Article

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Abstract: Food waste disposal to landfills practice contributes to greenhouse problem due to the emission of gases such as methane to the atmosphere. Shelf-life extension of food products and further valorization of food wastes such as the conversion to activated carbon are tested nowadays as alternative techniques. The development and use of biobased active coatings against food oxidation/bacterial deterioration is an environmentally friendly technique. In this study, a novel food active coating was successfully developed based on a chitosan (CS)/poly-vinyl alcohol (PVOH) matrix activated with a nanohybrid of natural thymol (TO) adsorbed in activated carbon (AC) derived from spent coffee. The results shown that, compared to the pure CS/PVOH polymeric matrix, the coating with 15% wt. TO@AC nanohybrid concentration achieved +23.5 % Young's modulus value, +20.1% ultimate strength value, increased barrier of +50.2% for water and +74.0 % for oxygen, +69.0% antioxidant activity, increased antibacterial activity of +5.5% against *Escherichia coli*, +17.8% against *Salmonella enterica,* +42.5% against *Staphylococcus aureus,* and +2.5% against *Listeria monocytogenes*. A visual evaluation of this coating shown a delay to fresh bananas enzymatic browning and significantly decrease their weight loss. This indicates that it could be potentially used to extend the shelf-life of fresh fruits.

Keywords: shelf life; biomass valorization; spent coffee; activated carbon; thymol; chitosan; poly-vinyl-alcohol; nanohybrids; control release; active coatings; bananas preservation

1. Introduction

Nowadays, a worldwide effort is making to disengage the global economy from fossil fuels and their petrochemical derivatives [1]. One huge category of such derivatives are the petrochemical polymer plastics which due to their non-biodegradable nature contributes significantly to environmental pollution [1,2]. To reach the zero-carbon footprint, researchers suggest the replace of the fossil fuel-made polymers with biopolymers, which can be extracted or synthesized from biomass. Plastic polymers are used in the food industry as packaging materials because of their flexible, rigid, or semi-rigid nature [3,4]. For a transition to sustainable and "green" food industry, the researchers recommend the use of biobased polymers as food packaging materials [5,6]. Cellulose, starch, gelatin, alginate salts, and chitosan are some of the suggested natural abundant biopolymers which can replace the petrochemically derived polymers used in the food packaging industry [7]. Chitosan (CS) holds a great potential for use in food packaging applications due to its non-toxicity, biodegradability, film-forming properties, gas barrier properties, and significant antibacterial activity against various food pathogens [8]. Moreover, it can be produced from chitin which is the second

most abundant biopolymer after cellulose, and can be extracted from crustacean waste, such as crabs, shrimps, and crayfish [9].

Another significant global trend is the food waste management which can enforce the sustainability and the green economy [10,11]. Large amounts of food are wasted due to the modern lifestyle which increases the demand for availability of all kinds of food throughout the year. Active packaging could be a potential sustainable solution to reduce foods waste by increasing their preservation period and their nutritional characteristics [12]. CS-based films have been suggested as promising fruit and vegetable coatings [13]. Because of their antibacterial properties and their controllable CO_2 and O_2 barrier properties, CS films could regulate fruit and vegetable respiration and extend their shelf-life period. Polyvinyl alcohol (PVOH) is a synthetic water-soluble biodegradable polymer which incorporates excellently with CS, to develop CS/PVOH composites with enhanced tensile, barrier, and antibacterial properties [14,15]. On the other hand, essential oils (EO) and their derivatives are promising candidates to replace antioxidant and antibacterial additives used to preserve fruit and vegetables [16]. EO can be incorporated into a biopolymer such as CS creating bioactive hydrogels and, exhibiting controlled antioxidant and antibacterial activity, be used as fruit and vegetable active coatings [16]. To minimize the reduction of EO's antioxidant and antibacterial activity, because of their volatile nature, it is suggested that such bioactive compounds be adsorbed into nanocarriers such as montmorillonite and halloysites nanoclays [17–19]. Recently, natural zeolites were suggested as an alternative nanocarrier for such bioactive compounds, to controlled release EOs in active food packaging systems [20–22]. Under the same spirit, activated carbon (AC) could be a novel alternative nanocarrier and a controlled release platform for EOs in active packaging systems because it can be produced almost from all kinds of biomass and food waste, and due to its very high specific pore surface area [23].

Browning of raw fruits is a major problem in the food industry and is believed that it will be one of the main reasons for quality loss during after harvest handling and processing. It will also be one of the main reasons for dramatically increase of food waste [24]. Bananas are rich in nutritional components but are highly sensitive to enzymatic browning. Thus, is a particularly problematic fruit. This enzymatic browning reduces banana's preservation time, deters consumers from purchasing them, and increases banana waste [25]. Last years studies shown that CS-based active coatings could extend bananas' preservation period and protect them from enzymatic browning [26].

Recently, AC originated by pyrolysis process of spent coffee was modified with a thymol (TO)-rich fraction of thyme essential oil to create a TO@AC nanohybrid. The TO@AC nanohybrid was successfully incorporated into LDPE active packaging films [27]. In the present study, the as prepared TO@AC nanohybrids were incorporated into a CS/PVOH matrix at 5, 10, and 15 wt.% contents to develop novel CS/PVOH/xTO@AC active films/coatings (where x=5, 10, and 15) via a solution casting method. For comparison, pure AC from spent coffee was incorporated into a CS/PVOH matrix to develop CS/PVOH/xAC active film/coatings (where x =5, 10, and 15). The as-prepared CS/PVOH/xAC and CS/PVOH films were characterized with XRD analysis, FTIR spectroscopy, and SEM images to investigate the relaxation and dispersion of the pure AC and TO@AC nanohybrid within the CS/PVOH matrix. Tensile, water/oxygen barrier properties were investigated, while total antioxidant activity and antibacterial properties against two gram-positive and two gram-negative food pathogens were studied. The most active CS/PVOH/xAC and CS/PVOH/xTO@AC films were successfully applied as coatings to extend the preservation time of fresh bananas. The overall study shown that these novel CS/PVOH/xAC and CS/PVOH/xTO@AC active films could potentially be used as fresh fruit and vegetable active coatings.

The innovation of this work is that by following the spirit of bioeconomy, a biodegradable active coating for the preservation of fresh fruits such as fresh bananas was first time developed. This new active packaging was becoming by combining CS, which was derived by shellfishes' and crustaceans' byproducts, and AC, which was obtained from spent coffee, a water-soluble biopolymer such as PVOH and TO which was a component of naturally available EOs.

