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Research Article

A Multilayered Edible Coating to Extend Produce Shelf Life

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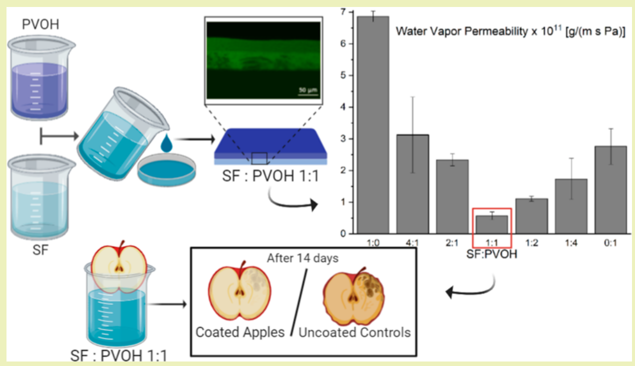
ABSTRACT: In this study, a new edible coating material with enhanced mechanical and gas barrier properties was studied by coupling silk fibroin (SF) with poly(vinyl alcohol) (PVOH). SF and PVOH water suspensions were mixed at different ratios to form multilayered membranes that, after a phase separation, assembled on the surface of fresh-cut produce upon dip coating. The effects of the mixing ratio on transparency, mechanical properties, water vapor, and oxygen permeability of the films were investigated. Higher PVOH fractions corresponded to an increased ductility (increased elongation at break and decreased Young's modulus), which is essential for a food packaging material. A coating with SF:PVOH weight ratio 1:1 presented the minimum water vapor permeability and was selected to perform perishable food preservation studies. Weight loss and color changes of coated fresh-cut apples over 14 days of storage at 4 °C were significantly lower than those of uncoated controls. The addition of ascorbic acid to the coating material was also investigated to obtain an active food coating with oxygen scavenging properties. The obtained results demonstrated the ability of SF:PVOH blends to assemble into bilayered edible coatings that extend the shelf life of fresh-cut produce.



KEYWORDS: *Silk fibroin, Poly(vinyl alcohol), Fresh-cut produce, Edible coatings, Food packaging, Food preservation*

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■ INTRODUCTION

Reduction of food waste plays a pivotal role in increasing the robustness of the global food system by positively affecting food security while mitigating environmental impact. The Food and Agriculture Organization (FAO) of the United Nations estimated that one-third of the food produced for human consumption is lost or wasted globally.[1] It is estimated that the energy used for the production, harvesting, logistics, and packaging of wasted food generates more than 3.3 billion metric tons of carbon dioxide and that 25% of the world freshwater consumption is used to produce food that is never eaten.[1−3] Food is wasted along the whole supply chain, from agricultural production down to household consumption, with medium- and high-income countries wasting significant amount of food at the consumption level, while low-income countries waste it early in the supply chain.[3]

Of increasing interest is food waste in fresh-cut produce (FCP),[4,5] which are edible portions of fruits and vegetables cut in smaller pieces after removal of inedible parts.[6] Retail and food service sales of FCP in USA in 2018 were estimated at US $40 billion and accounted for 20% of total retail sales of fruit and vegetables by value.[7] Preparation of FCP adds value to a commodity by requiring minimal processing such as cleaning, washing, sanitizing, and packaging, although it brings challenges in terms of quality retention, shelf life preservation, and food safety.[8−10] For example, the shelf life of FCP is usually shorter than that of the whole product, due to the increased metabolism of the wound tissue, which increases water loss, softening, browning, and biotic spoilage.[11−13] To decelerate spoilage, packaging with tuned oxygen and water barrier properties is largely used; however, materials used in the packaging can immoderately increase the cost and the environmental impact.[14] In this scenario, edible coatings have been widely studied as a method to preserve FCP freshness[10] and reduce both the requirements for packaging materials and nonbiodegradable plastic packaging waste.

The use of edible coatings consists in the application of a solution of any film-forming edible material directly on the food surface[15] through dipping, brushing or spraying.[16] Once dried, the solution forms a thin membrane on the food surface which reduces gas and water vapor transfer, browning, and aroma loss and prolongs the shelf life.[10,17−19] The main requirements for edible coatings and films are transparency, low water vapor and oxygen permeability, adequate mechanical properties, and flexibility[20]; moreover, they should avoid any alteration of the organoleptic properties, being tasteless and odorless.[21]

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As a biopolymer that has obtained the self-designated Generally Recognized as Safe (GRAS) status by the US Food and Drug Administration, silk fibroin (SF) represents a good candidate for the development of edible coatings and films. For example, SF was applied as edible coating to prolong the shelf life of climacteric (i.e., fruits, such as apples and bananas, which continue to ripen after harvest through ethylene production and increased cell respiration) and non-climacteric fruits.[22] SF is a structural protein extracted from *Bombyx mori* silkworm cocoons, showing outstanding mechanical properties, non-toxicity, biodegradability, edibility, transparency, and versatility.[23,24] SF's polymorphism (i.e., the property that enables the protein to be stable in dried conditions with different secondary molecular structures such as random coil and β-sheet)[25] can be controlled through water annealing process or alcohol treatment, to modulate the mechanical and gas barrier properties.[26] Alternatively, these properties can be regulated by formulating blends and mixtures of SF with other polymers. Polymer blending and the design of multilayered structures can be used to achieve synergistic effects in manufacturing of membranes to regulate transport processes by leveraging the intrinsic properties of each constituent.[27] Typically, SF is mixed in suspension with other biopolymers (e.g., trehalose,[28] chitin,[29] chitosan,[30] collagen,[31,32] gelatin,[33] tropoelastin,[34] and keratin[35]) to form miscible biopolymer blends with tailored mechanical properties and biodegradation kinetics. Alternatively, SF can be suspended with polymers (e.g., poly(vinyl alcohol), PVOH) to form immiscible blends[36−38] of tunable drug release profile. PVOH is of particular interest as a food coating material[39] given its GRAS status and low oxygen permeability ($5-7 \times 10^{-15}$ cm^3_{STP} cm cm^{-2} s^{-1} $cmHg^{-1}$), although it is permeable to water vapor (WVP = 3.1×10^{-9} g m^{-1} s^{-1} Pa^{-1}).[40,41] PVOH has also good thermal and chemical stability, and it presents excellent flexibility and tensile strength.[42−46]

Here, we investigated the phase separation and self-assembly of SF and PVOH water suspensions into edible bilayered structures from blends with different materials ratios. The barrier properties of the two materials for oxygen and water vapor were combined to enable the formation of high-performing coatings by simple water evaporation. By exploiting the previously reported immiscibility between SF and PVOH,[36−38] we fabricated a bilayered coating with several tunable features required for the edible packaging industry, such as transparency, barrier properties, and mechanical robustness. We evaluated the efficacy of the coating on FCP as a proof-of-concept of the material performance. Additionally, utilizing the beneficial effects of both SF and PVOH in the preservation of labile biomolecules,[47] we investigated the addition of ascorbic acid to SF:PVOH blends to reduce oxidation in fresh-cut apple during cold storage.

