Research Article

Starch and starch/bacterial nanocellulose films as alternatives for the management of minimally processed mangoes†

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ABSTRACT

Improving the storage of minimally processed mangoes represents a substantial challenge for packaging. For this purpose, thermoplastic starch (TPS) and its composites with bacterial cellulose nanoribbons (TPS/BC) were used as wrapping materials to prolong the shelf life of minimally processed mangoes. Commercial polyvinyl chloride stretch (PVC*) films and unwrapped mangoes were used as the controls. The samples were stored at 75 % RH and 5 °C for five and ten days. The films were removed after storage and subjected to mechanical tests and physical evaluation. The weight loss, firmness, total soluble solids, and total titratable acidity in the mangoes were tested to monitor fruit ripening. ATR-FTIR was used as an alternative nondestructive technique to examine fruit quality through changes in the sugar and organic acid contents. The results showed that TPS films reduced mango weight loss until the fifth day (2.84 %), whereas the reduction in weight loss seen in mangoes wrapped with TPS/BC was even lower (13.18 %). Therefore, even though both TPS and TPS/BC films can be used to prolong the fruit shelf life for five days, the latter is more effective. The elongations at break of both film samples remained constant over time, which means that these films can be used under stress conditions.

Keywords: Thermoplastic starch, bacterial cellulose nanoribbons, wraps, minimally processed mangoes, ATR-FTIR.

1. Introduction

In recent years, global organizations such as the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have recommended that their member countries work to change their population's eating habits to improve their welfare and health [1-2]. These recommendations suggest increasing the consumption of fruits and vegetables [3-4].

Mango is one of the most popular fruits in the world because of its delicious flavor and nutritional value [3]. Initially, mangoes are acidic, astringent and rich in ascorbic acid (vitamin C). During the ripening process, the level of vitamin C decreases and the levels of pro-vitamin A, vitamins B1 and B2, sucrose (the major sugar), fructose, and glucose increase [5]. One method for increase the commercialization and consumption of a fruit is to reduce its processing, especially offering fruit with the peel removed via minimal processing [6-7]. Minimal processes (washing, peeling, cutting, and packaging) physically damage the fruit, increasing fruit respiration rate and decreasing shelf life. Various methods have been used to maintain the quality of fresh-cut fruit and minimize adverse effects [3, 8]. One such method is the addition of agents such as antioxidants (ascorbic acid) or firming agents (calcium derivatives) [9]. Despite the effectiveness of these treatments [10], some consumers are demanding a reduction in the use of chemical methods for extending the shelf lives of fresh fruit products. Another alternative is focusing on the fruit atmosphere, for example, the application of a modified atmosphere (MA) or controlled atmosphere (CA) [11]. However, these methods may cause changes in flavor because of the concomitant increase in anaerobic respiration and ethanol production. In addition, modified atmospheres can accelerate fruit maturation. Thus, these methods are also not ideal [10, 12-14]. To avoid the challenges associated with the aforementioned methods, the use of plastic protective films is increasing due to their accessibility and ease of manipulation. These types of packaging can extend the shelf life of minimally processed fruits by reducing moisture, respiration, and physiological changes [13, 15] and by preventing changes in aroma, taste, texture, and appearance [16].

However, some consumers have become increasingly concerned about both fresh-cut fruits and the negative environmental impact of packaging materials [14]. This trend represents a significant challenge for packaging researchers studying ecofriendly materials. Ecofriendly materials based on polysaccharides such as starch, alginate, and cellulose can be useful for packing [4]. Studies have shown that biofilms and coatings are helpful for some fruits, such as whey protein coatings for apples [17], potato starch-based edible coatings for guavas [13], and edible hydroxypropyl methylcellulose-lipid composite coatings for plums [18].

Although polysaccharide-based films present ecofriendly characteristics, such as biodegradability, some of these films, such as starch-based films, have poor mechanical properties due to retrogradation effects related to the reassembling of solubilized starch granules. This phenomenon can be controlled by starch modification, blending with other materials or reinforcing with renewable biodegradable materials [19-20].

Both bacterial (BC) and vegetal cellulose (VC) have good mechanical properties; thus, they can be used to reinforce thermoplastic starch (TPS) films [21]. Bacterial cellulose nanoribbons are even more ecofriendly than vegetable cellulose due to the absence of noncellulosic components, such as lignin, that have to be removed using chemical treatments. Unfortunately, few studies have investigated the use of bacterial cellulose nanoribbons, especially those obtained from agroindustrial wastes, to reinforce thermoplastic potato starch films [22-23].

In this context, the goal of this study was to extend the shelf life of minimally processed mangoes using two polysaccharide-based films, TPS and a thermoplastic starch reinforced with bacterial cellulose nanoribbons (TPS/BC). Mangoes were wrapped with these films and stored for five days and ten days at 5 °C. The fruit ripening was evaluated by weight loss and changes in firmness, total soluble solids (TSS), and total titratable acidity (TA). ATR-FTIR was used as a nondestructive alternative evaluation technique. Furthermore, the physical integrity of the films during storage was evaluated through mechanical tests and morphological analysis. The samples for analysis were removed from the films covering the fruits during the shelf-life tests.

