Physico-chemical and microstructural properties of fish gelatin/agar bio-based blend films

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Abstract

This study was conducted with the aim of improving the physico-chemical properties of fish gelatin (FG) based films. For this purpose, FG was blended with agar (AG) in different compositions to acquire biodegradable films (100:0, 80:20, 60:40, 50:50 & 0:100, FG:AG). The obtained results showed that the AG addition strongly increased the film rigidity and resistance to fracture, while reducing the film stretchability, mainly at 50FG: 50AG ratio. AG incorporation greatly reduced the water vapor permeability (WVP) and solubility of gelatin films, as this decline for the blend film with a 50:50 ratio of biopolymers has been about 41% and 66%, respectively ($p < 0.05$). Additional advantages of AG inclusion to FG films are the reduction of the UV-transmittance. Both polymers showed good compatibility, as demonstrated by scanning electron microscopy (SEM) and atomic force microscopy (AFM) images. Therefore, the blend composition influenced the properties of FG/AG bio-based films.

Keywords: Bio-based films, Fish gelatin, Agar, Physico-chemical properties, Film structure.

1. Introduction

Packaging is an important and effective way to protect food against unfavorable chemical and microbial changes (Shen, Wu, Chen, & Zhao, 2010; Bourtoom, 2008). Increasing environmental concerns about waste problems and depletion of natural resources caused by non-biodegradable petroleum-based plastic packaging materials triggered an increased interest in the development of edible bio-based films for food packaging (Gómez-Guillén, Pérez-Mateos, Gómez-Estaca, López-Caballero, Giménez, & Montero, 2009; Hosseini, Rezaei, Zandi, & Farahmandghavi, 2015). Among the materials used for the preparation of edible films and coatings, polysaccharides and proteins are good options because of their favorable structural and mechanical characteristics (Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011). Gelatin is a biodegradable polymer with the protein nature that is obtained by changing the thermal nature or chemical and physical degradation of collagen (Boanini, Rubini, Panzavolta, & Bigi, 2010). Fish gelatin (FG) has advantages other than mammal's gelatin that include lower gel strength and melting temperature because of the lower levels of proline and hydroxyproline (Otoni et al., 2012). Also, among fish-based gelatin, cold water fish gelatin has a lower melting and gelation temperature than warm water fish (Otoni et al., 2012). This biopolymer shows good film-forming properties, yielding transparency, more or less colorless and very flexible films (Gómez-Estaca, Montero, Fernández-Martín, & Gómez-Guillén, 2009).

However, the main problems of FG-based films as packaging material are permeability toward water and relatively weak mechanical properties (Hosseini, Rezaei, Zandi, & Farahmandghavi, 2013; Vejdani, Ojagh, Adeli, & Abdollahi, 2016). One of the most effective strategies to have new material with the desired properties is polymer blending (Rao, Kanatt, Chawla, & Sharma, 2010). Films formed by mixing polymers usually results in modified physical and mechanical

properties compared to films made of individual components (Kanatt, Rao, Chawla, & Sharma, 2012).

Agar (AG), a gelatinous polysaccharide that is extracted from marine red algae such as *Gelidium* and *Gracilaria* spp., is one of the most promising polysaccharide for developing biodegradable packaging films (Kanmani & Rhim, 2014). It has been widely used for the preparation of bio-based films due to its high mechanical strength and moderate water resistant properties (Giménez, Lopez de Lacey, Perez-Santín, Lopez-Caballero, & Montero, 2013). Moreover, AG-based films were found to be heat-sealable (Phan, Debaufort, Voilley, & Luu, 2009). Upon considering the best characteristics of FG and AG separately, we speculated that their combination would lead to better films than those formed by each individual material alone.