EXPERIMENTAL SECTION

Materials. SF was extracted from *B. mori* cocoons (Tajima Shoji Co., Ltd., Yokohama, Japan) through a degumming process elsewhere described,[48] which consisted in boiling 5 g of silk cocoons during 30 min in 2 L of 0.02 M sodium carbonate (Sigma-Aldrich, St. Louis, CO) solution. After being rinsed and let dry overnight, SF was then dissolved in a 9.3 M lithium bromide (anhydrous, 99%, Alfa Aesar, Thermo Fisher Scientific, Ward Hill, MA) solution for 4 h in a laboratory water bath (VWR, Radnor, PA) at 60 °C. In order to remove LiBr, the obtained SF−LiBr solution was dialyzed in a cassette (12−30 mL capacity, 3500 MWCO, Thermo Scientific, Rockford, IL) against 2 L of DI water during 48 h. The dialyzed SF solution (40 mL) was centrifuged twice at 4700 rpm for 20 min, to remove the impurities which settled at the bottom of the centrifuge tubes.

Poly(vinyl alcohol) (PVOH, average molecular weight, MW = 13 000−23 000, degree of hydrolysis 98%) was purchased from Sigma-Aldrich (St. Louis, CO). A 6% w/v PVOH solution was prepared by dissolution in DI water at 95 °C under magnetic stirring until complete dissolution (about 1 h).

Film Preparation. After preparing two 6% w/v solutions of SF and PVOH, different blends were obtained by carefully mixing the solutions at different SF:PVOH ratios (1:0, 4:1, 2:1, 1:1, 1:2, 1:4, 0:1). Films were obtained by casting the mixed aqueous solutions on a flat poly(dimethylsiloxane) (PDMS) surface. The solutions were spread on the mold using a spatula and dried overnight at room temperature. Film thickness (X) was measured using an electronic digital micrometer (Chicago Brand, Medford, OR) with an accuracy of 0.001 mm. The measurements were repeated three times on different spots of the films.

Scanning Electron Microscopy (SEM). To observe the microstructure of the films, the samples were cut into 10 mm × 10 mm pieces using a razor blade (VWR, Radnor, PA). The cross-section of all the blend films was coated with a 10 nm gold layer (EMS Q150T ES coater) and observed using a Zeiss Merlin High-resolution SEM (Zeiss, Thornwood, NY) with an acceleration voltage of 2 kV.

Fluorescence Microscopy. To detect the presence of phase separation between SF and PVOH in the films, the samples (n = 3) were cut into 10 mm × 30 mm specimens using a razor blade. An epifluorescent microscope (Eclipse TE2000-E, Nikon, Tokyo, Japan) under fluorescein isothiocyanate (FTIC) lightning configuration was used to acquire micrographs of the surface and cross-section of the films. The thickness of the two layers was quantified by analyzing the cross-section images showing clearly different fluorescent contrasts from two polymer materials.

UV−Vis Spectroscopy. The visible and ultraviolet (UV) light barrier properties of the films were measured at selected wavelengths (220−1000 nm) using a UV−vis spectrophotometer (VWR, Radnor, PA). The measurements were taken by carefully placing a strip of 2 mm × 20 mm of each films in a semimicro Vis cuvette (Eppendorf, Hamburg, Germany) which fitted the instrument sample holder; empty cuvettes were scanned as background before each measurement. The absorbance and transmittance of the films were evaluated by performing tests in triplicate.

Mechanical Tests. Tensile strength and elongation at break were determined according to the ASTM D 882-18 standard.[49] The films were cut into 5 mm × 25 mm strips and tension tests were performed using a tensile testing machine (5943 Instron, Norwood, A) equipped with a 1 kN load cell at a crosshead speed of 2 mm min^{-1}. The gauge length was set at 15 mm. The tests were performed in triplicate.

Fourier-Transform Infrared Spectroscopy (FTIR). FTIR spectrometer (Spectrum 65, 399 PerkinElmer) equipped with an universal attenuated total reflection (ATR) sampling accessory (diamond/ZnSe crystal) was used to evaluate the composition of the two surfaces of SF:PVOH films and the surface of the apple coating. SF:PVOH blend films were cut into strips of 10 mm × 30 mm. The background was first scanned, and then spectra were acquired over a range of 4000 to 500 cm^{-1} with an accumulation of 32 scans. After edible coating, apples were freeze-dried. Spectra of the outermost surfaces of samples were collected at a wavelength range of 4000 to 650 cm^{-1}, with a resolution of 4 cm^{-1} and an accumulation of 64 scans.

Thermal Analysis. For the differential scanning calorimetry (DSC) analysis, samples were heated in a Discovery DSC (TA Instruments, New Castle, DE) in nitrogen gas flow. To remove surface moisture, samples were stored under vacuum at room temperature and preheated at 105 °C for 15 min in the instrument. After the sample was cooled to 30 °C, standard mode DSC measurements were performed until 270 °C with a heating rate of 5 °C min^{-1}. Thermogravimetric analysis (TGA) was conducted for samples without storage in vacuum using a Discovery TGA (TA Instruments, New Castle, DE). Samples were heated from 30 to 800 °C with a heating rate of 10 °C min^{-1} in nitrogen.

