text{ \AA}$ .<sup>37,38</sup> On the other hand, the observed  $d$ -spacing for Star/Vee-Chit nanocomposite samples have higher  $d$ -spacing values, indicating that glycerol might not be the only species intercalated. Since the intensity of the intercalation diffraction peak in the prepared samples when the clay content is increased, it could be inferred that the starch chains may be the responsible for layer expansion of clay particles. Since starch chains are not linear as cellulose chains,<sup>1</sup> it is not possible to identify the conformation of these glycosidic macromolecules after intercalation. Due to the high hydrophilicity of starch, water molecules could be absorbed by the samples, and moved to the interlayer domain during the moisture equilibration period. Even if an intercalation signal is detected, it is not possible to determine through the XRD technique if exfoliation or intercalation is dominant. Furthermore, the broadness of the intercalation peak indicates a wide range of interlayered species caused by the presence of huge non-linear macromolecules and glycerol.<sup>39</sup>

#### Thermogravimetric analysis (TGA)

TGA curves of the precursors and the produced starch-based samples are shown in Figure 3. At the temperature utilized in the extrusion process ( $130^\circ\text{C}$ ), glycerol exhibits



**Figure 3.** TGA curves of (a) glycerol; (b) Star/Vee-Chit 2.5%; (c) Star/Vee-Chit 5.0%; (d) TPS. Synthetic airflow of  $50 \text{ mL min}^{-1}$ . Inset: TGA curves of (1) Pristine Veegum<sup>®</sup> HS clay; (2) chitosan-modified Veegum clay.

a mass loss of approximately 4.5 wt.% (Figure 3a), indicating that its presence in the extruded materials might be lower than the nominal value due to its partial evaporation. In addition, due to the low shear force of the twin-screw extruder and moderate low temperatures used to obtain the samples, it was not expected to achieve chemical modification of starch chains with glycerol.<sup>40</sup>

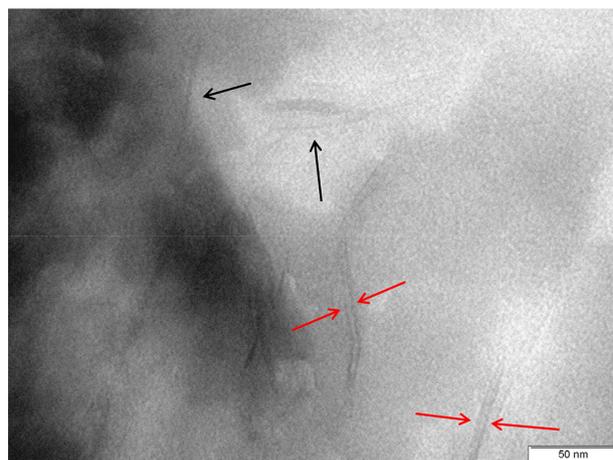
Up to 500 °C, Veegum® HS clay exhibits one mass loss event caused by the release of moisture initially present in the inorganic material. This event ranges from the beginning of the heating ramp up to 150 °C, yielding a mass loss of 6.5 wt.% (Figure 3 inset). Chitosan-modified clay displays a more complex mass loss process in comparison to the pristine clay. Similarly, the first mass loss event occurs up to 150 °C (Figure 3 inset) and involves the release of water molecules present in the hybrid sample. Since chitosan has affinity for water molecules due to the capability of hydrogen bond interactions, water is kept intercalated/adsorbed in chitosan-modified clay increasing the mass loss value. According to chitosan-modified clay TGA curve, about 8.0 wt.% of H<sub>2</sub>O is lost in the first event. At higher temperature values, intercalated chitosan macromolecules undergo non-oxidative decomposition at about 200-300 °C and eventually goes through oxidative decomposition of the remaining carbonaceous residue up to 700 °C (Figure 3 inset).<sup>34,35</sup>