2. Materials and Methods

2.1. Materials

Chitosan (CS) with a molecular weight of 100,000–300,000 was purchased from Acros-Organics company (Zeel West Zone 2, Janssen Pharmaceuticalan 3a, B2440, Geel, Belgium). Polyvinyl alcohol, 86-89% hydrolyzed, low molecular weight, was purchased from Thermo Scientific Chemicals Co., (168 Third Avenue, Waltham, MA USA 02451). Activated carbon (AC) produced from spent coffee of students café of University of Ioannina was prepared with a pyrolysis process and a treatment with KOH according to previous report [28]. The Brünaüer–Emmett–Teller surface area of AC was 1372 m^2/g and the micropore volume fraction was 84.6% [28]. Acetic acid (CAS Number: 64-19-7) was supplied by Sigma-Aldrich (Co., 3050 Spruce Street, St. Louis, MO, USA, 314-771-5765).

2.2. Preparation of TO@AC nanohybrid

The modification of pure AC with rich in thymol (TO) fraction of thyme oil was carried out with a recently developed "green" distillation/adsorption process to obtain TO@AC nanohybrid [27]. According to this report the obtain TO@AC nanohybrid absorbed 51 wt% TO fraction while this TO fraction is rather physiosorbed than chemisorbed [27]. For controlled release applications, physisorption of such TO molecules in AC is preferred than chemisorption.

2.3. Preparation of CS/PVOH/xAC and CS/PVOH/xTO@AC films

For the preparation of CS/PVOH/xAC and CS/PVOH/xTO@AC films a solution casting method was used [29]. Firstly, a 1 v/v in acetic acid 2 wt.% CS aquatic solution of was prepared and a 10 wt.% aquatic solution of PVOH [29,30]. For each film 90 ml of the 2 wt.% CS solution and 12 ml of the 10 wt.% PVOH solution was mixed. In the obtained CS/PVOH solution 0.21 g, 0.44 g and 0, 71 g of AC or TO@AC powder was added to achieve 5, 10, and 15 wt.% AC and TO@AC final nominal content. All the CS/PVOH/xAC and CS/PVOH/xTO@AC mixtures were homogenized with stirring for 5 min at 18,000 rpm. Approximately 50 ml of obtained solutions were casted in two plastic petri dishes with 10 cm diameter and dried at 25 °C for three-four days. The obtained CS/PVOH/xAC and CS/PVOH/xTO@AC (see Figure 1) were piled off from petri dishes and stored at 25 °C and 50% RH for further use and characterization.
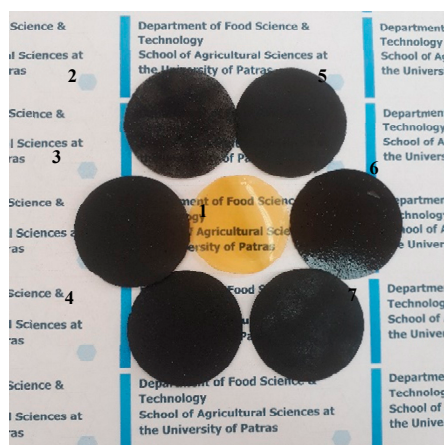

Figure 1. Obtained (1) pure CS/PVOH, (2) CS/PVOH/5AC, (3) CS/PVOH/10AC, (4) CS/PVOH/15AC, (5) CS/PVOH/5TO@AC, (6) CS/PVOH/10TO@AC and (7) CS/PVOH/15TO@AC.

2.4. XRD analysis of CS/PVOH/xAC and CS/PVOH/xTO@AC films

The obtained CS/PVOH/xAC and CS/PVOH/xTO@AC films were characterized with XRD analysis by using a Brüker D8 Advance X-ray diffractometer instrument (Brüker, Analytical

Instruments, S.A., Athens, Greece) and according to the experimental condition described recently [29].

2.5. FTIR spectroscopy of CS/PVOH/xAC and CS/PVOH/xTO@AC films

The relaxations of AC and TO@AC with CS/PVOH matrix were investigated with FTIR spectroscopy measuraments. For the measuraments an FT/IR-6000 JASCO Fourier transform spectrometer (JASCO, Interlab, S.A., Athens, Greece) was employed according to the experimental conditions described recently [29].

2.6. Tensile measuraments of CS/PVOH/xAC and CS/PVOH/xTO@AC films

Tensile properties of obtained CS/PVOH/xAC and CS/PVOH/xTO@AC films as well as pure CS/PVOH were carried out according to the ASTM D638 method and the methodology described recently [22,29]. For the tensile measurements a Simantzü AX-G 5kNt instrument (Simandzu Asteriadis, S.A., Athens, Greece) was employed.

2.7. Water Vapor Transmission Rate Measurements and Water Diffusion Coefficient Calculation

The Water Vapor Transmission Rate (WVTR g/cm^2.s) for all obtained CS/PVOH/xAC and CS/PVOH/xTO@AC films, as well as pure CS/PVOH film was measured according to the ASTM E96/E 96M-05 method at 38 ^{o}C and 95 %RH by using a handmade apparatus and employing. The calculated WVTR values were transformed to water vapor diffusivity (D_{wv}) values according to the theory and equations described in detail in previous publications [20,31].

2.8. Oxygen Transmission Rate Measurements and Oxygen Permeability Calculation

Oxygen Transmission Rate (OTR) values (cc O_2/m^2/day) for all obtained CS/PVOH/xAC and CS/PVOH/xTO@AC films, as well as pure CS/PVOH film were measured according to the ASTM D 3985 method at 23 ^{o}C and 0% RH. For the measurements an oxygen permeation analyzer (O.P.A., 8001, Systech Illinois Instruments Co., Johnsburg, IL, USA) was employed. From the measured OTR values the oxygen permeability coefficient values (PeO_2) were calculated according to the theory and equations provided in detail in previous publications [20,31].

2.9. Total antioxidant activity of CS/PVOH/xAC and CS/PVOH/xTO@AC films

The total antioxidant activity of all CS/PVOH/xAC and CS/PVOH/xTO@AC films was estimated according to the diphenyl-1-picrylhydrazyl (DPPH) method. For the experiments a 40 ppm ethanolic solution of DPPH stock solution was prepared. Inside a dark glass bottle 10 ml of DPPH stock solution and 300 mg of each film were putted and incubated for 24 hours. The absorbance at 517 nm wavelength of the DPPH solution was measured in the biggening (0 h) and in the end (24 h) of the incubation using a Jasco V-530 UV-vis spectrophotometer. For comparison the absorbance of a 10 mL of ethanolic DPPH solution without the addition of any film was measured at 517 nm and considered as the blank sample.