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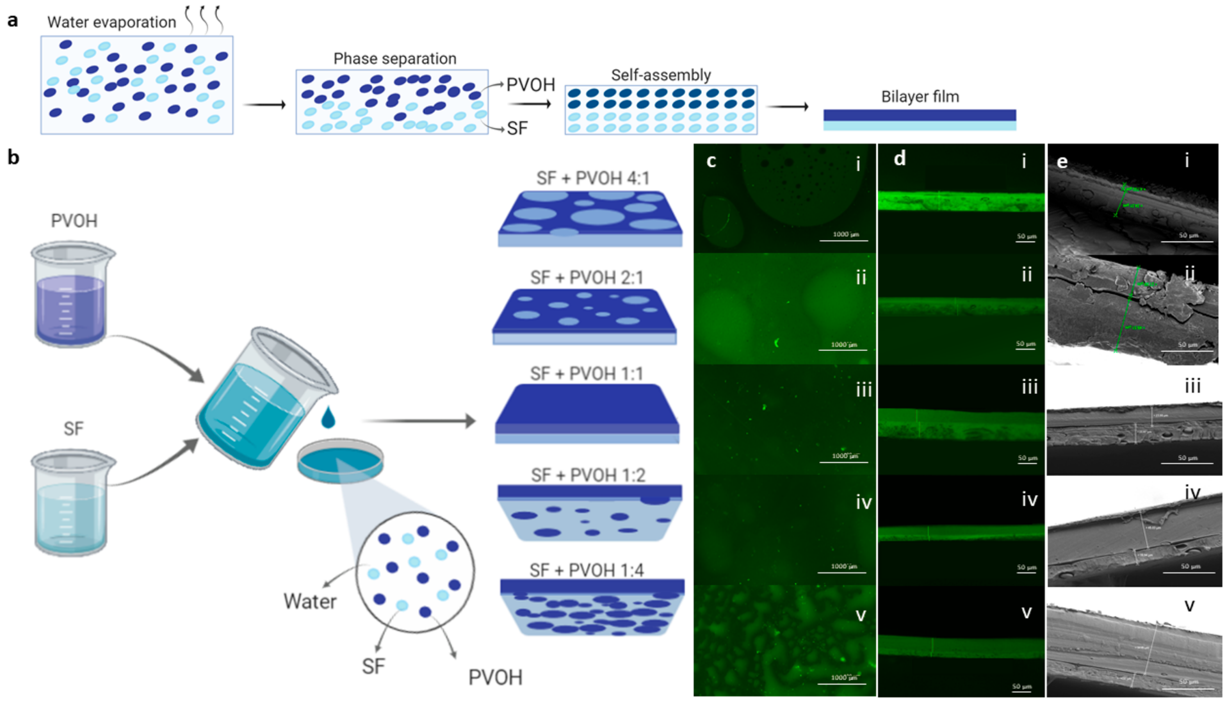

Figure 1. Preparation schematic and morphological characterization of SF and PVOH blend films. (a) Starting from a water suspension, phase separation occurs during drying and SF and PVOH self-assemble and spontaneously form a bilayered structure. (b) SF and PVOH aqueous solutions are mixed at different ratios. Blend films are obtained by solvent casting. (c) Fluorescence micrographs of the surface of SF:PVOH films with ratios (i) 4:1, (ii) 2:1, (iii) 1:1, (iv) 1:2, and (v) 1:4. SF is depicted in green given the protein autofluorescence. SF:PVOH blend films show superficial phase separation. Superficial phase separation in SF:PVOH 4:1 and 1:4 films is the most evident, while it is reduced in SF:PVOH 2:1 and 1:2 films. SF:PVOH 1:1 films show no superficial phase separation. (d) Fluorescence micrographs of the cross-section of SF:PVOH blend films with (i) 4:1, (ii) 2:1, (iii) 1:1, (iv)1:2, and (v) 1:4 ratios. SF:PVOH blend films are characterized by the presence of two layers, the top layer is constituted by PVOH, while the bottom layer is constituted by SF. Increasing PVOH content in the blend films, the thickness of the top layer increases and the bottom layer decreases. (e) SEM images of the cross-section of SF:PVOH blend films with ratios (i) 4:1, (ii) 2:1, (iii) 1:1, (iv) 1:2, and (v) 1:4.

Water Vapor Permeability. Water vapor permeability (WVP) was evaluated using the test cup methods described in ASTM E96-E96 M.[50] To perform the test, glass vials with PP hole cap (McMaster-Carr, Elmhurst, IL) were used. The films (n = 3) were held between the glass vials and the cap by using two silicon rubber O-rings. Caps were sealed to the glass vials using vacuum grease (Dow Corning, Midland, Michigan) over the circular opening and enveloped with Parafilm to minimize gas leakage. The glass vials were placed in a hermetic container equilibrated at 75% RH and 22 °C using a NaCl saturated solution. A hygrometer (HygroSet, Quality Importers Trading Company, Weston, FL) was placed in the container to verify the RH value. Each glass vial was filled with 2 g of $CaCl_2$ anhydrous (Sigma-Aldrich, St. Louis, CO) in order to keep a 0% RH inside the vial. The vials were weighed every 2 h, and the water vapor transmission rate (WVTR) was calculated by performing a linear regression analysis of weight gain of the test cup versus time.

WVP was then calculated according to the following eq 1:

$$\mathrm{WVP} = \frac{\mathrm{WVTR} \times X}{A \times S \times (\mathrm{RH}_2 - \mathrm{RH}_1)} \tag{1}$$

where X is the thickness of the films (mm), A is the permeation area (5×10^{-5} m^2), S is the saturation vapor pressure of water (2645 Pa at 22 °C), RH_1 is RH in the container (75% RH), and RH_2 is RH in the vials (0% RH).

Oxygen Permeability. The oxygen permeability of the films was measured according to the ASTM F3136-15 standard.[51] To perform the measurements, a stand-alone fiber optic oxygen meter Fibox 4 (PreSens, Germany) and an oxygen permeation cell with an integrated oxygen sensor type PSt3 (PreSens, Germany) were used. The cell has a cylindrical shape (outer diameter = 11.7 cm; inner diameter = 9 cm) and is divided into two chambers, each with two gas connectors. The upper chamber has a volume of 110 cm^3 and includes an optical window, where the optical oxygen sensor is integrated. The optical oxygen sensor signal in the optical window of the upper chamber is read by the polymer optical fiber, which is connected to the oxygen meter. The upper chamber was flushed with nitrogen, while the lower chamber was left open in order to contain air with known oxygen concentration. The film was fixed between those two chambers. Oxygen permeation from the lower chamber, through the film and into the upper chamber was evaluated with the oxygen meter. The oxygen permeability of the film can be calculated from the increase in oxygen concentration over time in the upper chamber. Oxygen concentrations were measured at 10 min intervals, and the measurements were repeated on three specimens of each type of film. The oxygen permeability calculation method is described by Abdellatief et al.[51,52] and has been reported in the Supporting Information.

Edible Coating of Apples. Based on the obtained results for optical, mechanical and barrier properties, SF:PVOH 1:1 blend film was identified as the most promising material to be tested as an edible coating. In order to obtain a coating with antioxidant properties, an antioxidant agent, ascorbic acid (AA), was added to this formulation. Increasing AA content was obtained by mixing 20 mL of SF:PVOH 1:1 and 10 mL of DI water and AA solution ([AA] = 0.01−0.1%).