Star/Vee-Chit nanocomposites show a distinct mass loss associated to the release of water molecules (Figures 3b and 3c) when compared to TPS (Figure 3d). The almost linear TPS mass loss from room temperature up to 280 °C is associated to strong hydrogen bonds established between the pristine starch chains and water (also glycerol) molecules,<sup>41</sup> delaying their evaporation process. Above 280 °C, TPS undergoes decomposition. For samples prepared in the mini-extruder, a high amount of plasticizer content was required (30 wt.%) due to a torque limitation of the screw system caused by the high viscosity of the molten phase. When starch-based films are obtained through casting method, lower plasticizer contents can be used to produce plastic thin films, with typical values usually lower than 20 wt.% due to the presence of water within the gelatinization step acting as a co-plasticizer for starch.<sup>42</sup> As a direct result of the larger presence of glycerol, TPS sample obtained through extrusion process exhibits lower decomposition temperature of the organic phase in comparison to TPS materials obtained through casting process (300 °C), containing as much as 13% of glycerol as plasticizer.<sup>43</sup> Star/Vee-Chit nanocomposites, even though containing similar plasticizer content as extruded TPS, display a significant anticipation in the decomposition event (onset temperature at 260 °C). Produced starch-based samples containing glycerol and pristine Veegum® HS clay

(without chitosan) also showed a decreasing in the onset temperature of the organic decomposition mass loss (Supporting Information, Figure S1) with a registered temperature of 270 °C. Several other studies showed no improvement on the thermal properties of starch/clay composites in comparison to TPS.<sup>20,44,45</sup> The first derivative of TGA curves of TPS and Star/Vee-Chit 5.0% nanocomposite are shown in Supporting Information, Figure S2.

#### Transmission electron microscopy (TEM)

Transmission electron microscopy images complement the XRD data and can shed additional understanding on the arrangement of the clay particles over the polymer matrix. Figure 4 shows the TEM image of the Star/Vee-Chit 5.0% nanocomposite. Light grey and grey background accounts for the presence of the organic phase, such as starch chains and glycerol. Moreover, wavy-like grey entities are presented homogeneously throughout the visualization area, and they are associated to the chitosan-modified clay particles. In this image, the inorganic phase possesses up to 150 nm length and each individual layer is seen as darker lines as pointed by the arrows.



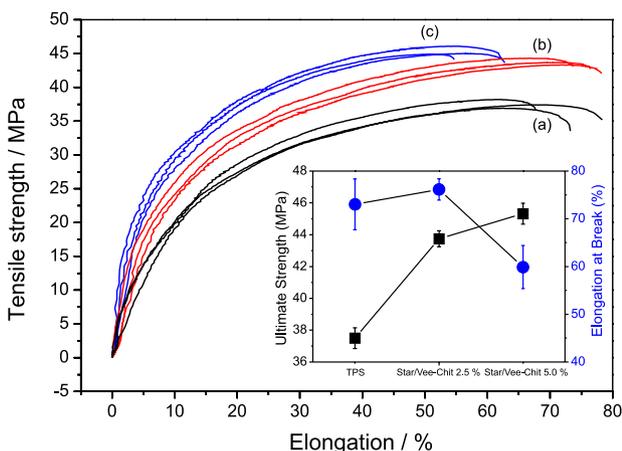
**Figure 4.** TEM image of Star/Vee-Chit 5.0% nanocomposite.

It is also possible to identify different sets of inorganic particles distributed over the starch matrix. In the first set, indicated by black arrows (Figure 4), the presence of the chitosan-modified clay single layers is observed, indicating that shear force during the extrusion process along with the composition of the organic and inorganic parts used to produce Star/Vee-Chit composites were enough to yield a fraction of exfoliated particles. On the other hand, another set of clay-stacked layers were observed as indicated by the red arrows in the electronic version of the article (Figure 4). In this case, only two adjacent inorganic layers were observed and, according to the XRD data previously discussed, it

corresponds to an intercalation process. The presence of few stacked particles, that are responsible for the broad low angle intercalation peak present in the nanocomposites, indicates that not only the shear force of the extrusion process had adequate energy to break down the Vee-Chit phase tactoids, but also that the interaction between this phase and the polymer matrix was strong enough to prevent restacking of the layers. Additionally, the homogeneous distribution of chitosan-modified clay particles over the starch matrix was observed not only at the macroscopic level, where no aggregation of Vee-Chit over the produced samples was identified, but also at the microscopic level.

