

Starch Modified With Chitosan and Reinforced With Feather Keratin Materials Produced by Extrusion Process: An Alternative to Starch Polymers

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Starch (potato), chitosan, and feather keratin are used for processing biodegradable films produced by extrusion. The morphology of the films is examined with a scanning electron microscope and showed the excellent dispersion of keratin. The dispersion is the result of compatibility between the polysaccharides and proteins, as well as the proper operation of the extrusion process. Water solubility of the starch-chitosan films decreased with an increase of keratin materials. The storage modulus increased up to 137% for the composites with unmodified ground quill, and by 192% for composites with modified ground quill. In a tensile test, the composites with unmodified and modified quill reached outstanding increments up to 8160 and 7250% in elastic modulus, respectively, compared to the matrix. They also reached up to 3800% and 3150% in maximum strength, respectively, compared to the matrix. The lysozyme test showed relevant changes in the degradability rate, because the weight loss of the films at 3 weeks decreased from 53% for starch-chitosan matrix and up to 34% for composites with 5 wt% of modified quill. The results corroborated that chicken feather materials can be useful for the development of a manufacturing process for starch composites, and the decomposition of starch-chitosan composites can be controlled depending on the content and type of keratin.

1. Introduction

Extrusion compounding is one of the most promising methods for the industrial processing of natural polymers, due to the easy scale-up and possibility of further molding of the materials.^[1] It is also used in the food and pharmaceutical industries to affect product microstructure, chemistry, or the macroscopic shape of products.^[2] The process can involve any, or all, of the following operations: heating, cooling, feeding, conveying, compressing, shearing, reacting, mixing, melting, homogenizing, amorphous sizing (converting polymer crystalline domains to amorphous domains), cooking, and shaping. Extrusion processes are applied to polysaccharides for specific purposes, such as physical modification or chemical modification (reactive extrusion), manufacture of confectionary gels and encapsulation of flavors or drugs.^[2–3] Hence, extrusion technology has become an attractive option to produce biodegradable films due to its high

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productivity and minimal space requirements. By volume, the most widely extruded food material is starch.^[3–4]

Starch has attracted considerable attention as a biobased, biodegradable, low-cost, and naturally abundant material. These qualities make starch a desirable structural platform for the manufacture of sustainable and biodegradable plastic packaging. Starch has been used as an important raw material in biodegradable plastics. Its gelatinization process has attracted much attention, because it represents an important and unique characteristic in the processing of starch-based materials.^[5–6] Also, its use as one of the raw materials for manufacturing sustainable and biodegradable “green” plastic products is of great interest.^[7] Starch has some disadvantages, including poor water resistance due to its hydrophilic nature, and also its brittle nature in the absence of plasticizers. Besides the addition of unsuitable plasticizers softens and weakens the starch, resulting in deterioration of the mechanical properties.^[8–10] Thus, starch needs to be blended with other polymers to eliminate these disadvantages. Biopolymers, in particular, represent an alternative to synthetic polymers. Starch is combined with hydrophobic and ductile biopolymers, usually present in continuous form, whereas starch represents the discontinuous phase.^[11]

The second polysaccharide involved in this research is chitosan, which is considered a biopolymer due to its biodegradability, biocompatibility, and non-toxicity.^[12–13] Several important reviews of chitosan have been written, taking into account the variability of its inherent properties and their potential applications with other materials.^[14–19] Similar to starch, chitosan lacks water resistance and has poor mechanical properties.^[20] However, several studies have shown that forming miscible composites with other hydrophilic polymers might allow for compatibility and improvement of the final composite properties. Consequently, chitosan has been shown to favor the tensile properties of different starch-based composites.^[20]

Alternatively, polysaccharides like chitosan and starch have been studied when combined with synthetic or natural polymers. Hence, the development of new materials using natural fibers has become an area of great interest, due to the importance of improved materials that can be used in everyday life. Natural fibers to reinforce composites could be found in other non-plant sources. For instance, keratin fibers could be obtained from wool or feathers. Keratin fibers from feathers are nonabrasive, eco-friendly, biodegradable, renewable, insoluble in organic solvents, chemically unreactive, and pliable. They also have hydrophobic behavior, warmth retention, and low cost. Interestingly, they can recover their original mechanical properties, with little loss, after repeated deformations, and have a highly organized hierarchical structure. The presence of a honeycomb structure, present in the chicken feather quill, allows low density, high flexibility, excellent compressibility and resilience, and good capacity to absorb sound. On the other hand, keratin self-assembly and hierarchical structure is responsible for numerous features in birds, such as mechanosensory, ornamental, flight, and thermal regulation, to name a few. These characteristics make the keratin obtained from chicken feathers a suitable material for use as a reinforcement. It is also a material capable of modifying the physical-chemical properties of natural matrices, such as the starch-chitosan matrix.^[17,21–22]

There are several studies focused on the development of films based on starch-chitosan. Most of them only involve processing by casting,^[7,15,17–19] which is an unscalable process at the industrial level. Thus, the aim in this study is to evaluate starch-chitosan composites using the extrusion technique. Composites will be made with byproducts of the poultry industry, such as chicken feathers (keratin), in a natural matrix of starch modified with chitosan. This process is environmentally friendly due to the reuse of a by-product and production of new, green materials. The effect of the reinforcement type and the concentration of keratin was evaluated for the following: water content; water solubility; thermal, thermomechanical, mechanical, and structural properties; and the decomposition time of these films. The three natural polymers processed by extrusion gave an alternative to reinforced starch polymers.