2. Materials and Methods

2.1 Materials

Mangoes (*Mangifera indica* L.) were obtained from a local market (Antioquia, Colombia). The fruits were selected according to their maturity using the NTC 5139 (2002) standard [24] and converted into minimally processed fruit. One of the most common sources of starch in Colombia is derived from potato. Almicor Industries Ltd. (Bogotá, Colombia) supplied the samples of potato starch used in this work. The composition of the dry starch was 32.8 wt % amylose and 67.1 wt % amylopectin. Glycerol and potassium sorbate, which are generally recognized as safe (GRAS) and meet the requirements of the United States Pharmacopeia (USP), were provided by Protokimica (Medellín, Colombia).

Bacterial cellulose nanoribbons were produced in pineapple culture medium using a previously developed methodology [25]. The *Komagataeibacter medellinensis* strain used herein was isolated from homemade vinegar purchased from the local wholesale marketplace [25]. Pineapple juice was autoclaved at 120 °C and $10.35x10^4$ Pa for 15 min, and the liquid extract was allowed to reach room temperature. Aerobic fermentation was achieved by adding 10 vol. % inoculums to the medium and statically incubating the samples at 28 °C for 13 days. The collected pellicle was washed with water and homogenized in a Waring blender for 5 min to afford the nanoribbons. The nanoribbons were treated with 5 wt % KOH solution for 14 h, rinsed until pH 7, and homogenized in water for 5 min. The chemical composition of the obtained dried bacterial cellulose nanoribbons is consistent with cellulose. Less than 1 wt % corresponds to minerals, especially Mg or K salts. The average diameter of the nanoribbons was approximately 50 nm.

Commercial polyvinyl chloride stretch films (PVC*) were provided by Colombiana *Empack* S.A.S, Medellín, Colombia, and the material had a tensile strength of 14 MPa and Young’s modulus of 100-200 MPa.

TPS and TPS/BC Film Production

TPS and TPS/BC films were produced by casting according to the method presented by Montoya et al. [21]. The starch solution was prepared using 4 wt % starch, 1.3 wt % glycerol and 0.2 wt % potassium sorbate. The starch solution was added to Petri dishes by casting to produce TPS films. Then, the plates were dried in a forced convection oven at 50 °C for three days.

For the TPS/BC films, 20-70 nm wide BC ribbons at a concentration of 7.5 wt % were added to the starch solution, and the mixture was homogenized. The TPS/BC solution was cast and dried under the same conditions as the TPS solution.

2.2 Methods for Film Characterization

For the minimal processing of the fruit, the selected mangoes (as described in Section 2.1) were cleaned, peeled, segmented into cubes 30-mm in size, and cleaned again. During cleaning, the samples were washed in 10 ppm sodium hypochlorite, rinsed with ultrapure water, and dried with absorbent laboratory paper. For the wrapped samples, the individual cubes were enclosed using a 200-mm piece of film. The wrapping process helped to enclose the mangoes samples inside the films. For each type of film, three cubes were analyzed for each evaluation period. To analyze the shelf life of the fruit, minimally processed mangoes were wrapped with different films (TPS, TPS/BC and PVC*) and stored for 5 and 10 days at a fridge temperature of 5 °C. All experiments were performed in triplicate. The film samples for testing were isolated from the films used during the shelf-life tests after both storage periods.

Mechanical Properties

The tensile testing of the films was performed with an Instron 5582 Universal Testing Instrument equipped with a 50 N load cell. All samples were tested in accordance with the ASTM D882-09 standard with a cross-head speed of 25 mm/min using 5 mm by 19 mm rectangular strips. Mechanical tensile data were gathered from nine different specimens and then averaged. Samples were extracted from each wrapped film.

Morphological Analysis

SEM using a Jeol JSM 5910 LV instrument operated at 10 kV was used to study the film morphology after cryo-fracture. All films were coated with gold/palladium using an ion sputter coater for 5 min.

2.3 Physicochemical Characterization of Mangoes

Several physicochemical aspects of the minimally processed mangoes wrapped with different films (TPS, TPS/BC and PVC*) and unwrapped mangoes (UWM) were evaluated after the shelf-life test at a fridge temperature of 5 °C on the 5th and 10th days. Nonexposed peeled mangoes (UWM0) were used as a reference. The sugar and organic acid contents were measured by attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR).

Weight Loss

The weight loss of each mango piece was measured with an analytical balance *OHAUS Adventurer™* immediately after slicing and on the 5th and 10th days, and the values were determined according to equation (1). The results were compared and are expressed as percentages.

$$\%\, wl(d) = \frac{w0 - w(d)}{w0}\, x\ 100 \qquad \text{Eq. (1)}$$

where *% wl (d)* is the weight loss percent at the stored time (days); *w0* is the initial sample weight; and *w (d)* is the sample mass at the studied time.

Firmness (N)

The firmness was measured with a *Force Test™ FTK100* penetrometer. The force required to penetrate the cut surface of the mango piece with a 2-mm stainless steel probe was determined (the mango was held perpendicular to the probe). The results are expressed in *N*.