Until recently, FG and AG were studied alone or in combination with other biopolymers (Hosseini et al., 2013; Phan et al., 2009; Wu, Geng, Chang, Yu, & Ma, 2009) for edible films and coating applications, however, the fabrication of the biocomposites from cold water fish skin gelatin and agar has not been elucidated. Hence, the objectives of the present work were to examine the effect of different ratios of FG/AG on the functional characteristics of the blend films such as mechanical properties, water vapor permeability (WVP), film solubility, and transparency. The films were also characterized in their thermal stability (TGA) and morpho-structural (FTIR, SEM and AFM) features.

2. Materials and methods

2.1. Materials

Gelatin from cold water fish skin (CAS # 9000-70-8) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Agar-agar (CAS # 9002-18-0), glycerol (CAS # 56-81-5), and Tween 80 (CAS # 9005-65-6) were purchased from Merck Chemicals Co. (Darmstadt, Germany).

2.2. Bio-based film preparation

A series of FG/AG blends were processed into films by a casting method through varying the agar concentration from 0% to 50%. Pure FG and AG films were prepared as control. Single FG film forming solution (3%, w/v) was prepared by dissolving 3 g gelatin in 100 ml distilled water for 30 min and then heated at 45 °C for 30 min under continuous stirring (Hosseini et al., 2013). Neat AG film was also obtained by dissolving agar (1.5 g/100 ml) at 95 °C for 30 min under a 600-rpm magnetic stirring (Phan et al., 2009). The solutions of FG and AG were carefully mixed at various ratios (100:0, 80:20, 60:40, 50:50 & 0:100, FG:AG). All mixtures were warmed and stirred at 70 °C for 30 min to obtain a good blend. Glycerol (0.3 g/g dry matter) was added as a plasticizer and solutions were again warmed and stirred at 70 °C for 15 min. Finally, aliquots of 15 g of film-forming solutions were poured in polystyrene petri dishes (8 cm of diameter) and the mixture was evaporated in an oven at 45 °C for 24 h to obtain the casting films. Films were conditioned at 25 °C and a relative humidity (RH) of $50 \pm 4\%$ RH for 48 h for further analysis.

2.3. Characterization of the films

2.3.1. Mechanical properties

A universal testing machine (TVT-300Xp, Perten, Sweden) was used to determine the tensile strength (TS), elongation-at-break (EAB) and elastic modulus (EM) of the films according to the ASTM standard method D 882-09 (2009). Films strips of 110×20 mm were conditioned at 23 ± 2 °C and $53 \pm 2\%$ RH for 48 h in an environmental chamber before testing. Initial grip separation and mechanical crosshead speed were set at 50 mm and 5 mm/min, respectively. Each type of film was tested by at least five replicates.

2.3.2. Water vapor permeability (WVP)

WVP values of the films were calculated gravimetrically via the ASTM E96-05 method (2005). Circular glass cups with a diameter of 50 and a depth of 10 mm were applied. The samples were cut into circles and sealed onto the cup mouth containing 6 ml distilled water (100% RH, 2.337×10^3 Pa vapor pressure at 20 °C), placed in a desiccator at 20 °C and 0% RH (0 Pa water vapor pressure) containing silica gel. The water transported through the film was measured every 2 h during 10 h by the weight loss of the glass permeation cell. The measured WVP of the films was determined using Eq. (1):

$$\text{WVP} = \frac{\text{WVTR} \times L}{\Delta P} \quad (1)$$

where WVTR is the water vapor transmission rate (g mm/kPa h m^2) calculated from the slope of the straight line divided by the exposed film area (m^2), L is average film thickness (mm), and ΔP is the partial water vapor pressure variance (kPa) through two sides of the film. Three replicates of each film were tested.

