Efficient gas barrier properties of multi-layer films based on poly(lactic acid) and fish gelatin

Seyed Fakhreddin Hosseini*, Zahra Javidi, Masoud Rezaei

*Department of Seafood Processing, Faculty of Marine Sciences, Tarbiat Modares University,
P.O. Box 46414-356, Noor, Iran*

***Corresponding author:** Seyed Fakhreddin Hosseini **Tel:** +98 1144553101-3 **Fax:** +98 1144553499 **E-mail addresses:** hosseinisf@modares.ac.ir , hosseinisf@gmail.com (S. F. Hosseini).

Abstract

Multi-layer film structures of poly(lactic acid) (PLA) and fish gelatin (FG), prepared using the solvent casting technique, were studied in an effort to produce bio-based films with low oxygen (OP) and water vapor permeability (WVP). The scanning electron microscopy (SEM) images of triple-layer film showed that the outer PLA layers are being closely attached to the inner FG layer to make continuous film. The OP of multi-layer film ($5.02 \text{ cm}^3/\text{m}^2\cdot\text{day}\cdot\text{bar}$) decreased more than 8-fold compared with that of the PLA film, and the WVP of multi-layer film ($0.125 \text{ g mm/kPa h m}^2$) also decreased 11-fold compared with that of the FG film. Lamination with PLA profoundly increased the water resistance of the bare gelatin film. Meanwhile, the tensile strength of the triple-layer film ($25 \pm 2.13 \text{ MPa}$) was greater than that of FG film ($7.48 \pm 1.70 \text{ MPa}$). At the same time, the resulting film maintains high optical clarity. Differential scanning calorimetry (DSC) analysis also revealed that the materials were compatible showing only one T_g which decreased with FG deposition. This material exhibits an environmental-friendliness potential and a high versatility in food packaging.

Keywords: Multi-layer film, Poly (lactic acid), Fish gelatin, Oxygen permeability, Water vapor permeability, Film properties.

1. Introduction

Today's increasing knowledge of sustainability has switched the focus from traditional plastic materials to more environmentally friendly options in different fields of application including food packaging [1]. In accordance with a new market research report, the biodegradable plastics market is projected to grow from more than USD 2.0 Billion in 2015 to USD 3.4 Billion by 2020, at a CAGR (Compound Annual Growth Rate) of 10.8% between 2015 and 2020 [2].

Poly(lactic acid) (PLA) can offer a sustainable alternative for food packaging across a wide range of commodity applications in response to consumers' requests and market tendencies in the use of renewable resources [3,4]. PLA is one of the most promising materials owing to its high mechanical strength (similar to polystyrene (PS) and poly ethylene terephthalate (PET)), biodegradability and biocompatibility [5,6]. Additionally, PLA has been approved by the U.S. Food and Drug Administration (FDA) for its application in food-contact material [7]. It is among the most widely available bio-based thermoplastics, with a production capacity of over 150,000 metric ton/year [8], and it is anticipated to grow as it is responding to the sustainable demands connected to the reduction of civic solid waste.

However, more attempts are still required to improve the properties of PLA so as to make it competitive with petrochemical-based plastics in demanding functions, such as the packaging of oxygen-sensitive food [1]. For instance, according to Auras et al. [9] the oxygen permeability coefficient (OPC, 11×10^{-18} kg m/m² s Pa at 40 °C and 0% RH) of PLA requires it to be decreased up to 10-fold before it can compete with PET, a most commonly used plastic for this purpose.

Although a number of techniques have been examined to improve the gas barrier properties of PLA [1], an effective approach is the inclusion of impermeable layer via a multi-layer system

design [2]. Multi-layer films have gained relevance in many industrial applications, especially in the fresh food packaging industry, due to its extending the shelf life of food products [10].

Polymers originating from natural products, such as carbohydrates and proteins, offer the greatest opportunities as components of multi-layer structures since their biodegradability and environmental compatibility are assured [10]. Protein films are excellent oxygen and aroma barriers at low relative humidity with a potential to replace synthetic oxygen-barrier components in multi-layer formulations such as EVOH [10]. Among all the protein sources, gelatin, obtained by the controlled hydrolysis of the insoluble fibrous collagen, has also been extensively studied for its film forming ability and has good barrier properties against oxygen and light, and prevents dehydration and lipid oxidation [11].

Fish gelatin (FG) has come under increasing consideration in current years as an encouraging alternative to mammalian gelatin since it can be isolated from by-products of the fish processing industry and has no health related issues for consumer safety concerns [12]. FG has advantages to other mammals gelatin that include lower gel strength and melting temperature due to the lower levels of proline (Pr) and hydroxyproline (Hyp) [13]. Also, the less amounts of these amino acids (Pr and Hyp) increase the gelatin hydrophobicity. However, like other protein-based films, gelatin has a relatively poor water barrier characteristic and mechanical strength, which is the main drawback of applying gelatin films as packaging material [12].

One approach to solve this issue is, lamination, which enhances the efficiency of polymeric films by integrating the properties of several types of polymer layers into one multi-layer film in which the hydrophilic layer (proteins, polysaccharides, etc.) is sandwiched between hydrophobic layers such as PLA polymers thus preventing the plasticization and swelling of the inner layer [10,14]. Although in recent years noteworthy works have been carried out in the progress of protein films

as packaging materials, and despite the superior gas barrier characteristics of these films, they, nevertheless, tend to have higher water vapor permeability and are mechanically weaker compared to most synthetic polymers.

Therefore, the aim of this work was to prepare a multi-layer film by combining PLA films as outer layers with FG as inner layer through the solvent casting method, in order to improve the barrier and mechanical properties of individual films, which will help in competing with non-biodegradable plastic (e.g., PE, PET) in food packaging application.

