Heliyon 11 (2025) e41430

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Designing and characterization of bilayer films based on bitter vetch seed protein and polylactic acid for enhanced food packaging

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ARTICLE INFO

Keywords:
Bilayer film
Bitter vetch seed protein
Polylactic acid
Physicochemical properties
Morphological characteristic

ABSTRACT

Packaging films based on natural biopolymers often suffer from inadequate barrier and mechanical properties. To address these challenges, multilayer films have emerged as potential solutions. In this study, we prepared bilayer films using bitter vetch seed protein (BVSP) and polylactic acid (PLA). The barriers, mechanical, physical, chemical, thermal and morphological properties of the prepared films were evaluated. The bilayer films exhibited robust adhesion between the BVSP and PLA layers without any chemical modification or additional components. This physical interaction between the layers contributed to the overall film properties. The addition of the PLA layer to BVSP films led to reduced solubility and moisture content in the bilayer films compared to pure BVSP films. The bilayer films demonstrated good tensile strength, ranging from 13.46 to 15.51 MPa, surpassing the BVSP films (ranging from 5.66 to 6.25 MPa). Additionally, the bilayer films exhibited better elongation at break, ranging from 17.91 % to 27.68 %, compared to pure PLA films (ranging from 14.95 % to 23.28 %). The bilayer films exhibited reduced water vapor permeability (WVP), ranging from 1.40×10^{-10} to 6.55×10^{-10} g/Pa.s.m, in contrast to the BVSP film (ranging from 1.02×10^{-9} to 1.57×10^{-9} g/Pa.s.m). This improvement was attributed to the hydrophobic nature of the PLA layer. Thermal decomposition curves revealed that the BVSP/PLA bilayer film exhibited greater stability than the pure BVSP film. Based on our results, bilayer films with superior properties compared to pure BVSP films are promising candidates for food packaging applications.

1. Introduction

Polymeric films based on petroleum are used in different industries, especially food applications. In recent centuries, these plastics have increased environmental and health problems. As a result, various bio-based polymers with good biodegradable and physicochemical properties have been used in the last decades [1]. Scientists try to replace non-degradable plastics with renewable biopolymers that possess unique properties of plastic materials, such as effective processing, to extend the shelf life of food products, protect them from microbial spoilage and reduce environmental pollution [2]. Among all biopolymers, those with antioxidant and antimicrobial properties have attracted significant attention due to their potential for use in the preparation of active films. Many biodegradable biopolymers like starch, chitin, lignin, cellulose, proteins and polylactic acid (PLA) have been used in different fields

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https://doi.org/10.1016/j.heliyon.2024.e41430
Received 28 June 2024; Received in revised form 20 December 2024; Accepted 20 December 2024

[3].

Among the natural biomaterials, proteins are suitable options to replace petroleum-based polymers [4]. However, only some commercial products using proteins as raw material have been produced. Some protein sources like several species of the leguminous family are low-priced and usually used for animal feeding. One of these species, bitter vetch (*Vicia ervilia*) is generally cultivated throughout the mild regions of Europe, western and central Asia, the Americas and North Africa for using its seeds and hay. Bitter vetch (BV) is an annual grain legume crop grown only for fodder due to its high nutritional value. It has several desirable characteristics like high yields, low price and high protein content. Seeds of BV contain up to 25 % protein. Protein of BV was studied as an available source to produce edible coatings, biodegradable films and biodegradable shaped containers [5]. The primary amino acids found in BV seeds are glutamic acid, aspartic acid, arginine, lysine and leucine. In contrast, BV seeds are poor in sulfur amino acids like methionine and cysteine.

Protein films usually suffer from high water vapor permeation and weak mechanical properties. There are some methods to improve these problems of protein films like composite and multilayer films. Multilayer systems have achieved importance in various applications such as food packaging, to increase the shelf life of foods [6]. PLA was chosen as the second layer of bilayer films to improve the properties of protein films in this study.

PLA is the most popular thermoplastic material for food packaging due to its low cost, flexibility and biodegradable properties. Furthermore, PLA has attracted researchers' attention because of its high molecular weight, good workability and high-water solubility resistance [7]. PLA is an aliphatic, linear and thermoplastic polyester produced from lactic acid. Lactic acid is generated from the microbial fermentation of glucose gained from biomaterials such as sucrose or lactose in corn, sugar beets or wheat starch [8]. PLA can develop firm and high-transparency films with low water vapor permeability (WVP). Nevertheless, PLA shows limitations, as it displays brittleness, mechanical fragility and limited oxygen barrier properties [9]. In recent years, researchers developed bilayer films based on PLA/cassava starch [10], soy protein isolate (SPI)/PLA [11], gliadin and PLA [12], fish gelatin (FG) and PLA [13].

PLA/cassava starch bilayer films showed better resistance to moisture and tensile strength in comparison to starch monolayer films [10]. Weak mechanical and barrier properties of protein films improved after SPI/PLA bilayer films preparation [11]. Improved moisture barrier and mechanical properties of gliadin film and oxygen barrier property of PLA film were the advantages of gliadin/PLA films [12]. FG/PLA films showed less moisture permeability in comparison to pure gelatin film [13]. Arabestani et al. [14] designed bilayer films based on bitter vetch seed protein (BVSP) and zein. Their results showed that designed bilayer films had better mechanical and moisture resistance in comparison to both pure protein films.

This study aims to prepare biodegradable bilayer films with improved mechanical and physical properties from BVSP and PLA for the first time. BVSP was extracted and used for the fabrication of bilayer film with PLA. Glycerol was used as a plasticizer in preparing BVSP films. The morphological, physicochemical and mechanical properties of mono and bilayer films were evaluated.

2. Materials and methods

2.1. Materials

BV seeds were purchased from a local market (Isfahan, Iran). Pellets of PLA were obtained from Chemiekas Gmbh Co. (Wien, Austria). DPPH (1,1-diphenyl 2-picrylhydrazyl), glycerol and chloroform were bought from Merck Company (Darmstadt, Germany). All other chemical materials had analytical grades.