2.3.3. Film solubility

Film solubility was tested according to the method reported in the literature (Gontard et al., 1992). Three pieces ($1 \times 4 \text{ cm}^2$) of film were weighed to the nearest 0.0001 g and afterwards dried in a forced-air oven at 105 °C for 24 h. At this time, films were regained and re-weighed to calculate their initial dry weight (W_i). Then, the samples were submerged in distilled water (30 ml) and mildly shaken (100 rpm) for 24 h at an ambient temperature. The samples were then filtered using Whatman No. 1. The filter paper plus undissolved portions was dried in an air-circulating oven (105 °C, 24 h) and weighed (W_f). The film solubility (FS%) was calculated using Eq. (2):

126
$$FS\% = \frac{W_i - W_f}{W_i} \times 100$$

127 (2)

128 W_i = initial dry film weight (g)

129 W_f = final dry film weight (g)

130 *2.3.4. Water contact angle (CA) measurement*

131 The wettability of the films was determined from contact angle (CA) measurement using a
132 goniometer (Krüss G10, Hamburg, Germany) with the sessile drop technique, by depositing a
133 drop of a liquid onto the film surface. A drop of about 5µL of ultrapure water was carefully
134 placed on the surface of film with a microsyringe. The θ angle at the water/film interface was
135 measured after 5 s to the nearest degree (Möller, Grelier, Pardon, & Coma, 2004). Five
136 measurements on each film were performed at random positions.

137 *2.3.5. Light transmission and opacity*

138 The barrier properties of the films against ultraviolet (UV) and visible light were measured at a
139 selected wavelength between 200 and 800 nm using the method as described in our previous
140 study (Hosseini et al., 2013). Film portions of 10 × 40 mm were placed into the test cell of a UV-
141 1650 spectrophotometer (Model PC, Shimadzu, Kyoto, Japan), using an empty cell as the
142 reference. The measurement was done in triplicate and the average of three spectra was
143 calculated. The transparency of the films was determined by the following equation:

144
$$\text{Transparency (A/mm)} = -\log T/x \quad (3)$$

145 where A = absorbance at each wavelength, T = transmittance (%) at each wavelength, x = film
146 thickness (mm). According to the equation, high transparency indicates opaque.

The opacity of the films was also calculated using the following equation:

$$O = \text{Abs}_{600} / x \quad (4)$$

Where Abs_{600} is the value of absorbance at 600 nm and x is the film thickness (mm).

2.3.6. Fourier transform infrared with attenuated total reflection (FTIR-ATR)

FTIR-ATR spectroscopy was used to identify the chemical structure of the FG and AG films and possible interactions between their components. FTIR-ATR spectra of the films were recorded with KBr pellets at wavenumber range of 400-4000 cm^{-1} using 8400S spectrometer (Shimadzu, Japan). For each spectrum, 16 scans were co-added. Before analysis, the film samples were kept in desiccator with silica gel for 1 week at room temperature to obtain the most dehydrated films.

2.3.7. Thermo-gravimetric analysis (TGA)

TGA analysis was performed with a Perkin-Elmer PYRIS I thermogravimetric analyzer (USA). Samples of 10-15 mg was placed in the TGA furnace and the measurements were carried out under nitrogen atmosphere with a heating rate of 10 $^{\circ}\text{C}/\text{min}$ from 25 to 600 $^{\circ}\text{C}$. Weight losses of samples were measured as a function of temperature.

2.3.8. Scanning electron microscopy (SEM)

The morphological examinations of the films were performed by scanning electron microscopy (SEM) (VEGA II, TESCAN, Czech Republic). Strips of dry films (obtained by maintaining in a desiccator with silica gel for 2 weeks) were immersed in liquid nitrogen and cryo-fractured manually. Then, the specimens were stuck onto a cylindrical aluminum stub by a double-sided tape. The stubs with the film were sputtered with a thin layer of gold in an ion sputter coater (K-450X, EMITECH, England) and placed into a scanning electron microscope to see the cross-

section morphology of the films. The surface of the films was also investigated. Micrographs were obtained for sample surface and cross-sections at 1000× magnification.

2.3.9. Atomic force microscopy (AFM)

The surface morphology of the films was analyzed using an atomic force microscope (Dual Scope TM DS95-50, DME, Denmark) with a 50 × 50 micron scan size at a resolution < 0.1 nm. Two statistical parameters, associated with sample roughness, were calculated: average roughness (R_a), and root-mean-square roughness (R_q). Three images were analyzed in each formulation.

