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Development and characteristics of biodegradable aloe-gel/egg white films

Marco Antonio López-Mata,[1] Gerardo García-González,[1] Edith Valbuena-Gregorio,[1] Saúl Ruiz-Cruz,[2] Paul Baruk Zamudio-Flores,[3] Silvia Elena Burruel-Ibarra,[4] Gloria Guadalupe Morales-Figueroa,[5] Luis Quihui-Cota[5]

[1]Departamento de Ciencias de La Salud, Universidad de Sonora, Campus Cajeme. Blvd. Bordo Nuevo S/N, Antiguo Providencia, Apdo. Postal 85040. Tel 01(644) 410 52 70. Cd. Obregón Sonora, México

[2]Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora, 5 De Febrero 818 Sur, Apdo. Postal 85000, Cd. Obregón, Sonora, México

[3]Centro de Investigación en Alimentación y Desarrollo A.C, Av. Río Conchos S/N Parque Industrial, Apdo. Postal 31570, Cuauhtémoc, Chihuahua, México

[4]Departamento de Investigación en Polímeros y Materiales, Universidad de Sonora, Unidad Centro, Apdo. Postal 83000, Hermosillo, Sonora, México

[5]Departamento de Nutrición Pública y Salud, Coordinación de Nutrición, Centro de Investigación en Alimentación y Desarrollo, A.C. Carretera al Ejido La Victoria Km 0.6, Apdo. Postal, 83304, Hermosillo, Sonora, México

Correspondence to: M. A. López-Mata (E-mail: marco.lopez@cajeme.uson.mx)

ABSTRACT: The use of synthetic nonbiodegradable polymers has led to environmental damage. This has encouraged the interest to the development of new renewable and biodegradable matrices. The potential of egg white (EW) protein for the development of bioplastic materials has been published. However, the mixture of EW with *Aloe*-gel (AG) for film formation has not been documented. In this study, films with different EW and AG combinations are manufactured and their properties are analyzed. In general, the AG/EW films are homogeneous, smooth, with no pores and with cumulus of protein on the surface with better extensibility, plasticity, and low tensile strength. In addition, they are yellow colored, UV-light blocker, with high solubility (2.2 times) and high Water Vapor Permeability (4.17 times) compared with the control (EW film). The AG/EW films showed higher percentage of soluble protein and antibacterial activity than the control. © 2016 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 44067.

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INTRODUCTION

The increased use of synthetic nonbiodegradable polymers in various applications like packaging, coating, and films has led to serious environmental damage.[1] This has encouraged the interest of the development of new matrices (renewable and degradable) based on biopolymers. For the development of coating and films, compounds such as polysaccharides, lipids, proteins, or combinations have been used.[2] The proteins of plants (soy, wheat, gluten, corn, and cottonseed) and animals (gelatin, milk, casein, and collagen) are available source for the elaboration of bioplastics. Previous studies had already shown the potential of egg white (EW) protein (ovomucin fibers and numerous globular protein) for the development of bioplastic materials.[3,4] Although the mechanism of film formation remains hypothetical, the inter and intramolecular disulfide bonds and free sulfhydryl groups plus the secondary structure α and β (in low frequency) and ovalbumin (coil polypeptide), have been considered as primary factors that provide the film's forming-ability.[5]

Studies on films based on EW have shown excellent mechanical and structural properties, such as high transparency and nutritionally functional, but they exhibit high sensitivity to moisture and high water vapor permeability (WVP).[6] Other films based on proteins are usually brittle and susceptible to cracking because of the strong cohesive energy density of their polymers, but the addition of plasticizer with sorbitol improves their extensibility and viscoelasticity. However, when glycerol is added, a lower plasticizing effect was observed accompanied with lower elasticity properties.[7]

On the other hand, *Aloe vera* is a tropical plant of the family *Liliaceae*, recognized for centuries as a healing of different diseases. *Aloe vera* gel [*Aloe*-gel (AG)] is the portion of the parenchyma tissue of mucilaginous-gel appearance that can be obtained by filleting of its leaves.[8] This gel is composed of 99% water and the remaining compounds are glucomannans, amino acids, lipids, sterols, and vitamins. Because of the epithelizing action, AG has been used to heal the damaged skin tissue (small wounds and burns), anti-inflammatory, antimicrobial capacity, and other human health benefits.[9,10] In addition, the use of AG has increased dramatically in the cosmetic, pharmaceutic, and food industries.[11]

The low solid content of AG difficult to use as material for films but combined with other hydrophilic biomaterials can increase its mechanical and functional properties. For instance, the AG combined with biopolymers of polysaccharides as chitosan[8,12] and alginate[13] can form membranes as dressing materials or edible films with excellent advantages. Few studies have examined the combination of AG with animal protein for films manufacturing. Cheng-Pei *et al.*[14] produced edible films of *Aloe*/gelatin with excellent antimicrobial properties (Gram positive and negative bacteria).