### Mechanical properties

Figure 5 shows the mechanical properties of starch-based materials evaluated through tensile tests. Regarding the elongation values, statistical difference was observed only for Star/Vee-Chit 5.0% nanocomposite in comparison to the other two analyzed materials. Elongation at break values registered for TPS and Star/Vee-Chit 2.5% were  $73 \pm 5$  and  $76 \pm 2\%$ , respectively, while for Star/Vee-Chit 5.0%, it was obtained  $60 \pm 4\%$ . A decrease in the elongation of starch composites containing clays is commonly reported in the literature.<sup>46,47</sup> Due to the establishment of stronger clay-starch and starch-chitosan interactions in comparison to starch-starch connections, slippage of the polymer chains under stress is decreased in the nanocomposites.



**Figure 5.** Stress-strain curves of (a) TPS; (b) Star/Vee-Chit 2.5%; (c) Star/Vee-Chit 5.0%. Inset: mean values and standard deviation of ultimate strength and maximum elongation at break. (■) Ultimate strength; (●) elongation at break.

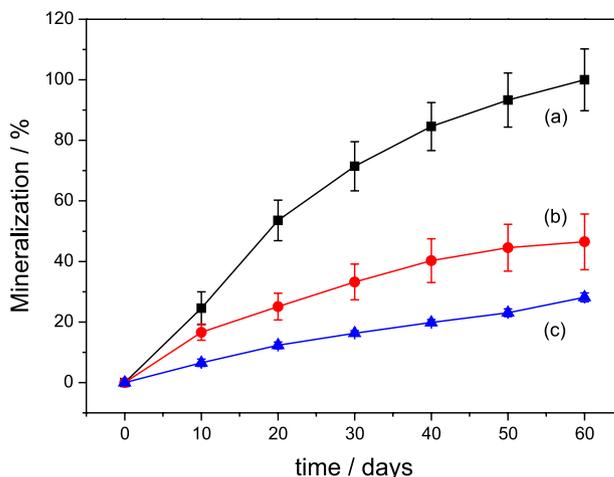
In contrast, tensile strength exhibits a drastic increase when chitosan-modified clay particles are incorporated into the starch formulations. Maximum tensile strength values registered for TPS, Star/Vee-Chit 2.5% and Star/Vee-Chit 5.0% were  $38 \pm 1$ ,  $44 \pm 1$  and  $45 \pm 1$  MPa, respectively.

Statistical differences of values were observed for TPS and the starch nanocomposites even though no statistical difference was observed when the amount of added clay is changed.

The improvement of tensile strength for the clay-containing composites in comparison to TPS is also associated to the presence of strong interactions of starch chains with either clay surface and/or with chitosan chains.<sup>1</sup> For these materials, to disrupt the intense macromolecular and organic-inorganic interactions, it is required to apply higher tensile forces than for TPS sample. The Young's modulus, obtained from the elastic deformation portion of the tensile strength *versus* elongation plot, also indicates the presence of strong interactions in a sample. Young's modulus of Star/Vee-Chit nanocomposites are higher than that of TPS ( $242 \pm 12$  and  $291 \pm 14$  MPa for composites containing 2.5 and 5.0% of chitosan-modified clays, respectively, and  $213 \pm 2$  MPa for TPS). These values point out a larger resistance of the polymer chains to move in the samples containing modified clay nanoparticles.

### Direct measurement respirometer (DMR)

Samples prepared in this work were submitted to simulated biodegradation tests to evaluate the influence of chitosan-modified clay filler over starch biodegradation. Figure 6 shows the mineralization results obtained for pristine cassava starch, TPS and Star/Vee-Chit 5.0% composite. In the interval of 30 days, pristine starch granules undergo biodegradation by soil microorganisms and lose more than 70% of its initial mass (Figure 6a). After 60 days, all carbon from the starch was converted to  $\text{CO}_2$ .



**Figure 6.** Mineralization curves of (a) pristine cassava starch; (b) TPS; (c) Star/Vee-Chit 5.0% nanocomposite.

In comparison to pristine starch, TPS is decomposed at a slower rate. After 30 days, TPS sample has its initial

carbon content reduced by 35% and after 60 days, it is observed only an increment totalizing 45%. One important parameter to be taken account is the difference of surface area between these two samples.<sup>48</sup> Pristine starch comprises a fine powder of granules in micrometer scale, while TPS material possess particles with lesser than 2 millimeter in the longest dimension. Thus, even though both samples were employed using the same mass, the larger surface area for pristine starch and a novel structure formed when starch was gelatinized in the presence of glycerol can be considered the main factors to explain the difference on the mineralization process of these two samples. Nevertheless, there is a statistical difference between the mass losses on all the analysis performed after 20 days.