2. Materials and methods

2.1. Materials

Poly (lactic acid) (PLA), 2002 D, was purchased in pellet from Natureworks® Co., (USA). Cold water fish skin gelatin was obtained from Sigma-Aldrich (St. Louis, MO, USA). Also, chloroform and glycerol (analytical grade) were obtained from Merck Chemicals Co. (Darmstadt, Germany).

2.2. Preparation of multi-layer films

Pristine PLA films were provided by solvent casting method as reported in our previous study [6]. At first, a solution of 3.5% (w/w) PLA was prepared in chloroform under magnetic stirring (400 rpm) at room temperature (~ 25 °C), after which the solution was poured onto a glass plate and the solvent was allowed to evaporate in a hood in a dark place over 3 days at room temperature. FG films were also prepared by solution casting method as reported in our earlier work [15]. Briefly, FG solution was provided by dissolving 3 g gelatin in 100 ml distilled water for 30 minutes and then heated at 45 °C for 30 minutes while continuously stirring. Glycerol (0.3 g/g gelatin) was added as a plasticizer and solutions were again warmed and stirred at 45 °C for

15 minutes. Finally, a certain amount of film-forming solution was poured into polystyrene petri dishes and dried at an ambient temperature for 3 days.

Multi-layer films were prepared by combining the PLA films as the outer layers and FG film as the inner layer according to the method of Rhim et al. [16] with some modifications. First, one outer PLA layer was prepared by casting PLA solution (1.5%, w/w) and drying (24 h, at ambient temperature); then an inner layer of FG solution (1.5%, w/w) was cast over it and drying (48 h in an oven at 45 °C), followed by the casting of other PLA outer layer solution (1.5%, w/w) and then left to dry in an oven at 45 °C for 24 h. After that, the three types of films (PLA, FG and PLA/FG/PLA) were peeled from the plate and placed in a sealed container containing silica gel until time of use. Thickness of the films was measured at nine random points along the specimens by a micrometer (Mitutoyo Manufacturing Co. Ltd., Tokyo, Japan) at an accuracy of 0.001 mm, and average values were used in calculations.

2.3. Films characterization

2.3.1. Scanning electron microscopy (SEM)

Cross-sectional images of pristine PLA, FG and their multi-layer films, were taken by scanning electron microscopy (SEM) (VEGA II, TESCAN, Czech Republic). Strips of dry films were immersed in liquid nitrogen and cryo-fractured manually. The specimens were then 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.

2.3.2. Atomic force microscopy (AFM) imaging

AFM (CP-R, Veeco Instruments, USA) imaging was also used to characterize the surface morphology and roughness of the films with a $1.0 \times 1.0 \mu\text{m}$ scan size. These images were scanned in tapping mode under ambient conditions by triangular cantilever with a spring constant of 50 N/m. The roughness value was determined from the height image and is presented as a root-mean-square (RMS) value.

2.3.3. Oxygen permeability (OP)

The oxygen transmission rate (OTR) was determined using a gas permeability tester (Brugger GDP-C, Munich, Germany). The measurements were performed at 23 °C and 50% RH according to ASTM D 3985-05 [17]. The sample area was 14 cm^2 , and the pressure of the oxygen was 1.5 bar. The OTR was normalized with respect to the pressure and material thickness and converted to the oxygen permeance and oxygen permeability (OP), respectively.

2.3.4. Water vapor permeability (WVP)

The WVP of films were determined according to a modification of E96-05 gravimetric method¹⁸ proposed by McHugh et al. [19] using a glass permeation cup (4.9 cm internal diameter and 1.1 cm height). The film samples were sealed onto the cup's mouth containing 6 ml distilled water (100% RH; $2.337 \times 10^3 \text{ Pa}$ vapor pressure at 20 °C), located in a desiccators at 20 °C and 0% RH (0 Pa water vapor pressure) including silica gel. The water transported through the film and adsorbed by the desiccant was controlled from the weight loss of the glass permeation cell (measured every 2 h during 10 h). Weight loss over time was plotted to obtain the slope ($r^2 \geq 0.99$). Then the WVP of the film was calculated using the following equation:

$$WVP = (WVTR \times L)/\Delta p \quad (1)$$

Where WVTR was the measured water vapor transmission rate (g mm/kPa h m²) through a film, L was the average film thickness (mm), and Δp was the partial water vapor pressure variance (kPa) across the two sides of the film. Three measurements were made for each film, and the mean value is reported.

2.3.5. Swelling ratio and water solubility of the films

Swelling ratio (SR) of the films was determined gravimetrically. Since, proteins are susceptible to heat-induced crosslinking, to avoid heat curing, film specimens (2 × 2 cm) were desiccated by silica gel for 3 days to determine initial dry matter. Then, they were placed in closed beakers containing 30 ml of distilled water and stored at room temperature. Films were then removed after 2 h and weighed immediately after wiping off the excess water on the surface with a filter paper. The SD was calculated according to the following equation:

$$SR (\%) = [(W_t - W_0) / W_0] \times 100 \quad (2)$$

Where, W_0 and W_t are the weight of the films before and after immersion, respectively.

Water solubility (WS) of the films was tested in order to determine the resistance of the films in water. Pre-weighed desiccated films (2 × 2 cm) were immersed in 30 ml of distilled water in a beaker at room temperature, covered with Parafilm and kept for 24 h. Then, films were removed from the beaker and oven dried (105 °C for 24 h) to determine final dry matter. In the case of fish gelatin film, the samples were passed through a weighed filter paper (Whatman No.1), and the filter paper together with unsolubilized fraction was subsequently dried in an oven and weighed. The WS was calculated by the following equation:

$$WS (\%) = [(W_i - W_f) / W_i] \times 100 \quad (3)$$

Where, W_i and W_f are the weight of the initial and final dry matter of the films, respectively.