Although the filmogenic ability of EW is well recognized, the mixture of EW with AG for film formation has not been previously reported or published. Therefore, it would be interesting to investigate the intrinsic properties of a new AG/EW film, which might give it characteristics to be used for practical applications. Thereby, the aim of the present work was to evaluate the physicochemical, antibacterial, mechanical, and optical properties of the AG/EW films. Our results showed that the biodegradable AG/EW films exhibited homogeneous surfaces, smooth, nonporous,and insoluble protein material. In addition, the higher solubility and WVP (WVP), the better extensibility-plasticity of the films. All films were yellow colored with efficient capacity for UV-light-blocking. In addition, the films showed high content of soluble protein and antibacterial activity against *E. coli* O157:H7 and *S. aureus*.

EXPERIMENTAL

Materials

The AG was extracted of fresh whole leaves of Aloe (*Aloe vera* L.), obtained from a botanic garden of northwest México. The leaves were selected randomly and obtained from a 3 year-old plant with 30–35 cm high and with no pathological event or apparent damage. EW was obtained of *Gallus gallus domesticus*, acquired in a local commercial market (northwest México). Only high analytical grade reagents were used in this study.

Extraction of AG and EW

Previously washed leaves of *Aloe vera* using distilled water were filleting to remove the skin and the parenchyma. Then, parenchyma was ground in a conventional blender (Oster®, México) to obtain a malleable gel which was filtered using a nylon-mesh to remove the remaining plant debris. Once filtered, the malleable gel was centrifuged at 3000*g* during 10 min at 25 °C to remove the air bubbles and remaining leaves. The AG obtained was stored at −20 °C until analysis. The egg used was Bachoco®, medium (52–61*g*) traditional white type and obtained from a local market in Cd. Obregón, (Sonora, México). The eggs were selected discarding mechanical or pathological injuries and then washed. After the EW was carefully drained through a hole at the top of the eggshell (≈1 cm) to avoid yolk contamination. The EW obtained was stored at −20 °C until analysis.

Preparation of the Films

The AG-EW film forming solutions were prepared using the casting method[15] making AG-EW combinations as follows: AG/EW: 50:50, 40:60, 30:70, 0/100 (control) [w/w], with glycerol (3.7% w/w). All solutions were shaken at 80 RPM for 5 min and then were filtered using a nylon-mesh. The filtered solutions were decanted (10 mL) in a silicone mold (6 cm of diameter) and subjected to drying process using a vacuum oven (Binder-FD53) at different temperatures and times cycles. The used temperatures and times were initially 30 °C for 22 h; then 80 °C for 1 h; and finally 40 °C by 20 h. The resulting films were preserved under NaBr (52% relative humidity) at 25 °C in a desiccator until analysis. Dried films were used until testing procedures.

Film Thickness

The film's thickness was measured using a manual micrometer (Mitutoyo, Japan) with precision of 0.001 in. Five measurements for each film were randomly taken and the thickness mean was calculated and used to estimate the values of WVP and transparency.

Scanning Electron Microscopy

The microstructure of the films was examined with a scanning electron microscope (JEOL JSM-5410LV) (Tokyo, Japan) equipped with an INCA system and an Energy Dispersive X-Ray (EDS) microanalysis detector (Oxford Instruments, Buckinghamshire, UK) and operated at 20 kV. The films were cut into a proper size and placed on a cylindrical copper support (1 cm in diameter), during the Scanning Electron Microscopy (SEM) analysis. Gold was used to cover the samples to allow electric conduction and to prevent the accumulation of charge under electron bombardment. The analysis of the sample was performed using a secondary electron detector.

Solubility

The film solubility was performed by cutting the films into squares (20 × 20 mm) and placed in a vacuum oven (70 °C, 35 kPa for 24 h) to dry and to determine their weight. Next, the films were put and immersed in Petri dishes with 10 mL of tri-distilled water kept at 25 °C for 24 h with occasional agitation. Then, the films were dried again following the same procedure until they reached a constant weight to determine the weight of undissolved matter.[16] The solubility was calculated based on the following equation:

$$\text{Solubility } (\%) = \left(\frac{MI - MF}{MI}\right) \times 100 \quad (1)$$

where *MI* represents the initial mass and *MF* the final mass.