The % antioxidant activity after 24 h incubation of films was calculated according to the following equation:

$$\textbf{\% Antioxidant activity = (Abs}_{\textbf{blank}} \textbf{ - Abs}_{\textbf{sample}}\textbf{)/Abs}_{\textbf{blank}} \textbf{ x 100} \qquad (1)$$

2.10. Antibacterial Activity Tests of CS/PVOH/xAC and CS/PVOH/xTO@AC films

The antimicrobial activity of the films was evaluated using the well diffusion method against four foodborne pathogenic bacteria. These bacterial strains, including *Escherichia coli* (ATCC 25922) and *Salmonella enterica* subsp. *enterica* (DSMZ 17420) (Gram-negative bacteria), as well as *Staphylococcus aureus* (DSMZ 12463) and *Listeria monocytogenes* (DSMZ 27575) (Gram-positive bacteria), were acquired from the Institute of Technology of Agricultural Products, ELGO-DEMETER, located in Lykovryssi, Greece.

To initiate the experiments, the bacterial strains were cultured in Mueller Hinton Broth at a temperature of 37 °C for 24 h, facilitating growth and achieving a bacterial concentration ranging from 10^7 to 10^8 colony-forming units per milliliter (CFU mL^{-1}). Subsequently, the bacterial cultures were evenly spread on Müeller-Hinton agar plates by rotating the plates at 60-degree intervals, ensuring uniform growth of bacterial colonies.

To create wells for testing, a cork borer dipped in alcohol and flamed was used to cut 6-mm wells into the agar surface of the plates. These wells were then filled with 100 μl of the studied suspensions, from which the final films were formed. Following this, the plates were incubated overnight at 37 °C.

After the incubation period, the diameters of any clear zones surrounding the wells containing the antimicrobial suspensions were measured using calipers. This measurement aimed to evaluate the extent of antimicrobial activity demonstrated by the suspensions against the tested bacteria. The entire experimental procedure was conducted in triplicate to ensure consistent and reliable result.

2.11. Application in fresh bananas preservation

For the application of the obtained CS/PVOH/xAC and CS/PVOH/xTO@AC films as bananas coating the following procedure was followed. With the procedure described hereabove in 2.3 section 100 ml of pure CS/PVOH, CS/PVOH/15AC and CS/PVOH/15TO@AC hydrogel coatings were prepared. Next, twelve (12) banana samples without diseases and pests, mechanical damage, and the same maturity were selected from a local fruit market. The samples were divided into four groups with three bananas each. The first group with three bananas was the uncoated group. The second group with three bananas was the group coated with CS/PVOH coating. The third group with three bananas was the group coated with CS/PVOH/15AC coating and the fourth group with three bananas was the group coated with the CS/PVOH/15TO@AC coating. Then all the coated and the uncoated bananas were stored in room conditions (15-20 °C and 65% relative humidity (RH)) for 8 d. Then, the quality changes of bananas at 0, 1, 2, 3, 4, 5, 6, 7, and 8 d of storage were monitored by taking photos and measuring the weight loss of them.

2.12. Statistical analysis

The statistical software IBM, SPSS ver. 25, was used to treat the resulting tensile, water/oxygen barrier, antioxidant activity, antibacterial activity, and weight loss of fresh bananas measurements of three pieces of every sample. Values presented in Tables 1–3, Figures 5 and 7 are the final mean values of these three measurements for every property, and the plus/minus (±) standard deviation is tabulated on the right of each value. A confidence interval of C.I. = 95% was assumed in every case. Hypothesis tests assuming a statistical significance level of $p = 0.05$ was carried out for every case to ensure that different mean values of a property for different samples are also statistically different. The non-positive normality tests implied the non-parametric Kruskal–Wallis method for such investigations, and statistically equal mean values are indicated in tables with the same superscript index.

3. Results

3.1. XRD analysis of CS/PVOH/xAC, CS/PVOH/xTO@AC films

In Figure 2 the XRD plots of pure CS/PVOH film as well as of all CS/PVOH/xAC and all CS/PVOH/xTO@AC films are observed in the range of 2 to 30° 2 theta.
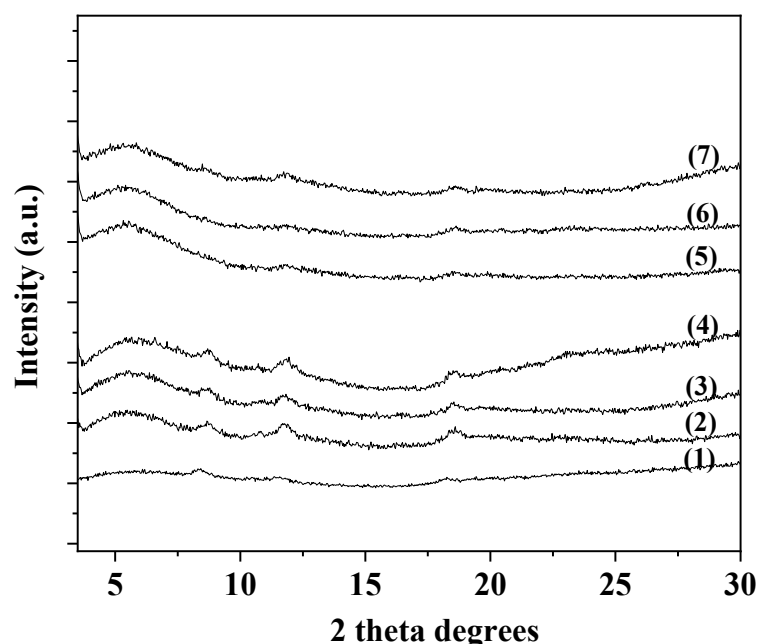
Figure 2. XRD plots of (1) CS/PVOH, (2) CS/PVOH/5AC (3) CS/PVOH/10AC, (4) CS/PVOH /15AC, (5) CS/PVOH /5TO@AC, (6) CS/PVOH/10TO@AC, and (7) CS/PVOH /15TO@AC obtained films.

In the XRD plots of pure CS/PVOH composite film (see line (1) in Figure 1) broad peaks at around 8.5°, 11.5° and 18.5° are observed. Peaks at 8.5° and 11.5° indicate the CS's hydrated crystallite structure because of the insertion of water molecules in the CS's crystal lattice [14,31]. The third peak at 18.5° is assigned to the CS's regular crystal lattice [14,31]. As it is mentioned recently, the peaks at 8.5°, 11.5°, and 18.5° indicate that PVOH enhances the hydrated structure of CS [15,31].

In the case of CS/PVOH/xAC films the peaks at 8.5°, 11.5° and 18.5° (see plot lines (2), (3) and (4) in Figure 2) are slightly increased in comparison to the pure CS/PVOH film. On the contrary, in the case of CS/PVOH/xTOAC films the same peaks at 8.5°, 11.5° and 18.5° (see plot lines (5), (6) and (7) in Figure 2) are almost disappeared. This suggests a higher miscibility and dispersion of modified TO@AC nanohybrid as compared to pure AC with CS/PVOH matrix.