2. Experimental Section

Potato starch was acquired from National Starch, Co. (Hammond, IN, USA). The potato starch contained 0.24% protein, 0.06% lipid, 0.24% ash, 8.74% humidity, and was 99.46% pure. Chitosan (9012-76-4) (75% deacetylated) was obtained from Sigma–Aldrich and sorbitol from “Golden Bell Reactivos.” Acetic acid (9508–05) was obtained from J.T. Baker, and chicken feathers were donated by Pilgrims Company (Queretaro, Mexico). Chicken feathers were modified according to the methodology described by Flores-Hernandez et al. in 2017.^[23] The reagent grade (>98%) sodium hydroxide (1310-73-2) was purchased from Sigma–Aldrich. Lysozyme (BP535-1) employed in this work was acquired from Fisher BioReagents.

2.1. Sample Preparation

2.1.1. Blend Preparation

Before extrusion, solutions of the individual polymer components were prepared in order to favor the compatibility of the components. The solutions were subsequently mixed and a homogenous composite was obtained by solution casting. Starch solutions were made with 3.8 g of starch in 100 mL of water, with sorbitol as a plasticizer (1% v/v). Chitosan solutions were prepared by dissolving 0.2 g of chitosan in 100 mL of acetic acid solution (1% v/v) and stirring at room temperature. Solutions were heated beyond their gelatinization temperature (90 °C) for 10 min, under stirring, and afterward were cooled to 30 °C. Both solutions were mixed directly, and short keratin biofibers and ground quill (modified and unmodified) were added at 5% and 10% (w/w). Equal volumes of the mixtures were spread on poly(tetrafluoroethylene) dishes and dried for 48 h at room temperature. Each film removed from the dishes was finely milled in a hammer Pulvex mill, using a 4 mm diameter orifice in the mesh sieve.

2.1.2. Sodium Hydroxide (NaOH) Treatment

Keratin biofiber was placed in a glass vessel, and 100 mL of NaOH 0.1 M solution was added to the vessel and stirred well. The keratin biofiber was immersed for 5 h and heated to 50 °C.

Later, the alkaline treated biofibers were washed thoroughly in distilled water to remove the excess NaOH (pH = 11). Thereafter, the fibers were dried at 35 °C for 48 h (fiber humidity = 9.2%) before making the composites. Chemical conditions to modify the keratin material were selected, taking into account the methodology described by Flores-Hernandez et al. in 2017.^[23] The conditions described here showed changes on the surface, but the internal structure of the keratin was not impacted.^[24–27]

2.2. Extrusion Process

An experimental laboratory single-screw extruder with an *L/D* ratio of 20:1, screw compression ratio of 3:1, and 2.9 mm internal diameter die-nozzle was used. The temperature during the feeding was 40 °C, the temperature in the intermediate zone was 70 °C and the die temperature was 75 °C. The three heating zones were electrically heated independently and air-cooled. The screw speed was 40 rpm. The extruded films were stored in desiccators at room temperature (30 °C) for 24 h, prior to taking physical and mechanical measurements. Films were processed based on previous studies with starch.^[28] **Table 1** shows the nomenclature for the composites with modified and unmodified keratin.

2.3. Lysozyme Tests

To evaluate the degradation of the composites, a solution 1 L of phosphate buffer solution (PBS) was prepared with the following quantities: 8 g NaCl, 0.20 g KCl, 0.14 g KH₂PO₄, and 0.91 g of Na₂PO₄. Reagents were added, one by one, to distilled water and stirred until they were completely dissolved. The pH was adjusted to 7.4, using 1N NaOH and/or 1N HCl. The NaN₃ (0.02%) was added to the solution before sample incubation. Composites for the enzymatic tests were cut into approximately 1.0 × 1.0 cm squares. The samples were made in quadruplicate and weighed prior to contact with PBS at a pH of 7.4. The degradation in vitro was conducted in test tubes containing 5 mL of PBS with NaN₃ and 5 μg mL⁻¹ lysozyme. The composites were placed in test tubes and incubated for different periods of time (1 day, and 1, 2, and 3 weeks) at 37 °C. After completing the incubation time for each sample, the polymer films were washed with deionized water, dried in a vacuum oven at room temperature, and weighed again. The degradation was evaluated according to the weight loss. Lysozyme was dialyzed and lyophilized from 3× crystalline lysozyme. This material was suitable for the analysis of bacterial cell walls during enzyme purification and for the hydrolysis of mucopolysaccharides.

2.4. Physical Properties

2.4.1. Water Content (WC)

WC was determined through the weight loss. The film samples (1 × 3 cm²) were weighed (*w*₁), dried at 90 °C for 24 h and weighed (*w*₂) again. WC was determined as the percentage of the initial film weight lost during drying and reported on a wet basis according to Equation (1).^[29] The temperature was chosen to avoid loss of plasticizer. The WC measurement was performed in triplicate.

$$\text{Water content (\%)} = \frac{[\text{initial weight (W1)} - \text{final weight (W2)}]}{\text{initial weight (W1)}} \times 100 \quad (1)$$

2.4.2. Water Solubility (WS)

The films (2.5 cm²) were dried at 90 °C for 24 h in a laboratory oven. The temperature was chosen to avoid loss of plasticizer. The pre-weighed film (initial dry weight (*w*_i)) samples were immersed under constant agitation in 50 mL of distilled water for 6 h at 30 °C. After that period, the remaining pieces of the films were filtered and dried at 90 °C to constant weight (final dry weight (*w*_f)). The weight of the solubilized dry matter was calculated as the water solubility percentage of the films according to Equation (2).^[29]

$$\text{Water solubility (\%)} = \frac{(\text{initial dry weight (W}_i) - \text{final dry weight (W}_f))}{\text{initial dry weight (W}_i)} \times 100 \quad (2)$$

2.5. Thermal Properties

2.5.1. Differential Scanning Calorimetry (DSC)

DSC was conducted in a TA Instruments model Q200. Samples were heated at a rate of 10 °C min⁻¹ from 15 to 400 °C.

2.5.2. Thermogravimetric Analyses (TGA)

TGA were carried out by TA Instruments SDT 2960, under a nitrogen atmosphere in the range of 30 °C to 600 °C, and with a heating rate of 5 °C min⁻¹.