Water Vapor Permeability

The WVP was determined gravimetrically based on the ASTM E96-92 method.[17] The films were sealed in the upper part of a

50 mL glass cup with 10 mL of distilled water (100% RH; 3.168 kPa of WVP at 25 °C). Subsequently, they were placed in a glass desiccator with anhydrous calcium sulfate at 25 °C and 0% RH (0 kPa of WVP). The cups were weighed at 3-h intervals for 12 h. Ideal status and uniformity of the WVP conditions were assumed with constant air circulation outside of the test cup using a miniature fan inside the desiccator. The lost weight of the cup was plotted in relation to time to estimate the slope by a linear equation (correlation coefficient was 0.99). Using the estimated slope, the water steam transmission rate (*WVTR*) was calculated by the following equation:

$$WVTR = \frac{\text{Slope}}{\text{Film area}} \quad (2)$$

The *WVP* of the films was calculated multiplying the *WVTR* and the average thickness of the film (*G*) and divided by the difference in partial pressures inside (*PA*1) and outside (*PA*2) of the cup, as follows:

$$WVP = \frac{WVTR}{(PA1 - PA2)} G \quad (3)$$

Optical Properties

The color was measured using a spectrophotometer (X-rite SP-64) calibrated with a white standard plate. Three films of different concentration were chosen randomly, and the average of 5-color-measurements was taken. The film color was expressed based on the CIE model (L^* a^* and b^*). The transparency was determined according to the established method by Han and Floros,[18] by cutting the films into rectangles (9 × 45 mm) which were placed inside of the spectrophotometer cell (Cintra 10E, Australia). The transmittance was read at 600 nm and the film transparency was calculated by the following equation:

$$\text{Transparency} = \log\left(\frac{T600}{G}\right) \quad (4)$$

where *T*600 is the transmittance at 600 nm, and *G* represents the film thickness (mm).

The UV-vis light (200–800 nm) transmission properties through the films were also determined.

Mechanical Properties of Films

The standard method ASTM-D882-95 (41) was used to measure the tensile strength (TS), % elongation (% E) and elasticity modulus (EM). Films were cut into rectangles (6 × 1 cm) and conditioned at 52% relative humidity (RH) for 24 h before testing using NaBr. After, the films were mounted and clamped with grip of Texturometer TAXT-Plus (Stable Micro Systems, Surrey, UK). The results were analyzed with the Exponent Lite Software (Version 4.0). The conditions of the test were: 30 kg load, an initial gauge 4 cm length and stretched using a cross-head speed of 20 mm min^{-1}. TS was expressed in MPa and calculated dividing the maximum load by the initial cross-sectional area of the film. The %*E* was calculated as the ratio of increased length to initial length ratio of the film and expressed as percentage. EM was evaluated using the slope of stress-strain lineal behavior. All tests were repeated five times per type of film.

Soluble Protein Content (%)

The films (100 mg) were weighed and placed in 10 mL of water for 20 h and homogenized at 12,000 RPM in an Ika Ultra-turrax T 18 basic (Germany). After centrifugation (1000*g* for 5 min), the precipitate was discarded (insoluble portion of the film) and the supernatant (soluble portion) was collected for protein quantification using the Bradford method,[19] as follows: 25 μL of sample and 225 μL of Coomassie assay reagent were mixed and incubated at room temperature for 5 min. After, the absorbance was measured at 595 nm using a micro-plate iMark Bio-Rad (Japan). The analysis was performed by triplicate with lesser than 5% of variability. A bovine serum albumin standard curve was built ($R^2 = 0.994$) and the data were expressed as soluble protein content (%).

Antibacterial Activity

Escherichia coli O157:H7 (ATCC 43890) and *Staphylococcus aureus* (ATCC 9144) strains were used and they were kept in tryptone soy broth (TSB) with glycerol (20%) and stored at −40 °C until use. A loopful of bacteria was transferred to 10 mL of TSB and incubated at 37 °C overnight. Then, another loopful of the overnight culture was transferred to TSB. The culture was grown at 37 °C until the desired amount of colony forming unit per mL (cfu/mL) for the inhibitory analysis. An inhibition zone assay was performed adding 100 μL of inoculate containing 10^6 cfu/mL of each tested strain and streaked out over the surface of Muller-Hinton agar plates (Difco). Different films were disc shape cut (6 mm diameter), and placed on inoculated agar and incubated at 37 °C for 24-48 h.[20] The EW film, and AG and EW liquids impregnated on filter paper discs were used as controls. The results were expressed as antibacterial activity by contact area when the area of the agar directly underneath of the film disc showed no bacterial growth, as described by Altiok *et al.*[21] Analysis was performed by triplicate.

Statistical Analysis

The AG/EW proportions of the different concentrations were used as independent factors and the solubility, WVP, transparency, color, mechanical properties, and percentage of soluble protein content were considered as response variables. ANOVA was used to test for difference among means and the Duncan test ($\alpha = 0.05$) to determine whether three or more means differed significantly in the analysis of variance. All the statistical analysis was performed using the NCSS statistical package, 2000.