3.2. FTIR spectroscopy of CS/PVOH/xAC, CS/PVOH/xTO@AC films

FTIR spectra of all obtained CS/PVOH/xAC and CS/PVOH/xTO@AC films as well as pure CS/PVOH film are plotted in Figure 3.
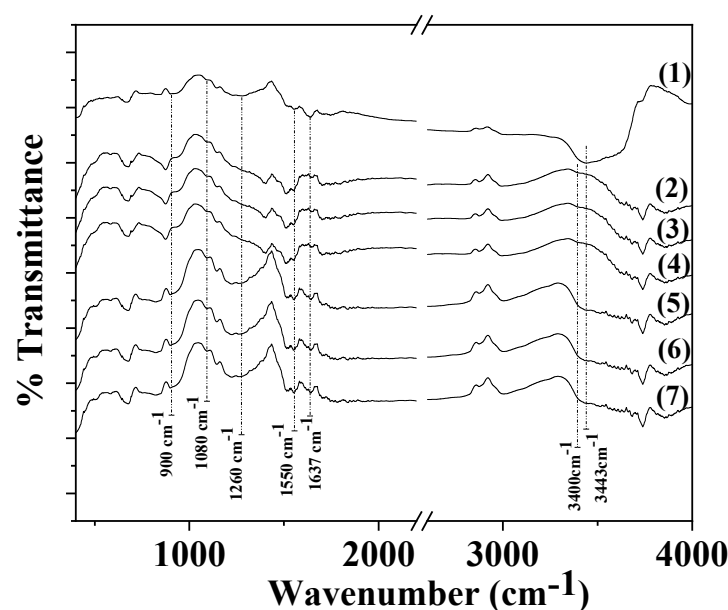
Figure 3. FTIR plots of (1) CS/PVOH, (2) CS/PVOH/5NZ (3) CS/PVOH/10NZ, (4) CS/PVOH /15NZ, (5) CS/PVOH /5TO@NZ, (6) CS/PVOH/10TO@NZ, and (7) CS/PVOH /15TO@NZ obtained films.

In the FTIR plot of pure CS/PVOH film (see line (1) in Figure 3) there are obtained reflections of both CS and PVOH polymers. The large band at 3443 cm^{-1} is assigned to stretching vibration of hydroxyl groups of both CS and PVOH [29]. At 3400 cm^{-1} is assigned the primary stretching vibration of amino groups of CS [29]. The reflections at 1637 cm^{-1}, 1550 cm^{-1}, 1260 cm^{-1}, 1080 cm^{-1}, 900 cm^{-1} are red shift of C-H stretching, C-O stretching, N-H bending and O-H stretching of CS due to the strong relaxation with PVOH chains [32]. In the case of both CS/PVOH/xAC (see lines (2), (3) and (4) in Figure 3) and CS/PVOH/xTO@AC (see lines (5), (6) and (7) in Figure 3) FTIR plots the characteristic reflections of AC are observed in advance to CS/PVOH matrix reflections. The band at 1260 cm^{-1} is assigned to the stretching vibration of C-O groups. The bad with a maximum at about 3420–3440 cm^{-1} is assigned to the O–H stretching mode of hydroxyl groups of the adsorbed water molecules [28]. With a careful glance in CS/PVOH/xAC and CS/PVOH/xTO@AC FTIR plot it is resulted that in the case of CS/PVOH/xTO@AC the reflection at 1260 cm^{-1} is increased implying a bigger relaxation and interplay between CS/PVOH N-H groups and TO@AC C-O groups. In advance in the case of CS/PVOH/xTO@AC FTIR plots the reflection of the primary stretching vibration of amino groups of CS at 3400 cm^{-1} are more intense than in the case of CS/PVOH/xAC FTIR plots where the same reflection almost disappeared. This is also implying the higher relaxation of N-H groups of CS/PVOH matrix with the O-H groups of TO@AC nanohybrid.

Thus, by both XRD analysis and FTIR spectroscopy it is resulted the higher dispersion and relaxation of TO@AC nanohybrid with CS/PVOH matrix in comparison to pure AC with CS/PVOH matrix.

3.3. Tensile properties CS/PVOH/xAC, CS/PVOH/xTO@AC films

In the Figure 4 the representative stress-strain curves of all CS/PVOH/xAC and CS/PVOH/xTO@AC films as well as of the pure CS/PVOH film are plotted for comparison. From these stress strain curves the Young's (E) modulus, ultimate tensile strength (σ_{uts}), and % strain at break (ε_b) have been calculated and listed in Table 1.
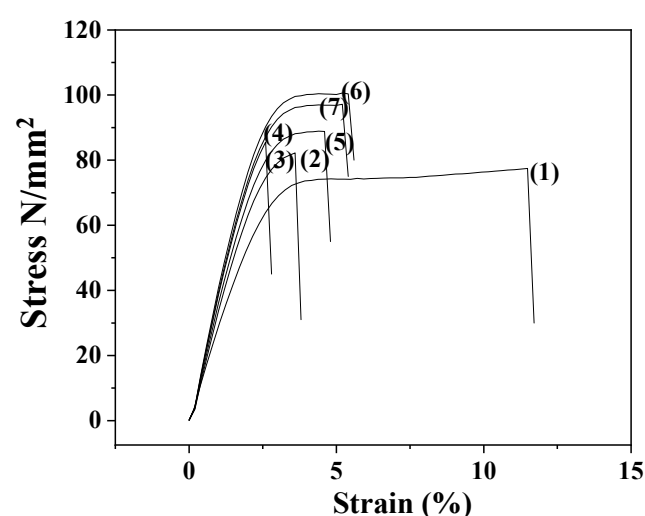
Figure 4. Stress-strain curves of (1) CS/PVOH, (2) CS/PVOH/5AC (3) CS/PVOH/10AC, (4) CS/PVOH /15AC, (5) CS/PVOH /5TO@AC, (6) CS/PVOH/10TO@AC, and (7) CS/PVOH /15TO@AC obtained films.

Table 1. Calculated values of Young's (E) modulus, ultimate tensile strength (σ_{uts}), and % strain at break (ε_b).

	E	σ_{uts}	$\varepsilon\%$
CS/PVOH	2249.3(100.3)	71.2(1.8)	11.8(0.9)
CS/PVOH/5AC	2511.0(43.0)	79.0(5.7)	4.1(0.7)
CS/PVOH/10AC	2881.3(63.2)	86.1(6.1)	3.4(0.1)
CS/PVOH/15AC	2789.0(64.4)	82.1(4.5)	5.4(0.9)
CS/PVOH/5TO@AC	2692.0(54.2)	81.8(7.4)	5.2(1.5)
CS/PVOH10TO@AC	3041.3(79.2)	104.3(8.0)	6.1(0.9)
CS/PVOH/15TO@AC	2938.5(60.5)	88.0(6.1)	5.9(0.8)

As it is obtained from the Young's (E) modulus, ultimate tensile strength (σ_{uts}), and % strain at break (ε_b) values of Table 1 the addition of both pure AC and modified TO@AC nanohybrid in CS/PVOH matrix enhanced the tensile stress and strength values while reduces the elongation at break values of all CS/PVOH/xAC and CS/PVOH/xTO@AC films. Higher stress and ultimate strength values are obtained for all CS/PVOH/xTO@AC films in comparison to CS/PVOH/xAC films. The film with the highest stress and ultimate strength is the CS/PVOH10TO@AC film.