2.3.10. Statistical analysis

Analysis of variance (ANOVA) was used to compare mean differences of the samples and the differences between the means were determined by the least significant difference test at $p \leq 0.05$. The statistical software package (SPSS ver. 17.0, SPSS Inc., Chicago, IL, USA) was used to analyze the experimental data.

3. Results and discussion

2.3.1. Mechanical properties of the films

Generally, mechanical strength and flexibility are needed for a packaging film to keep its integrity and tolerate external stress (Hosseini et al., 2015). Values of tensile strength (TS), elongation-at-break (EAB) and elastic modulus (EM) of the films can be seen in Table 1. Generally, the addition of AG caused a significant increase ($p < 0.05$) in TS and the EM, while a significant decrease on EAB was observed. Neat FG film exhibited lower TS (1.77 MPa) but higher EAB (76.73%) values, being in the same range reported by other authors (Denavi et al., 2009; Hosseini et al., 2013). Conversely, pure AG film showed substantially higher ($p < 0.05$)

TS value (18.69 MPa) and a reduced EAB (29.92%), when compared with FG film. The TS value reported for AG film is similar to that reported by Giménez et al. (2013) (18.48 MPa), however, the EAB value is markedly lower than that reported by these authors (62.35%). As presented in Table 1, in the blend films, the addition of AG induced a significant increase in the TS, leading to stronger films as compared with gelatin film, but significantly ($p < 0.05$) decreased the EAB. The mechanical properties of the blend film are dependent on the intermolecular forces, the hardness of polymer chains, as well as molecular symmetry of each polymer (Suyatma, Copinet, Tighzert, & Coma, 2004). According to Kester and Fennema (1986), increasing structural cohesion, results in the reduction of the film flexibility and thus percentage of the EAB. A Similar behavior was observed by Wu et al. (2009), who studied films manufactured from potato starch and agar.

Elastic modulus (EM), a measure of intrinsic film stiffness, is the slope of the linear range of the stress-strain plot (Mauer, Smith, & Labuza, 2000). EM of the resulting biocomposite films was also affected by the addition of AG in the film formulation, causing an improvement of up to 20-fold in the ratio of 50FG:50AG with respect to the control FG film (Table 1). It seems the better tensile strength of polysaccharide films (Wang et al., 2007) as well as good compatibility between both biopolymers (i.e. FG and AG), is the reason that the TS and EM of the films were significantly increased with increasing amounts of AG in the composite films.

2.3.2. WVP

One of the most important functions of biodegradable films that are used in food packaging is to prevent moisture transfer between the environment and food product. For this purpose, the biopolymer-based films used in packaging should have the least amount of water vapor permeability (Gontard, Guilbert, & Cuq, 1992). As shown in Table 1, neat AG film exhibited

213 better water vapor barrier properties than FG one (0.56 vs. 1.49 g mm/ kPa h m²). The thickness
214 of the films could have an effect on the WVP measurement. Gelatin films with a higher amount
215 of protein and thickness could adsorb more water from the environment, due to the presence of a
216 wide range of hydrophilic amino acids in the gelatin (McHugh, Avena-Bustillos, & Krochta,
217 1993). The WVP of FG film were significantly reduced when AG was added (Table 1), as this
218 decline for the blend film with a 50FG:50AG ratio has been about 42% ($p < 0.05$). It was
219 assumed that, when AG was added, strong intermolecular interaction such as hydrogen bonds
220 and hydrophobic and electrostatic interactions was formed between protein and polysaccharide
221 chains (Guerrero, Kerry, & de la Caba, 2014), which minimized free volume and intermolecular
222 distance in the films. Consequently, water molecules diffused more difficulty in the compact
223 network and the lower WVP value resulted. According to Guerrero et al. (2013), agar may act as
224 a reinforcing agent, forming a compact and homogeneous three-dimensional network gel prior to
225 setting of the film.