RESULTS AND DISCUSSION

It has been previously recommended that the material used as natural matrix structural for elaboration of films, should be available, functional, low cost, and biodegradable. The egg "hen" was selected as starting material because of its high commercial production in worldwide, easy availability and low cost (≈$0.07 USD in Mexico). In addition, EW is a complex system of ovomucin fibers in an aqueous solution of numerous globular protein and adequate as matrix of protein for film elaboration. Previous studies have pointed out that the EW protein, mainly consists of the ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), lysozyme (3.5%), and ovomucin (3.5%),

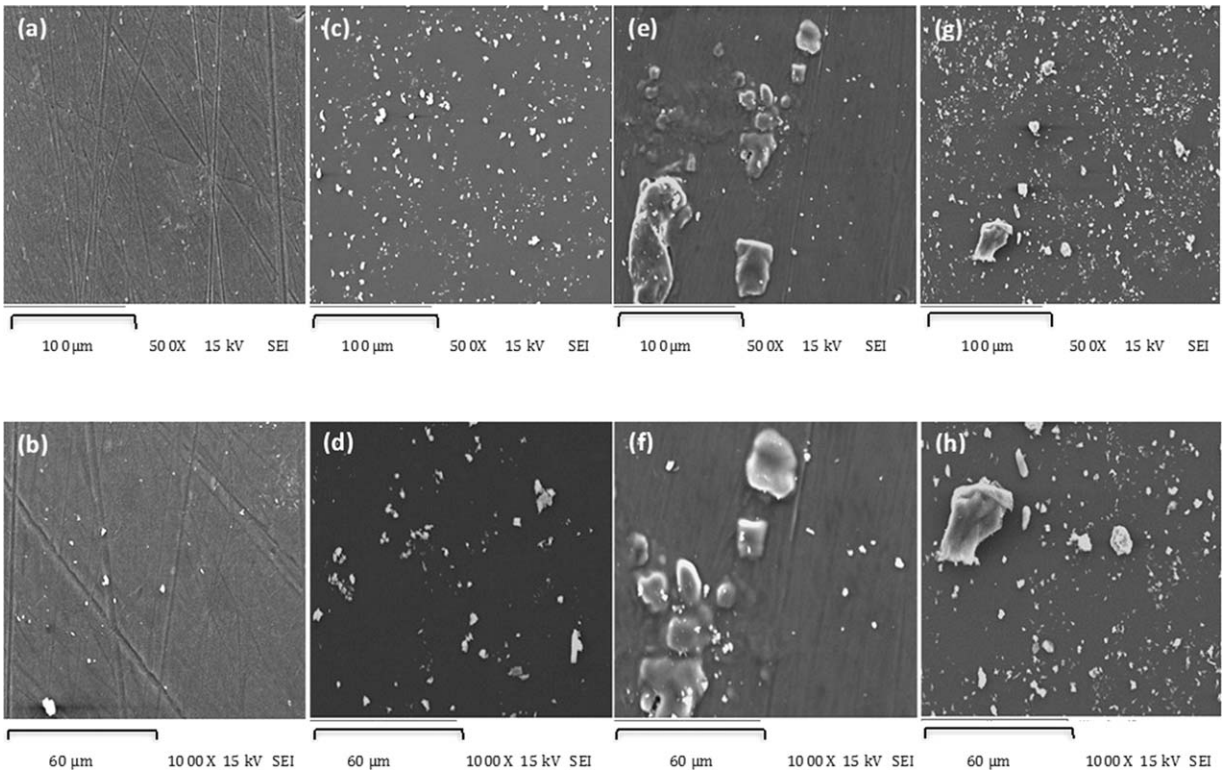

Figure 1. Surface morphology of AG/EW films observed by SEM: film columns show control (a,b), AG/EW 30/70 (c,d), 40/60 (e,f), and 50/50 (g,h).

and others minor proteins.[22] The ovalbumin and lysozyme have been extensively studied, being the former the major egg-white protein fraction (45 kDa) phosphorylated and glycosylated.[23] On the other hand, *A. vera* is an adaptable plant to different environments (hot and humid climates), making cultivation easy in diverse parts worldwide with minimum care requirements. This plant can be harvested every 6–8 weeks by removing 3–4 leaves per plant and obtaining the gel.[24]

Scanning Electron Microscopy

The morphological characterization of biomaterial features such as surface is important for strength, flexibility and the probable interactions with cells.[25] The surface morphology of our film is shown in Figure 1. The SEM showed on the control surface [Figure 1(a,b)] smooth sections with channels containing some white material. These channels have been reported as grooves and river marks in the EW with glycerol, which is attributed to the realignment of proteins EW network.[4] The channels also appeared in the 40/60 films [Figure 1(e,f)] but with major undissolved material. These channels disappeared in the 30/70 films [Figure 1(c,d)] and the 50/50 films [Figure 1(g,h)]. A homogeneous, smooth, and nonporous surface was observed but with more white material, probably, as a result of cumulus of concentrated insoluble proteins of the protein network in the EW films, as previously described by Lee *et al.*[4]

