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# Eco-innovative Bilayer Films of *Eremurus spectabilis* Root Gum and Polyvinyl Alcohol Enriched with *Cuminum cyminum* husk Extract for Beef Preservation

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**Abstract**

This study focused on the development and characterization of a novel active bilayer film composed of *Eremurus spectabilis* root gum (ESRG), polyvinyl alcohol (PVA), and *cuminum cyminum* husk extract for potential application in beef preservation. The bilayer system was fabricated by casting PVA as a structural support layer and ESRG incorporated with cumin husk extract as the active layer. Physicochemical characterization revealed that the incorporation of cumin husk extract increased film thickness while reducing water solubility and water vapor permeability, thereby improving barrier properties. Structural analyses (FTIR, SEM, and DSC) confirmed successful integration of bioactive compounds and enhanced thermal stability. Mechanical tests demonstrated reduced tensile strength but increased flexibility upon extract incorporation. When applied to beef fillets stored at 4 °C for 12 days, the active bilayer film significantly inhibited microbial growth (aerobic plate count, *Enterobacteriaceae, Pseudomonads*, and *psychrotrophs*) compared with both the control and simple bilayer films. Additionally, the film effectively delayed lipid oxidation (TBARS) and protein degradation (TVB-N), while maintaining lower pH and improved sensory quality. Overall, the active bilayer PVA/ESRG film with cumin husk extract demonstrated promising potential as a sustainable and effective biodegradable packaging material for extending the shelf-life and preserving the quality of fresh beef.

**Keywords:** *Eremurus spectabilis* root, PVA, Cumin husk extract, bilayer film, beef preservation.

## 1. Introduction

In contemporary society, the widespread utilization of petroleum-based plastic packaging for the preservation of diverse food items has raised significant concerns regarding its environmental impact. As a result, numerous countries have implemented stringent regulations on its use, highlighting the urgent need for alternative solutions in our daily lives (Heydari-Majd et al., 2023; Bahrami et al., 2022; Abdolshahi et al., 2022). To address this problem, the creation of biodegradable polymers derived from environmentally friendly material is greatly advantageous for food packaging, assuming they can match the effectiveness of existing plastics in safeguarding food from physical damage, bacterium contamination, and chemical reactions such as oxidation (Sharayei et al., 2025). Currently, there is a renewed focus on agricultural waste, enhancing the technology involved in the production of biopolymers and food packaging to satisfy consumer expectations for high-quality, healthy, and safe food products (Niazmand et al., 2025; Heydari-Majd et al., 2024; Heydari Majd et al, 2025; Salarbashi et al., 2020; Heydari-Majd et al., 2019[c]). *Eremurus spectabilis*, commonly known as 'serish or sirish', is a flowering *Asphodelaceae* plant distributed from the eastern Mediterranean to the Caucasus. This perennial herb is native to areas much of Iran, Iraq, and Lebanon-Syria. This aromatic herb is a valuable source of edible vegetables, a rich source of antioxidants and phenolic compounds along with minerals that are consumed in various forms in Turkey. The species is of medicinal importance to treat fungal diseases, diabetes, hepatitis, liver disorders, celiac disease, stomach problems, and some cancers (Tahmouzi et al., 2026). It produces derivatives of specialized adhesives from its processed roots. *E. spectabilis* root has been used in various forms for traditional uses such as jaundice, liver issue, stomach irritation, skin ailments, and bone cracks and is used industrially to produce adhesives (Hoseinpour et al., 2025a). The roots contain many oligo- and polysaccharides, mainly consisting

of arabinogalactan, in addition to galactomannan, along with chemicals that represents phenolics with distinctive high antioxidant and antimicrobial potential (Alhalak & Sekerler, 2025). *E. spectabilis* root gum (ESRG) is a potential natural polymer of agricultural waste for production of biodegradable film. An important strategy to improve the application of polymer packaging, such as those derived from ESRG, is to activate them with natural active ingredient such as plant-extracts, antimicrobials, and antioxidant, to extend the food shelf-life.

Plant extracts can be great natural additives/preservatives for food as they provide a high amount of polyphenolic materials that have antimicrobial and antioxidant properties (Marvdashti et al., 2023; Heydari Majd et al, 2019[b]; Abdolshahi et al., 2018; Majd et al., 2014). Different essential oils and extracts from different plants, such as *Zataria multiflora*, *Menthe piperita*, *Pycnocycla bashagardiana* and *Ferula asafoetida*, have also been tested in functional films, exhibiting good antibacterial activity (Aldarraji et al., 2025; Niazmand et al., 2020).