Honeycrisp apples were freshly picked and purchased from a local orchard (Shelburne Farm, Stow, MA). Apples of approximately the same size and maturity were rinsed and cut into 12 equal slices using a sharp knife. Each slice was then cut into pieces with a truncated pyramid shape of similar size.

Fresh-cut apples were immediately dipped into each solution for 5 min, followed by a drying at room temperature for 4 h. Apple pieces were then stored by placing one piece in each well of 12-well plates

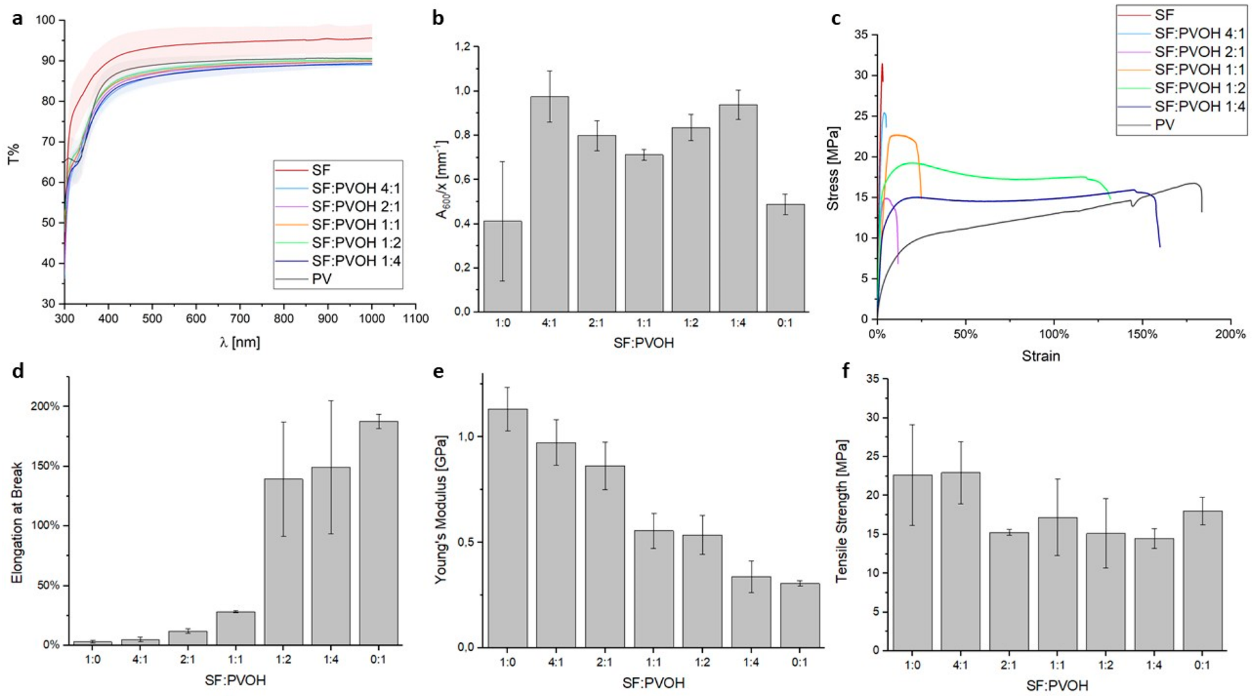

Figure 2. Optical and mechanical properties of SF:PVOH films. (a) Light transmittance of SF:PVOH blend films. All the films show high transmittance (above 85%) in the visible range [400−780 nm] (b) $A_{600\ \mathrm{nm}}/X$. SF:PVOH 1:1 presents significantly lower ($p < 0.05$) values with respect to SF:PVOH 4:1 and 1:4, no statistical difference is found with respect to SF:PVOH 2:1 and 1:2. (c) Stress−strain characteristic curves of SF:PVOH films. Pure SF films show the characteristic curve of brittle materials, while pure PVOH films show the characteristic curve of ductile materials. SF:PVOH blend films show properties intermediate between those of SF and PVOH. (d) Elongation at break of SF:PVOH blend films. PVOH content affects the elongation at break of SF:PVOH blend films, an increase in PVOH content corresponds to an increase in the elongation at break. (e) Young's modulus of SF:PVOH blend films. PVOH content affects Young's modulus; an increase in PVOH content corresponds to a statistically significant decrease in Young's modulus of blend films. (f) Tensile strength of SF:PVOH blend films.

(VWR, Radnor, PA) under refrigerated conditions (4 °C) for the following 14 days. For the estimation of coating layer thickness, apples were thin sliced using a razor blade and imaged using the fluorescence microscopy. In addition, coated apples were freeze-dried, and the outermost coated surfaces were analyzed to collect ATR-FTIR spectra as described above.

Moisture Loss and Colorimetric Measurements. Moisture loss of fresh-cut apples was determined through gravimetric analysis. The weight of the apple pieces was evaluated with a standard laboratory scale at days 1, 2, 5, 9, 12, and 14. The experiments were performed in triplicate. A colorimeter (WR10−8, FRU Instruments, Shenzhen, China) was used to measure the CIELAB color parameters, L^* (lightness), a* (redness), and b^* (yellowness) at days 1, 2, 5, 9, and 14. Each measurement was taken at three locations for each sample piece.

The browning index (BI), defined as brown color purity, was also determined using the following equation:[13,53−55]

$$\mathrm{BI} = \frac{100(x - 0.31)}{0.172} \tag{2}$$

where

$$x = \frac{a^* + 1.75L^*}{5.646L^* + a^* - 3.12b^*} \tag{3}$$

In addition, changes in color and shape of fresh-cut apples were evaluated through time-lapse images acquired at days 1, 7, and 14.

Statistical Analysis. Statistical significance of the measurements was evaluated via the one-way ANOVA test followed by pairwise comparison testing to determine significant differences at a significance level of $p < 0.05$. Bonferroni's correction was applied.