2.3.6. Tensile properties

A universal test machine (TVT-300Xp, Perten, Sweden) was used to determine the tensile strength (TS), elastic modulus (EM) and elongation-at-break (EAB) of the films according to the ASTM D882-02 standard procedure [20]. Film samples (rectangular strips of 100×20 mm) were conditioned in an environmental chamber for 48 h at 23 ± 2 °C and $53 \pm 2\%$ RH before testing. Tests were performed on at least five samples of each material with an initial grip separation and mechanical crosshead speed was set at 50 mm and 5 mm/min, respectively.

2.3.7. Color and transparency

Surface color of the films was measured using a colorimeter (BYK Gardner, USA). A white standard color plate ($L=94.61$, $a=-0.89$ and $b=0.57$) was used as a background for color measurements. Hunter color values were averaged from five readings of each sample. The total color difference (ΔE) was calculated as follows:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

Where ΔL , Δa , and Δb are the differences between each color values of standard color plate and film specimen, respectively.

The opacity of a material is an indication of how much light passes through it. The higher the opacity, the lower the amount of light that can pass through the material [21]. The opacity of the films was also calculated by the following equation:

$$O = \text{Abs}_{600} / x$$

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

2.3.8. Thermal characterization

Differential scanning calorimetry (DSC) experiments were carried out in a DSC 500-B device (Faith, China). Samples of approximately 5 mg were placed into aluminum pans, sealed and scanned over the range of 25 to 200 °C with a heating rate of 10 °C/min. The glass transition temperature (T_g), crystallization temperature (T_c), and melting temperature (T_m) of the films were measured.

The thermal stability of the film samples was tested with thermogravimetric analysis (TGA; Perkin-Elmer PYRIS I thermogravimetric analyzer, USA). About 10-15 mg of each sample was heated from room temperature to 600 °C at a heating rate of 10 °C/min under nitrogen atmosphere to obtain TGA data.

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

FTIR-ATR spectra of the films were recorded with KBr pellets at wavenumber range of 400-4000 cm^{-1} using 8400S spectrometer (Shimadzu, Japan). Before analysis, the film samples were kept in desiccator with silica gel for 1 week at room temperature to obtain the most dehydrated films.

2.3.10. 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 (LSD) test at $p \leq 0.05$.

3. Results and discussion

3.1. Morphology of multi-layer film

3.1.1. Microstructural analysis by SEM

The primary objective with this work was to make uniform multi-layer films of FG onto PLA to enhance its gas barrier properties. In a detailed study, the importance of whether FG structure could be formed on PLA substrates was regarded. At a macroscopic level, a good adhesion of the FG coating on the PLA substrate with a good integrity was observed. The microstructure of films was qualitatively studied using scanning electron microscopy (SEM), and high-resolution SEM cross-section images of PLA, FG, and PLA/FG/PLA triple-layer films are shown in Fig. 1. Pure PLA film showed dense, compact and relatively continuous phase (Fig. 1a), whereas neat FG film showed a smooth and more homogeneous structure (Fig. 1 b). Through lateral cryo-fracture of multi-layer structure (Fig. 1c), the FG interlayer could be easily identified as a relatively thick inner layer in comparison to the overall thickness of multi-layer, exhibiting strong adherence to the outer matrices (i.e. PLA). In the present study, a good adhesion between each layer of the triple-layer structure was formed without surface modification methods such as corona discharge, flame and plasma treatments, and chemical etching [22]. Similar results were observed in multi-layer films based on PLA and soy protein isolate (SPI) [16]. These authors demonstrated that each layer possibly worked as an adhesive between film layers, i.e., the middle layer worked like water-borne adhesive and the outer PLA layer worked as a solvent-borne adhesive. It can be assumed that the strong bond strength was induced by means of hydrogen interaction between carbonyl groups in PLA and amino groups of gelatin.

3.1.2. AFM images

Topographical imaging was conducted by AFM to measure the surface roughness of film sample. Fig. 2 shows AFM surface images of pure PLA, FG, and designed multi-layer films, which clearly shows the difference in surface morphology of the films. Both PLA (Fig. 2a) and FG (Fig. 2b) films exhibit smooth surfaces with the low RMS values (3.6 and 1.5 nm, respectively). The root-mean-square value of the PLA/FG/PLA multi-layer film (3.2) was not markedly different from virgin PLA and FG, which indicates that the PLA layers were evenly coated over the FG film.

3.2. Gas barrier properties

3.2.1. Oxygen barrier properties

Where food packaging is included, packaged goods will have additional requirements for packing materials including approval for contact with the food and compostability as a disposal attribute [23]. For example, there must be no change in food composition and organoleptic properties (e.g., change in taste) generated by gases, aromas, or water vapor permeating through the packaging. Gas permeability of packaging materials is of great significance for food preservation. Barrier to oxygen in a packaging system can increase the shelf-life of food products and also improve the quality of food [24].