226 2.3.3. Water solubility and wettability

227 Solubility is an important property of edible films as they are used as protective layers on food.
228 Water sensitivity of gelatin-based films is an important challenge for the replacement of
229 traditional plastics, especially for packaging applications. The poor resistance of gelatin-based
230 films towards water is due to the hydrophilic nature of the protein (presence of polar peptides)
231 and to the substantial amount of hydrophilic plasticizer (glycerol) added to provide enough film
232 elasticity (Hosseini, Javidi, & Rezaei, 2016). As can be seen in Table 1, the water solubility of
233 the control FG film is relatively high, around 62%, which was in accordance with the values
234 reported by Hosseini et al. (2013) for the films elaborated from fish skin gelatin (~64%) and
235 were considerably lower than those reported for cod skin gelatin films (~92%) (Núñez-Flores et

al., 2012). The addition of AG drastically improved the water resistance of FG films in the mixtures (80:20, 60:40 and 50:50, FG:AG), with pronounced changes produced from the 50FG:50AG ratio onwards (around 65%), indicating an AG proportion of 50% to be the optimum proportion for producing substantial physical interference in the entanglement of gelatin polypeptide chains within the film matrix. Such interference, which might have led to a significant blockade of gelatin ability to interact with water molecules, would mainly be responsible for reducing the water solubility in the composite films (Hosseini et al., 2013). Giménez and co-authors (2013) reported a lower solubility in the agar film containing 33% gelatin compared with pure agar film.

The contact angle (CA) of water droplet deposited onto the edible film surface has usually been used to describe their hydrophobicity. Low CA ($\theta < 65^\circ$) indicates a hydrophilic surface and high CA ($\theta > 65^\circ$) are characteristic of hydrophobic surfaces (Vogler, 1998). The CA of neat FG film was about 50° , indicating a high hydrophilicity of gelatin chains. Compared with FG film, CA values of FG/AG bio-based blend films improved greatly ($p < 0.05$) and reached to a maximum value of 83° when the agar content was 50% (Table 1). This implies a decrease in surface hydrophilicity of FG films, which is mainly attributed to the formation of intermolecular hydrogen bonds between hydroxyl groups of agar and amino groups in protein (Guerrero, Garrido, Leceta, & de la Caba, 2013), that resulted in a reduced ability to establish links with water molecules.

2.3.4. Light transmission and opacity of the films

Transparency is an important feature of the edible films that affects the appearance of the coated product. On the other hand, it can affect the speed of the lipid oxidation and ultimately the product quality (Rao et al., 2010). Light transmission of all films at selected wavelengths are

shown in Fig. 1a. In the UV range of 200-280 nm, pure FG and its composite films exhibited excellent UV barrier properties, which cause oxidative deterioration of packaged foods, leading to nutrient losses, discoloration and off-flavors (Martins, Cerqueira, & Vicente, 2012). This result is in agreement with previous reports on fish gelatin-based films (Hosseini et al., 2015). The ability of the protein-based films to absorb radiation may be associated with a high content of aromatic amino acids such as tyrosine and tryptophan in the protein structure (Aitken & Learmonth, 1996). Although, light transmission of neat AG films was negligible at 200 nm, but was relatively high at 280 nm (52%). The result suggested that the blend films effectively prevented the UV light. In general, light transmission in visible range (350-800 nm) for parent FG and AG films was in the range of 84.9-92.7%, while for the blend films it was in the range of 47.2-80.2% (Fig. 1). Therefore, the blend films showed a lower light transmission than the control FG and AG ones in visible range, indicating that FG/AG blend films were low in transparency. It can be concluded that AG with a high light transmission barrier most likely contributed to limited light transmittance of the blend films at both UV and visible ranges.