Total Soluble Solids (TSS) and Total Acidity (TA)

Samples were biochemically analyzed (TSS and TA). To measure these parameters, mango juice was prepared by blending the pieces of mango. TSS contents were assessed with a digital *Abbe Refractometer B&C 32400* calibrated with distilled water; the results are reported as degree Brix (° Brix). Acidity was determined by titration of the pure mango with 0.1 N NaOH solution until the pH reached a value of 8.1 (using a pH meter). The total acidity is expressed as the percentage (%) of citric acid.

Attenuated Total Reflection Fourier-Transforms Infrared Spectroscopy (ATR-FTIR)

Infrared spectroscopy experiments were used to verify the chemical changes that occurred during the ripening of the stored mangoes. The assays were performed using an FTIR Nicolet 6700 series spectrometer equipped with a single-reflection ATR and a type IIA diamond crystal tungsten carbide tip. The diamond ATR has an estimated sampling area of 0.5 mm^2, and a reproducible pressure was applied to the samples each assay. The infrared spectra were collected with a 4 cm^{-1} resolution, and a total of 64 scans were collected. The data were processed (i.e., construction of baseline and normalization) using OMNIC software.

Statistical Analysis

The results were statistically compared by analysis of variance ANOVA using the program *Origin Pro 8.5®*. Means were compared using Tukey's test to examine if the differences among variables were significant ($p \leq 0.05$).

3. Results and Discussion

3.1. Film Characterization

The mechanical properties of the samples obtained from the wrapping films after the evaluation of the shelf life of the fruits were measured. Figures 1a to 1c show the tensile strength, Young's modulus and elongation at break, respectively, of the TPS and TPS/BC films after 5 and 10 days of exposure. Even after environmental exposure, the mechanical properties of the test films are still in the range of the mechanical properties of films based on potato starch [26]. After 5 days of exposure, the tensile strength of the TPS/BC film (see Figure 1a) was higher than that of the TPS because of the reinforcing effect of the BC. However, after 10 days of exposure, this behavior is different. It is possible that the mechanical behavior at these exposure times is influenced by the recrystallization process of the starch [27-28]. This effect is particularly evident in the case of the TPS because in the TPS/BC film, the presence of the BC controls the behavior and could affect the modifications of the starch structure. Changes in the starch structure was supported by the observations reported by El Halal et al. [19] when analyzing the mechanical properties of potato starch films; they concluded that the observed changes were related to the recrystallization of the amylose molecules in the starch. In the case of the modulus (see Figure 1b), again the effect of BC reinforcements and the alterations in the starch structure, possibly by the recrystallization of the starch, can be observed. These results are in concordance with the information presented in Figure 1c; reductions in the elongation were observed for both types of film at both exposure times. Even when the elongation at break is reduced, the lower elongation was still adequate for a fruit-protecting material. These results show that the TPS and TPS/BC films are useful for the preservation and storage of minimally processed mangoes.

As mentioned before, in the TPS/BC materials, the process could be countered by the presence of BC nanoribbons in the film. The strong hydrogen bonding interactions between the BC and starch could improve the interfacial interactions and reduce TPS recrystallization [27, 29-30]. Additionally, BC may increase the capacity of the film to capture water from the surrounding environment; that is, the water acts as a plasticizer, affecting the molecular interactions and weakening the mechanical properties of the film [25].

SEM images of the tensile fracture surfaces of the TPS and TPS/BC films are shown in Figure 2. While the TPS film fracture surface is uniform, indicating a common rupture of a brittle surface (see Figure 2a), the TPS/BC film fracture surface is rough due to the presence of BC nanoribbons (see Figure 2b). The fracture shows no evidence of aggregates, indicating that the BC nanoribbons were homogeneously distributed throughout the matrix. Furthermore, good compatibility between the reinforcing BC and the TPS matrix (good fiber-matrix bonding) is observed based on the absence of pulling out BC nanoribbons. These results are in agreement with previous studies showing that the use of *in situ* BC reinforcement provided a smooth TPS fracture surface, while for nanocomposites, the BC ribbons were homogeneously distributed throughout the matrix, and strong interfacial adhesion was observed between the BC and the TPS matrix [31]. Nonsignificant variations on the edge of the fracture surfaces were observed, possibly related to the degree of swelling of the films during the test. These results are likely related to the intermolecular interactions inside of the films and alterations of the microstructure as they affect the mechanical properties, and these structural features can help preserve the integrity of the film during the test. Authors such as Araujo et al. [32] have suggested the aforementioned explanation during a swelling test of the starch films. A substantial amount of work remains to be done in this field as highlighted by the small number of scientific publications analyzing the micro- and nanostructure of starch films under fridge conditions.

The mechanical behavior of PVC* samples after being subjected to fridge temperature (5 °C) for five and ten days could not be evaluated due to the significant change in film consistency, which prevented the isolation of test samples.