RESULTS AND DISCUSSION

Microstructure. We designed aqueous blends of SF and PVOH that, after phase separation, can assemble and spontaneously conform on complex organized geometries forming bilayered structures (Figure 1, parts a and b). The resulting material optimizes performance as an oxygen and water barrier and enables the facile fabrication of food edible coatings from a ternary system made of water and the two polymers. Tables S1 and S2 report the morphological characterization of SF:PVOH films. Fluorescence microscopy was used to investigate both the surface and the cross-section of the blend films obtained by solvent casting, as the intrinsic autofluorescence of SF allows to visualize the spatial distribution of the two polymers (Figure 1, parts c and d). SEM (Figure 1e) was used to further investigate the film microstructure. Together, the two analyses depicted the formation of films where the two polymers are clearly separated and form a binary structure in which SF settles at the bottom and PVOH is on the top (Figure 1d). This spatial distribution should be due to the different density of the two polymers (ρ_{silk} = 1.30−1.38 g cm^{-3}[56] and ρ_{PVOH} = 1.19−1.31 g cm^{-3}[57]). At blending ratios other than SF:PVOH 1:1, the presence of superficial phase separation between SF and PVOH was detected, and films showed a homogeneous layer of the dominant material and a heterogeneous layer as a mixture of the two polymers (Figure 1b). The appearance of the heterogeneous layer was most evident in SF:PVOH 4:1 and 1:4, while it was reduced in SF:PVOH 2:1 and 1:2. More interestingly, SF:PVOH 1:1 films formed a bilayered structure that consists of two distinct homogeneous layers.

Phase separation in SF:PVOH blend films was previously investigated by Tanaka et al., who found that the ternary system made of H_2O, SF, and PVOH resulted in the formation of macrophase (with SF:PVOH ratios 90:10 to 60:40) and microphase separation regions (with SF:PVOH ratios 60:40 to 10:90), depending on the relative concentration of the two polymers.[37] Consequently, Tanaka et al. related a lower phase separation to a lower SF content but did not identify a minimum superficial phase separation in the blend films SF:PVOH 1:1 as well as the presence of two clearly distinct layers, which has not been reported in the literature before. Our results suggest that, in well-mixed water suspensions of SF and PVOH, phase separation occurs during drying procedures and an organized bilayered structure spontaneously forms under our experimental conditions (i.e., materials concentration, time scales for solvent evaporation, and membrane thickness) (Figure 1a). This result is further validated by ATR-FTIR analysis, which showed that the two surfaces of the films present two clearly different spectra (Figure S1). Moreover, DSC analysis showed that glass transition temperatures of SF and PVOH films (onset points at ∼175 °C for silk and ∼70 °C for PVOH) did not change in SF:PVOH 1:1 blend film, further supporting that the two polymers are separated and form two homogeneous layers (Figure S2). Measures of the thickness of the bottom and top layers of SF:PVOH blend films, further correlates the proposed mechanism (Tables S1 and S2). A decrease in SF content corresponded to a statistically significant ($p < 0.05$) decrease in the thickness of the bottom layer, except for the case of SF:PVOH 1:2 and 1:4 films. Concurrently, an increase in PVOH content corresponded to a statistically significant ($p < 0.05$) increase in the thickness of the top layer, except for the case of SF:PVOH 2:1 and 4:1 blend films.

Optical Properties. Transmittance of visible light (400−780 nm) for SF films ranged from 83.4 ± 5.8% at 400 nm to 94.9 ± 3.5% at 780 nm (Figure 2a and Table S3), similar to values previously reported in literature for pure SF.[58,59] Transmittance of PVOH films ranged from 71.5 ± 4.8% at 400 nm to 90.4 ± 1.0% at 780 nm, similar to what was previously reported.[43] SF:PVOH blend films presented lower values of transmittance, ranging from 70.9 ± 3.3% to 89.9 ± 0.5%, than those of pure SF or PVOH films, being probably due to the presence of superficial phase separation, which may result in light-scattering that decreases the transmittance.

Several studies reported the values of absorbance at 600 nm ($A_{600\ nm}$) divided by the thickness of the specimens to obtain the absorbance value per unit of thickness related to an higher transparency when the ratio is low;[60,61] in this way, it is possible to evaluate the transparency of the films without the influence of the thickness (X) of the specimens. SF:PVOH 1:1 showed a significantly lower ($p < 0.05$) $A_{600\ nm}/X$ value compared to SF:PVOH 4:1 and 1:4 blend films (Figure 2b, Table S3). The lower value of $A_{600\ nm}/X$, and consequently the higher transparency, of SF:PVOH 1:1 compared to the other blend films may be caused by the formation of a bilayered structure which consists of two homogeneous layers. It is worth to notice that transparency of SF:PVOH blends is similar to data reported for polymers commonly used to fabricate transparent packaging (e.g., poly(ethylene terephthalate) PET)[62] and higher than the ones reported for edible coatings made with other structural biopolymers, such as whey protein isolate and pullulan.[60]

Mechanical Tests. Mechanical properties of edible coatings are strongly related to coating durability and ability to preserve food's mechanical integrity.[63] Uniaxial tensile tests on pure SF and PVOH films show a brittle and a ductile behavior, respectively (Figure 2c). The elongation at break, Young's modulus, and tensile strengths of SF:PVOH films are shown in Figure 2d−f. By increasing the PVOH content in the blend films, the elongation at break increases (Figure 2d) while Young's modulus decreases (Figure 2e). However, the mixing ratio of SF and PVOH did not have a significant effect on the tensile strength (Figure 2f). Despite the native SF fibers' outstanding mechanical properties, materials obtained from regenerated SF solutions can be brittle due to the absence of secondary and hierarchical structure which characterize native fibers.[64] The addition of PVOH has been applied by different authors in order to enhance the mechanical properties of other biopolymers.[65−67] In this study, SF:PVOH blend films with PVOH content ⩾50 wt % show a ductile behavior, which is required for edible coating materials, having higher elongation at break and lower tensile strength than those of previously reported edible coatings made with different biopolymers (e.g., methylcellulose, whey protein isolate, alginate, etc.).[68−70]

Water Vapor Permeability. Generally, a series model can be used to predict the permeability properties of multilayer films from the properties of the individual layers and the rule of mixture can be applied to predict the properties of blended materials.[71] Figure 3a reports the WVP value for SF:PVOH blend films, which was modulated by changing the SF:PVOH mixing ratio. Pure SF presents the highest WVP and WVTR values (WVP = 6.86×10^{-11} g m^{-1} s^{-1} Pa^{-1}; WVTR = 5.88×10^{2} g m^{-2} day^{-1} with X = 20 μm), while PVOH exhibits significantly ($p < 0.05$) lower WVP and WVTR (WVP = 2.76 $\times 10^{-11}$ g m^{-1} s^{-1} Pa^{-1}; WVTR = 2.37×10^{2} g m^{-2} day^{-1} with X = 20 μm). By increasing PVOH content in the films up to 50%, WVP significantly ($p < 0.05$) decreases, with SF:PVOH 1:1 films showing the minimum WVP and WVTR values (WVP = 5.7×10^{-12} g m^{-1} s^{-1} Pa^{-1}; WVTR = 4.90×10^{1} g m^{-2} day^{-1} with X = 20 μm) among the different ratios, which is 1 order of magnitude lower than the WVP of pure SF films. By further increasing the PVOH content, WVP significantly ($p < 0.05$) increases. These results suggest a decreased WVP in SF:PVOH 1:1 due to the formation of an effective water barrier at the interface between SF and PVOH and that the commonly used series model cannot be applied to predict the barrier properties of the multilayer coating. The enhanced barrier properties of SF:PVOH 1:1 blend films when compared to the other analyzed ratios may be attributed to the homogeneous bilayered structure of the 1:1 blended material, which minimizes the formation of superficial phase separation between SF and PVOH that could compromise barrier properties by favoring diffusion and permeation.[27] Interestingly, SF:PVOH 1:1 films also showed a lower WVP with respect to different edible coating materials reported in the literature, such as whey protein isolate and pullulan blends (7×10^{-11} g m^{-1} s^{-1} Pa^{-1}), whey protein isolate and chitosan blends (1.5×10^{-11} g m^{-1} s^{-1} Pa^{-1}), and pure methylcellulose (5×10^{-11}g m^{-1} s^{-1} Pa^{-1}),[60,68,72] indicating great promise for the use of this material for food coating applications.