The opacity values of the control films along with those of the blend films are illustrated in Fig. 1b. FG film transparency was 0.58 A₆₀₀/mm, which was similar to the value reported by Hosseini et al. (2013) for fish gelatin film. As shown in Fig. 1b, the addition of AG into FG matrices led to significant increase ($p < 0.05$) in opacity of the resultant films. The increase in opacity may have been the result of compactness of the film matrix in which the polymer inter-chain spacing was decreased, permitting less light to pass through the film (Yang, Paulson, & Nickerson, 2010). The contraction in structure can be evidenced by the decrease in thickness of the films added with agar. However, transparency values for the biocomposite films in the present study were lower than the values measured for low-density polyethylene (4.26

A600/mm), which is a commercial plastic intended for packaging (Guerrero, Nur Hanani, Kerry, & de la Caba, 2011).

2.3.5. *FTIR-ATR spectroscopy*

FTIR-ATR spectra of the control FG, AG and their blends are shown in Fig. 2. The spectrum of FG film showed characteristic bands at approximately 3278 cm^{-1} (amide-A, NH-stretching), 1635 cm^{-1} (amide-I, C=O stretching), 1535 cm^{-1} (amide-II, N-H bending) and 1242 cm^{-1} (amide-III, C-N and N-H stretching), (Hosseini et al., 2013; Staroszczyk, Pielichowska, Sztuka, Stangret, & Kolodziejska, 2012). The characteristic peak appeared at 2916 cm^{-1} corresponds to the C-H stretching vibration (Hosseini et al., 2015). The band situated at the wavenumber of $\sim 1041 \text{ cm}^{-1}$ was found in all film samples, corresponding to the glycerol (-OH group) added as a plasticizer (Bergo & Sobral, 2007).

In the spectrum of neat AG film, the characteristic broad absorption band at about 3278 cm^{-1} is attributable to hydroxyl (O-H) groups, which are able to form hydrogen bonds with O-H and amino (N-H) groups of protein (Guerrero et al., 2013). The characteristic absorption peaks at 1056, 1033 and 925 cm^{-1} indicates C-O stretching group of 3,6-anhydro-galactose (Kanmani & Rhim, 2014). Also, the peak at 871 cm^{-1} is due to the C-H stretching residual carbon of β -galactose (Wu et al., 2009). After the addition of AG in protein matrices some of the peaks were shifted to lower and higher frequency in resultant blend films. For example, the amide-I peak was shifted from 1635 (FG film) to 1643 cm^{-1} in the biocomposite films with ratios of 60:40 and 50:50 (FG:AG). Meanwhile, with the replacement of 50% gelatin with agar (i.e. the ratio 50FG:50AG), the peak of amide II was shifted from 1535 to 1542 cm^{-1} . The spectra changes suggested the presence of protein-protein and protein-polysaccharide interactions via hydrogen

bond (Wu et al., 2009). FTIR spectra result reconfirmed the interaction between protein and polysaccharide, which was governed by FG/AG ratio.

2.3.6. Thermal behavior

TGA test shows thermal stability of the films through measurement of their weight loss during temperature increase. Generally, the thermal stabilities of the composite films were improved as compared with those of neat FG film. This hypothesis is supported by the fact that replacement of 20-50% of FG with AG increases the onset temperatures of thermal degradation by 8-12 °C (Table 2). The weight loss (TG) and derivative (DTG) curves for the TGA tests of FG/AG bio-based blend films at different ratios are shown in Fig. 3a and b, respectively. From TG curve (Fig. 3a), it could be seen that the control FG film showed the two-step thermal degradation processes, while the neat AG and the blend films exhibited three distinctive weight loss regions. The first weight loss at 60-120 °C is probably due to the vaporization of adsorbed and bound water. The second degradation occurred at around 170-360 °C, which was attributed to the volatilization of glycerol (used as film plasticizer) and thermal degradation of biopolymers (Reddy & Rhim, 2014). The third stage of weight loss occurred at above 370-440 °C, which was due to the thermal degradation of more stable AG polymer molecules. The maximum mass loss corresponding to $T_{d\ max}$ was clearly regarded as a peak in a derivative TG, or so-called DTG, thermogram (Fig. 3b); for example, T_d of the FG film was 323.3 °C. The $T_{d\ max}$ of FG/AG composites took place at temperatures lower than that for pure FG film (Table 2).