2.2. Extraction of BVSP

Proteins were extracted from the BV seeds according to the method of Shrestha et al. [15] with some modifications. The BV seed powder was solubilized in alkaline water (1:10 w/v) at pH 11 under a magnetic stirrer (IKA® RH basic 2, Germany) for 1.5 h at room temperature (25 °C). The prepared mixture was centrifuged at 5000×*g* for 15 min at 25 °C (Hermle Z 36 HK, Germany). The resultant supernatant was collected and the pH was adjusted to 5.4 by HCl (1 N). Eventually, the obtained proteins were dried with a freeze-dried (Sanat Pardaz Dena Co., Ltd., Iran).

2.3. Preparation of bilayer films

The bilayer film based on PLA and BVSP was prepared according to the method of Kamali et al. [16] with some modifications. Various concentrations of BVSP (2.5, 3 and 3.5 %) and PLA (1, 1.5 and 2 %) were used for preparing bilayer films based on the casting method. PLA solution was prepared by dissolving PLA pellets into the chloroform under magnetic stirring for 8 h. Then, the developed solutions were poured uniformly into glass Petri dishes and dried at room temperature for 4 h. Afterward, the BVSP solution was prepared by dissolving BVSP powder in distilled water (pH = 11) under constant stirring for 90 min. The dispersion was heated at 85 °C for 20 min. Glycerol (40 % w/w of BVSP) was added as a plasticizer and stirring was carried out for 30 min. These dispersions were poured into the glass Petri dishes containing the PLA layer and finally dried at room temperature for 72 h. BVSP and PLA pure films were produced with the same method. Finally, all the films were peeled off and stored in a desiccator containing $Ca(NO_3)_2$ solution (RH = 55 %).

2.4. Films characterization

2.4.1. Thickness

Film thickness was characterized using a micrometer (Mitutoyo-Co, Japan) with a sensitivity of 0.001 mm. The average thickness of each film sample was calculated from 10 random points [4].

2.4.2. Water solubility

The water solubility of film samples was determined according to the method of da Silva Bruni et al. [17]. Film pieces (2 × 2 cm^2) were dried in an oven (Memmert, Germany) at 105 °C for 24 h and weighed (W_i). Then, they were immersed in a beaker containing 30 mL distilled water and shaked at 200 rpm for 18 h at 25 °C. The insoluble samples of films were separated by filter paper (Whatman, grade 41) and dried in the oven at 105 °C to obtain the final dry weight (W_f). The solubility of films was calculated by equation (1):

$$\text{Water Solubility (\%)} = \frac{(\mathrm{W_i} - \mathrm{W_f})}{\mathrm{W_i}} \times 100 \tag{1}$$

2.4.3. Moisture content

The moisture content of prepared films was determined according to the method of Dodange et al. [18]. The weight of small film pieces (2 × 2 cm^2) was measured before (W_i) and after (W_d) drying in an oven at 105 °C for 24 h. The moisture content of films was calculated using equation (2):

$$\text{Moisture content (\%)} = \frac{(W_i - W_d)}{W_i} \times 100 \tag{2}$$

2.4.4. Color and opacity

The color parameters (L*, a* and b*) of the film samples were determined using a colorimeter (Nippon Denshoku model ZE 6000, Japan). The absorbance of the film samples (1 cm × 4 cm) was measured at 600 nm to measure the opacity. Then, equation (3) was used [7]:

$$\text{Opacity} = \frac{A}{t} \tag{3}$$

where, A is the absorbance of film samples at 600 nm wavelength and t is the films mean thickness (mm).

2.4.5. Mechanical properties

Mechanical properties of film samples, including tensile strength (TS), elongation at break (EB), and Young's modulus (YM), were measured using a Universal Testing Machine (BONGSHIN Model DBBP-100, Iran). For this purpose, film samples with dimensions of 4.5 cm × 1.5 cm were conditioned at 50 ± 5 % relative humidity for 48 h, and then placed between two grips (45 mm gauge length and 50 mm/min speed) at room temperature. TS, EB, and YM were calculated using equations (4)–(6), respectively [19].

$$Tensile\ strength = \frac{F_{max}}{A} \tag{4}$$

$$Elongation\ at\ break = \frac{L_{max}}{L} \times 100 \tag{5}$$

$$Young's\ modulus = \frac{(F \times L_0)}{(A \times \Delta L)} \tag{6}$$

where, F_{max} is the maximum load (N), A is the cross-section area (m^2), L_{max} is the extension at rupture (m), L_0 is the initial length of the film sample strips (m), F is the force (N) and ΔL is changes in the length (m).

2.4.6. Water vapor permeability

WVP of films was characterized according to the method of Maroufi et al. [20]. The test was done using glass cups (4.5 cm height and 1.5 cm diameter) containing 3 g of anhydrous calcium chloride ($CaCl_2$). Film samples were attached to an 8 mm hole in the glass cup lid. The initial weight of each cup was determined and then placed into a desiccator containing distilled water (RH = 100 %) at 25 °C. The weight of absorbed water vapor by anhydrous $CaCl_2$ in cups was gravimetrically checked every 24 h for 7 days. The WVP (g/Pa.s.m) was calculated using equation (7).

$$\text{WVP} = \frac{(WVTR \times X)}{(A \times \Delta P)} \tag{7}$$

where, WVTR is the water vapor transmission rate (g/s), x is the thickness of films (m), A is the surface area of the films exposed to moisture transfer (m^2) and ΔP is the partial pressure difference of water vapor (Pa).

2.4.7. Contact angle

The water contact angles of film samples were carried out using a goniometer (Kruss G10, Germany). One droplet of deionized water was dropped on the film with a special injection syringe and contact angle was measured.

2.4.8. Antioxidant activity

The method described by Dodange et al. [4] with slight modifications, was used for measuring the antioxidant activity of the selected bilayer film (containing 2 % PLA and 3 % BVSP). Film sample (80 mg) was agitated with distilled water at ambient temperature (25 °C) and 200 rpm for 24 h to extract the antioxidant compounds from the films. Subsequently, 100 μL of the supernatant was added to a 3.9 mL ethanolic solution of DPPH (25 mg/L). The solution was kept in a dark place for 30 min and then the absorbance was measured at 517 nm. The antioxidant activity of the films was determined based on their ability to scavenge DPPH radicals using equation (8).

$$\textit{Ability of DPPH scavenging} = \frac{(A_{DPPH} - A_s)}{A_{DPPH}} \times 100 \tag{8}$$

where, A_{DPPH} and A_s show absorbance of DPPH solution (blank sample) and the sample, respectively.