Cumin (*Cuminum cyminum L.*) is a recognizable annual herb in the *Apiaceae* family. Cumin offers outstanding properties that are known to be valuable for prolonging the shelf-life of materials. Cumin is a phytogenic plant that has been used in traditional medicine and cooking, and is best known for its bioactive compounds that can include but are not limited to phenolics, terpenoids, and flavonoids. Cumin is known to have superior antioxidant and anti-inflammatory properties (Kumar et al., 2025). Cumin processing generates husk by-products during harvesting and post-harvest operations, which represent an underutilized agricultural resource. Although this amount of production is extensive, there is a research gap, that will explore cumin husk phenolic-rich active compounds for use in biodegradable films (Hoseinpour et al., 2025b).

Conversely, biopolymer films made from natural agricultural waste, like those from ESRG, frequently face challenges such as weak resistance to water, difficulty in processing, and

inadequate thermal-mechanical characteristics. However, combining different biopolymers is considered one of the most effective methods to address these issues.

Blending ESRG film with mechanically robust poly(vinyl alcohol) (PVA) is one fitting way to address the challenges outlined in the project proposal. Further, the blend of these biopolymers should dramatically increase the physicochemical film properties and utilize the ideal characteristics of ESRG material (Jebel et al., 2025).

PVA is a water-soluble, biodegradable polymer that has gained substantial interest in advanced packaging applications due to its nontoxic nature and properties that make it different from other polymers. PVA is a colourless polymer and a highly abundant chemical that also has good film-forming ability, solid chemical stability to a variety of aqueous solutions, highly crystalline nature, and hydrophilic traits among other factors. PVA as a synthetic polymer provides environmentally friendly alternatives for sustainable packaging that also provides some degree of chemical stability and is ultimately biodegradable (Saini et al., 2025).

Beef meat is prone to microbial growth as a result of usually occurring or contaminating microorganisms, which can lead to economic and health issues during refrigerated storage. In recent years, strategies to prevent spoilage and pathogenic bacteria have been implemented, with active edible films being effective at promoting longer shelf-life and safety (Bahrami et al., 2025). Edible films are widely used on many types of food but can also be applied to beef meat to improve shelf-life. While considerable work has been done in the area of food applications, no literature is available regarding the use of cumin husk extract within bilayer ESRG /PVA film, specifically in the context of helping preserve beef meat. Therefore, beef meat serves as a relevant test system for evaluating active bilayer films with both antioxidant and antimicrobial activity.

A bilayer design was created to provide functional separation in the film system. The semi-crystalline nature of the PVA layer gives the structure the mechanical integrity and oxygen barrier characteristics required to create the film, while the cumin husk extract-enriched ESRG layer contains the biological phase required to deliver the antimicrobial and antioxidant effects. Unlike single-layer mixtures, which often face issues like phase mismatch and unregulated release of bioactive materials, the bilayer setup forms a complex route for diffusion. This setup helps slow down the movement of phenolic substances and reduces the direct contact of the hydrophilic PVA layer with surrounding moisture. Therefore, the bilayer configuration increases performance by enabling structural and functional synergy rather than just mixing the components together.

Therefore, the present work aimed to developed an active film based on cumin husk extract/ESRG and fabricate this active film on PVA to create a bilayer cumin husk extract/ESRG and PVA composite film with increased barrier and antibacterial activity using a new natural formulation. Finally, we investigated the ability of this new formulation film to control the lipid oxidation, microbial spoilage, and sensorial change of fresh beef fillet during chilled storage.



## 2. Materials and methods

### 2.1. Chemicals and samples

*Eremurus spectabilis* roots were sourced from local cultivators in the Binalud Mountains region Khorasan Razavi Province, Iran by local farmers. The collection of this plant material for research purposes is in accordance with customary local practice and does not require a specific permit. The species was taxonomically identified by Dr. Reza Azarbayejani, botanist at the National Center for Genetic and Biological Resources of Iran (IBRC). A voucher specimen was deposited

at the Herbarium of the National Center for Genetic and Biological Resources of Iran, Tehran, Iran, under the voucher number IBRC P1007041.

The collection of plant material was conducted in accordance with relevant institutional and national guidelines and regulations. Since Eremurus spectabilis is not listed as an endangered or protected species, no special permits were required for its collection.