Char at 600 °C, which is the amount of residue left after final thermal decomposition, of the FG/AG blend films was more than that of the pristine FG film. The char at 600 °C of the FG film was 21.83%, but it increased to 24.32-27.33% depending on the agar concentration of the blend films (Table 2). This is mainly due to higher ash content of agar than the FG (Reddy & Rhim,

2014). Nonetheless, previous studies have shown that the high value of residue rate may imply the good flame retardance of the material (Jiang, Zhao, Hou, 2016).

2.3.7. Films morphology

Scanning electron microscopy (SEM) observations were carried out to contribute to a better understanding in the homogeneity and in the microscopic structure of the biocomposite films. SEM micrographs of the surface and cross-section of neat FG, AG and their blend films are illustrated in Fig. 4. Pure FG film displays smooth and homogeneous surfaces, without pores and with excellent structural integrity (Fig. 4a). Meanwhile, the cross section of the FG films (Fig. 4a₁) is even, which indicates a homogeneous structure. On the one hand, the pristine AG film micrograph showed a relatively smooth surface and continuous or dense cross section structure (Fig. 4b & b₁), comparable with the results reported by Wu et al. (2009) on agar-based films. In the case of the blend films, no distinct separation was observed (Fig. 4c₁-e₁), indicating the compatibility of both biopolymers. Meanwhile, it should be noted that the in equal ratios of the FG and AG (i.e. 50FG:50AG) completely homogeneous and continuous surface and cross section was observed, which confirmed a compact and dense structure (Fig. 4e₁). The obtained structure can be due to the intermolecular biopolymer associations or may be due to the good compatibility between FG and AG (Tian, Xu, Yang, & Guo, 2011). Both FG and AG chains have hydroxyl functional groups available; therefore, intermolecular interactions by hydrogen bonds should occur in the blend films, which improved the miscibility of the two phases. This may be explained by the improved mechanical and barrier properties of the composite films.

2.3.8. Surface morphology analysis by atomic force microscopy

AFM allow the determination of roughness parameters that can be calculated for the surfaces of films. Moreover, AFM allows a histogram to be plotted of the relative height of every pixel recorded during the scan (Ghasemlou et al., 2013). Fig. 5 showed that the surface morphology varied markedly between the FG/AG blend films with different ratios, probably due to the different arrangement of the protein matrix, caused by the agar replacement. Pure FG and AG films showed the relatively homogeneous surface morphology with the surface peak height of 62.2 and 52.7 nm, respectively (Fig. 5a & b). As can be seen from Fig. 5a, the surface of the control FG film was smoother as indicated by lower R_a and R_q values (3.55 and 5.03 nm, respectively), while for the FG/AG blend films, the surface was rougher (i.e., more wrinkles), presenting a more complex superficial topography (Fig. 5c-e). The aggregation of FG and AG chains would influence the surface morphology of FG/AG films. The nodules on the film surface result from the macromolecules aggregation due to the strong hydrogen bonding interaction between FG and AG chains (Guerrero et al., 2013). The surface of the 60FG:40AG film was rough and uneven, showing the highest R_a , R_q and peak height values of 7.54, 10.16 and 92.5 nm, respectively (Fig. 5d). The height profiles showed that the blend film with the replacement of 50% FG by agar (i.e. ratio 50FG:50AG) had a minimum vertical distance of 51.4 nm (Fig. 5e). The decrease of the peak height when compared with pure FG (62.2 nm) and FG/AG bio-based blend films in the other two ratios (69.6 and 92.5 nm, respectively, for 80:20 and 60:40, FG:AG) (Fig. 5c & d), may indicate the establishment of some interactions between FG and AG by electrostatic forces, hydrogen bonding, etc. (Oses et al., 2009).