In contrast, when Star/Vee-Chit 5.0% sample is compared to TPS, a decrease in the rate of mineralization of the sample containing chitosan-modified clay is observed ( $p < 0.05$ ) in the range between 10 and 60 days. Since both samples were similarly prepared and possess close surface area values, a direct comparison seems simple. For Star/Vee-Chit 5.0% composite, after 30 days around 15% of its initial carbon content mass was converted to  $\text{CO}_2$ , while after 60 days, 28% was observed. During all the measurements, Star/Vee-Chit 5.0% sample showed lower mineralization values in comparison to TPS, indicating that the presence of the chitosan-modified clay is actively

reducing the microbiological population around the buried sample, decreasing its rate of biodegradation.

The decrease in the biodegradation rate of the Star/Vee-Chit 5.0% samples could be attributed partially to the clay particles since they can interact with the cell membrane of microorganisms, disrupting the viability of growth, and also by capturing essential ions from the medium, known as corona effect, decreasing the availability of nutrients.<sup>49</sup> However, clay particles also present a positive effect of buffering the pH of the surroundings close to the neutrality, and thus, from this perspective, it can assist on bacteria proliferation.<sup>50</sup> Hence, chitosan can be attributed as the key component in the reduction of mineralization owing to its intrinsic antimicrobial activity.<sup>27</sup> The chitosan content on Star/Vee-Chit 5.0% sample was around 1 g / 100 g of sample, showing that even in small content it could decrease the rate of starch biodegradation.

A visual impact of the microbiological population can be achieved by observing the jars after 60 days of experiment (Figure 7). Pristine starch-filled jar contained a large surface holding mold (Figure 7a), indicating that the conditions of microbial proliferation was adequate; the same was observed for TPS sample but to a lesser extent (Figure 7b) while control did not contained any visual presence of mold. When compared to Star/Vee-Chit-filled jar, only a few spots containing mold were



**Figure 7.** Interior of jars after 60 days of DMR experiments: (a) pristine cassava starch; (b) TPS; (c) Star/Vee-Chit 5.0%; (d) control.

observed, corroborating with the mineralization results (i.e., microbiological population in this medium may not possess conditions to proliferate and hence biodegrade starch).

## Conclusions

Starch/chitosan-modified clay nanocomposites were successfully prepared by melt extrusion. Star/Vee-Chit samples have both intercalated and exfoliated clay particles on the polymer matrix, according to XRD and TEM techniques. Even though X-ray diffraction peaks related to intercalated material were identified and electron microscopy images showed only the presence of few stacks of clay, indicating a homogeneous dispersion of inorganic layers over starch. Thermal properties of starch-based samples are often reduced in comparison to pristine starch due to the presence of high glycerol content required to assist starch gelatinization as a plasticizer during extrusion. The presence of clay particles in the obtained starch-based composites did not improve their thermal properties in comparison to TPS. On the other hand, incorporation of Veegum-chitosan particles increases the tensile strength when compared to TPS. An improvement of over 20% in this parameter is observed for Star/Vee-Chit 5.0% sample against pristine thermoplastic starch. Due to strong interfacial interactions between both phases, the movement of polymer chains under stress was limited, yielding lower elongation values and higher Young's modulus. Biodegradation of starch was severely affected by the presence of chitosan owing to its intrinsic bactericide nature, decreasing the rate of transformation of the carbon content from polymer to CO<sub>2</sub>. After 60 days, Star/Vee-Chit 5.0% sample had a mineralization percentage almost 40% lesser than TPS. The longer lifespan for the nanocomposite suggest potential applications in agricultural sector, like in mulch films to control or alter the soil temperature, preserve the soil humidity, maintain the raised-bed soil structure, reduce seed germination time, decrease weeds and plant diseases, provide efficiency in the usage of water and fertilizers, increase yields and improve product quality.

## Supplementary Information

Supplementary data (TGA and DTG curves) are available free of charge at <http://jbcs.sbq.org.br> as PDF file.

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