2.4.9. Biodegradability

Film samples were cut into 2 × 2 cm^2 and buried under 2 cm of compost (purchased from the local market in Isfahan, Iran, with a relative humidity of 57 % and pH = 6.3). The samples were kept at ambient temperature under aerobic conditions. At specific time intervals (10, 20, 30 and 60 days), the films were retrieved from the compost and photographs were taken [21].

2.4.10. Chemical structure

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy (Bruker model Tensor 27, Germany) was used for studying functional groups and probably chemical interactions of the obtained films. All spectra were recorded over the wavenumber range of 600–4000 cm^{-1}.

2.4.11. Thermal analysis

Thermal gravimetric analysis (TGA) (NETZSCH STA 449F3 Jupiter®, Germany) was used to determine the thermal characteristics of pure BVSP, PLA and bilayer of PLA/BVSP films. Film samples (5 mg) were encapsulated in an aluminum pan and heated from room temperature to 500 °C at a heating rate of 10 °C/min to determine the thermal behavior and the mass loss of samples at different temperatures.

2.4.12. Morphology

The surface and cross-section microstructure of pure PLA, BVSP and bilayer films were investigated by scanning electron microscope (FE-SEM) (ZEISS, SIGMA, Germany). Film samples were fractured by immersion in liquid nitrogen to generate cross-sections. Before analysis, the film samples were mounted on an aluminum holder and allowed to sputter with a thin layer of gold to make the film samples conductive.

2.5. Statistical analysis

The effect of different contents of PLA and BVSP (Table 1) on the properties of the prepared bilayer films was studied in a completely randomized design and all measurements were performed in three replications. Statistical analysis was done using the ANOVA method with SAS software (version 9.4, 2016, USA). The means were compared using Duncan's multiple range tests ($p < 0.05$).

Table 1
Different contents of BVSP and PLA in the preparation of bilayer films.

Sample	PLA (%)	BVSP (%)	Sample	PLA (%)	BVSP (%)
$PLA_0,Pro_{2.5}$	0	2.5	$PLA_{1.5},Pro_{3.5}$	1.5	3.5
PLA_0,Pro_3	0	3	$PLA_2,Pro_{2.5}$	2	2.5
$PLA_0,Pro_{3.5}$	0	3.5	PLA_2,Pro_3	2	3
$PLA_1,Pro_{2.5}$	1	2.5	$PLA_2,Pro_{3.5}$	2	3.5
PLA_1,Pro_3	1	3	PLA_1,Pro_0	1	0
$PLA_1,Pro_{3.5}$	1	3.5	$PLA_{1.5},Pro_0$	1.5	0
$PLA_{1.5},Pro_{2.5}$	1.5	2.5	PLA_2,Pro_0	2	0
$PLA_{1.5},Pro_3$	1.5	3			

3. Results and discussion

3.1. Properties of designed bilayer films

3.1.1. Thickness

Thickness is one of the most important physical properties of films which impacts the water vapor permeation of the prepared films. The thickness of pure and bilayer films with different amounts of PLA and BVSP are shown in Fig. 1A. The results showed that the thickness of pure PLA and pure BVSP films increased from 0.05 to 0.1 mm and 0.16–0.25 mm, respectively, by increasing the solid contents of the films. The thickness of bilayer films was 0.20–0.35 mm. Among film samples, the films produced with only PLA displayed the lowest thickness while the highest thickness belonged to bilayer films. The thickness of all film samples increased with enhancing the amounts of PLA and BVSP which is related to more solid matter in the film matrix [4]. Since no other compound was used in the preparation of PLA films, interactions between PLA molecules were strong, resulting in a compact structure. In contrast, glycerol was employed as a plasticizer during the preparation of protein films. Glycerol molecules interpose between the protein chains, reducing intensive intermolecular interactions among them. Consequently, the compression of the protein films decreased, leading to thicker films compared to PLA films [22].

3.1.2. Water solubility

High solubility in water is a crucial feature defining the biodegradability of films used for food product packaging. Conversely, lower solubility of packaging film indicates that the packaging will exhibit good stability when placed in a moist environment. The water solubility of bilayer and pure films based on BVSP and PLA are shown in Fig. 1B. The pure BVSP films and PLA films exhibited the highest and lowest solubility, respectively. The high solubility of BVSP films results from the hydrophilic structure of protein and the presence of abundant hydroxyl groups in BVSP film. In contrast, films based on PLA are hydrophobic. Increasing the concentration of BVSP and PLA had no significant effect on the solubility of the pure films ($p > 0.05$). The solubility of bilayer films ranged from 21.77 to 26.10 %. The reduction in water solubility in bilayer films compared to BVSP films is attributed to the hydrophobic PLA layer [8]. Edible films with low water solubility are suitable for protecting foods with high and intermediate humidity [23]. The decreased water solubility of bilayer films compared to pure protein films is an advantage of the prepared bilayer film for food packaging. Similar results were observed by González and Igarzabal [11] who reported that the solubility of pure SPI film was higher (83.4 %) compared to SPI/PLA bilayer films (40.1 %). Our results demonstrate better water resistance in both pure BVSP and bilayer films which can be attributed to the more hydrophilic structure of SPI compared to BVSP. Additionally, Gürler et al. [8] reported a similar trend for starch/PLA bilayer film.

3.1.3. Moisture content

The moisture content of bilayer and pure films based on BVSP and PLA is presented in Fig. 1B. The results indicate that the moisture content of protein films was higher than other films. The primary reason for the elevated moisture content in BVSP films lies in the presence of glycerol as the plasticizer. Glycerol can form hydrogen bonds with water molecules, effectively retaining water within the film structure. The moisture content of bilayer films decreased upon the addition of PLA layer (acting as a hydrophobic material) to the protein layer. However, the effect of PLA and BVSP amounts was not statistically significant ($p > 0.05$). PLA exhibits lower water absorption and water solubility compared to protein due to its hydrophobic nature. These findings align with Gürler et al. [8] study on bilayer films based on PLA and starch (moisture content of starch, starch/PLA and PLA were 22.22, 9.51 and 0.48 %, respectively) and Scaffaro et al. [1] investigation of bilayer films based on PLA and MaterBi (moisture content of PLA, MaterBi and bilayer films were 0.46, 0.71 and 0.65 %, respectively).