The oxygen permeability (OP) of neat PLA, FG and multi-layer films are displayed in Fig. 3. As mentioned before, in spite of the hydrophilic nature, most protein films are good oxygen barriers at low-to-intermediate relative humidity (RH) [16]. Generally, a remarkable decrease in OP was observed for the FG-coated PLA substrates. The OP of the neat FG film is $0.011 \text{ cm}^3\cdot\text{mm}/\text{m}^2\cdot\text{day}\cdot\text{bar}$, which is markedly lower (~ 18 times) than values reported for extruded beef skin gelatin films containing 0.2% glycerol ($0.195 \text{ cm}^3\cdot\text{mm}/\text{m}^2\cdot\text{day}\cdot\text{bar}$) [25], and is

comparable to the value reported for ethylene vinyl alcohol (EVOH) ($0.001\text{--}0.01\text{ cm}^3\cdot\text{mm}/\text{m}^2\cdot\text{day}\cdot\text{atm}$), which is considered as a high-barrier biodegradable polymer against oxygen and gas [26]. The OP was measured to $5.02\text{ cm}^3/\text{m}^2\cdot\text{day}\cdot\text{bar}$ for the triple-layer film compared with $38.25\text{ cm}^3/\text{m}^2\cdot\text{day}\cdot\text{bar}$ for the pristine PLA, corresponding to a reduction of 8-fold. This superior oxygen barrier is comparable to multi-layer structures consisting of PLA/SPI prepared by Rhim et al. [16]. The enhanced oxygen barrier property of the PLA films may presumably link to the impermeable FG layer coated on the polymer substrate, which act as physical obstacles retarding movement of the gas through the film [27]. Besides impermeability of FG layer, another possible reason for the improved barrier of multi-layer system is the electrostatic attractions between the oppositely charged constituents, which results in a more tightly packed layer [28].

This multi-layer film also shows one of the lowest oxygen permeability reported for bio-based materials. In this regard, it is very valuable when compared to some commercial plastic packaging materials, the OP value of PLA/FG/PLA triple-layer film is several times less than those reported for polypropylene (PP) ($50\text{--}100\text{ cm}^3\cdot\text{mm}/\text{m}^2\cdot\text{day}\cdot\text{atm}$), and polyethylene (PE) ($50\text{--}200\text{ cm}^3\cdot\text{mm}/\text{m}^2\cdot\text{day}\cdot\text{atm}$), as well as polystyrene (PS) ($100\text{--}150\text{ cm}^3\cdot\text{mm}/\text{m}^2\cdot\text{day}\cdot\text{atm}$), and is comparable to that of poly ethylene terephthalate (PET) ($1\text{--}5\text{ cm}^3\cdot\text{mm}/\text{m}^2\cdot\text{day}\cdot\text{atm}$) [26], which is considered to be a good barrier to oxygen. The present study demonstrated that high performance barrier films can be fabricated by using multi-layer technique.

3.2.2. Water vapor barrier properties

The prepared multi-layers have also been tested for water vapor permeability (WVP) (Fig. 4). As mentioned previously, the purpose of designing the current triple-layer structure was using the benefits of each of the films alone (PLA and FG). Similarly to oxygen permeability, the multi-

layer structure shows a significant reduction in WVP, but this time compared to stand-alone FG film. A pristine PLA and FG films exhibited a permeability of 0.065 ± 0.005 and 1.40 ± 0.09 g mm/kPa h m², respectively, which is comparable to the reported values in the literature [15,29]. Fabrication of multi-layer system improved water vapor barrier properties of the FG films dramatically as evidenced by a decrease of up to 91% in WVP value. This means that the water vapor barrier of multi-layer film improved 11-fold in comparison with that of neat FG films and was comparable to that of PLA film. This improvement could be attributed to the structure reached in the multi-layer films and the better barrier properties of PLA film. In spite of the water uptake capacity of fish gelatin, in this multi-layer system, the FG interlayer was protected by the PLA layers, avoiding the plasticization of the protein which could have a negative effect on barrier properties.

Literature describes a multi-layer system which exhibits a similar effect produced by PLA layers in SPI [16]. As far as synthetic polymers are concerned, the WVP values for multi-layer film is 2 orders of magnitude lower than that of permeability value noticed in cellophane films (0.248 g mm/kPa h m²), but higher than that observed in low-density polyethylene (LDPE) films (0.0072 g mm/kPa h m²) [30].

This multi-layer system is, therefore, of great interest since the outer layer (PLA film) provided the hydrophobic character needed to avoid the plasticization of the FG interlayer and the subsequent increase in water vapor permeability, whereas the inner layer (FG film) contributed to improve oxygen barrier properties of these PLA materials, enhancing barrier properties of the obtained multi-layer structures.

3.3. Water resistance

The knowledge of the swelling is also important for the design of packages and predictions of stability and quality changes that may occur during storage [31]. As shown in Table 1, the swelling ratio (SR) of PLA film is very low ($\sim 2\%$), which is attributable to the hydrophobicity of PLA [16]. In the case of the FG film, due to the complete disintegration and/or dissolution of gelatin in the first few minutes, it was not possible to measure the SR of the FG film. So, it is worth noting that the designed triple-layer film kept their shape even after immersion in water for several hours, demonstrating that lamination with hydrophobic PLA layer was effective in restricting gelatin water absorption [10]. However, the swelling degree percentage of the three-layer film (about 41%) was significantly higher than that of neat PLA which may be linked to the water absorption of the inner gelatin layer. Indeed, when water molecules reach the inner hydrophilic gelatin layer through the unprotected edges of the sample, gelatin swells and eventually dissolves conducting to the separation of the external PLA outer layers [10].

Water sensitivity of gelatin-based films is an important challenge for the replacement of traditional plastics, especially for packaging applications. The poor resistance of gelatin-based films towards water is due to the hydrophilic nature of the protein (presence of polar peptides) and to the substantial amount of hydrophilic plasticizer (glycerol) added to provide enough film elasticity [32]. The water solubility (WS) was determined to evaluate the integrity of the multi-layer film in aqueous environment and results are presented in Table 1. While, both PLA and multi-layer films maintained their integrity after incubation in water for 24 h, individual FG film disintegrated and/or dissolved almost completely after soaking in water already in 2 min. As can be seen in Table 1, water resistance of the FG film increased profoundly by the lamination with PLA layers, i.e., the WS decreased down to about 54% for triple-layered film. The solubility of

the multi-layer film was mainly attributed to the solubilization of hydrophilic inner FG layer due to the penetration of water molecules into the interfacial zone of the film specimen, as formerly concluded from the SR test [10].