Root samples were carefully cleaned with water to remove surface contaminants, shade-dried on cotton cloth, and processed into powder using a semi-industrial mill. Green cumin pods were obtained as waste material from a local cumin processing facility in Mashhad and underwent similar preparation procedures including washing, shade-drying, and grinding to powder form. Both powdered materials were stored in cool, dark conditions to preserve their bioactive properties. PVA with molecular weight 146,000-186,000 g/mol and 99% purity, along with all other analytical-grade chemical reagents, were procured from Sigma Aldrich (Germany) and used without additional purification. Malondialdehyde (1,1,3,3-Tetraethoxypropane, MDA), glacial acetic acid, and 2-thiobarbituric acid (TBA), were obtained from Merck (Darmstadt, Germany). For microbiological analyses, specialized culture media were sourced from Quelab Laboratories Inc. (Montreal, Quebec, Canada), encompassing Plate Count Agar (PCA) for general bacterial enumeration, *Pseudomonas* Agar for selective isolation of *Pseudomonas* species, and Violet Red Bile Glucose (VRBG) Agar for detecting *Enterobacteriaceae*. These standardized materials provided the necessary foundation for conducting accurate biochemical and microbiological assessments throughout the study.



### 2.2. *Cuminum cyminum* husk extract preparation

Hydroalcoholic extraction of cumin husk was performed using a solid-to-solvent ratio of 1:6 (w/v). The extraction medium consisted of 75% ethanol (96% purity) and 25% distilled water. Cumin husk powder (50 g) was mixed with the solvent system and subjected to extraction in a shaking water bath at 45°C for 3 hours. The resulting extract was clarified through vacuum filtration, and ethanol was subsequently removed using rotary evaporation (Laborota 4000 efficient, Germany). The concentrated extract powder was stored under refrigerated conditions (4°C) until further use in film preparation (Tuzcu et al., 2017).

### 2.3. Bilayer active film preparation

A bilayer film system was developed by combining PVA and ESRG-based active layers. The PVA layer was prepared by dissolving 3% (w/v) PVA powder (87-89% alkalization degree) in distilled water at 70°C for 2 hours, followed by incorporation of 40% (w/w) glycerol as plasticizer. The solution was cast in 10 cm diameter plates and dried at 40°C for 15 hours (Lara et al., 2019).

For the active layer, ESRG powder extract was dissolved in distilled water using magnetic stirring (1500 rpm) for 1 hour at ambient temperature, then heated to 85°C for 10 minutes to ensure complete dissolution. Glycerol (40% w/w) was added as plasticizer, followed by *Cuminum cyminum husk* extract at 40 w/w dry matter. After mixing at 40°C for 30 minutes and ultrasonic (Model-GE 750, Sonics and Materials, Newtown, CT) degassing, 40 ml portions were cast and dried at 40°C for 18 hours.

The bilayer structure was achieved by laminating the two films using commercial edible adhesive (Tutkal brand), with both surfaces coated and pressed together until complete adhesion at room temperature. Interfacial adhesion between layers is promoted by hydrogen bonding between hydroxyl-rich PVA and ESRG chains, with glycerol acting as a molecular bridge. The polysaccharide-based edible adhesive further enhances polymer interdiffusion across the interface,

resulting in continuous bonding without visible interfacial voids, as later confirmed by SEM observations.

### 2.4.Evaluating the characteristics of films

#### 2.4.1. Film thickness

Film thickness measurements were conducted at three randomly selected locations using a digital hand micrometer (Mitutoyo, Tokyo, Japan) with a precision of 0.001 mm. The mean value of these three measurements was calculated and subsequently employed for evaluating both mechanical characteristics and barrier properties of the films.

To ensure that thickness did not dominate functional outcomes, casting volume, polymer concentration, and drying conditions were kept constant for all samples. Furthermore, variations in mechanical and barrier properties did not follow linear thickness-dependent trends, indicating that matrix structural modifications and intermolecular interactions contributed more significantly than thickness differences alone.



#### 2.4.2. Water vapor permeability (WVP)

Water vapor permeability (WVP) was evaluated using the standard gravimetric method, which provides reliable measurements of moisture barrier properties. The experimental setup involved placing anhydrous calcium chloride ($CaCl_2$) granules in test cups to create a moisture-free environment (0% relative humidity). Circular film specimens were then secured over the cup openings, and the assembled units were positioned within a desiccator containing distilled water to maintain 100% relative humidity conditions. Weight measurements were recorded at 12-hour intervals over a 72-hour period to monitor moisture uptake through the film samples. The water vapor transmission rate (WVTR) was determined by calculating the slope of weight gain (grams)

versus time (hours) and normalizing this value by the effective film area (square meters). Water vapor permeability was subsequently calculated using the following relationship, expressed in units of g·mm/m²·h·kPa (Hajinezhad et al., 2020):

$$WVP = \frac{WVTR - X}{\Delta P \times S} \quad (1)$$

where WVTR represents the water vapor transmission rate (g/h), X denotes the film thickness (mm), S corresponds to the film area (m²), and ΔP indicates the vapor pressure differential across the film (kPa). This approach enables quantitative assessment of the film's barrier performance under controlled humidity gradients.