Solubility

Solubility is a measure of the film resistance to water. The control film (EW film only) showed the lowest solubility (20%), which increased when AG was added ($P < 0.05$) [26.6% (30/70), 42.4% (40/60), and 33.3% (50/50)] (Figure 2). These values were 1.33, 2.12, and 1.66 times higher than the control; however, they were lower than those reported by Handa *et al.*[26] in EW films (44.9% and 50.9%). The values found in this study are probably the result of efficient formation of covalent disulfide (S-S) bonds in the protein network during the drying process and this influenced that hydrophilic groups on protein were lesser accessible to water. On the other hand, the protein denatured by heat applied during the formation of films could have induced an unfolding process leaving the amino groups with

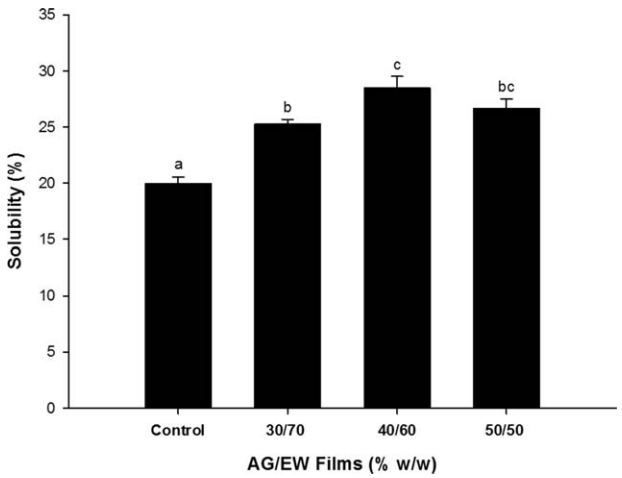

Figure 2. Solubility percentages values of AG/EW films. *Different letters on bars indicate significant differences ($P < 0.05$).

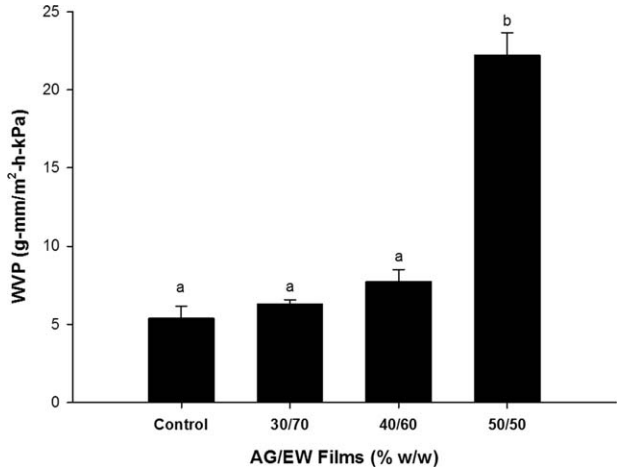

Figure 3. Water vapor permeability (WVP) of the AG/EW films.*Different letters on bars indicate significant differences ($P < 0.05$).

sugars of AG more exposed and developing new complexes at the same time by Maillard reaction[27] which can explain the increased solubility of AG/EW. However, in our study, the water solubility of films increased with the increase of AG proportions. This behavior was also previously observed by Pereira *et al.*[13] who elaborated alginate/AG films and they observed that water solubility of the films increased significantly from 5.6% to 8.4% accompanied with increase of AG from the proportions 95/5 to 75/25 (v/v). The increase of solubility of the films with higher AG may be because of the presence of soluble polysaccharides present in the AG as acemannan, galactan, and glucomannan.[28]