Table 1. Composition and nomenclature of starch-chitosan (ESCh)/keratin-reinforced composites.

Percentage of keratin reinforcement (wt%)	Type of Keratin reinforcement			
	Short biofiber	Ground quill	Treated short biofiber	Treated ground quill
5	ESCh-SB05	ESCh-GQ05	ESCh-SBM05	ESCh-GQM05
10	ESCh-SB10	ESCh-GQ10	ESCh-SBM10	ESCh-GQM10

2.6. Mechanical Properties

2.6.1. Dynamic Mechanical Analysis (DMA)

The thermomechanical response of the films was measured using a dynamic mechanical analyzer (DMA), Perkin Elmer DMA 8000, under the flexural mode of testing. The dimension of the specimens was $22 \times 5 \times 0.18$ mm. The heating rate was set at 5°C min^{-1} , and the samples were tested between 30 and 250°C . Two samples of each composite were analyzed for thermal, mechanical and thermomechanical characterization.

2.6.2. Tensile Test

The tensile tests were carried out on a Mechanical Zwick/Roell model Z005 tester, with load cell of 5000 N, and at 50 mm min^{-1} . Five specimens were made, on average, per sample according to ASTM D638.

2.7. Structural Properties – Scanning Electron Microscopy (SEM)

The morphology of the composites was observed by scanning electron microscopy (SEM), using a JSM-6060LV JEOL microscope at an accelerating voltage of 20 kV. Samples of all composites were fractured using a Zwick/Roell Z005 universal testing machine, to observe the behavior of the reinforcement and matrix. Fractured samples were mounted on metal stubs and were vacuum-coated with gold at 7×10^{-2} mB using argon in an EMS 550, sputter coater.

3. Results and Discussion

3.1. Moisture Content and Water Solubility

The moisture content and water solubility for the reinforced films at different keratin percentages (5–10 wt%), types of reinforcement (fiber and ground quill) and surface nature (with and without modification) are shown in **Table 2**. In most composites reinforced with fiber and quill without modification, the moisture content decreased. This can be attributed to the hydrophobic character due to the keratin materials.^[21] Additionally, composites with fiber and quill chemically modified with NaOH showed a slight increase in the moisture content with respect to the starch-chitosan matrix, and this behavior can be attributed to the effect of chemical modification of the keratin materials. The hydrophobic character of the keratin was slightly diminished, and therefore the moisture content of the films increased. These results are opposite with the findings of cellulose fibers^[30–31] because the hydrophilic nature of those fibers was reduced due to the alkali treatment. Thus, keratin materials provide other properties to starch films and diversify the possible applications.

Also, an understanding of the water solubility is important for films based on biopolymers, because water impacts their properties. Thus, the evaluation of the film integrity, considering

Table 2. Water content and solubility of starch-chitosan- (ESCh)/keratin-reinforced composites.

Nomenclature	Water content (%)	Water solubility (%)
ESCh	6.9 ± 0.19	13.4 ± 0.16
ESCh-SB05	6.9 ± 0.15	13.2 ± 0.33
ESCh-SB10	6.8 ± 0.11	12.0 ± 0.57
ESCh-GQ05	6.7 ± 0.23	13.4 ± 1.02
ESCh-GQ10	6.8 ± 0.22	6.4 ± 0.53
ESCh-SBM05	7.2 ± 0.54	13.3 ± 1.09
ESCh-SBM10	7.2 ± 0.22	12.6 ± 0.12
ESCh-GQM05	7.0 ± 0.28	13.6 ± 0.67
ESCh-GQM10	7.0 ± 0.19	7.7 ± 0.50

the water resistance, is important. In this research, the water solubility of reinforced films decreased with the increase of keratin concentration. The decrease in water solubility in this type of starch film is a required property, because the solubility value indicates the film integrity in an aqueous system. If the solubility is lower, the material shows resistance to being dissolved in water. For example, the films used in the coating of fruits and vegetables require a low solubility.^[32] As these results indicate, quill produced more water resistant composites than fiber, but both materials improved the water resistance of the starch films.

3.2. Thermal Analysis

The curves obtained from differential scanning calorimetry (DSC) are shown in Figures 1a and 1(b). **Figure 1a** shows the behavior of composites reinforced with keratin fiber (5 wt% and 10 wt%) and ground quill (5 wt% and 10 wt%). The thermograms presented endothermic peaks around 70 – 150°C , which are related to the evaporation of water in keratin, chitosan, and starch, at around 69, 100, and 150°C , respectively.^[17–18,33–34] It is important to mention that the endothermic peaks in the majority of the composites with reinforcement shifted to the right, and higher temperatures than the matrix were obtained. Consequently, these composites showed more thermal stability in this step. Additionally, the second transition, located around 300°C in the thermograms, could be due to the following two reasons: 1) the polymer decomposition of chitosan and starch, dehydration of saccharide rings and the decomposition of acetylated and deacetylated units and 2) the thermal degradation of keratin with disruption of disulfide bonds and denaturation of helical structures.^[17,35] DSC results for composites reinforced with treated fiber and ground quill are shown in **Figure 1b**. The curves are very similar and show the same results as the composites with untreated fiber and ground quill. First, the endothermic peak appeared around 90 – 130°C , and is associated with loss of water due to the hydrophobic character. Second, over 310°C , dehydration of saccharide rings, destruction of disulphide bonds and denaturation of the helix structure of keratin occurred.