4. Conclusions

A series of fish gelatin/agar blend films were fabricated by a solution casting method. Experimental results suggest hydrogen bonding interactions existed between protein and

polysaccharide as evidenced by a decrease in surface hydrophilicity and WVP of FG films. Agar addition greatly increased the strength and rigidity of the FG films, while decreasing their extensibility. The FG/AG biocomposite films exhibited a high UV barrier property that implies the blend films have a high potential for being used to protect food products from UV light induced photo-chemical reactions. Meanwhile, TGA analysis indicated that the presence of AG improved thermal stability of FG film. The blending of FG and AG rendered films of homogeneous structure, due to the high compatibility of both polymers, as revealed by microscopy images. Hence, it seems the blending of FG with AG is a suitable approach to obtain environmentally friendly packaging films. However, further studies are needed to investigate potential performance improvement for industrialized use of the film.

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Table 1
Tensile strength (TS), elongation-at-break (EAB), elastic modulus (EM), water vapor permeability (WVP), film solubility, and contact angle (CA) of FG/AG bio-based blend films with different proportions.

FG/AG ratio	TS (MPa)	EAB (%)	EM (MPa)	WVP (g mm/kPa h m ²)	Film solubility (%)	CA (°)
100:0	1.77 ± 0.14 ^d	76.73 ± 17.31 ^a	9.05 ± 3.52 ^c	1.490 ± 0.031 ^a	62.12 ± 1.97 ^a	50.32 ± 1.46 ^b
80:20	3.69 ± 0.68 ^d	45.17 ± 8.03 ^b	21.61 ± 5.96 ^c	1.206 ± 0.046 ^b	47.00 ± 0.93 ^a	74.86 ± 0.74 ^b
60:30	7.04 ± 0.47 ^c	40.22 ± 3.75 ^b	45.55 ± 24.7 ^c	0.932 ± 0.014 ^c	22.25 ± 0.25 ^b	80.02 ± 2.60 ^b
50:50	13.62 ± 0.64 ^b	38.89 ± 2.40 ^b	186.80 ± 8.12 ^b	0.873 ± 0.022 ^d	20.85 ± 0.86 ^c	83.16 ± 2.52 ^b
0:100	18.69 ± 3.92 ^a	29.92 ± 12.92 ^b	361.67 ± 44.87 ^a	0.562 ± 0.024 ^e	25.23 ± 1.06 ^c	74.28 ± 1.73 ^a

Values are presented as means ± standard deviation. Different letters in the same column indicate significant differences (p < 0.05).

Table 2
 Onset temperature and $t_{d \text{ max}}$ (maximum decomposition temperature) of FG/AG
 bio-based blend films with different proportions.

FG/AG ratio	Onset temperature (°C)	$T_{d \text{ max}}$ (°C)	Residue at 600 °C (%)
100:0	240	322	21.83
80:20	248	292	24.32
60:30	251	318	27.15
50:50	252	316	27.33
0:100	246	292	20.18

Figure captions

Fig. 1. (a) Light transmittance (%) and (b) opacity values of FG film, AG film and FG/AG bio-based blend films with different proportions.

Fig. 2. FTIR spectra of FG film, AG film and FG/AG bio-based blend films with different proportions.

Fig. 3. (a) TG and (b) DTG thermograms of FG film, AG film and FG/AG bio-based blend films with different proportions.

Fig. 4. SEM micrographs of the surface and cross-section of (a and a₁) FG film, (b and b₁) AG film, and bio-based blend films with different proportions: (c and c₁) 80FG:20AG, (d and d₁) 60FG:40AG, (e and e₁) 50FG:50AG.

Fig. 5. Three-dimensional AFM topographic images (left) and profile of the height values (right) of (a and a₁) FG film, (b and b₁) AG film, and bio-based blend films with different proportions: (c and c₁) 80FG:20AG, (d and d₁) 60FG:40AG, (e and e₁) 50FG:50AG.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

(a)

(b)

(c)

(d)

(e)

Fig. 5.