Oxygen Permeability. As shown in Figure 3b, oxygen permeability was very similar for all the samples tested with different SF and PVOH mixing ratio and was in the range of

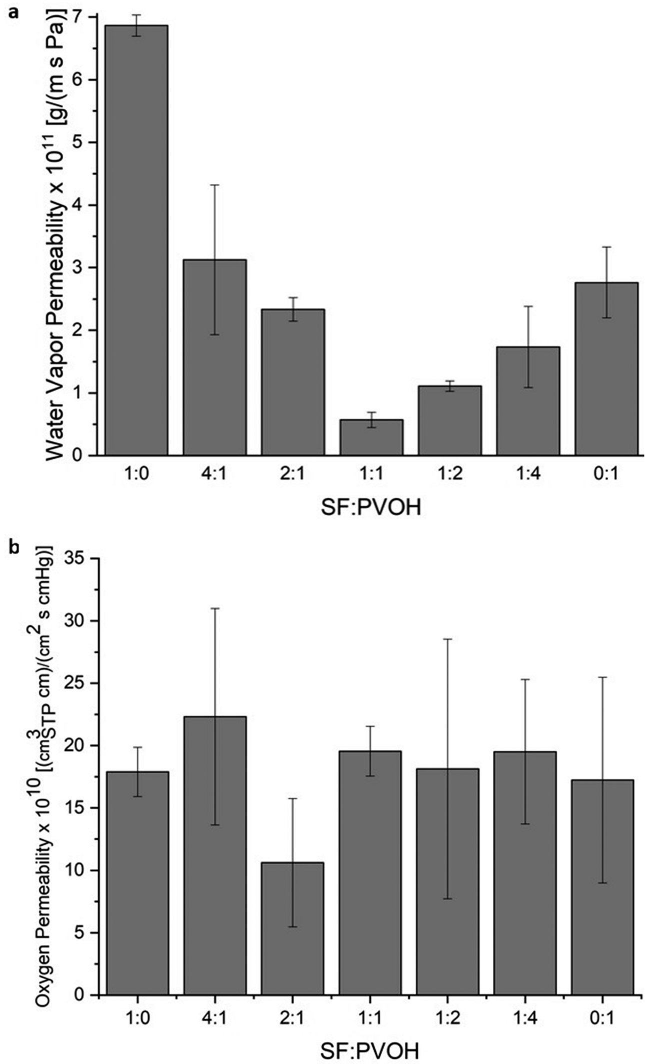

Figure 3. (a) Water vapor permeability of SF:PVOH blend films. PVOH relative content influences WVP; an increase of PVOH content up to 50% corresponds to a statistically significant decrease of WVP. A further increase of PVOH content from 66% up to 100% corresponds to a statistically significant increase of WVP. (b) Oxygen permeability of SF:PVOH blend films. PVOH addition does not have a statistically significant effect on oxygen permeability.

$10-22 \times 10^{-10}$ $cm^3{}_{STP}$ cm cm^{-2} s^{-1} $cmHg^{-1}$ (OTR in the range of $3.48-7.32 \times 10^4$ cm^3 m^{-2} day^{-1} with $X = 20$ μm). The measured oxygen permeability for SF films was very similar to previously reported values for SF materials without water annealing treatment.[22] PVOH films, however, have been reported to have a much lower oxygen permeability than the one here measured.[57] The high values here reported are probably related to the manufacturing process of PVOH films by solvent casting, which may impart micro defects in the film structure and could compromise the material oxygen barrier properties.

Application of SF:PVOH Bilayered Coating to Apples. The SF:PVOH 1:1 film showed the most improved WVP by forming a unique coating of two homogeneous layers. We then applied this blend on fresh-cut apples and compared it with coatings of pure SF and PVOH suspensions. To estimate the thickness of the coating layer, the coated apples were sliced and imaged under the fluorescence microscope (Figure S3). SF:PVOH 1:1 and pure SF coated apples had a shallow coating layer (200−300 μm) at the outermost surface, depicted by the stronger intrinsic fluorescence of SF. The PVOH coating layer was not visible due to the lack of intrinsic fluorescence. The images show that SF and SF:PVOH suspensions infiltrated the flesh of apples through the cut tissue and coated the fibers of the apple upon drying. Pure SF solution infiltrated deep into the flesh. In SF:PVOH 1:1, PVOH was narrowly distributed on the surface of the cut apples, while SF both formed a coating layer underneath PVOH and infiltrates the fruit tissue. SF was then able to both assemble on the fresh-cut apples surface and to coat the apple fibers deeper in the cut apple flesh, while PVOH settled on SF and formed a homogeneous outer layer. This is supported by the fact that FTIR spectra of the outermost layer of SF:PVOH coated apple show a lack of peaks corresponding to amide II and III peaks from SF (Figure S4). To gain a deeper understanding of the structure of SF:PVOH layer on the apple coating, further investigations will be required. In this study, we will focus more on the bilayered coating effect on the preservation of apple freshness as discussed in the following sections.