TGA results of composites reinforced with untreated and treated ground quill are shown in **Figures 2a** and **b**,

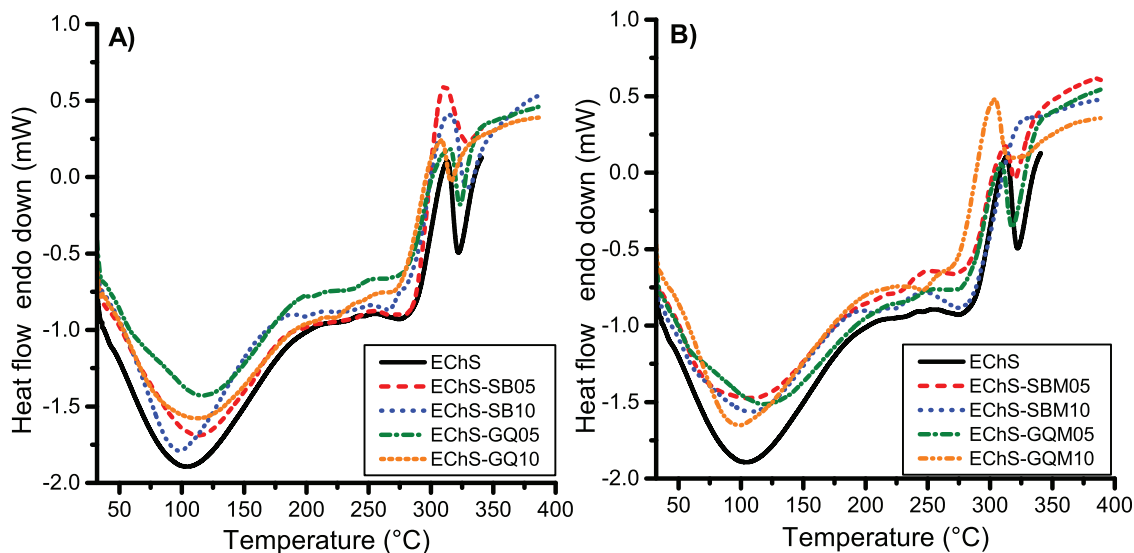


Figure 1. Differential scanning calorimetry (DSC). Curves for composites with 5–10% of a) biofiber and ground quill and b) treated biofiber and treated ground quill.

respectively. The thermal behavior observed for these composites can be described in three main steps. The first mass loss (7%), from 70 to 160 °C, was due to the loss of water. The second, from 210 to 400 °C, and with mass decreasing from 7 to 83%, was associated with a complex process. This process included dehydration of the saccharide rings, depolymerization and decomposition of the chitosan units, denaturation of the helix structure and thermal pyrolysis of the chain linkages and peptide bridges of keratin.^[17–18,33] The third, from 450 up to 600 °C, was associated with the greatest weight loss and corresponded to the complete degradation of the polymer.^[16,36]

The thermal behavior of the composites changed significantly during the third step due to keratin materials being included as reinforcements. The thermal stability in this last step was higher in the composites compared to the starch-chitosan curve (SCh). Also, the curves for the composites with modified keratin were more homogeneous in the third step than curves for the unmodified keratin composites and the matrix, indicating again the contribution of modified materials to the properties of these composites. The reinforced composite with the 10% treated quill had the lowest weight loss (59%), in comparison with the matrix (73%). The largest degradation for the composites was found in the composite with 5 wt% of unmodified biofiber (72%). The

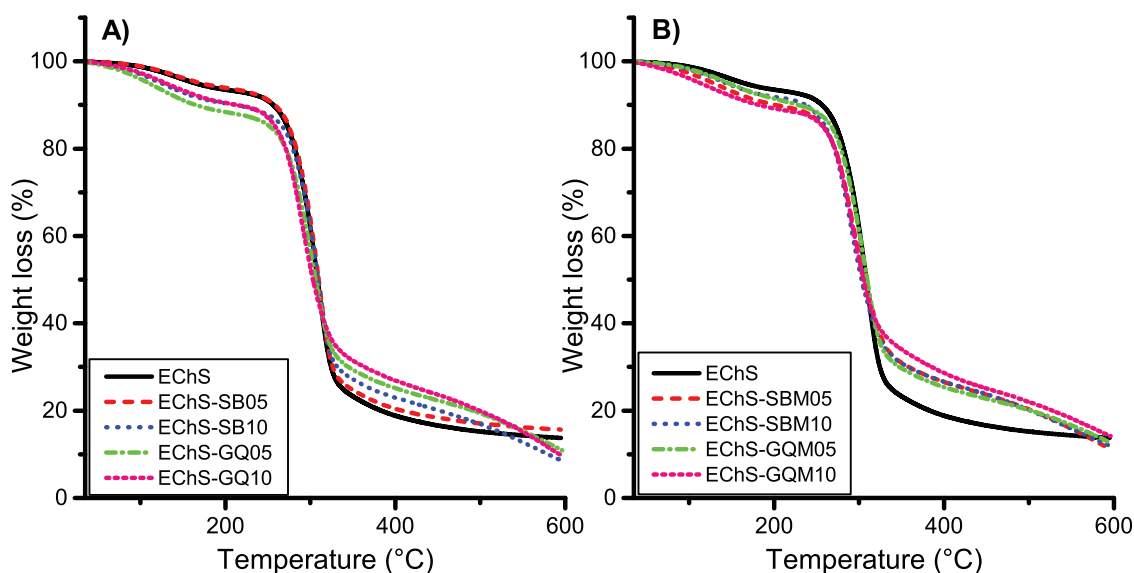


Figure 2. Thermogravimetric analysis (TGA) curves for composites with 5–10% of a) biofiber and ground quill and b) treated biofiber and treated ground quill.

degradation behavior in this part of the curve for the unmodified keratin composites was higher for the biofiber composites than the quill composites. This behavior may be associated with the inherent variations of natural materials.^[17]

3.3. Dynamical-Mechanical Analysis

DMA was performed to study the stiffness of the starch-chitosan composites reinforced with feather keratin, from room temperature to 200 °C. **Figure 3a** shows the storage modulus (E') curves and the effect of keratin content with respect to polymer matrix (starch-chitosan). The initial modulus ($E' = 1018$ MPa at 34 °C) for the starch-chitosan composite increased up to 40% ($E' = 1430$ MPa) for the samples containing up to 5 wt% ground quill. It increased up to 137% ($E' = 2411$ MPa) for the samples with 10 wt% of ground quill. This enhancement can be produced because the ground quill (feather keratin) improved the matrix stiffness, and also it had a high compatibility with the matrix.^[17,37] In general, all of the composites performed better than the matrix alone. Composites with biofiber also showed increments in storage modulus. For example, ESCh-SB05 improved 23% ($E' = 1257$ MPa) with respect to the polymer matrix, and ESCh-SB10 improved 119% ($E' = 2226$ MPa).