Water Vapor Permeability

The WVP indicates the water lost rate through the films. Figure 3 shows the WVP of the AG/EW films. The results indicated that the AG addition in the proportion 50/50 (22.40 ± 1.40 g mm kPa^{-1} h^{-1} m^{-2}) increased significantly (4.17 times) the WVP values in comparison to control (5.37 ± 0.70 g mm kPa^{-1} h^{-1} m^{-2}), the 30/70 and 40/60 AG/EW film proportions. The WVP for control was the lowest than that reported by Hand *et al.*[26] in films of EW (≈3 g mm kPa^{-1} h^{-1} m^{-2}) and (≈2 g mm kPa^{-1} h^{-1} m^{-2}) for gelatin-EW.[29] The increase of WVP with added AG into EW films (50/50 proportion) may explain the greater free volume between the EW and AG compounds. The increase of insoluble protein (white material) on the film's surface evidenced by the SEM picture [Figure 6(g,h)] was responsible for the reduction of hydrophobic protein in the network during the film formation procedure. We expected that all films with AG added showed a higher value of WVP by hydrophilic nature, which was not true for the 30/70 and 40/60 film proportions. Previous studies have revealed that AG is rich in hydroscopic polysaccharides such as acemannan that shows a good water uptake skill.[8] Probably this induced swelling to the films and avoided the water permeation through the films. Other explanation is an interaction of –CHO (sugar) and $-NH_3^+$ (protein) which may induce the formation of a compact structure with efficient moisture barrier activity (Mallaird reaction), as previously described by Kamboj *et al.*[30] However, it is possible that both swelling and Mallaird reactions have affected the WVP values.

Mechanical Properties of Films

The TS, %*E*, and EM are the most common indicators used to know the mechanical properties of films. TS, is considered the maximum tensile strength that a film can sustain. %*E* is the ability of film to stretch before breaking and EM is a measure of film stiffness, represented by the stress to strain ratio over the linear part of stress–strain curve.[31] Because of its high fragility and low malleability, it was not possible to use an AG film control during its mechanical assessment.

Because of the better film forming properties, the EW was used as comparison reference (control) for mechanical properties (Table I). Although, the 50/50 proportion film was assessed for different properties in our study, its high adhesiveness and fragility made difficult the mounting process on the grips of the tensile testing machine and analysis was not possible. On the other hand, the control film showed the highest TS or resistance which was higher than the film's values of gelatin-EW (5.5 MPa) and EW (4.65 MPa) published previously by Giménez *et al.*[29] and Gennadios *et al.*[3] In addition, the same control film showed the lowest extensibility and plastic properties in relation to %*E* and EM. This may be explained by the presence of channels observed in the SEM picture [Figure 1(a,b)]. The addition of AG into EW was associated with a significant reduction in the TS, observed in the 30/70 and 40/60 AG/EW film proportion. On the contrary, the %*E* and EM significantly increased in the 30/70 AG/EW film proportion that showed association with higher extensibility, plasticity, and low tensile strength in comparison with the control. In addition, an increase of AG in the 40/60 AG/EW film proportion was associated to reduced tendency of TS, %*E*, and EM. This finding was also observed by Zhang *et al.*[32] in films of protein soybean glycosylated with glucomannan. Probably, the higher the addition of polysaccharides in the film forming solution, the higher is the interaction between glucomannan and soluble protein, inducing a

Table I. Mechanical Properties of AG/EW Films

AG/EW films	TS (MPa)	%*E*	EM (MPa)	Thickness (mm)
Control	22.88 ± 3.04^a	6.97 ± 1.98^a	16.04 ± 1.77^a	0.22 ± 0.01^b
30/70	4.22 ± 0.58^b	30.86 ± 4.28^b	98.59 ± 10.65^b	0.22 ± 0.02^b
40/60	1.80 ± 0.30^b	27.91 ± 7.21^b	32.06 ± 5.70^c	0.28 ± 0.01^b
50/50	—	—	—	0.36 ± 0.05^a

Different letters in the same column indicate significant differences ($P < 0.05$).

Table II. Color Parameters of AG/EW Films

AG/EW Films (%w/w)	L^a	a^a	b^a
Control	84.78 ± 0.03^a	2.37 ± 0.02^a	41.13 ± 0.05^a
30/70	84.05 ± 0.01^a	-1.15 ± 0.01^c	41.00 ± 0.03^b
40/60	85.44 ± 0.01^a	-1.66 ± 0.01^d	28.87 ± 0.01^d
50/50	69.68 ± 0.02^d	8.86 ± 0.02^b	39.38 ± 0.02^c

[a] Different letters in the same column indicate significant differences ($P < 0.05$).

Table III. Light Transmittance (%) and Transparency Values of AG/EW Films

	Wavelength (nm)								
AG/EW films	200	280	350	400	500	600	700	800	Transparency value[a]
Control	0.02	0.10	13.54	48.11	75.72	84.82	86.55	87.35	2.57 ± 0.03^d
30/70	0.03	0.15	2.74	12.08	50.76	67.65	73.51	75.33	2.48 ± 0.03^a
40/60	0.09	0.30	2.78	9.38	46.37	61.37	67.40	69.25	2.33 ± 0.02^b
50/50	0.02	0.11	0.93	2.69	25.83	37.58	43.51	45.30	2.04 ± 0.06^c

[a] Different letters in the same column indicate significant difference ($P < 0.05$).

displacement of insoluble fractions of protein associated to reduced mechanical properties of the films.