3.1.4. Color and opacity

Color parameters play a crucial role in various applications, including food packaging. The color of packaging films is influenced by several factors such as film structure, temperature and pH during film preparation. Generally, higher temperature and alkaline pH

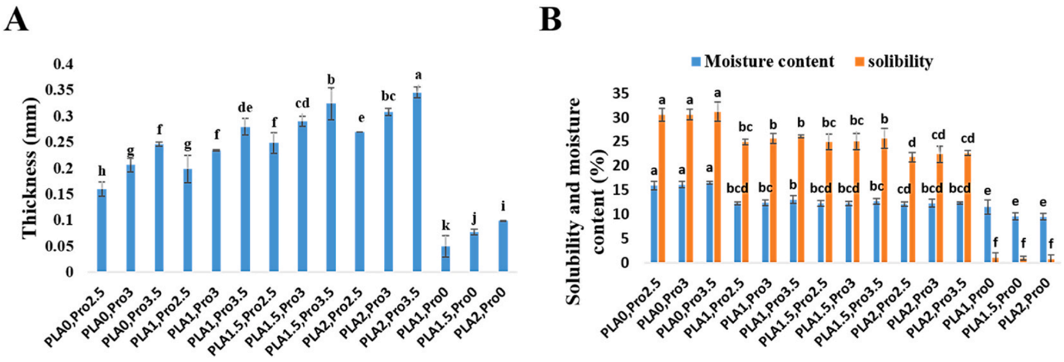


Fig. 1. Thickness (A), water solubility and moisture content (B) of PLA, BVSP and PLA/BVSP bilayer films.

cause darker films because basic solvents tend to extract more colorants compared to other solvents during film preparation. Additionally, the velocity and intensity of colorant extraction increase at higher temperatures. The color parameters of L* (lightness/darkness), a* (greenness/redness) and b* (blueness/yellowness) of pure films and bilayer films with varying amounts of PLA and BVSP are shown in Table 2. PLA films were colorless. Among all the prepared films, the PLA film exhibited the highest L* (ranging from 92.02 to 96.01), but the lowest a* (0.00–0.06) and b* values (1.05–1.74). In contrast, BVSP films displayed red to brown colors. Their L*, a* and b* values were 36.23–42.22, 33.82–34.75 and 56.51–57.17, respectively. Similar color characteristics for BVSP films were reported by Arabestani et al. [14]. The dark color of BVSP films can be attributed to the high temperature and used pH during their preparation. In bilayer films, the PLA layer led to increase in L* (39.57–56.46) and decrease in a* (21.52–28.62) and b* values (51.84–53.63) compared to pure BVSP films. The effects of PLA and BVSP on L* and a* were statistically significant ($p < 0.05$) while the effect on b* was not significant ($p > 0.05$). Similar results were reported by Nilsuwan et al. [24] who found that the L* and a* values of PLA film (90.53 and −1.26, respectively) were higher than those of FG film (89.82 and −1.43).

Opacity values of bilayer films compared to BVSP and PLA pure films are shown in Table 2. The opacity of pure BVSP films (ranging from 2.91 to 3.19) was significantly ($P < 0.05$) higher than that of the other films. Notably, the opacity value for pure PLA films (ranging from 0.25 to 0.78) was low, rendering them transparent. Furthermore, bilayer films containing PLA exhibited greater transparency than pure BVSP films, with an opacity range of 2.28–2.56. However, the impact of varying amounts of PLA and BVSP on the opacity of bilayer films was not statistically significant ($p > 0.05$). Generally, the addition of a PLA layer led to decreased opacity and increased transparency in bilayer films. These findings align with the results reported by González and Igarzabal [11] who observed that SPI/PLA bilayer films were more transparent than SPI film.

3.1.5. Mechanical properties

Desirable mechanical properties play a crucial role in safeguarding food products from production to consumption. Packaging films with high TS and enhanced flexibility are particularly well-suitable for food packaging applications. Mechanical properties including TS, EAB and YM of pure PLA, BVSP and bilayer films with varying amounts of BVSP and PLA are shown in Fig. 2A, B, and C. Notably, pure PLA films exhibited the highest TS (ranging from 33.20 to 43.90 MPa) and YM (ranging from 1109.64 to 1374.32 MPa) compared to the other film types. Increasing the PLA content led to a statistically significant ($p < 0.05$) increase in both TS and YM and decrease in EAB, indicating that PLA contributes to the formation of rigid, more resistant and less elongated films. Similar results were demonstrated by Lee et al. [25] who reported that PLA films had higher TS (40.58 MPa) than olive flounder skin gelatin (OSG) (31.14 MPa) and OSG/PLA bilayer films (37.56 MPa). Additionally, Nilsuwan et al. [13] found that the TS of PLA films (31.79 MPa) exceeded that of PLA/FG bilayer films (27.23 MPa).

The pure BVSP films exhibited the lowest TS ranging from 5.66 to 6.25 MPa and the highest EAB ranging from 58.92 to 89.97 % when compared to both bilayer and pure PLA films. These results can be attributed to the plasticizing effect of glycerol in the protein-based films. Generally, plasticizers enhance the mobility of polymer chains and film stretchability but often weaken the TS by reducing intermolecular forces [22]. Notably, BVSP films displayed no cracks or holes (as observed in Fig. 5), contributing to their high EAB. In contrast, the bilayer films exhibited higher TS and YM but lower EAB compared to BVSP films. Interestingly, the varying amounts of PLA and BVSP had no statistically significant effects on the mechanical properties of the bilayer films ($p > 0.05$). The obtained results highlight that the presence of PLA layer directly influenced the strength of the bilayer films. This behavior can be attributed to the lower stretchability of the PLA layer which ultimately enhanced the overall strength of the prepared bilayer films. Similar results were reported by Sanyang et al. [26] who observed an increase in the TS of sugar palm starch (SPS)/PLA bilayer films with higher PLA content.