Figure 3b shows the storage modulus (E') for SCh composites with modified keratin. In these composites, reinforced with modified biofiber, there is no clear tendency with respect to the quantity of reinforcement used. The initial modulus of ESCh-SM05 increased 132% ($E' = 2359$ MPa) in comparison with the matrix. If 10 wt% of biofiber was added, E' only increased 83% ($E' = 1863$ MPa) at room temperature, considering the matrix reference. The values of the storage modulus for starch-chitosan reinforced with modified ground quill presented a tendency with respect to reinforcement quantity. The improvements with respect to the matrix are: ESCh-GQM05 increased up to 14% ($E' = 1158$ MPa) and ESCh-GQ10 increased up to 192% ($E' = 2972$ MPa). However for the modified fiber composites, there is

no tendency for the E' values. The maximum values for the storage modulus were reached for the sample reinforced with 10 wt% modified quill, and all of the composites were also above the matrix at room temperature. Therefore, it was demonstrated that the addition of either of biofiber or ground quill, after modification, improved the storage modulus of the starch-chitosan polymer blend. This corroborates the results obtained by other techniques, described before (SEM, TGA, and DSC), where notable changes in properties indicated that interaction at the interface level was achieved in these materials.^[38–39]

The ratio of loss (E'') and storage modulus (E') is called $\tan \delta$. Also known as “damping,” $\tan \delta$ reflects the movement of polymer chains and the energy loss by dissipation, in addition to measuring the imperfection in the elasticity.^[40] **Figures 4(a)** and **(b)** depict this parameter for the evaluated composites. It is possible to observe that the maximum $\tan \delta$ values are higher in the composites (except for the 10 wt% unmodified quill case) in comparison with starch-chitosan films, indicating that more energy was dissipated by the displacements of polymer chains and keratin materials. However, the polymer chains in the keratin composites started to move at lower temperatures than in the matrix. This was deduced by the temperature maximum of $\tan \delta$, related to T_g .^[39] It is known that T_g values in starch-chitosan mixes depend on many factors, such as the deacetylation degree of chitosan, percentage of each polysaccharide, plasticizer quantity (glycerol, sorbitol), and water content. Thus, they are difficult to compare with other starch-chitosan materials. For instance, values of maximum $\tan \delta$ have been obtained from starch-chitosan materials (50 wt.% of each polysaccharide and approximately 6.2 wt% of plasticizer) at around 185 °C^[41] and also at around 89 °C^[42] (66 wt% of starch and 33 wt% of chitosan, considering 10 wt% of plasticizer). Thus, the polymer starch-chitosan (5–95 wt% and 1 wt% of plasticizer) in this research presented a T_g around 104 °C, and as mentioned earlier, this value tended to decrease in the samples with keratin. This behavior has been shown with keratin in other natural polymers,^[37,43] indicating that keratin modified the properties of

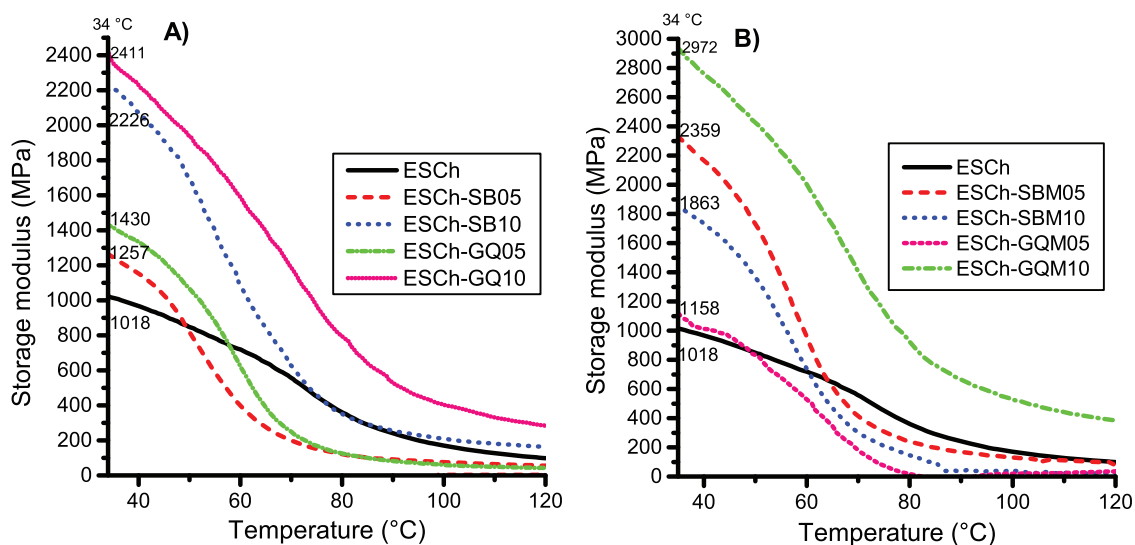


Figure 3. Storage modulus (E') for composites with 5–10 wt% of a) biofiber and ground quill and b) treated biofiber and ground quill.