The efficacies of the TPS and TPS/BC films for covering fruit were evaluated by storing the wrapped mangoes at a fridge temperature of 5 °C for five and ten days. PVC* film was used as a commercial reference, and unwrapped mango pieces were used as a control (UWM). Figures 3 and 4 show the appearance of the fruit after 5 and 10 days of storage, respectively. After 5 days, the mango pieces wrapped with PVC* and TPS/BC seem to have maintained their appearance. However, the morphologies of the mangoes wrapped with TPS and the UWM changed during storage. After 10 days, only the mangoes wrapped in PVC* had maintained their original morphology. The pieces wrapped in the other films and the UWM, the changes in fruit appearance may have been caused by dehydration during storage.

3.2 Mango Physicochemical Characterization

Table 1 shows that the amount of weight lost from the pieces of mango increased over time because of the moisture lost during the ripening process. The mango pieces wrapped with TPS and the UWM presented high weight losses, reaching approximately 70 % and 80 % on the 5th and 10th days of storage, respectively. TPS/BC films significantly reduced ($p \leq 0.05$) the weight loss to 50 % by the 5th day, but on the 10th day, the weight losses matched those of the TPS wrapped and UWM pieces (80 %). These results indicate that TPS/BC films are more effective for short periods of time, such as five days. Although PVC* wrapping slowed the weight loss during both evaluation periods, this material could reduce the exchange of gases with the surrounding environment. The presence of anaerobic microorganisms that affect the maturation process and the fruit quality can be promoted, as mentioned by authors such as De Roever [33] or Beuchat [34] in their analyses of the importance of the adequate protection and manipulation of the fruit, but the promotion can be even more dramatic when a large amount of surface area is exposed, as is the case of the fruits evaluated in this work. Further studies in this area are necessary due to the increasing use of synthetic polymer-based wrapping films for fruits.

The average firmness values of the mango pieces changed significantly ($p \leq 0.05$) during storage (see Figure 5). An increase in hardness was observed due to dehydration of the mango pieces regardless of wrapping. After 5 days of storage, no significant differences were observed between the pieces wrapped in TPS/BC and those wrapped in PVC*. Wrapping with TPS/BC decreased senescence and dehydration of the fruit because of the water-holding capacity of the bacterial cellulose [35]. As a result, the permeability was controlled, and the fruit moisture loss was decreased, allowing the fruit to retain its soft texture. After 10 days of storage, no significant differences between the mango samples wrapped with TPS or TPS/BC and the UWM was observed; only wrapping with PVC* film preserved the fruit firmness. Mango pieces wrapped with TPS and the UWM had greater losses of firmness.

The ATR-FTIR spectra of mangoes pieces acquired between 4000 and 600 cm^{-1} showed two absorption bands: 3800–2800 cm^{-1} and 1800–800 cm^{-1} (see Figure 6). The first band was assigned to hydroxyl groups (OH stretch at 3380 (peak 1)). In the spectra of the mango samples corresponding to UWM0 and PVC*, peak 1 shifted to longer wavenumbers, and their vibrations are at 3400 cm^{-1}. This shift could be related to two factors: the higher water content and the fruits being less mature. On the other hand, in the spectra of the samples wrapped in TPS, peak 1 shifted to shorter wavenumbers, and the vibration appeared at 3375 cm^{-1}, which suggests a change in the maturity of the fruit as proposed by Jiao et al. [36] when evaluating mango maturity. The absorbance at 1640 cm^{-1} (peak 4) can be used to determine the moisture content in the mangoes during the ripening process [37-38]. Peak 4 in the spectra of the UWM0 and PVC* fruit samples is slightly shifted to longer wavenumbers relative to the same peak in the spectra of the other samples. These results, combined with the previous observations of peak 1, suggest the change in the moisture content was dependent on the type of film used for wrapping. These results are also in concordance with the results presented in Table 1 and Figure 4 and the above discussion of water loss in the fruit samples. More studies, such as on vapor permeability, are required to further clarify these results.

The broad 2930 cm^{-1} C-H stretching band (peak 2) and the 1740 cm^{-1} C=O stretching band (peak 3) correspond to the fatty acids in the fruit [39-40], and these bands were more intense in spectra of the mangoes wrapped with TPS and the UWM because ripened more quickly [41]. The band at 1380 cm^{-1} (peak 6) corresponds to asymmetrical flexing of the methyl groups due to the presence of pro-vitamin A, and this peak was small on the 10th day of storage.

The second band, 1800-800 cm^{-1}, which corresponds to the absorptions of different types of sugar, was due to C-O stretching, OH bending, C-O stretching, and C-C stretching deformations (see Figure 6). In addition, characteristic absorption peaks of glycosidic linkages at 996 cm^{-1} (peak 11) and 868 cm^{-1} (peak 13) were observed [42-43]. During mango maturation, the accumulated starch in the chloroplasts was hydrolyzed into sucrose, fructose, and glucose [15, 44-45]. The sucrose band at 1050 cm^{-1} (peak 10) was the most intense. The fructose bands at 1418 cm^{-1} (peak 5), 1260 cm^{-1} (peak 7) and 924 cm^{-1} (peak 12) correspond to C-O bending and C-OH stretching [42]. The increase in the glucose content caused changes in the bands at 1140 cm^{-1} (C-OH bending, peak 8) and 1105 cm^{-1} (CH_2-related modes, peak 9) [46]. The increase in the intensities of the bands in the second zone was attributed to the ripening of the mango during storage, and these results confirmed the observation discussed above suggesting that the type of film used for wrapping influenced the fruit maturation. It is important to highlight that ATR-FTIR spectroscopy is a nondestructive alternative for evaluating mangoes quality parameters, especially during fruit storage.