3.4. Water – oxygen barrier properties of CS/PVOH/xAC, CS/PVOH/xTO@AC films

The obtained water vapor transmission rate (WVTR) and the oxygen transmission rate (OTR) values for all CS/PVOH/xAC and CS/PVOH/xTO@AC films as well as pure CS/PVOH film are listed in Table 2. From these values the water diffusivity (Dw) and the oxygen diffusivity (PeO2) values are calculated and are listed in Table 2 also for comparison.

Table 2. Film thickness, water vapor transmission rate (WVTR), water diffusivity (D), oxygen transmission rate (OTR), and oxygen diffusivity (PeO$_2$) values of pure CS/PVOH film as well as CS/PVOH/HNT and CS/PVOH/TO@HNT films.

	Water Vapor Transmition Rate film thickness (mm)	Water Vapor Transmition Rate (10^{-6} g/cm².s)	Diffusion Coefficient (10^{-4} cm²/s)	Oxygen Transmition Rate -film thickness (mm)	Oxygen Transmition Rate (ml/m².day)	Pe$_{O2}$ (10^{-7} cm²/s)
CS/PVOH	0.14±0.01	1.339±0.024	4.36±0.11	0.15±0.01	38.20±1.91	5.73±0.29
CS/PVOH/5AC	0.15±0.01	1.240±0.025	4.32±0.11	0.15±0.01	19.10±0.96	2.87±0.14
CS/PVOH/10AC	0.17±0.01	0.896±0.021	3.53±0.10	0.09±0.01	27.50±1.38	2.48±0.12
CS/PVOH/15AC	0.16±0.01	0.924±0.014	3.43±0.07	0.16±0.01	10.10±0.51	1.62±0.08
CS/PVOH/5TO@AC	0.12±0.01	0.902±0.018	3.18±0.07	0.14±0.01	18.50±0.93	2.59±0.13
CS/PVOH/10TO@AC	0.13±0.01	0.735±0.017	2.99±0.09	0.10±0.01	17.50±0.88	1.75±0.09
CS/PVOH/15TO@AC	0.14±0.01	0.802±0.012	2.17±0.09	0.15±0.01	10.30±0.50	1.49±0.07

As it is obtained from Table 2. both pure AC and modified TO@AC nanohybrid decrease the D_w and PeO$_2$ values of obtained films. Higher the AC and TO@AC wt.% content lower the obtained D_w and Pe$_{O2}$ values. TO@AC nanohybrid succeed to decrease farther the obtained D_w and PeO$_2$ values than pure AC. This result is corelated with the higher dispersion and relaxation of TO@AC nanohybrid shown hereabove in XRD analysis and FTIR spectrometry measurments.

3.5. Total antioxidant activity of CS/PVOH/xAC and CS/PVOH/xTO@AC films

The % total antioxidant activity of all CS/PVOH/x**AC** and CS/PVOH/xTO@**AC** films as well as pure CS/PVOH after 24 hours of incubation are plotted in the Figure 5. As it was expected pure CS/PVOH and all CS/PVOH/x**AC** films shown small values of tota antioxidant activity due to the presence of CS which is known to has an antioxidant activity [33]. For CS/PVOH/xTO@AC films significant % total antioxidant activity values are obtained due to the presence of TO molecules [34]. As the wt.% content of TO@AC used increase the obtained % total antioxidant activity values are increased farther. It must be also underlined that the values obtained here for CS/PVOH/xTO@AC are much higher from the values reported recently for CS/PVOH/xTO@AC films with the same method implying the superiority of AC against HNT as TO molecules nanocarrier for control release applications [29].
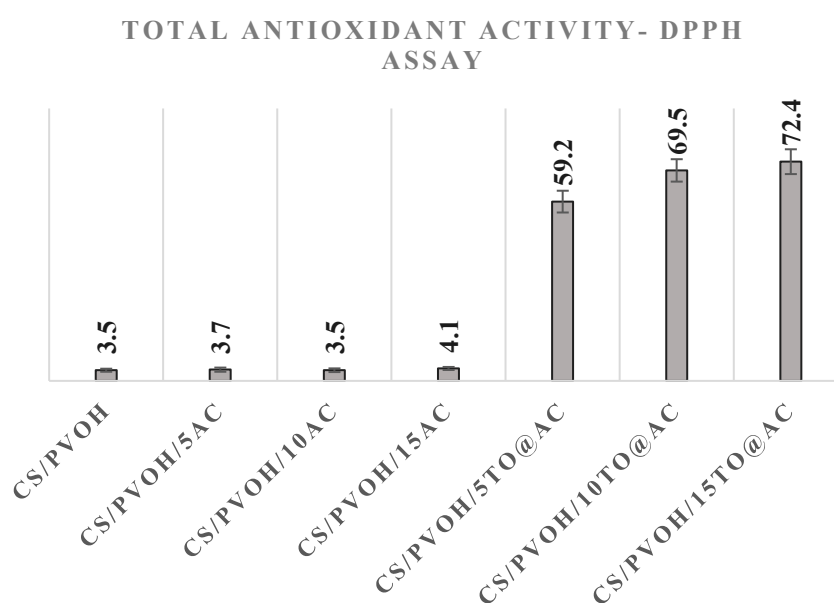

TOTAL ANTIOXIDANT ACTIVITY- DPPH ASSAY

3.5 3.7 3.5 4.1 59.2 69.5 72.4

CS/PVOH CS/PVOH/5AC CS/PVOH/10AC CS/PVOH/15AC CS/PVOH/5TO@AC CS/PVOH/10TO@AC CS/PVOH/15TO@AC

Figure 5. % Total antioxidant activity of all obtained CS/PVOH/xAC and CS/PVOH/xTO@AC films.

3.6. Antibacterial properties of CS/PVOH/xAC and CS/PVOH/xTO@AC films- Agar diffusion test

The antibacterial activity of various film materials against four bacterial strains (*E. coli*, *S. aureus*, *S. enterica*, and *L. monocytogenes*) was investigated in this study. The films tested included CS, CS/PVOH, CS/PVOH/5AC, CS/PVOH/10AC, CS/PVOH/15AC, CS/PVOH/5TO@ AC, CS/PVOH/10TO@ AC, and CS/PVOH/15TO@ AC. The inhibitory activity of the film materials against the bacteria was determined by measuring the clear zone diameters. The results showed varying levels of antibacterial activity among the film materials, indicating the potential for improved efficacy through the addition of specific components.