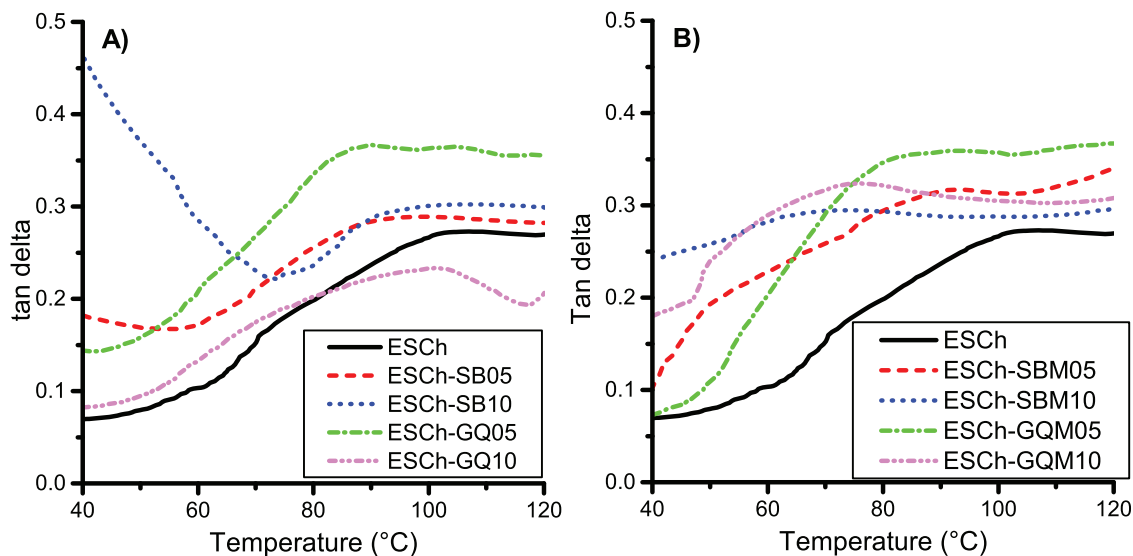


Figure 4. Tan δ curves for composites with 5–10 wt% of a) biofiber and ground quill and b) treated biofiber and ground quill.

starch-chitosan films, and the effect is more evident in the samples with modified keratin. The Tan δ values were modified according to the load and modification, indicating that the type and amount of reinforcement are parameters that can be used to produce a significant change in the movement of polymeric chains interacting with the reinforcement.

3.4. Tensile Properties of Films

Figure 5 shows the stress versus strain curves obtained for the composites with unmodified and modified keratin materials, in comparison with the starch-chitosan matrix. Considering the curve slopes, it is clear that chemical modification for the

reinforcements had the most relevance in the composites with the lower concentration. The composites with 5 wt% of modified keratin, either with quill or biofiber, showed a clear increment in the slope related to the modulus and the maximum strength, in comparison with the composites with the same concentration of unmodified keratin. However, the effect for 10 wt% keratin in the composites, either in modulus and maximum strength, was very similar for unmodified and modified keratin composites. Even though 10 wt% modified keratin in the composites does not show the same tendency than composites with 5 wt% of modified keratin, it is important to also consider the other characterization results. The DMA results showed that the composites with 10 wt% modified keratin had a higher storage modulus than the composites with unmodified keratin. **Table 3**

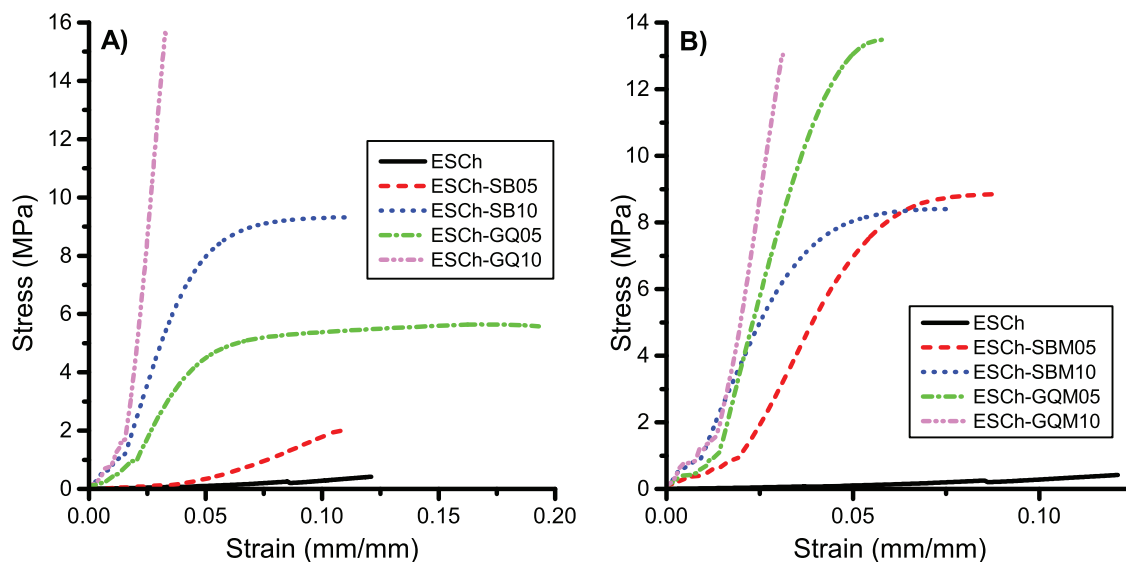


Figure 5. Stress-strain curves for composites with 5–10 wt% of a) biofiber and ground quill and b) treated biofiber and ground quill.

Table 3. Elastic modulus of starch-chitosan/keratin composites

Nomenclature	Elastic modulus [MPa]	Increase rate of elastic modulus (%)	Stress max [MPa]	Increase rate of stress max (%)
ESCh	10	–	0.4	–
ESCh-SB05	29	190	2.0	400
ESCh-SB10	194	1840	9.3	2225
ESCh-GQ05	134	1240	5.6	1300
ESCh-GQ10	826	8160	15.6	3800
ESCh-SBM05	164	1540	8.8	2100
ESCh-SBM10	200	1900	8.4	2000
ESCh-GQM05	343	3330	13.5	3275
ESCh-GQM10	735	7250	13.0	3150

shows the elastic modulus of the composites with modified and unmodified keratin. Thus, the addition of the reinforcements suggests that keratin materials modified superficially increase stiffness in starch-chitosan films, as reported for other synthetic polymers.^[8,32,44] In addition, it is important to note that in spite of the increased stiffness, the usefulness of the composite

materials increases in comparison with the extruded starch-chitosan matrix.