Optical Properties

Color is also an important property that describes the appearance of the biopolymer films. Table II shows the parameters of color of AG/EW films. All films showed a yellow coloration ($+b^*$ values). The highest yellowness was exhibited by the control and the 30/70 AG/EW film proportion ($P < 0.05$). The yellow color was lesser intense in the 40/60 film proportion. The color intensity did not show a linear behavior and its reduction may be associated to the concentration of polysaccharides and anthraquinones which are difficult to remove during the AG production.[12] The anthraquinones are air and light sensitive compounds and its interaction with other components may cause a reduction in the color intensity. On the other hand, the values of luminosity and brightness ($^*L$ values) were similar among the 30/70 and 40/60 AG/EW proportions and control films, but they were different for the 50/50 AG/EW film proportion. These results indicate that L^* values may be in function of EW protein content. Handa *et al.*[26] published that protein films of EW (desugared) had higher $^*L$ values ($^*L \approx 95$) than those found in our study's control film, probably associated to sugars in the EW during the forming film solution. Sugars formed complexes of Maillard type that darkened the film.[33] We suggested that among the factors that may induce color changes are the displacement of insoluble protein and the increase of water content. The UV and visible light transmission of the AG/EW films are shown in Table III. All films transmitted low UV light at 200 nm (0.02–0.09 range) and 280 nm (0.10–0.30 range). These results showed that the UV light is efficiently blocked by the films. In agreement to Manzocco *et al.*,[34] the EW is a UV light-blocker, but under light exposure, some EW proteins can be modified to lysozyme and ovotransferrin predominantly, inducing unfolding/aggregation phenomena and protein backbone cleavage. It has been shown that gelatin films of fish skin can act as high UV light-blockers compared to synthetic films.[35] Similarly, AG can act as a UV light-blocker through the *Aloe*-specific anthraquinones, polysaccharides, and vitamins.

Transparency of biopolymer films is an important property that helps us to understand how clearly we can see through it. Table III shows that all AG/EW films are slightly opaque. The control film showed significant reduced transparency values ($P < 0.05$) in

Figure 4. Images of AG/EW films obtained by casting method. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

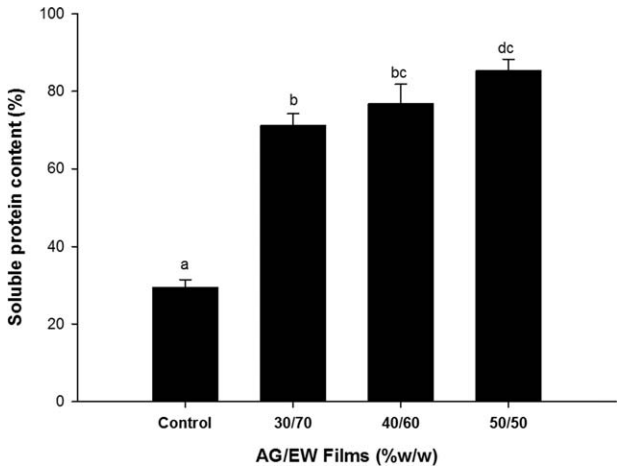

Figure 5. Percentage of soluble protein content of EW/AG films. *Different letters on bars indicate significant differences ($P < 0.05$).

Table IV. Antibacterial Activity of AG/EW Films

	Inhibitory effect by contact[a]	
AG/EW films	*E. coli* O157:H7 (ATCC 43890)	*S. aureus* (ATCC 9144)
EW liquid (control)	+	−
AG (control)	+	−
EW film (control)	+	+
30/70	+	+
40/60	+	+
50/50	+	+

[a] Contact area inhibition; positive (+) and negative (−).

association to AG addition. The 50/50 AG/EW film proportion showed the highest transparency value (Figure 4). The transparency values in our study were higher than those published in films of gelatin-chitosan (0.62–0.99 range) formed as bi-layer films.[36]

Soluble Protein Content (%)

The soluble protein content of films was determined by the Bradford method, because protein determination is not affected by the most common nonprotein compounds present in the biological sample. In addition it is rapid and sensitive.[33] The 50/50 (85%), 40/60 (76%), and 30/70 (71%) AG/EW film proportions showed the highest soluble protein concentrations than the control (29%) (Figure 5). The low content of soluble protein in the control may be associated to the heat's effect applied during the drying process of the formed film solution, that probably increased the SH groups on surface inducing more covalent S–S bridges formation (cross-linked) and reducing the accessibility of the hydrophilic groups of the protein to water.[26] The increased soluble protein content of the AG/EW films were 2.4 (30/70), 2.6 (40/60), and 2.9 (50/50) times higher than the control. This may be explained by the higher accessibility of the hydrophilic groups to water when the polysaccharides of the AG reduced the S–S bridges formation.