3.4. Mechanical properties

Table 1 displays tensile parameters (TS: tensile strength, EM: elastic modulus, EAB: elongation at break) for control PLA, FG and their multi-layer films. The evaluation of these criteria is an important issue, since films intended for food packaging must be resistant to the usual pressure that occurs in transport, application and handling to keep the properties and the integrity of food [5]. As previously mentioned, protein-based films, especially films manufactured from fish gelatin are known to have a rather low mechanical strength. The low tensile strength of FG films was one of the reasons preventing their use as free standing films for food packaging applications. The TS, EM and EAB of neat FG film were 7.48 ± 1.70 MPa, 0.11 ± 0.02 MPa and $80.26 \pm 0.19\%$, respectively, which are in good agreement with previously reported values for FG films [12,33]. As presented in Table 2, the pristine PLA film showed superior mechanical strength but displayed low flexibility value analogue compared to former observations available in literature [5,29,34]. The presence of PLA outer layers on both sides of gelatin interlayer increased the TS more than 3-fold and EM about 15-fold in comparison with the bare FG film (Table 1), indicating that these multi-layer structures were more resistant. Similar effects were observed in multi-layer structures prepared with PLA and SPI [16]. Meanwhile, the TS of triple-layer film is comparable to the values of widely used plastic films, such as LDPE (15.2-78.6 MPa), HDPE (17.9-33.1 MPa) [35] and film grade polypropylene (20-152 MPa).

3.5. Optical properties

Color and transparency are essential parameters in many applications, especially in food packaging, where visual inspection of the contents is of key relevance that could influence the decision of consumers [34]. Apparent color properties, percent transmittance at 600 nm (T_{600}), and opacity values of the multi-layer films along with those of single components are shown in Table 2. Visually, both pristine films as well as triple-layer film were clear and transparent. However, some differences in the CIELab coordinates (L^* , a^* , b^*) and full color variation (ΔE) between all films were observed (Table 2). Apparent color of the film samples determined by Hunter color values indicate that the ΔE of the neat PLA film slightly decreased after formation of multi-layer system (Table 2), which is mainly due to the increase in Hunter L - and a -values and decrease in b -values. Furthermore, UV-vis spectroscopy reveals that these films exhibit promising transparency at 600 nm (Table 2). The light transmittance for pure PLA film was 78.43%, while FG and triple-layer films exhibited a light transmission of 91.83% and 85.48%, respectively, at 600 nm. So, the addition of the FG transparent interlayer significantly increased the transmittance values of these multi-layer structures ($p < 0.05$).

In terms of opacity, this parameter decreased from 2.90 ± 0.36 for the control PLA film to 1.54 ± 0.08 for multi-layer films ($p < 0.05$) (Table 1). Given the fact that no significant differences ($p > 0.05$) were reported in thickness values, the markedly decreased opacity value of the triple-layer films compared to that of pristine PLA film gave indirect evidence of the compatibility between neighboring layers [16]. Moreover, transparency is a typical characteristic of protein-based films/coatings and it is one of the advantages of using gelatin in multi-layer structure. With regard to synthetic polymers, PLA/FG/PLA multi-layer film opacity was lower than those reported by Dias et al. [36] for LDPE films, which is one of the most common polyethylene

types used for food packaging. This result suggested that multi-layer systems based on PLA and FG were suitable for the manufacture of transparent films for food packaging.

3.6. Thermal characterization

3.6.1. Differential scanning calorimetry (DSC)

DSC analysis was conducted in order to evaluate the fish gelatin interlayer effect on the glass transition temperature (T_g), crystallization (T_c) and melting (T_m) phenomena of PLA/FG/PLA systems. The DSC curve of neat PLA film displayed the T_g at 44.3 °C [37], while the exothermic crystallization showed its maximum at T_c (84 °C) and the endothermic melting peak was observed at T_m (166.4 °C) (Fig. 5).

Thermograms of FG films exhibited T_g at about 29.8 °C, which is similar to that of film prepared from walleye pollock gelatin (29 °C) [38], but was lower than T_g value reported for cuttlefish skin gelatin film (60.3 °C) [39]. Differences in T_g between films could be due to the differences in type, the amount of plasticizer used and the source of gelatin. The T_m value for FG film was about 73.0 °C, which is very close to films made from tilapia skin gelatin [40], who found T_m of 76.5 °C. Rahman et al. [41] expressed that the endothermic peak for fish gelatin film was connected to the helix-coil transition and disruption of molecular ordered structure.

DSC curves for the multi-layer film showed a slight shift of T_g to lower temperature (41.6 °C) than those obtained in neat PLA, due to the plasticization effect of gelatin. It is worthwhile to note that the multi-layer film showed only one distinguishing T_g value ranged between those of the PLA (44.3 °C) and FG (29.8 °C) as noticed in DSC thermograms. This provides indirect evidence for the compatibility between the materials [16].

As indicated in Fig. 5, while the neat PLA film showed the exothermic crystallization peak at T_c (84 °C), the exothermic peak broadened gradually and shifted to right in multi-layer film, suggesting a slow rate of crystallization [42]. Finally, the appearance of tertiary peak of PLA film, which is assigned to the T_m of the material, did not show remarkable changes for the multi-layer film and T_m slightly decreased from 166.4 °C (neat PLA) to 165.5 °C in PLA/FG/PLA triple-layer film. From these DSC results, it can be concluded that FG interlayer deposition affected the structure of the final PLA-based multi-layer system, since glass transition temperature was partially reduced in relation to the neat PLA film. According to Pankaj et al. [43] it may be noted that T_g is an important parameter dictating the success in industrial processing of PLA polymer.