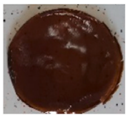
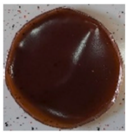
3.1.6. Water vapor permeability

WVP data for bilayer, pure BVSP and PLA films are presented in Fig. 2D. Low WVP values in packaging films indicate their superior ability to protect food from moisture. The WVP of BVSP films ranged from 1.02 to 1.57×10^{-9} (g/Pa.s.m). Decreasing BVSP content in both BVSP and bilayer films resulted lower WVP due to the hydrophilic nature of BVSP. Additionally, the abundance of –OH groups in the BVSP structure facilitated more hydrogen bonding with water molecules, leading to higher WVP compared to PLA. Similar findings were reported by Said et al. [27] for pectin films in comparison to PLA. The WVP of pure PLA films was lower than that of BVSP films, ranging from 2.67 to 6.43×10^{-11} (g/Pa.s.m). PLA's low water solubility and minimal water absorption ability contributed to this behavior. Interestingly, the WVP of PLA pure films remained unaffected by PLA content due to the dense and compact structure of PLA films which prevent penetration of water molecules. The preparation of bilayer films improved the BVSP film by reducing WVP ($1.40–6.55 \times 10^{-10}$ g/Pa s m). Notably, the WVP of bilayer films decreased as the PLA layer content increased and the BVSP layer content decreased ($p < 0.05$). This behavior can be attributed to the hydrophobic structure of PLA in comparison to BVSP, resulting in decreased WVP in the prepared bilayer films with higher PLA content. Generally, two primary factors influence the WVP of bilayer films: thickness and WVP of each layer [28]. These findings showed that laminating of PLA onto BVSP films can enhance the water vapor barrier properties of the resulting bilayer films compared to pure BVSP films. These results align with González and Igarzabal [11] who observed that the addition of PLA layer decreased the WVP of PLA/SPI bilayer films (2.3×10^{-11} g/Pa s m) compared to SPI film (14.9×10^{-11} g/Pa s m). Similarly, Nilsuwan et al. [13] reported that increasing the thickness of the PLA layer in FG/PLA bilayer film led to decreased WVP (from 4.67 to 2.65×10^{-11} g/Pa s m).

3.1.7. Contact angle

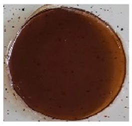
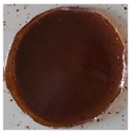
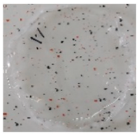
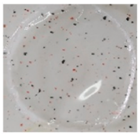
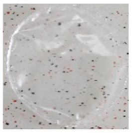
Contact angle measurement is a method commonly used to assess the wettability of a surface. By analyzing contact angle results, we can determine whether a surface is hydrophilic or hydrophobic. In our study, we measured the contact angle on both sides of the

Table 2
Appearance, color parameters and opacity of PLA, BVSP and PLA/BVSP films.

Sample	Appearance	L*	a*	b*	Opacity
$PLA_0,Pro_{2.5}$		42.23 ± 2.92^{fg}	33.82 ± 2.39^{a}	56.51 ± 2.04^{a}	2.91 ± 0.26^{a}
PLA_0,Pro_3		38.76 ± 2.67^{hi}	34.73 ± 1.38^{a}	57.07 ± 1.75^{a}	3.12 ± 0.36^{a}
$PLA_0,Pro_{3.5}$		36.23 ± 5.21^{i}	34.75 ± 1.32^{a}	57.17 ± 0.96^{a}	3.19 ± 0.40^{a}
$PLA_1,Pro_{2.5}$		56.46 ± 6.92^{c}	27.75 ± 1.22^{bc}	51.84 ± 2.38^{c}	2.28 ± 0.36^{b}
PLA_1,Pro_3		54.14 ± 2.33^{c}	27.95 ± 4.28^{bc}	52.77 ± 0.70^{bc}	2.49 ± 0.14^{b}
$PLA_1,Pro_{3.5}$		54.08 ± 6.69^{c}	28.63 ± 1.31^{b}	52.91 ± 0.73^{bc}	2.53 ± 0.23^{b}
$PLA_{1.5},Pro_{2.5}$		54.25 ± 2.36^{c}	24.90 ± 1.37^{d}	52.91 ± 2.15^{bc}	2.49 ± 0.17^{b}
$PLA_{1.5},Pro_3$		49.27 ± 1.77^{d}	26.50 ± 1.08^{cd}	52.93 ± 2.00^{bc}	2.50 ± 0.19^{b}
$PLA_{1.5},Pro_{3.5}$		43.70 ± 2.73^{ef}	28.22 ± 1.31^{bc}	53.13 ± 0.74^{b}	2.55 ± 0.07^{b}
$PLA_2,Pro_{2.5}$		45.57 ± 4.16^{e}	21.29 ± 2.72^{e}	53.39 ± 0.98^{b}	2.50 ± 0.21^{b}

(continued on next page)

Table 2 (*continued*)

Sample	Appearance	L*	a*	b*	Opacity
PLA$_2$,Pro$_3$		39.74 ± 3.05^{gh}	21.52 ± 1.82^{e}	53.53 ± 1.38^{b}	2.54 ± 0.07^{b}
PLA$_2$,Pro$_{3.5}$		39.58 ± 2.35^{gh}	21.52 ± 2.34^{e}	53.64 ± 0.72^{b}	2.56 ± 0.31^{b}
PLA$_1$,Pro$_0$		96.01 ± 0.48^{a}	0.06 ± 0.00^{f}	1.05 ± 0.51^{d}	0.25 ± 0.03^{d}
PLA$_{1.5}$,Pro$_0$		93.64 ± 0.39^{ab}	0.04 ± 0.00^{f}	1.65 ± 0.08^{d}	0.28 ± 0.00^{d}
PLA$_2$,Pro$_0$		92.03 ± 1.37^{b}	0.00 ± 0.00^{f}	1.74 ± 0.04^{d}	0.78 ± 0.05^{c}

Different small letters show significant differences in each column ($p > 0.05$).