Total Soluble Solids (TSS) and Total Acidity (TA)

During ripening, the TSS increased (see Figure 7) because of the degradation of the mango polysaccharides [7]. However, wrapping provided an additional barrier preventing interaction with the outside environment. The TSS decreased, resulting in a slower ripening process and, thus, a longer fruit shelf life. When TPS films were used, the TSS decreased by 25 %, whereas with the TPS/BC films, the TSS decreased by 36 %. For the UWM, the TSS increased by 172.73 %. While the protection capacity of the TPS/BC films was 18 %, the protection capacity of the PVC* was 14.4 %.

As shown in Figure 8, the TA significantly decreased ($p \leq 0.05$) during storage. On the 5th day, no difference was observed between the TA values of the mangoes wrapped with TPS and those wrapped with TPS/CB. In fact, a rapid decrease in TA was observed in both cases. On the 10th day, the mangoes wrapped with TPS, TPS/BC and PVC* films showed decreases in the initial value of citric acid (0.507 %) to 0.256 %, 0.314 %, and 0.363 %, respectively. The citric acid of the UWM decreased from 0.507 % to 0.258 %. In other words, there were no significant differences in the TA values obtained after wrapping with TPS/BC and PVC* by the 10th day of storage. The TA of mangoes wrapped with TPS/BC decreased by 19.3 % and by 14.4 % in PVC. This change corresponds to the normal ripening process in which an increase in pH indicates that the organic acids are being degraded into simpler compounds.

4. Conclusion

Wrapping with TPS and TPS/BC films maintained the physical integrity of the mango pieces under storage conditions, indicating that these films can be used as packaging material to preserve minimally processed mangoes. The TPS/BC films were the most efficient at maintaining the physical and chemical stability of the fruit for 5 days at 75 % RH and 5 °C. This result can be attributed to the addition of bacterial cellulose nanoribbons, which provided an additional barrier that decelerates water loss from the wrapped mangoes due to the water-holding capacity of the cellulose. Although ATR-FTIR indicated that the samples wrapped in PVC* films maintained their weight, these films alter the maturation of the fruit, possibly causing changes in the flavor or texture. The results of this work suggest that biodegradable TPS/BC films are a new option for the protection of minimal process fruits during short storage periods. This work also highlights the use of ATR-FTIR as an alternative technique for measuring the biochemical quality parameters of minimally processed mangoes.

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Table captions

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Wrapped	Weight loss of pieces mango (g)		
	0 days (no exposure)	5 days	10 days
TPS	7.79 ± 0.39 $^{a, b, c}$	2.31 ± 0.09 ^{a}	1.54 ± 0.08 ^{a}
TPS/BC	7.78 ± 0.22 $^{a, b, c}$	3.67 ± 0.24 ^{b}	1.73 ± 0.07 ^{a}
PVC*	7.45 ± 0.07^{a}	7.35 ± 0.07 ^{c}	6.21 ± 0.11 ^{b}
UWM	8.23 ± 0.04 ^{b}	2.40 ± 0.03 ^{a}	1.74 ± 0.03 ^{a}

Mean values with different letters (a, b, and c) in each column are significantly different ($p \leq 0.05$).

Figure captions

Figure 1. Mechanical properties of TPS and TPS/BC films at storage conditions of 75 % RH and 5 °C for 5 and 10 days. (a) Tensile strength, (b) Young´s modulus and (c) elongation at break.

Figure 2. SEM images of the tensile fracture surface of the films. The top part of the sample was close to the fruit: (a) TPS, (b) TPS/BC.

Figure 3. Images of minimally processed mangoes wrapped with (a) TPS, (b) TPS/BC, and (c) PVC* and UWM after storage at 75 % RH and 5 °C for 5 days.