3.6.2. Thermogravimetric analysis (TGA)

TGA analysis was also performed to evaluate the effect of FG interlayer on the thermal stability of multi-layer systems. The thermal decomposition behavior was determined by TGA analysis, and their TGA and DTG curves are shown in Fig. 6. Neat PLA film degraded in a single step process with a maximum degradation peak (T_{max}) centered at 365 °C, in agreement with values formerly reported [27,34]. The FG film showed a multi-step thermal decomposition with a first peak in the range of 50-120 °C, which was attributed to the vaporization of adsorbed and bound water; a second one with a two maximum degradation rate (T_{max}) at 216 and 243 °C associated to the loss of lower molecular weight protein fractions as well as glycerol [44], and a third peak centered at 322 °C assigned to the thermal decomposition of peptide bonds in the main chain of gelatin [10]. Finally, there were two main steps for multi-layer degradation. The first one appeared as a broad range from 50 °C to 180 °C and the second one from 220 °C to 370 °C. It seems the thermal degradation of polymers in multi-layers occurred at a lower temperature than

in the corresponding PLA monolayer film. This indicates that FG diffused from the interface inside the polymer matrices and the induced molecular interactions with both polymers affected their thermal degradation behavior [45].

3.7. FTIR-ATR analysis

The chemical structure of the obtained films was investigated by means FTIR-ATR analysis and the obtained spectra are showed in Fig. 7. In the neat PLA film spectrum (Fig. 7a), the bands at 2993 and 2947 cm^{-1} were assigned to the -C-H stretching [43]. At 1758 cm^{-1} the typical asymmetric stretching of carbonyl group (C=O) in PLA film is attributed to lactide [34]. On the other hand, a sharp peak centered at 1450 cm^{-1} assigned to the lactides -CH₃ group [34]. The negative bands at 1188 and 1087 cm^{-1} are due to the attenuation of PLA peaks due to the limited penetration depth of the IR signal by ATR [46]. The characteristic absorption peaks observed for the bare FG film (Fig. 7b) were: 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) [12]. The band situated at the wavenumber of ~1041 cm^{-1} was also corresponding to the glycerol (-OH group) added as a plasticizer [32]. The analysis of FTIR-ATR spectra of multi-layer films indicated the same spectrum as the bare PLA (Fig. 7c). This result could be considered to be because the penetration depth of the IR signal into the sample is typically between 0.5 and 2 micrometers, while the thickness of the PLA side in the laminated structure is at least 10 micrometers.

Conclusions

In summary, the PLA/FG/PLA multi-layer film was successfully synthesized by solution casting method. Scanning electron microscopy revealed a nearly perfect uniformity of the FG coating on

PLA, and light transmittance measurements showed good transparency of triple-layer films. The resulting multi-layer films showed significant reduction in oxygen (up to 87%) and water vapor permeability (up to 91%) compared with the neat PLA and FG films, respectively. Meanwhile, the water resistance of the virgin FG film was improved greatly via a multi-layer system design. DSC analysis revealed that the materials were compatible showing only one T_g that decreased with FG deposition. However, a shift to lower temperatures of degradation peak was detected in the presence of FG interlayer. On the basis of the obtained results, this super gas barrier film can be postulated as potential substitutes of synthetic oxygen-barrier layers used in multi-layer packaging such as EVOH. Multi-layer

Acknowledgements

The authors would like to thank the Iran National Science Foundation (INSF) (Project No. 93008916) for their financial support of this research. We would also like to thank Dr. Majid Jamshidian (Institut national de la recherche scientifique (INRS)) for the helpful discussions.

References

- [1] A.J. Svagan, A. Åkesson, M. Cárdenas, S. Bulut, J.C. Knudsen, J. Risbo, D. Plackett, Transparent films based on PLA and montmorillonite with tunable oxygen barrier properties, *Biomacromolecules*, 13 (2012) 397-405.
- [2] Robust growth projected for biodegradable plastics through 2020. <http://www.bioplasticsmagazine.com/en/news/meldungen/20150916-Biodegradable-plastics-to-show-robust-growth.php>, 2015 (accessed 16.09.2015).

- [3] J. Hughes, R. Thomas, Y. Byun, S. Whiteside, Improved flexibility of thermally stable polylactic acid (PLA), *Carbohydr. Polym.* 88 (2012) 165-172.
- [4] A. Marra, C. Silvestre, D. Duraccio, S. Cimmino, Polylactic acid/zinc oxide biocomposite films for food packaging application, *Int. J. Biol. Macromol.*, 88 (2016) 254-262.
- [5] I. Armentano, E. Fortunati, N. Burgos, F. Dominici, F. Luzi, S. Fiori, A. Jiménez, K. Yoon, J. Ahn, S. Kang, J. M. Kenny, Bio-based PLA_PHB plasticized blend films: Processing and structural characterization, *LWT-Food Sci. Technol.* 64 (2015) 980-988.
- [6] Z. Javidi, S.F. Hosseini, M. Rezaei, Development of flexible bactericidal films based on poly (lactic acid) and essential oil and its effectiveness to reduce microbial growth of refrigerated rainbow trout, *LWT-Food Sci. Technol.* 72 (2016) 251-260.
- [7] R.E. Conn, J.J. Kolstad, J.F. Borzelleca, D.S. Dixler, L.J. Filer, B.N. LaDu, M.W. Pariza, Safety assessment of polylactide (PLA) for use as a food-contact polymer, *Food Chem. Toxicol.* 33 (1995) 273-283.
- [8] Natureworks awards contract for front end engineering of its new ingeo production facility. <http://www.natureworkslc.com/News-and-Events/Press-Releases/2013/09-24-13> NatureWorks-awards-contract-for-engineering-new-facility, 2013 (accessed 24.09.2013).
- [9] R.A. Auras, B. Harte, S. Selke, R. Hernandez, Mechanical, physical, and barrier properties of poly (lactide) films, *J Plast Film Sheet* 19 (2003) 123-135.
- [10] J.F. Martucci, R.A. Ruseckaite, Biodegradation behavior of three-layer sheets based on gelatin and poly (lactic acid) buried under indoor soil conditions, *Polym. Degrad. Stab.* 116 (2015) 36-44.