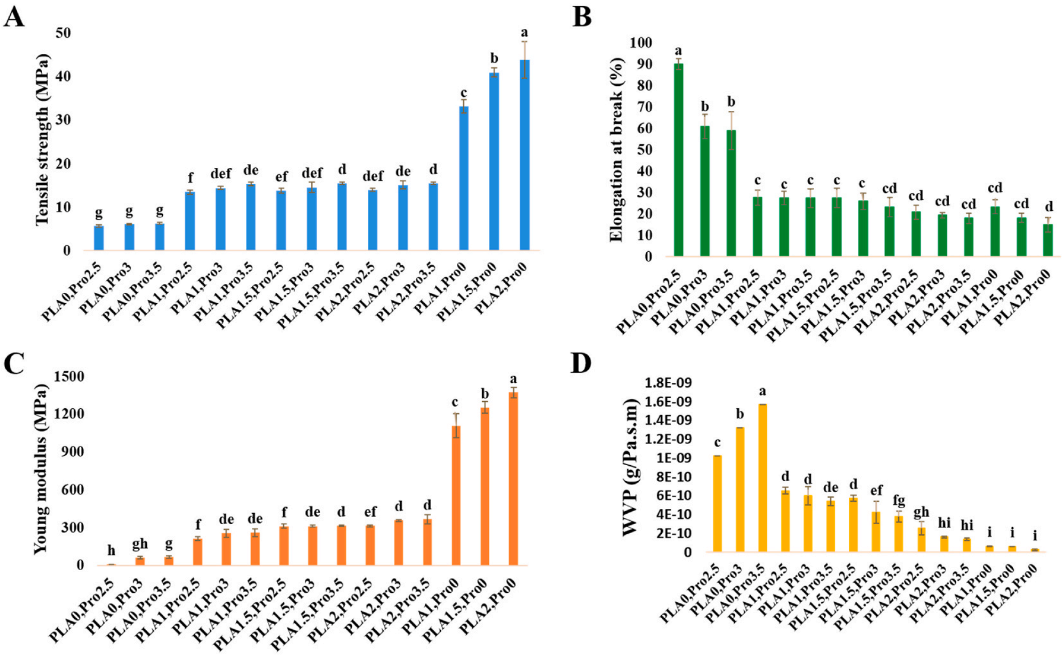


Fig. 2. TS (A), EAB (B), YM (C) and WVP (D) of pure PLA, BVSP and PLA/BVSP bilayer films.

bilayer film to understand any potential physical changes resulting from the addition of the second layer. The contact angle of pure PLA, BVSP and both sides of the bilayer films are reported in Table 4. The results indicate that the BVSP film is more hydrophilic than the PLA film. Interestingly, the presence of PLA in the bilayer films did not significantly affect the contact angle when compared to the pure BVSP film. This lack of significant change can be attributed to the physical arrangement of the two layers in the bilayer film which no chemical interactions occurred between the layers. These findings align with the results reported in the ATR-FTIR and FE-SEM analyses. Similar results were reported by Scaffaro et al. [1] who observed that the PLA layer in bilayer films was less hydrophilic than the MaterBi layer. Furthermore, the presence of each layer did not significantly impact the contact angle of the bilayer films. Additionally, according to the observations by González and Igarzabal [11], PLA films exhibit greater hydrophobicity than SPI films. They reported that the contact angle of pure SPI film and the SPI layer in bilayer films did not show significant differences.

3.1.8. *Antioxidant activity*

The obtained results revealed that the bilayer film (composed of 2 % PLA and 3 % BVSP) without any additional additive compounds exhibited antioxidant activity (24.58 ± 0.19 %). This finding can be attributed to the high levels of aromatic amino acids present in the BVSP structure. These amino acids have side chains that can react with free radicals. Additionally, the presence of acid

Table 3
Pictures of BVSP, PLA and bilayer films for 60 days storage under soil.

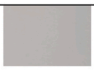
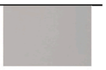
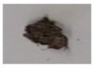
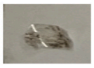
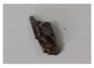
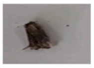
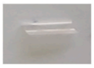
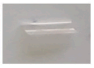
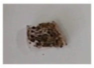
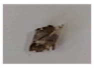
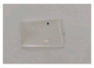
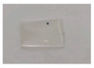
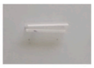
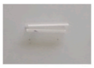
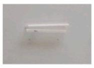
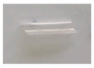
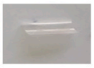
Sample	10th day	20th day	30th day	60th day
PLA$_0$,Pro$_{2.5}$				
PLA$_0$,Pro$_3$				
PLA$_0$,Pro$_{3.5}$				
PLA$_1$,Pro$_{2.5}$				
PLA$_1$,Pro$_3$				
PLA$_1$,Pro$_{3.5}$				
PLA$_{1.5}$,Pro$_{2.5}$				
PLA$_{1.5}$,Pro$_3$				
PLA$_{1.5}$,Pro$_{3.5}$				
PLA$_2$,Pro$_{2.5}$				
PLA$_2$,Pro$_3$				
PLA$_2$,Pro$_{3.5}$				
PLA$_1$,Pro$_0$				
PLA$_{1.5}$,Pro$_0$				
PLA$_2$,Pro$_0$				

Table 4
Contact angle of pure PLA, BVSP and both sides of PLA/BVSP bilayer films.

Sample	Contact angle (°)
Pure BVSP film	54.2 ± 3.12^b
BVSP surface of bilayer film	57.62 ± 0.33^b
PLA surface of bilayer film	71.66 ± 2.24^a
Pure PLA film	73.4 ± 5.89^a

Small letters indicate significant differences in each column ($p < 0.05$).

and basic amino acids in the BVSP allows them to chelate metal ions, contributing to the antioxidant activity of the bilayer films. These results align with a study by Arabestani et al. [14] which reported similar antioxidant properties for BVSP films.