Table 3 and Figure 6 provide an assessment of the antibacterial effectiveness of the studied nanoreinforced CS/PVOH based packaging films.

Table 3. Antimicrobial activity of active films against food pathogenic bacteria *E. coli*, *S. aureus*, *S. enterica* and *L. monocytogenes*.

Film material	*E. coli* Inhibition[1] (diameter of clear zone)	*S. aureus* Inhibition[1] (diameter of clear zone)	*S. enterica* Inhibition[1] (diameter of clear zone)	*L. monocytogenes* Inhibition[1] (diameter of clear zone)
CS	3.07 ± 0.22[a]	3.56 ± 0.43[a]	3.40 ± 0.32[a]	2.03 ± 0.26[a]
CS/PVOH	3.63 ± 0.35[ab]	3.72 ± 0.25[ab]	3.49 ± 0.11[ab]	3.78 ± 0.52[ab]
CS/PVOH/5AC	0 ± 0[c]	0 ± 0[c]	0 ± 0[c]	0 ± 0[ac]
CS/PVOH/10AC	0 ± 0[c]	0 ± 0[c]	0 ± 0[c]	0 ± 0[ac]
CS/PVOH/15AC	0 ± 0[c]	0 ± 0[c]	0 ± 0[c]	0 ± 0[ac]
CS/PVOH/5TO@AC	3.08 ± 0.26[abd]	5.43 ± 0.49[abd]	4.13 ± 0.68[abd]	2.66 ± 0.36[a]
CS/PVOH/10TO@AC	3.10 ± 0.29[abd]	3.63 ± 0.46[abd]	3.00 ± 0.16[abd]	3.44 ± 0.30[ad]
CS/PVOH/15TO@AC	3.84 ± 0.60[abd]	4.53 ± 0.36[abd]	6.07 ± 0.18[e]	3.86 ± 0.91[ad]

[1]Inhibitory zone surrounding wells measured in mm after the subtraction of the well diameter (6mm); Results expressed as mean ± standard deviation (n=3); Means in the same column baring different superscript letters are significantly different (p<0.5).
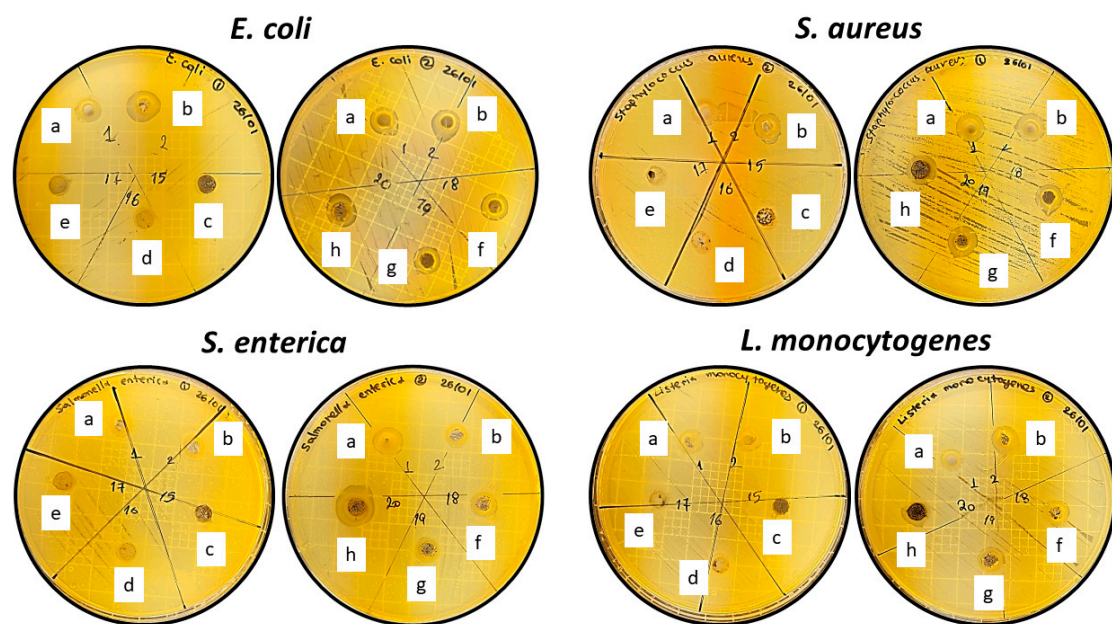

Figure 6. Petri dishes images of (a) CS, (b) CS/PVOH, (c) CS/PVOH 5%AC, (d) CS/PVOH 10%AC, (e) CS/PVOH 15%AC, (f) CS/PVOH/5TO@AC, (g) CS/PVOH/10TO@AC, (h) CS/PVOH/15TO@AC films against *E. coli*, *S. aureus*, *S. enterica*, and *L. monocytogenes*.

The results indicate that the film material composed of CS) exhibits some antimicrobial activity against the tested bacteria, with varying levels of effectiveness. Notably, it demonstrates moderate inhibition against *S. aureus* and *S. enterica*, while displaying lower activity against *L. monocytogenes*. However, its efficacy against *E. coli* is relatively limited. CS, is known for its antibacterial effects against a wide range of bacteria. This property is attributed to its cationic characteristics, specifically the positively charged ammonium (NH_4^+) groups that interact with the negatively charged elements of bacterial cell walls. Furthermore, the importance of chitosan's cationic nature in contributing to its antibacterial activity has been emphasized by Giannakas et al. (2022) and Unuabonah et al. (2018) [15,35].

The CS/PVOH film material displayed diverse antibacterial activity depending on the bacterial strain, with the greatest inhibitory activity against *L. monocytogenes*. Moreover, CS/PVOH films showed comparable inhibitory activity to CS against *S. aureus* and *S. enterica.* This suggests that the addition of PVOH to CS enhances the film's ability to inhibit bacterial growth.

On the other hand, the film materials containing AC in various concentrations (CS/PVOH/5AC, CS/PVOH/10AC, and CS/PVOH/15AC) show no antimicrobial activity against any of the tested bacteria. This indicates that the inclusion of activated carbon in the film formulation does not contribute to its antimicrobial properties.

However, when TO complexed with AC (TO@AC) is incorporated into the CS/PVOH film material, the antimicrobial activity is notably improved.