- [11] M.C. Gómez-Guillén, M. Pérez-Mateos, J. Gómez-Estaca, E. López-Caballero, B. Giménez, P. Montero, Fish gelatin: a renewable material for developing active biodegradable films, *Trends Food Sci. Technol.* 20 (2009) 3-16.
- [12] S.F. Hosseini, M. Rezaei, M. Zandi, F.F. Ghavi, Preparation and functional properties of fish gelatin–chitosan blend edible films, *Food Chem.* 136 (2013) 1490-1495.
- [13] C.G. Otoni, R.J. Avena-Bustillos, B.S. Chiou, C. Bilbao-Sainz, P.J. Bechtel, T.H. McHugh, Ultraviolet-b radiation induced cross-linking improves physical properties of cold-and warm-water fish gelatin gels and films, *J. Food Sci.* 77 (2012) 215-223.
- [14] M.J. Fabra, A. Lopez-Rubio, J.M. Lagaron, High barrier polyhydroxyalcanoate food packaging film by means of nanostructured electrospun interlayers of zein, *Food Hydrocolloid* 32 (2013) 106-114.
- [15] S.F. Hosseini, M. Rezaei, M. Zandi, F. Farahmandghavi, Fabrication of bio-nanocomposite films based on fish gelatin reinforced with chitosan nanoparticles, *Food Hydrocolloid* 44 (2015) 172-182.
- [16] J.W. Rhim, K.A. Mohanty, S.P. Singh, P.K. Ng, Preparation and properties of biodegradable multilayer films based on soy protein isolate and poly (lactide), *Ind. Eng. Chem. Res.* 45 (2006) 3059-3066.
- [17] ASTM, D 3985-05, Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulometric sensor, 2005.
- [18] ASTM, E 96-05, Standard test method for water vapor transmission of materials, 2005.

- [19] T.H. McHugh, R. Avena-Bustillos, J.M. Krochta, Hydrophilic edible films: modified procedure for water vapor permeability and explanation of thickness effects, *J. Food Sci.* 58 (1993) 899-903.
- [20] ASTM, D882-02, Standard test method for tensile properties of thin plastic sheeting, 2002.
- [21] B. Cuq, N. Gontard, J.L. Cuq, S. Guilbert, Functional properties of myofibrillar protein-based biopackaging as affected by film thickness, *J. Food Sci.* 61 (1996) 580-584.
- [22] J.W. Lee, S.M. Son, S.I. Hong, Characterization of protein-coated polypropylene films as a novel composite structure for active food packaging application, *J. Food Eng.* 86 (2008) 484-493.
- [23] H.J. Endres, A. Siebert-Raths, Performance profile of biopolymers compared to conventional plastics, in: M. Moeller, K. Matyjaszewski, (Eds.), *Polymer Science: A Comprehensive Reference*, Elsevier Science, Amsterdam Netherland, 2012, pp. 317-353.
- [24] A.L. Brody, B. Bugusu, J.H. Han, C.K. Sand, T.H. Mchugh, Innovative food packaging solutions, *J. Food Sci.* 73 (2008) 107-116.
- [25] Z.N. Hanani, J. McNamara, Y.H. Roos, J.P. Kerry, Effect of plasticizer content on the functional properties of extruded gelatin-based composite films, *Food Hydrocolloid* 31 (2013) 264-269.
- [26] J. Lange, Y. Wyser, Recent innovations in barrier technologies for plastic packaging-a review, *Packag Technol Sci* 16 (2003) 149-158.
- [27] J.W. Rhim, Preparation and characterization of vacuum sputter silver coated PLA film, *LWT-Food Sci. Technol.* 54 (2013) 477-484.

- [28] K. Halász, Y. Hosakun, L. Csóka, Reducing water vapor permeability of poly (lactic acid) film and bottle through layer-by-layer deposition of green-processed cellulose nanocrystals and chitosan, *Int J Polym Sci.* 2015.
- [29] Z.Ö. Erdohan, B. Çam, K.N. Turhan, Characterization of antimicrobial polylactic acid based films, *J. Food Eng.* 119 (2013) 308-315.
- [30] C. Péroval, F. Debeaufort, D. Despré, A. Voilley, Edible arabinoxylan-based films. 1. Effects of lipid type on water vapor permeability, film structure, and other physical characteristics, *J. Agric. Food. Chem.* 50 (2002) 3977-3983.
- [31] R. Andrade, R. Lemus, C. Perez, Models of sorption isotherms for food: uses and limitations, *Vitae Rev. Fac. Quimica Farm.* 18 (2011) 325-334.
- [32] S.F. Hosseini, M. Rezaei, M. Zandi, F. Farahmandghavi, Development of bioactive fish gelatin/chitosan nanoparticles composite films with antimicrobial properties. *Food Chem.* 194 (2016) 1266-1274.
- [33] J. Wu, H. Liu, S. Ge, S. Wang, Z. Qin, L. Chen, Q. Zheng, Q. Liu, Q. Zhang, The preparation, characterization, antimicrobial stability and *in vitro* release evaluation of fish gelatin films incorporated with cinnamon essential oil nanoliposomes, *Food Hydrocolloid* 43 (2015) 427-435.
- [34] M.P. Arrieta, J. López, A. Hernández, E. Rayón, Ternary PLA-PHB-Limonene blends intended for biodegradable food packaging applications, *Eur. Polym. J.* 50 (2014) 255-270.
- [35] L.R. Castilho, D.A. Mitchell, D.M. Freire, Production of polyhydroxyalkanoates (PHAs) from waste materials and by-products by submerged and solid-state fermentation, *Bioresour. Technol.* 100 (2009) 5996-6009.