3.1.9. Biodegradability

Biodegradability reflects the ability of material compounds to degrade over time. As shown in Table 3, pure BVSP films exhibited rapid degradation compared to other films. Notably, after 60 days, the pure BVSP films had undergone significant degradation, with complete decomposition observed by the 30th day. These findings align with a study by González and Igarzabal [11] which SPI films were completely decomposed in soil after just 19 days. In contrast, the pure PLA films did not exhibit significant changes even after two months, remaining largely intact. Other study which performed by Hosseini et al. (2022) investigated PLA film properties and reported that PLA films remained intact even after three months. Regarding the bilayer films, degradation occurred in two distinct steps. The initial step involved the decomposition of the BVSP layer which was fully degraded by the 60th day. However, the degradation of the PLA layer required additional time.

3.1.10. Chemical structure

The ATR-FTIR spectra of film samples are displayed in Fig. 3. As can be seen in the spectra of pure PLA and PLA layer of bilayer films, the band at 1741 cm^{-1} is attributed to the stretching vibration of the carbonyl group (C=O) of lactides. Also, the bands at 1080 and 1178 cm^{-1} are referred to as the C-O stretching vibration of the PLA. In addition, the band at around 870 cm^{-1} is related to the stretching vibration of the C-C single bond [29].

The critical bands, including amide-I, amide-II, amide-III, amide-A and amide-B appeared in the BVSP film and BVSP layer of bilayer film. The amide-I band appeared at 1635 cm^{-1}. The vibration mode of the amide-I is a C=O stretching vibration coupled with the C-N stretch, CCN deformation and in-plane N-H bending modes [13]. The BVSP and bilayer films showed a specific absorption band in the amide-II region at about 1541 cm^{-1}, related to N-H bending [11]. The band at around 1238 cm^{-1} displayed the amide-III band that referred to the combined bands between C-N stretching vibrations and the N-H deformation of amide bonds, as well as absorptions arising from wagging vibrations of CH_2 groups from the backbone of glycine and side chains of proline [11]. The amide-A band of BVSP film and BVSP layer of bilayer film appeared at 3280 cm^{-1}, showing the stretching vibrations of the N-H group. Amide-A indicates hydrogen bonding coupled with NH-stretching [13]. Amide-B of BVSP and bilayer films were observed at 3088 cm^{-1}, referred to as asymmetric stretching vibration of -NH_3^+ and = C-H. Both the BVSP and BVSP layers of bilayer films demonstrated bands at 2873 cm^{-1} (symmetrical) and 2925 cm^{-1} (asymmetrical) stretching vibrations of C-H groups in -CH_2 [13]. The band appeared at 1037 cm^{-1}, corresponding to the interactions between the film structure and plasticizer (OH group of glycerol) [30]. The results revealed that all

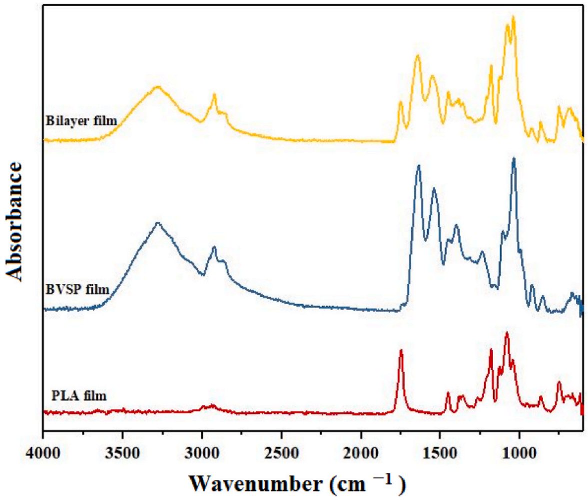


Fig. 3. ATR-FTIR spectra of pure PLA, BVSP and PLA/BVSP bilayer films.

characteristic bands observed in the ATR-FTIR spectra of BVSP and PLA pure films were also present in the bilayer film. Notably, the ATR-FTIR results indicated that no bonds were added or removed during the formation of the bilayer films. This finding suggests that the interaction between BVSP and PLA layers in the bilayer film was physical, with no chemical bonding occurring between the two layers. A similar finding is reported by Zhu et al. [11] for PLA/gliadin bilayer film.

3.1.11. Thermal analysis

The thermal decomposition curves of PLA, BVSP and bilayer films are depicted in Fig. 4. The thermal decomposition curve of the pure BVSP film exhibited three main weight losses. The first step occurred at 80 °C, corresponding to the evaporation of water. The second weight loss was observed in the temperature range of 170–260 °C which indicating the elimination of the plasticizer (glycerol) present in the BVSP film. Finally, the third and most significant weight loss occurred at temperatures exceeding 260 °C, signifying the thermal decomposition of BVSP. The thermal decomposition curve of the pure PLA film revealed an extreme weight loss at 358 °C, reflecting the decomposition and depolymerization of PLA [11]. The bilayer film demonstrated thermal decomposition results similar to those of the BVSP film. The main degradation process in the bilayer film occurred at a temperature comparable to that of the pure BVSP film.

Interestingly, the thermal decomposition of protein in the bilayer film occurred at a lower temperature than that of PLA [6]. Notably, the bilayer film exhibited better thermal stability up to 250 °C, as evidenced by lower weight losses at equivalent temperatures compared to the pure BVSP film. These results align with the findings of González and Igarzabal [11] who reported that the SPI/PLA bilayer film displayed a thermogram like the SPI film.

3.1.12. Morphology

FE-SEM micrographs from the surface and cross-section of bilayer film, BVSP and PLA films are shown in Fig. 5A-G. The PLA film showed a homogenous, smooth and regular surface, whereas pure BVSP film displayed a compact and smooth surface. This morphology of pure PLA film was related to its good dissolving ability which improves the interaction between PLA molecules [3]. These observations were consistent with the results of González and Igarzabal et al. [11] for the pure PLA film. Two layers of the bilayer film are shown in cross-section micrographs that include upper (BVSP) and lower (PLA) layers. The dense and compressed structure was observed from cross-section micrographs of individual and pure PLA and BVSP films. This result showed that the casting method used in this study for preparing films could be suitable for bridging between two layers of bilayer films. A similar result was reported for gelatin of tilapia skin/PLA multilayer film [31] and fish gelatin/PLA bilayer films [24] prepared by casting. In addition, Zhu et al. [12] reported that the micrographs of PLA/gliadin bilayer film showed a compact and homogenous structure.