Antibacterial Activity

The antibacterial activity by contact area is funded on the diffusion of antimicrobials in a film disk, which depends on the size, shape, and polarity of the diffusing molecule, chemical structure of the film and degree of molecular cross-linking.[37] Table IV shows the antibacterial activity of the AG/EW films against Gram positive and Gram negative bacteria. It was found that all AG/EW film proportion tested against *E. coli* O157:H7 and *S. aureus* produced inhibition by contact. Figure 6 shows the positive and negative inhibition. The EW liquid and the AG control were harmless against *S. aureus*, but active against *E. coli* O157:H7. However, previous studies have found antibacterial activity of the EW and AG films against both tested bacteria because EW contains ovotransferrin and lysozyme, recognized as antibacterial agents.[38] The antibacterial activity of ovotransferrin depends on its ability to sequester Fe^{3+} that is essential for bacterial growth. However, other studies have suggested that the ovotransferrin's antibacterial activity may result from its binding capacity to the bacterial surface. Lysozyme has been recognized as bactericidal agent by the effect of lipophilization with long-chain fatty acids and short-chain saturated fatty acids.[5] On the other hand, the AG has been shown to be an effective antimicrobial agent against *S. aureus* and *E. coli*, but the action's mechanism remains unknown. Other studies have suggested that the acemannan, anthraquinones, and salicylic acid may be responsible for that antibacterial activity.[8] The

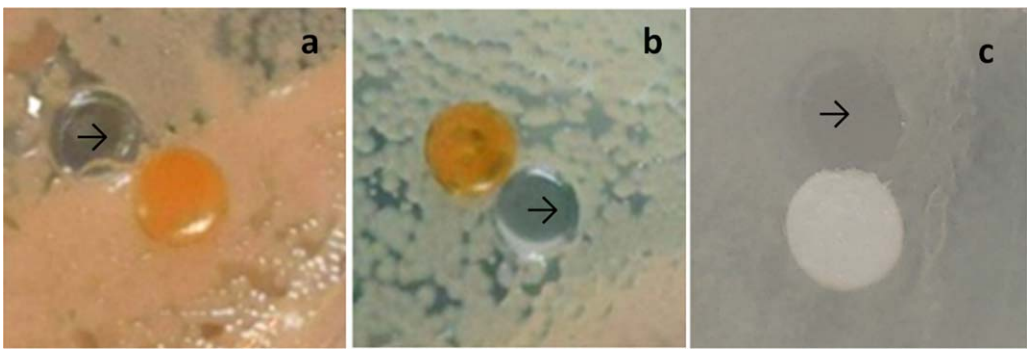

Figure 6. Inhibitory effect by contact of 30/70 AG/EW film (yellow disc) against *E. coli* O157:H7 (a), *S. aureus* (b) and EW liquid control (no effect by contact). The film was removed from the observed contact zone (arrow). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

limited activity against *S. aureus* and *E. coli* observed in this study may result of the low amounts of antibacterial compounds in the film.

The antibacterial activity of all the AG/EW films may result of the synergy effect from either the components or the concentration of bioactive compounds, because of the loss of water during the making process of films. The temperature applied during the films' preparation may also explain the antibacterial effect because of denaturation by heat of the lysozyme with loss of enzymatic activity, but there is an significant improvement of the antimicrobial activity against Gram-negative bacteria.[5] Few studies have investigated the combination of AG and protein matrix for film formation. Chen *et al.*[14] observed antibacterial activity of AG/gelatin against *E. coli*, *S. aureus*, *S. marcescens*, *C. freundii*, *E. aerogens*, and *B. cereus*. These authors found that an increased AG ratio in the composite film formulation was proportional to its antibacterial activity.

CONCLUSIONS

This study showed the potential of the AG addition to the EW for development of films. The AG/EW films showed structural homogeneous surfaces, smooth, nonporous, and insoluble protein material. When the solubility and the WVP increased, a better extensibility-plasticity of the films was observed although accompanied with reduced tensile strength. On the other hand, the films were slightly opaque, yellow colored with efficient capacity for UV-light-blocking. In addition, films showed a high content of soluble protein and all the AG/EW film proportion showed antibacterial activity against *E. coli* O157:H7 and *S. aureus*. It is recognized that additional studies on the AG/EW films are required. Future studies should determine the chemical composition of the EW and AG in an independent way and to elucidate the chemical interaction of the protein EW with AG compounds. In addition, characterizations (contact angle and glass transition temperature) and behavior of the films should be investigated with the use of other plasticizers. Also, this matrix could be tested as encapsulation system of bioactive compounds, drugs, cells, bacteriophages, wound dressings, and other materials of the pharmaceutical and food industries.

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