The clear zone diameters ranged from 2.66 to 6.07, with the highest inhibition observed against *S. enterica*. Specifically, **t**he presence of 5%TO@AC enhances the film's effectiveness against *S. aureus* and *S. enterica*. Increasing the concentration of TO@AC to 10% and 15% in CS/PVOH films displayed varying degrees of inhibitory activity against the bacteria, with CS/PVOH/15%TO@AC demonstrating the highest inhibitory activity against *S. enterica*, followed by *S. aureus*. The results clearly demonstrate an increased inhibitory effect on bacterial growth with higher concentrations of TO@AC nanohybrids. The results of the statistical analysis revealed significant differences in the antibacterial activity of the film materials against the tested bacteria ($p < 0.05$) and Tukey HSD post-hoc comparisons were conducted to identify the specific differences.

For the tested bacteria, pairwise comparisons showed that all CS/PVOH films containing TO@AC in different concentrations, exhibited significantly higher inhibitory activity compared to CS/PVOH films incorporated only with AC ($p < 0.05$). In the case of *S. enterica*, the CS/PVOH/15% TO@AC film displayed the highest inhibitory activity, with significant differences observed when compared to all the tested materials ($p < 0.05$). These statistical results confirm that the addition of thyme oil complexed with activated carbon (TO@AC) significantly enhances the antibacterial activity of the CS/PVOH films, particularly against *S. aureus* and *S. enterica*.

AC, also known as activated charcoal, is a highly porous form of carbon that has various applications due to its adsorption properties. It is extensively utilized in the field of air pollution control and wastewater treatment for the removal of various pollutants. This is primarily attributed to its remarkable characteristics, such as large surface area and high adsorption capacity.

While AC is commonly used for its ability to remove impurities, toxins and odors, its antimicrobial activity against bacteria is limited. A notable concern associated with AC is the formation of biofilm on carbon particles by bacteria, leading to increased resistance to disinfection processes and the potential for the carbon itself to act as a source of bacterial contamination [36,37].

AC primarily works by adsorbing (binding) substances to its porous surface. As mentioned, before it can effectively remove organic compounds, toxins, and certain gases through this adsorption process. However, its ability to kill or inhibit the growth of bacteria is not significant. The porous structure of AC provides a large surface area for bacteria to attach to, but it does not possess inherent antimicrobial properties. The adsorption process can physically trap bacteria within the carbon's pores, preventing them from spreading. This property is utilized in certain applications, such as water and air filters, to remove bacteria and improve cleanliness. However, it's important to note that activated carbon alone does not actively kill bacteria [38].

Based on the above, AC seems that may have the ability to limit the migration properties of an antimicrobial agent through adsorption. When an antimicrobial agent is incorporated or impregnated into activated carbon, the activated carbon's porous structure physically traps and binds the antimicrobial agent, preventing or limiting its release into the surrounding environment. In the present work, the antimicrobial activity of chitosan, is completely lost when it is incorporated into activated carbon. This suggests that the specific characteristics of chitosan and its interaction with activated carbon may lead to the loss of its antimicrobial properties.

There could be several reasons why chitosan loses its antimicrobial activity when incorporated into activated carbon. One possibility is that the adsorption process of chitosan onto activated carbon might alter its chemical structure or inhibit its interaction with microorganisms, thereby diminishing its antimicrobial effectiveness. Another reason could be related to the pore size or surface chemistry of the activated carbon. If the pore size of the activated carbon is not suitable for retaining chitosan or if the surface chemistry of the activated carbon hinders the interaction between chitosan and microorganisms, it could lead to a loss of antimicrobial activity.

However, our results demonstrated an increase in antimicrobial activity when thyme oil was incorporated into the CS/AC matrix. The presence of thyme oil led to a notable enhancement in the inhibitory effect against *S. aureus* and *S. enterica*. The antimicrobial efficacy observed suggests a synergistic effect between the constituents of the composite matrix, particularly chitosan, activated carbon, and thyme oil.

It's worth noting that the effectiveness of activated carbon in limiting the migration properties of an antimicrobial agent can vary depending on the specific characteristics of the antimicrobial agent and the properties of the activated carbon. In the case of chitosan, further investigation and experimentation would be required to understand why its antimicrobial activity is completely lost when incorporated into activated carbon.

In this study, CS served as the base material for the film, and its inherent antibacterial activity likely played a crucial role in controlling microbial growth, which was further amplified by the addition of thymol-modified activated carbon (TO@AC). The incorporation of specific components, such as thyme oil, can enhance the antibacterial efficacy of CS/PVOH films. However, the addition of activated carbon did not exhibit any inhibitory effects on the tested bacteria. These findings emphasize the need to optimize formulations and concentrations to maximize the antibacterial activity of film materials for various applications, including food packaging and biomedical devices. Azmi et al. (2022) provide insights into the various factors influencing the antibacterial properties of clay, which may be a significant avenue for future exploration if considering clay-based nanohybrids for antimicrobial applications [39].

3.7. Visual Evaluation of the obtained Active Coatings against enzymatic browning of fresh bananas

In the Figure 6 representative photos during the experimental period of 8 days of the uncoated bananas and bananas coated with CS/PVOH, CS/PVOH15AC and CS/PVOH/15TO@AC coatings are shown. It must be mentioned that the CS/PVOH15AC and CS/PVOH/15TO@AC samples are chosen to apply as bananas coatings as it was shown to have the highest water/oxygen barrier properties and the highest antioxidant activity. With a first glance it is obvious that all the CS/PVOH, CS/PVOH15AC and CS/PVOH/15TO@AC coatings succeed to protect bananas form the enzymatic browning in comparison to uncoated bananas [24]. This result is probably due to the CS/PVOH and

CS/PVOH/15AC hydrogel coatings, which probably succeeded in protecting the fresh bananas from enzymatic browning growth. With a more careful glance it is obtained that CS/PVOH/15AC and CS/PVOH/15TO@AC coatings are succeeded to protect more effectively the bananas form enzymatic browning. The extension of life duration was confirmed only by visible observations, and it was around 2 to 3 days longer for bananas coated with CS/PVOH/15AC and CS/PVOH/15TO@AC coatings as compared to the respective uncoated bananas and bananas coated with pure CS/PVOH coating.