Color Changes. Apples were cut and dipped either into SF, SF:PVOH, PVOH, or water with the addition of 0.1%, 0.05%, 0.01% and 0% of AA, and preservation studies were conducted during 14 days of storage (Figure 4a, Figure S2). When apples are cut, the tissue cells are broken and enzymes, such as polyphenol oxidases (PPOs), are liberated and brought into contact with their substrates, causing color changes in the fruit flesh. During this process, known as enzymatic browning and catalyzed by PPOs, phenolic compounds present in the apple flesh oxidize to form slightly colored *o*-quinones, which then polymerize to form pigments.[73,74] Color changes can be measured observing a decrease in lightness (L^*) of the samples and an increase of redness (a^*) and browning index (BI). Tissue yellowness (b^*) is also evaluated. The variation of the parameter (L^*) as a function of time for apple pieces is reported in Figure 4b. It is possible to notice that all the coatings, except for PVOH-based, have a significant ($p < 0.05$) effect in maintaining lightness when compared to uncoated slices, up to 14 days after fruit processing. Both SF:PVOH 1:1 ($L^* = 66.17 \pm 2.56$) and pure SF ($L^* = 64.30 \pm 1.05$) coating performed significantly better ($p < 0.05$) than pure PVOH ($L^* = 58.96 \pm 3.13$) coating. Uncoated apple slices 14 days post cut showed $L^* = 51.59 \pm 3.20$, which is comparable to other values reported in the literature for uncoated apples 10 days post cut.[75] In general, the addition of ascorbic acid (AA) has a slightly positive effect in preventing color changes, but the variation of the AA concentration does not cause significant differences (Figure S5a). This was probably due to the low AA concentration in the formulations here reported. However, the addition of higher amounts of AA caused pH-induced gelation of SF, making it not suitable for the processing and application of coatings.

The same trend was observed when evaluating the increase of a^* as a function of time (Figure 4c). In fact, all the coatings, except for PVOH coatings, mitigated the increase of a^* significatively ($p < 0.05$) with respect to uncoated controls. After 14 days post cut, samples coated with SF:PVOH 1:1 blends have a lower value of a^* when compared to samples coated with PVOH. The addition of AA, independent from its concentration, did not cause significant effects on PVOH coating performance (Figure S5b).

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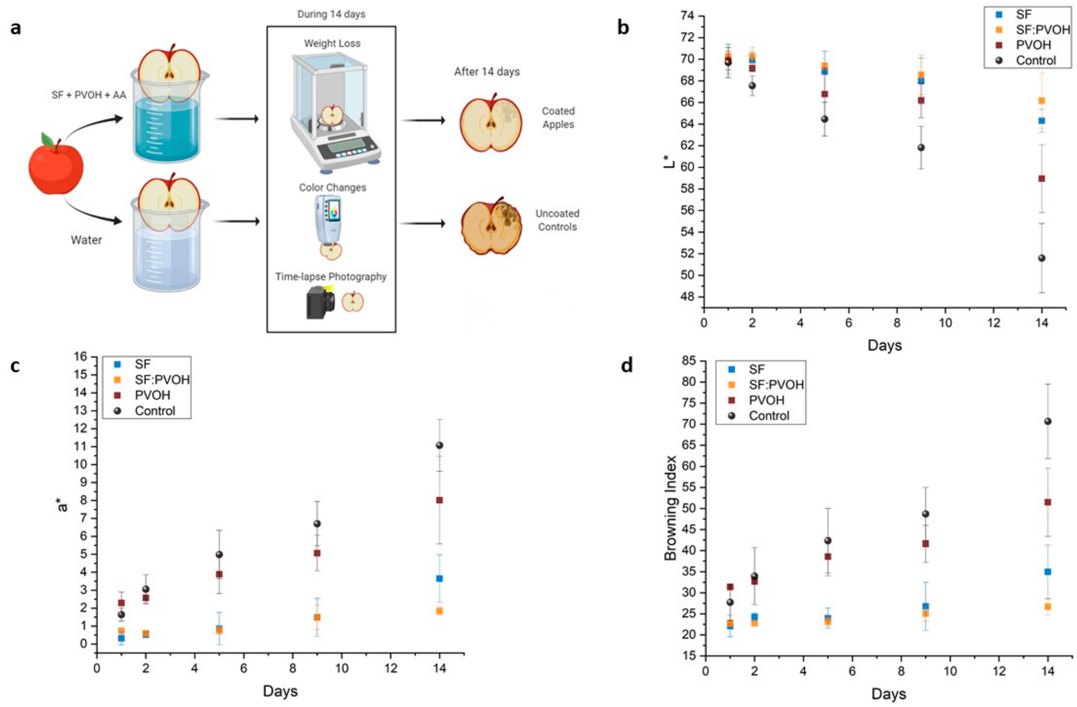

Figure 4. (a) Apple coating and preservation studies. (b) Lightness. (c) Redness. (d) Browning index of apple pieces dipped in SF, SF:PVOH, and PVOH. During 14 days of storage SF, PVOH, and SF:PVOH coatings showed a positive effect in maintaining apple pieces colors with respect to uncoated controls.

Finally, BI (Figure 4d), which represents the purity of the brown color and takes into account the three L^*, a^*, and b^* parameters, summarizes the effect of the color changes due to browning. BI of all the apple pieces at day 1 ranged between 22 and 31. Fourteen days post cut, uncoated controls presented a BI = 70.7 ± 8.8, while BI measured for apple slices coated with SF:PVOH 1:1 blend coatings was 26.7 ± 1.8. The incorporation of AA, independently from the concentration of AA solution, did not lead to significant difference in BI at day 14 (Figure S5c). Apple pieces coated with pure PVOH and pure SF showed BI = 51.49 ± 8.08 and BI = 34.96 ± 6.36, respectively. SF coating had a positive effect on browning reduction with respect to uncoated controls, while PVOH did not have a significative effect. BI is the most analyzed parameter in literature to measure color changes in fresh-cut apples. Olivas and co-workers applied an alginate-based coating after immersing apple pieces in a $CaCl_2$ aqueous solution.[53] The coated apple slices presented BI ranging between 25 and 31 at day 1 post cut, and BI ranging between 40 and 45 at day 8 post cut, while uncoated apples presented BI = 38 and BI = 50, respectively. These data suggest that apple slices coated with an alginate-based formulations at day 8 post cut have a higher BI when compared to apple slices coated with SF:PVOH 1:1 at day 14. Application of an aloe vera gel on sliced apples also show decreased antibrowning properties when compared to SF:PVOH blends[55] as BI = 32 was measured 12 days post cut. Addition of an antibrowning solution (i.e., cysteine, ascorbic acid, and citric acid) enhanced the antibrowning properties of the aloe vera coating and provided BI values similar to the ones measured for apple slices coated with SF:PVOH 1:1 in this study.