- [36] M.V. Dias, H.S. de Medeiros, N.D. Soares, N.R. de Melo, S.V. Borges, J.D. Carneiro, J.M. de Assis Kluge, Development of low-density polyethylene films with lemon aroma, *LWT-Food Sci. Technol.* 50 (2013) 167-171.
- [37] M. Llana-Ruiz-Cabello, S. Pichardo, A. Baños, C. Núñez, J.M. Bermúdez, E. Guillamón, S. Aucejo, A.M. Cameán, Characterisation and evaluation of PLA films containing an extract of *Allium* spp. to be used in the packaging of ready-to-eat salads under controlled atmospheres, *LWT-Food Sci. Technol.* 64 (2015) 1354-1361.
- [38] Z. Liu, X. Ge, Y. Lu, S. Dong, Y. Zhao, M. Zeng, Effects of chitosan molecular weight and degree of deacetylation on the properties of gelatine-based films, *Food Hydrocolloid* 26 (2012) 311-317.
- [39] M. Jridi, S. Hajji, H.B. Ayed, I. Lassoued, A. Mbarek, M. Kammoun, N. Souissi, M. Nasri, Physical, structural, antioxidant and antimicrobial properties of gelatin–chitosan composite edible films, *Int. J. Biol. Macromol.* 67 (2014) 373-379.
- [40] Y. Pranoto, C.M. Lee, H.J. Park, Characterizations of fish gelatin films added with gellan and κ -carrageenan, *LWT-Food Sci. Technol.* 40 (2007) 766-774.
- [41] M.S. Rahman, G.S. Al-Saidi, N. Guizani, Thermal characterisation of gelatin extracted from yellowfin tuna skin and commercial mammalian gelatin, *Food Chem.* 108 (2008) 472-481.
- [42] C. Xu, X. Luo, X. Lin, X. Zhuo, L. Liang, Preparation and characterization of polylactide/thermoplastic konjac glucomannan blends, *Polymer* 50 (2009) 3698-3705.

- [43] S.K. Pankaj, C. Bueno-Ferrer, N.N. Misra, L. O'Neill, A. Jiménez, P. Bourke, P.J. Cullen, Characterization of polylactic acid films for food packaging as affected by dielectric barrier discharge atmospheric plasma, *Innov Food Sci Emerg* 21 (2014) 107-113.
- [44] P. Kanmani, J.W. Rhim, Physical, mechanical and antimicrobial properties of gelatin based active nanocomposite films containing AgNPs and nanoclay, *Food Hydrocolloid* 35 (2014) 644-652.
- [45] R. Ortega-Toro, I. Morey, P. Talens, A. Chiralt, Active bilayer films of thermoplastic starch and polycaprolactone obtained by compression molding, *Carbohydr. Polym.* 127 (2015) 282-290.
- [46] F. Carosio, S. Colonna, A. Fina, G. Rydzek, J. Hemmerlé, L. Jierry, et al. Efficient gas and water vapor barrier properties of thin poly (lactic acid) packaging films: Functionalization with moisture resistant nafion and clay multilayers. *Chem. Mater.* 26 (2014) 5459-5466.

Figure (s)

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Table 1
Thickness, water resistance (swelling ratio (SR) and water solubility (WS)) and tensile properties (tensile strength (TS), elastic modulus (EM) and elongation-at-break (EAB)) of individual films and their multi-layer structure.

Film	Thickness (μm)	Water resistance		Tensile properties		
		SR (%)	WS (%)	TS (MPa)	EM (GPa)	EAB (%)
PLA	38.00 ± 2.00 ^a	2.10 ± 0.84 ^a	5.11 ± 0.90 ^a	47.18 ± 6.01 ^a	1.39 ± 0.15 ^a	2.82 ± 0.46 ^a
FG	41.66 ± 1.52 ^a	ND ^{*b}	100.00 ± 0.00 ^b	7.48 ± 1.70 ^b	0.11 ± 0.02 ^b	80.26 ± 0.19 ^b
Multi-layer	40.33 ± 3.05 ^a	41.02 ± 2.12 ^c	54.35 ± 1.69 ^c	25.00 ± 2.13 ^c	1.71 ± 0.03 ^c	1.57 ± 0.07 ^c

Different letters in the same column indicate significant differences ($p < 0.05$). *ND: Could not determined due to disintegration after immersion in water.

Table 2

Colour parameters (CIELab) and total color difference (ΔE), light transmission (%T), and opacity values of individual films and their multi-layer structure.

Film	Colour parameters				%T	Opacity (UA/mm)
	L^*	a^*	b^*	ΔE		
PLA	90.59 ± 0.45^a	-0.97 ± 0.07^a	3.04 ± 0.05^a	4.49 ± 0.59^a	78.43 ± 2.45^a	2.90 ± 0.36^a
FG	91.08 ± 0.31^a	-1.05 ± 0.01^a	3.61 ± 0.02^b	4.75 ± 0.18^a	91.83 ± 0.20^b	0.88 ± 0.06^b
Multi-layer	90.78 ± 0.52^a	-0.48 ± 0.07^b	2.42 ± 0.08^c	3.22 ± 0.25^b	85.48 ± 0.83^c	1.54 ± 0.08^c

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