As can be seen in Table 5, designed bilayer films in this study had acceptable barrier properties against water vapor and they showed better barrier properties in comparison to bilayer films based on PLA/pea starch [3], potato starch [7] and gelatin [25]. Also, among the different studies that are compared, designed bilayer films had the most tensile strength after the bilayer film based on olive flounder skin gelatin/PLA [25] and their elongation at break had an acceptable value among the reviewed studies. These results showed that designed bilayer films could overcome the disadvantages of pure BVSP film (weak mechanical and barrier properties), which can indicate that these films are considered suitable candidates for packaging a variety of food products.

4. Conclusion

In this study, we successfully prepared bilayer films based on PLA and BVSP. Our findings demonstrate that the PLA/BVSP bilayer films significantly improved the mechanical properties and opacity of BVSP films. Moreover, the bilayer films exhibited substantial reductions in WVP, solubility and moisture content compared to the BVSP films. These promising results position the prepared bilayer films as excellent candidates for food packaging applications. Furthermore, the thermal stability of the bilayer films surpassed that of pure BVSP films. ATR-FTIR analysis revealed physical interactions between the two layers, while the chemical nature of BVSP and PLA remained unchanged in the bilayer films. Our study underscores the potential of bilayer films to overcome the limitations of single-layer films commonly used in food packaging. These versatile bilayer films can find applications in the packaging industry including the incorporation of active compounds for improved shelf life and the use of pigments for intelligent packaging to monitor the quality of stored foods.

CRediT authorship contribution statement

Sona Dodange: Writing – original draft, Software, Formal analysis. **Hajar Shekarchizadeh:** Writing – review & editing, Supervision, Resources, Conceptualization. **Mahdi Kadivar:** Writing – review & editing, Supervision, Investigation.

Data and code availability

Data will be made available on request. For requesting data, please write to the corresponding author.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Sona Dodange reports financial support was provided by Iran National Science Foundation. If there are other authors, they

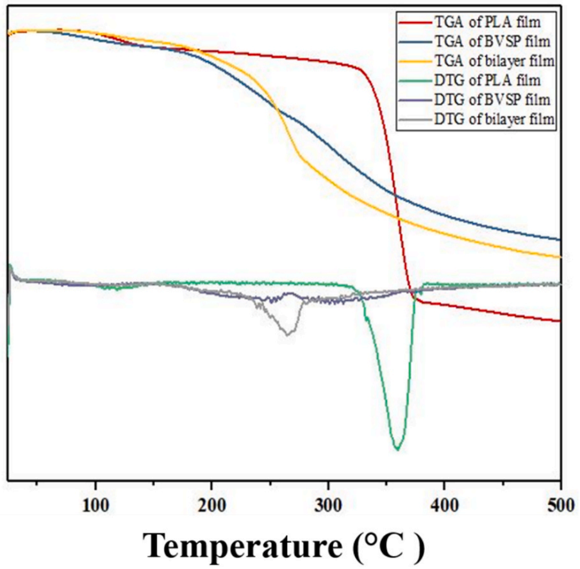


Fig. 4. TGA and DTG curves of pure PLA, BVSP and PLA/BVSP bilayer films.

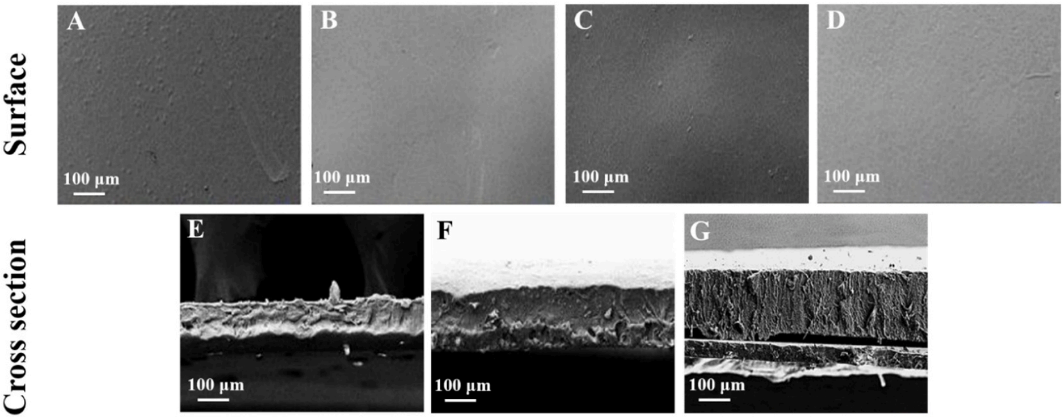


Fig. 5. FE-SEM micrographs of pure PLA (A), BVSP (B) and PLA/BVSP bilayer (C (PLA layer) and D (BVSP layer)) films' surface and pure PLA (E), BVSP (F) and PLA/BVSP bilayer (G (Upper layer: BVSP and Lower layer: PLA)) films' cross-section.

Table 5

Comparison of tensile strength, elongation at break and water vapor permeability of the bilayer films of this study, along with other bilayer films made from PLA.

Film composition	Tensile strength (MPa)	Elongation at break (%)	WVP (10^{-10} × g/Pa.s.m)	Reference
BVSP-PLA	13.46–15.50	17.91–27.68	1.4–6.55	–
Pea starch- PLA	8.45–11.76	15.21–44.83	2.50–8.92	[3]
Potato starch-PLA	10.92	21.94	14.26	[7]
Soy protein–PLA	8.57–13.69	1085–1579	0.23–0.34	[11]
Whey protein-PLA	9.25	159.94	–	[16]
Olive flounder skin gelatin-PLA	37.56	72.21	9.2	[25]
Sugar palm starch-PLA	10.74–13.65	15.53–26.40	0.21–0.42	[26]
Pectin-PLA	2.83–7.89	73.55–468.25	1.18–5.40	[27]

declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work is based upon research funded by Iran National Science Foundation (INSF) under project No (4000552) and Isfahan University of Technology (IUT).

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