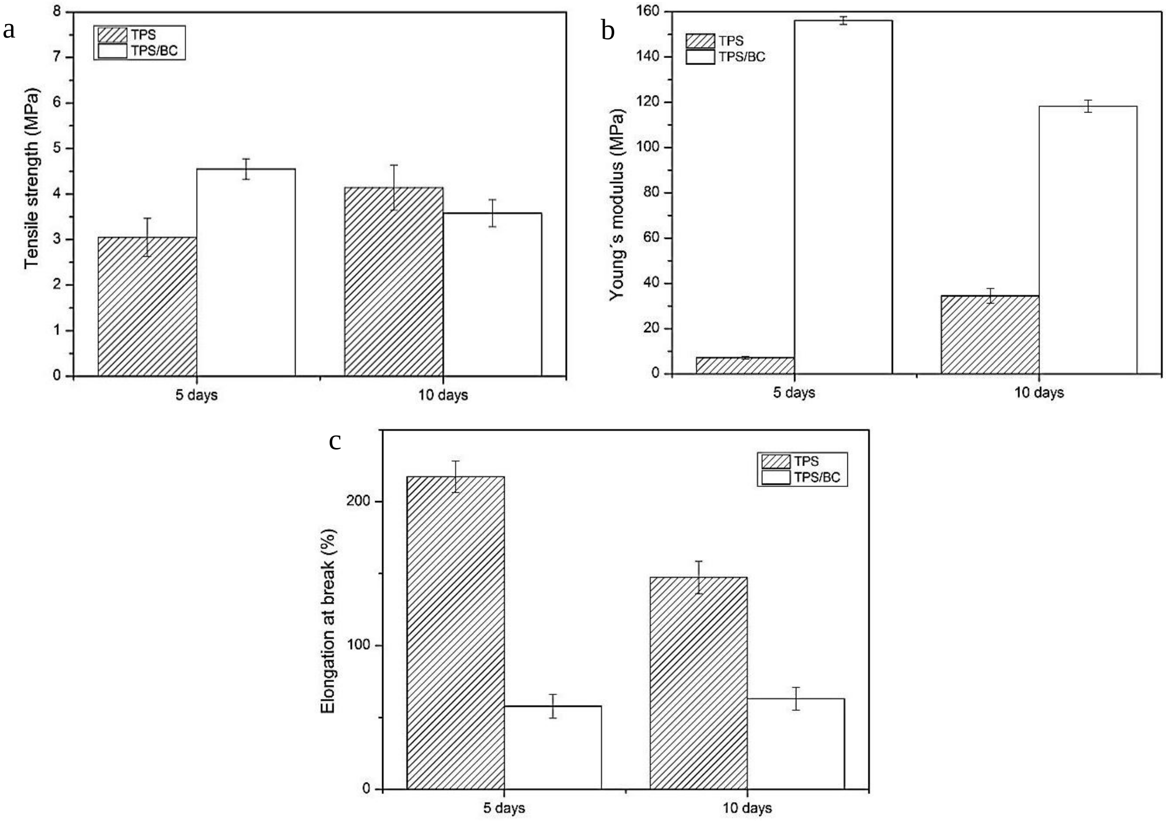
Figure 4. Images of minimally processed mangoes wrapped with (a) TPS, (b) TPS/BC, and (c) PVC* and UWM after storage at 75 % RH and 5 °C for 10 days.

Figure 5. Firmness values of pieces of mango wrapped with TPS, TPS/BC and PVC* and UWM after 0, 5 and 10 days at 75 % RH and 5 °C.

Figure 6. ATR-FTIR spectra of pieces of mango wrapped with TPS, TPS/BC and PVC* and UWM after 10 days of storage and UWM0 (initial mango samples).

Figure 7. TSS of pieces of mango wrapped with TPS TPS/BC, and PVC* and UWM after 0, 5 and 10 days of storage at 75 % RH and 5 °C.

Figure 8. TA of mangoes wrapped with TPS, TPS/BC and PVC* and UWM stored for 0, 5 and 10 days at 75 % RH and 5 °C.



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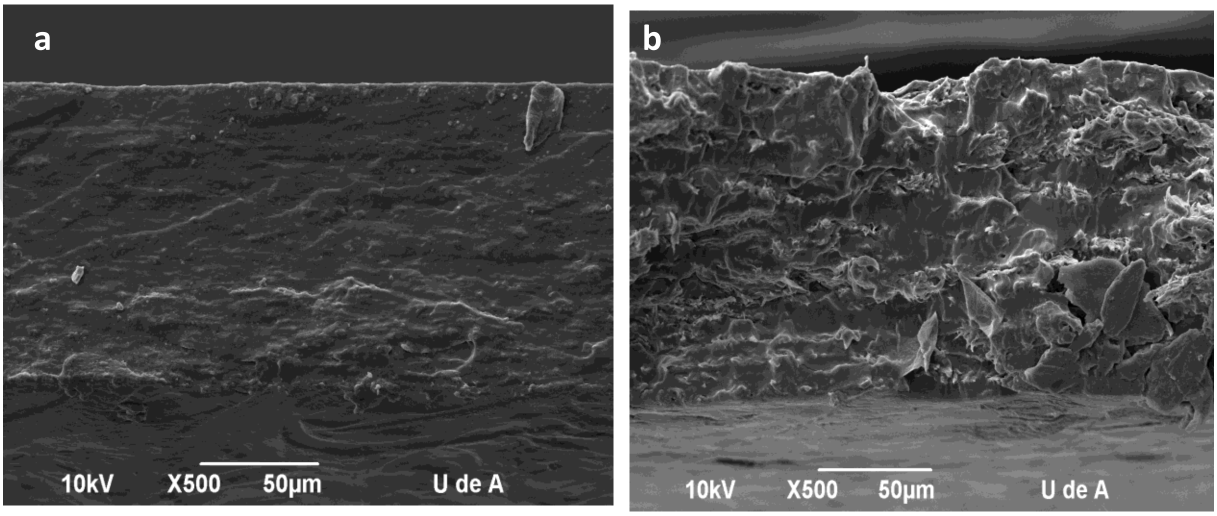