This is because the plastic region increased notably, and therefore the area under the stress versus strain curves was also higher for the composites, with the exception of the composites with 10 wt% quill. Those slopes indicated very rigid behavior. However, the mechanical tests again corroborated the adequate dispersion and interaction between the reinforcements and the matrix in the extrusion process, because relevant increments were reached with the keratin material. For example, up to 8160 and 7250% in the elastic modulus were reached, and up to 3800 and 3150% in maximum strength were reached, for composites with 10 wt% unmodified and modified quill, respectively. Also, the effect of chemical treatment was highlighted in composites with 5 wt%, also indicating the effects observed with this technique in other characterizations. The excellent interfacial adhesion between the reinforcement and the matrix is important to improve the mechanical strength of the composites.^[35]

3.5. Morphology of Composites Studied by Scanning Electron Microscopy

Figures 6 and 7 show the scanning electron micrographs of the fracture surface after tensile testing of the starch-chitosan composites, and the starch-chitosan composites reinforced with fiber and ground quill. Figures 6a and b show the matrix surface. These micrographs reveal streaks caused by a stress rupture, and there was no separation of phases between the two polymers, nor the formation of visible agglomerates, indicating structural integrity in the observed films.^[45] Figures 6c and d show the composites reinforced with fiber. These materials have a rough and irregular surface. In addition, it is

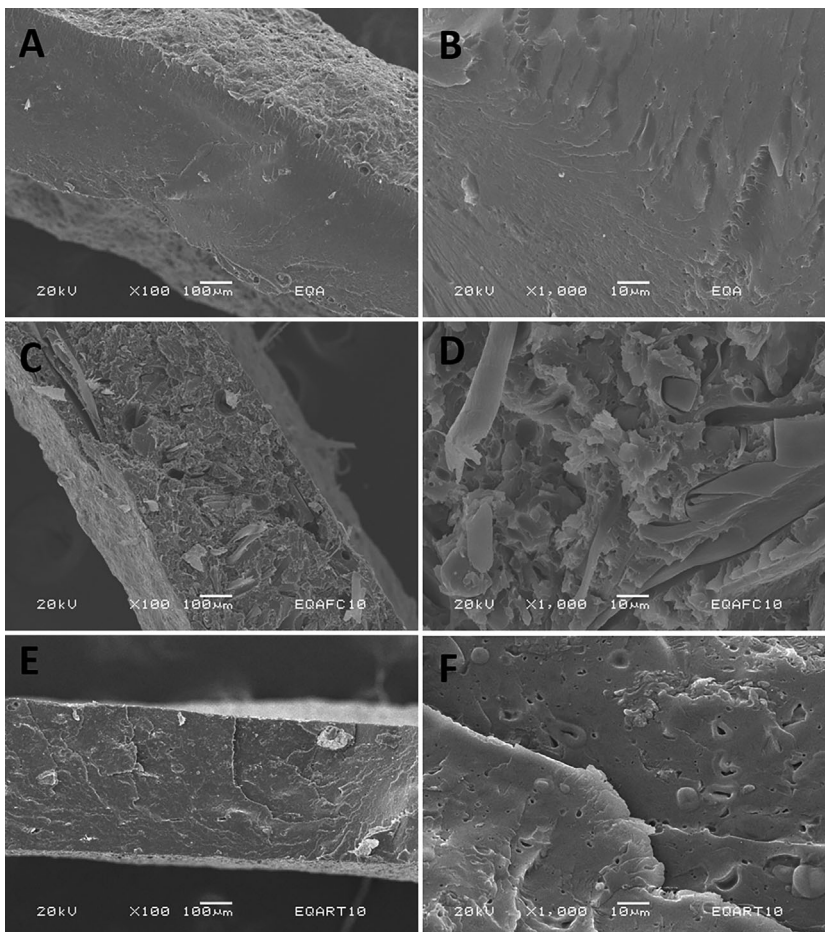


Figure 6. a,b) Micrographs of scanning electron microscopy (SEM) on fractured surfaces of starch-chitosan composites, c,d) composites reinforced with 10 wt% of keratin fiber (ESCh-SB10), and e,f) composites reinforced with 10 wt% of ground quill (ESCh-GQ10).

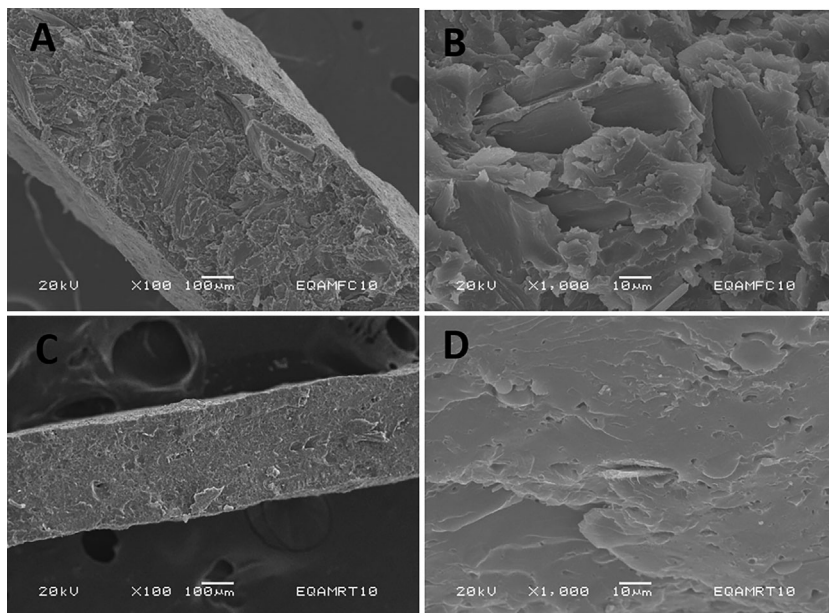


Figure 7. a,b) Composites reinforced with 10 wt% of treated keratin fiber (ESCh-SBM10); and c,d) composites reinforced with 10 wt% of treated ground quill (ESCh-GQM10).