Weight Loss. Figure 5a shows the time dependent weight loss of fresh-cut apple slices up to 14 days post cut. All the considered coatings (i.e., SF, SF:PVOH, and PVOH) had a significant ($p < 0.05$) effect on the time-dependent weight loss of apple slices compared to uncoated controls, which lost ca. 21% of their original weight at day 14 of cold storage. SF:PVOH 1:1 blend coating appeared to be the most effective coating in reducing weight loss as apple slices coated with SF:PVOH 1:1 lost 8.5% of their weight at day 14 post cut. Apple slices coated with PVOH lost ca. 13% of their original weight after 14 days. Apple slices coated with pure SF lost more than 15% of their weight at day 14 post cut.

Effects of edible coatings on the weight loss of fresh-cut apples was previously studied using a whey protein isolate and beeswax emulsion coating.[13] However, this strategy did not significantly reduce moisture loss over time. Similarly, moisture loss of apple slices coated with apple puree alone or in combination with citric acid and ascorbic acid did not significantly reduce moisture loss.[16] In contrast, our results show that SF:PVOH 1:1 edible coatings are an effective strategy to reduce fresh-cut apples weight loss.

Figure 5b shows the time-lapse images of apple pieces coated with SF, SF:PVOH 1:1, and PVOH, compared to uncoated controls. At day 7, apples coated with SF, SF:PVOH 1:1, and PVOH show a better appearance than uncoated control, which means that the coatings reduced enzymatic browning. The difference is even more remarkable at day 14. Among SF, SF:PVOH 1:1 and PVOH coatings, SF:PVOH 1:1 is the most effective in preserving apple pieces color and appearance at day 14.

■ CONCLUSION

This study demonstrated that SF:PVOH blend films are suitable materials as edible coatings to prolong the shelf life of FCP. SF and PVOH, after being mixed in solution, separate and form a bilayered structure during the evaporation of water, in which SF settles at the bottom and PVOH is present at the

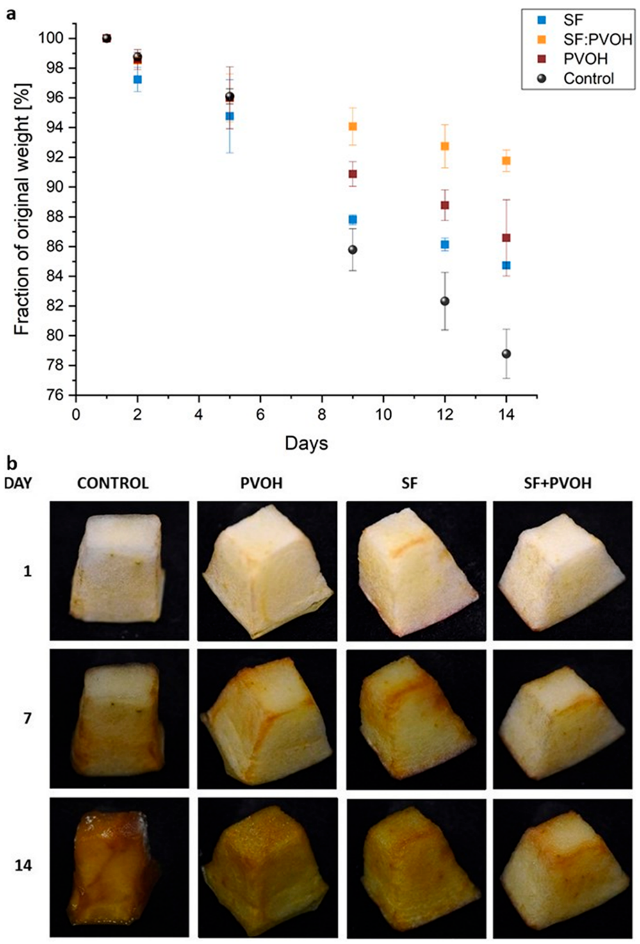

Figure 5. (a) Weight loss of apple pieces dipped in SF, SF:PVOH, PVOH, or water. During 14 days of cold storage SF, PVOH, and SF:PVOH coatings showed a positive effect on weight loss with respect to uncoated controls. (b) Time-lapse photographs of apple pieces coated with SF, SF:PVOH, PVOH and uncoated apple pieces. Apple pieces coated with SF:PVOH show reduced browning with respect to apple pieces coated with just SF and PVOH and uncoated controls.

top. Surface phase separation was visible for SF:PVOH mixing ratios other than 1:1, where SF and PVOH self-organized in a homogeneous, bilayered membrane. By changing the mixing ratio between SF and PVOH, it is possible to modulate the relative thickness of the two layers. Furthermore. SF:PVOH films show high transparency (transmittance >85%) and enhanced water barrier properties. SF:PVOH 1:1 self-assembled multilayer structures presented the highest barrier properties to water vapor, with a water vapor permeation of an order of magnitude lower than SF and PVOH alone. The addition of PVOH to SF also increased the ductility of the coating, as an increase in the relative amount of PVOH corresponds to an increase in the elongation at break and a decrease in Young's modulus. The positive effect of the combination of SF with PVOH makes it possible to enhance SF properties without the need for long and impractical water annealing process or alcohol treatment.

Based on the mechanical, transparency and water vapor permeability results, SF:PVOH 1:1 films resulted to be a valid candidate for edible food coating as demonstrated by the efficacy on fresh-cut apples. After 14 days post cut, apples slices coated with SF:PVOH 1:1 presented significantly lower weight loss with respect to uncoated controls and apples coated with pure SF. SF:PVOH 1:1 was also able to mitigate color changes in the apple slices as apple slices coated with SF:PVOH 1:1 presented a lower browning index with respect to uncoated controls and to apples coated with pure SF and PVOH after 14 days of storage. The higher efficacy of SF:PVOH 1:1 coating was probably due to the remarkable ability of the coating to form a bilayered structure, in which SF infiltrates in the apple's flesh and coats the apple fibers, while PVOH forms an outer protective layer.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.0c03365.

Oxygen permeability calculation method; FTIR-ATR spectra of SF:PVOH blend films (Figure S1); layer thickness of SF:PVOH blend films (Tables S1 and S2); absorbance and transmittance of SF:PVOH blend films (Table S3); DSC analysis of SF:PVOH blend films (Figure S2); fluorescence microscope images of apple coatings (Figure S3); FTIR spectra of apple coatings (Figure S4); and lightness, redness, and browning index of apple pieces coated with the addition of AA (Figure S5) (PDF)

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Author Contributions

#E.R. and D.K. contributed equally.

Notes

The authors declare the following competing financial interest(s): Benedetto Marelli is co-founder of a company called Mori, Inc. (formerly Cambridge Crops, Inc.) that uses silk-based technologies for food coating applications.

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