In Figure 7 they are plotted the average values of % weigh loss of uncoated and uncoated bananas. It is obvious that the group of bananas coated with CS/PVOH/15TO@AC coating exhibited the lower % weight loss during the experimental period of 8 days. The group of bananas coated with

CS/PVOH/15AC coating exhibited higher % weight loss values than bananas coated with CS/PVOH/15TO@AC coating but much lower % weight loss values than bananas coated with CS/PVOH coating. So, it is concluded that the % weight loss values sequence following the trend:

uncoated bananas>CS/PVOH coated bananas>CS/PVOH/15AC coated bananas> CS/PVOH/15TO@AC coated bananas. The results of % weight loss of fresh bananas during the 8 days period of storage agrees with the results of the visual evaluation of active coating in the protection of fresh bananas against the enzymatic browning deterioration process.
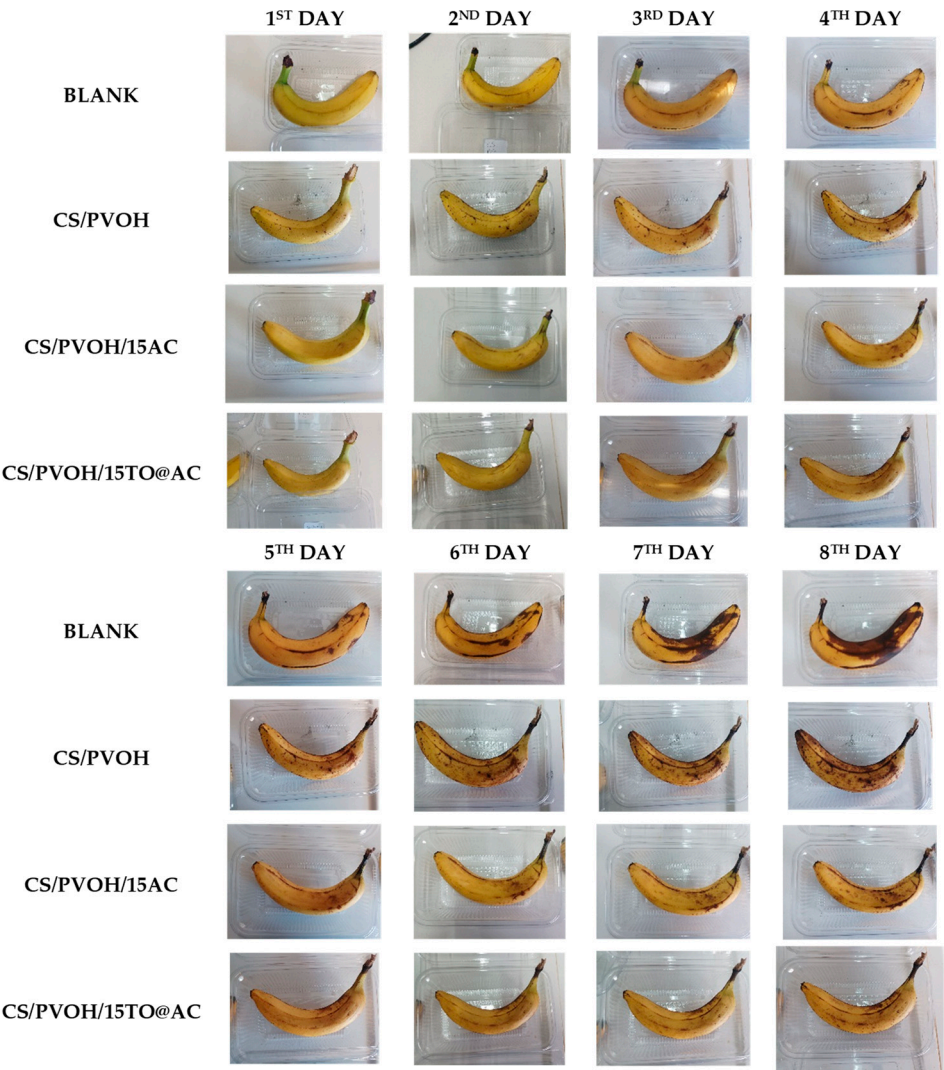

Figure 6. photos during the experimental period of 8 days of the uncoated bananas and bananas coated with CS/PVOH, CS/PVOH15AC and CS/PVOH/15TO@AC coatings.
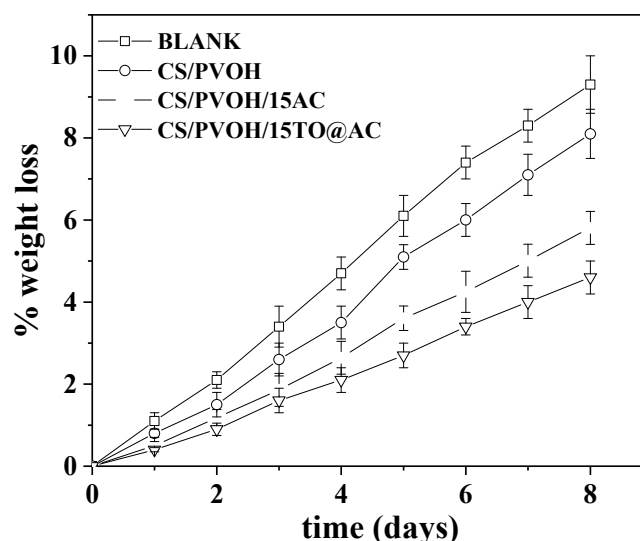
Figure 7. average % weight loss values of uncoated and bananas coated with CS/PVOH, CS/PVOH/15AC and CS/PVOH/15TO@AC coatings.

4. Conclusions

As it was shown hereabove from all the results of the analytical measurements studied the well-known CS/PVOH matrix can be transformed to a very promising active film/coating by the addition of pure AC and TO@AC nanohybrid. As it was shown both pure AC and TO@AC nanohybrid homogenously added in the CS/PVOH matrix in. content varying from 5 to 15 %wt. and resulted in CS/PVOH/xAC and CS/PVOH/xTO@AC films with higher tensile and water/oxygen barrier properties. TO@AC nanohybrid was shown to achieve higher dispersion in CS/PVOH matrix than pure AC and thus obtained CS/PVOH/xTO@AC films resulted in higher water/oxygen barrier properties than CS/PVOH/xAC films. Addition of TO@AC led to significant antioxidant activity of al obtained CS/PVOH/xTO@AC active films/coatings. In advance it was reported for first time that AC addition deactivates the antibacterial of CS/PVOH matrix while the addition of TO@AC nanohybrid resulted in enhanced antibacterial activity against four bacterial strains (E. coli, S. aureus, S. enterica, and L. monocytogenes) as compared to CS/PVOH matrix.

The overall conclusion is that the active coating CS/PVOH/15TGO@AC exhibited 23.5 % higher Young's modulus value, 20.1% higher ultimate strength value, 50.2% higher water barrier, 74.0 % higher oxygen barrier, 69.0% higher antioxidant activity, 5.5% higher antibacterial activity against *Escherichia coli*, 17.8% higher antibacterial activity against *Salmonella enterica*, 42.5% higher antibacterial activity against *Staphylococcus aureus*, 2.5% higher antibacterial activity against *Listeria monocytogenes* as compared to the pure CS/PVOH matrix. This active coating shown to slow down enzymatic browning and the weigth loss of fresh bananas and could be potentially used as active coating to extend the shelf-life of fresh fruits.

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