found that there was a good interfacial adhesion between the matrix and the reinforcement. This was attributed to fibers which were completely wetted by the matrix.^[17] Figures 6e and f present the micrographs of the composites reinforced with ground quill. Also, it was observed that the separation of phases between the starch-chitosan and ground quill did not occur. The micrographs show a few voids in the surface produced by some pull-out fibers, thus some fibers had little adherence to the polymer.^[17,32,44]

Micrographs of fractured surfaces of the composite reinforced with treated keratin are shown in Figures 7a–d. Figures 7a and b show the composites reinforced with 10 wt% of treated

keratin fiber, and Figures 7c and d show the composites reinforced with 10 wt% of treated ground quill. The four micrographs indicate the same results as the above composites, with untreated keratin fiber and untreated ground quill. The composites had good dispersion of the reinforcements. The matrix had striations and deformations around the reinforcement, indicating that the reinforcements were entirely wetted by the matrix.

3.6. Lysozyme Tests

The decomposition rate for the films obtained by extrusion was investigated by monitoring the change in weight loss during degradation in the phosphate buffer solution (PBS). **Figure 8** shows the behavior of weight loss for 3 weeks of degradation, using the buffer solution at 37 °C and pH 7. During the first week, the weight loss trend increases for all composites. Here, the starch-chitosan film had the highest percentage of decomposition. However, in the second week, the composite

reinforced with 5 wt% of fiber (ESCh-SB05) had greater weight loss. Finally, during week 3, the weight loss of the composite reinforced with 5 wt% of fiber was 73%, and had the highest percentage of decomposition. The matrix and composite reinforced with 5 wt% of ground quill (ESCh-GQ05) showed a weight loss of 53%. On the other hand, in the composites reinforced with 5 wt% of treated fiber (ESCh-SBM05) and 5 wt% of treated ground quill (ESCh-GQM05), degradation of 42 and 34% occurred, respectively. These results show that the composites reinforced with modified keratin were more resistant to degradation than the matrix, and even than the composites reinforced with untreated keratin. Thus, this corroborates that keratin can notably modify the properties of these polysaccharide materials. Also, the modification of keratin plays an important role in diversifying the properties of these composites.

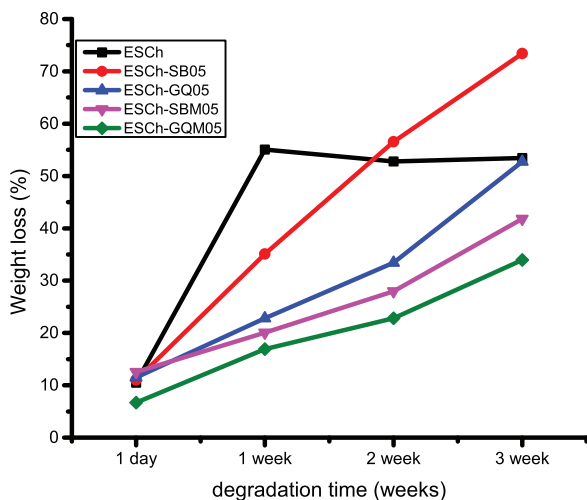


Figure 8. Enzymatic tests for composites reinforced with treated keratin in comparison with matrix (SCh) and composites reinforced with untreated keratin.

4. Conclusions

In this research, three natural materials processed by extrusion successfully produce green composites. Starch-chitosan matrix reinforced with materials obtained from waste-chicken feathers exhibit outstanding elastic modulus and stress maximum, in comparison with the matrix. In addition, the relevant increments in storage modulus and thermal behavior of composites support the possibility of using keratin materials (biofiber and ground quill) to improve the thermal and mechanical properties of polysaccharide mixes produced by semi-industrial extrusion machine.

Keratin materials concentration in the composites, also modify the water solubility of starch-chitosan, however, this effect is more significant with grounded quill. The modification in keratin surface affects the degradation rate of the starch-chitosan

films in the lysozyme test. Thus, keratin materials depending on the surface treatment could control degradation process of starch-chitosan composites.

The physical-chemical interactions between natural polymers in the composites are consequence to the chemical groups' affinity in the protein and polysaccharides involved in the casting process before extrusion. However, only physical interactions prevail after extrusion, due to the thermal and mechanical parameters involved in the processing.

The chitosan modifying the starch, together the keratin materials as reinforcements processed by extrusion, could be an important alternative to diversify and improve several properties analyzed in this research, for the starch films, based on natural reinforcements and manufactured in an industrial process as the extrusion.

Abbreviations

DMA, dynamical mechanical analysis; HCl, hydrochloride acid; KH_2PO_4 , potassium phosphate monobasic; L/D, length/diameter; M, molar concentration; mB, milliBar; mL, milliLiter; N, newton; NaCl, sodium chloride; NaN_3 , sodium azide; NaOH, sodium hydroxide; Na_2PO_4 , sodium phosphate; PBS, phosphate buffer solution; TGA, thermogravimetric analyses; WC, water content; Wf, final weigh; Wi, initial weigh; WS, water solubility.

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Conflict of Interest

The authors declare no conflicts of interest.

Keywords

chemical modification, chicken feathers, extrusion, keratin, starch-chitosan

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