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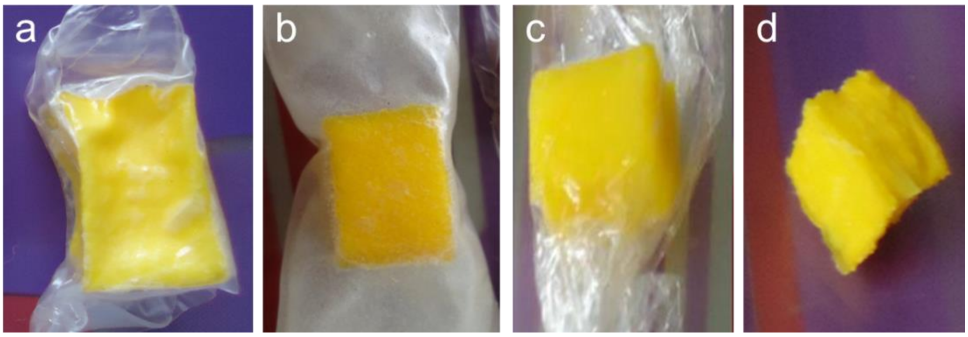

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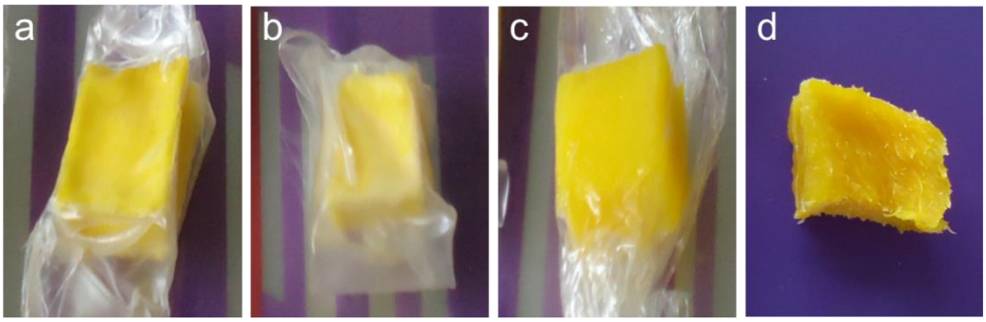

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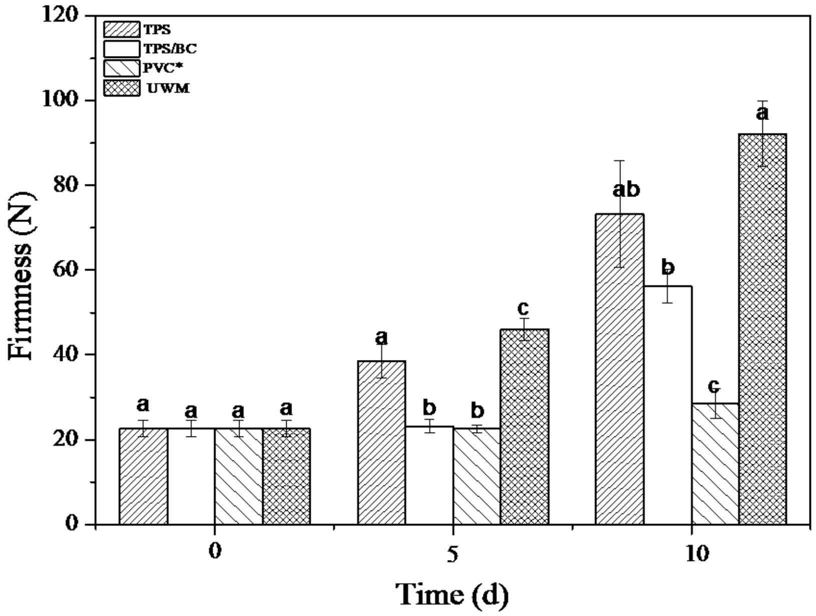

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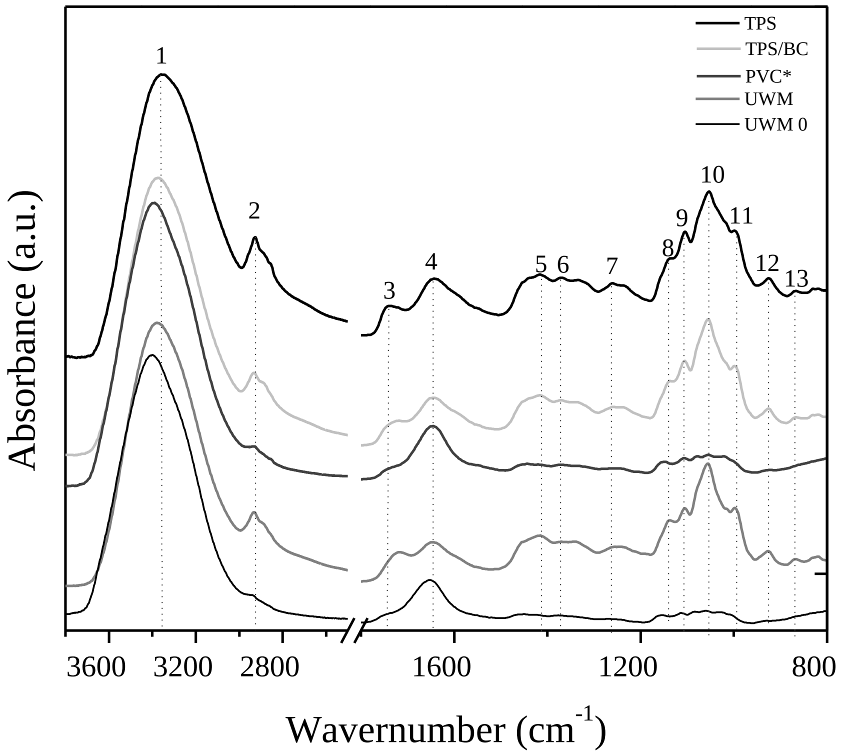

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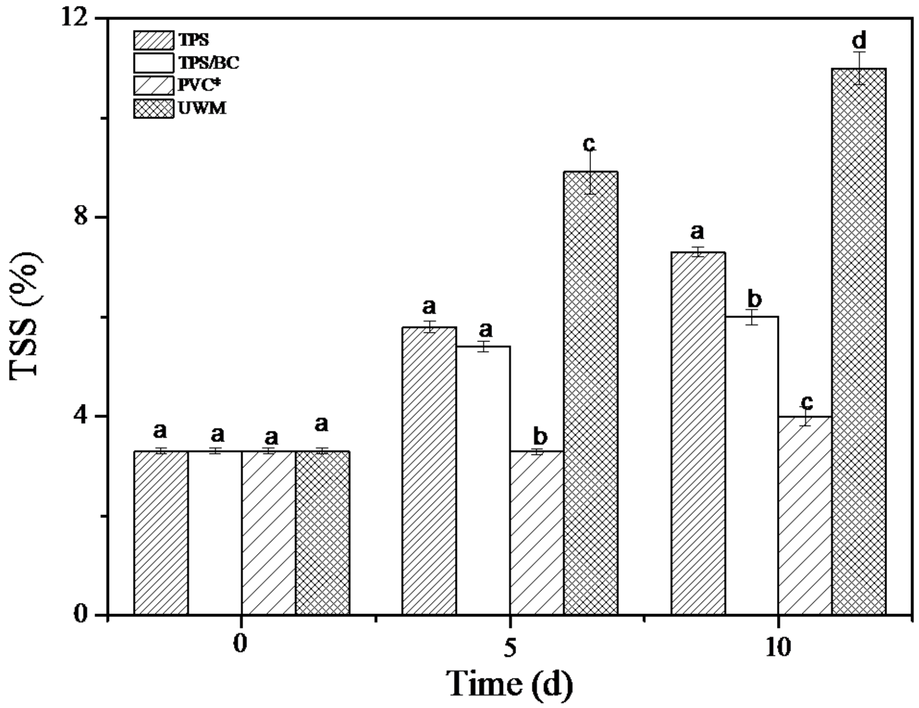

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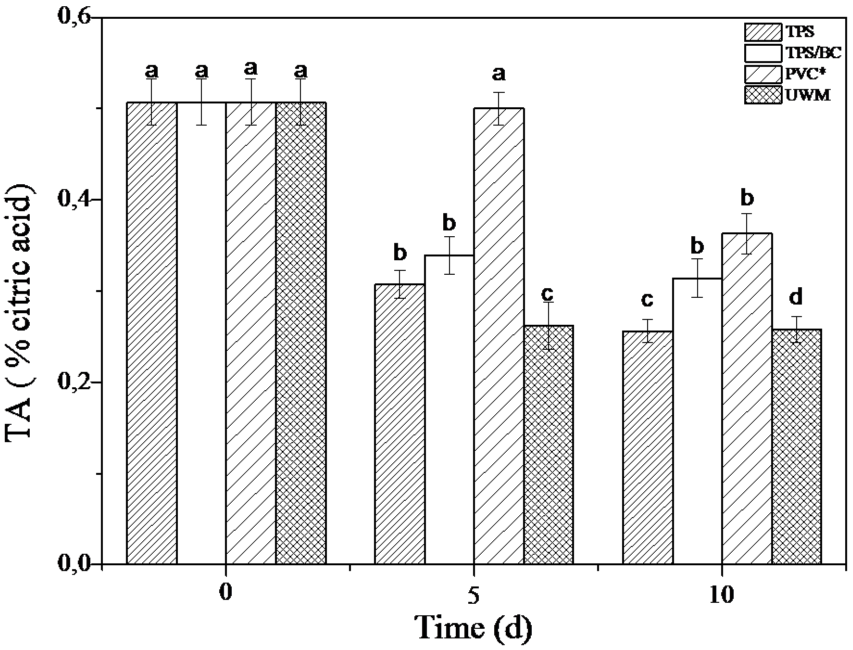

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