### 2.4.3. Solubility in water

To assess film solubility, specimens were initially cut into 2×2 cm squares and subjected to complete drying in an oven at 105°C for 24 hours to establish their initial dry weight ($W_1$). Subsequently, each film sample was immersed in 30 mL of distilled water and agitated using a mechanical shaker at 25°C for 6 hours to allow dissolution of water-soluble components. Following the dissolution period, the remaining film portions were carefully retrieved using sterile forceps and transferred to drying plates. Complete dehydration was achieved through oven drying at 105°C for 24 hours to obtain the final dry weight ($W_2$). The percentage solubility of each film sample was calculated using the following equation (Abdolshahi et al., 2022):

$$Solubility\ \% = \frac{(w_1 - w_2)}{w_1} \times 100 \quad (2)$$

### 2.4.4. Mechanical properties

Prior to testing, the film samples were conditioned at 50% relative humidity for more than 70 h and then trimmed into rectangular strips measuring 1 cm in width and 10 cm in length. Mechanical

properties, namely tensile strength (TS) and elongation at break (EB), were evaluated with a Testometric Machine (Model M350-10CT, Testometric Co. Ltd., Rochdale, England) following the ASTM D882 protocol (ASTM, 2012). The crosshead speed was adjusted to 50 mm/min with an initial grip separation of 50 mm. At least five replicates were performed for each film type. Since films were fabricated by solvent casting under static conditions without directional stretching, polymer chain orientation was minimal, and isotropic mechanical behavior was assumed. Therefore, tensile measurements in the principal casting direction are considered representative of bulk film properties.

### 2.5. Structural properties of films

#### 2.5.1. FTIR spectroscopy

To examine the structural interactions between components, Fourier-transform infrared (FTIR) spectroscopy was performed using a Bruker Alpha FTIR spectrometer (Bruker Corporation, USA). The infrared spectra of the film samples were acquired across the wavenumber range of 400–4000 $cm^{-1}$, with each spectrum representing an average of 15–16 scans collected at a resolution of 1 $cm^{-1}$ (Bahrami et al., 2023).



#### 2.5.2. Films microstructure

The microstructural characteristics of the films were analyzed using scanning electron microscopy (SEM) with a LEO 1450VP instrument (Carl Zeiss, Germany). Prior to examination, film samples were cryogenically preserved in liquid nitrogen and subsequently mounted onto aluminum stubs using double-sided adhesive tape. To enhance conductivity and image quality, specimens were sputter-coated with gold particles using an ion sputter coater (BAL-TEC AG, Balzers,

Liechtenstein) operated at 15 mA current for 60 seconds. Micrographs were then captured under high-vacuum conditions using an electron beam accelerated at 25.0 kV (Modaresi & Niazmand, 2021).

### 2.5.3. Thermal properties (DSC)

Thermal characterization of the film samples was conducted using Differential Scanning Calorimetry (DSC) with a Shimadzu instrument (Japan). The analysis was performed in an inert nitrogen environment maintained at a flow rate of 150 mL/min to prevent oxidative degradation during heating. Temperature scanning was carried out over a range of 20°C to 120°C with a controlled heating rate of 15°C/min to ensure accurate thermal transition detection (Hajinezhad et al., 2020).



### 2.6.Beef sample preparation and storage

Fresh red meat samples were procured from local retail establishments in Mashhad, Iran, and immediately transported to the Food Hygiene Laboratory at Ferdowsi University under aseptic conditions. Transportation was conducted using insulated expanded polystyrene containers filled with ice to maintain cold chain integrity throughout transit. Upon arrival at the laboratory facility, meat samples underwent thorough washing procedures before being portioned into uniform cubes measuring 3×3 $cm^2$ to ensure consistency across all experimental treatments. The prepared meat portions were individually wrapped with the respective film treatments and subsequently placed in sterile, coded polyethylene zip-lock bags to prevent cross-contamination. All packaged samples were stored under refrigerated conditions at 4-5°C throughout the designated storage intervals of 0, 3, 6, 9, and 12 days. Additionally, unpackaged meat samples were maintained under identical

storage conditions to serve as control specimens for comparative analysis of packaging effectiveness.

### 2.7. Microbiological analysis of beef samples

Microbial evaluation was conducted on 25 g meat samples collected aseptically at designated intervals and processed in stomacher bags (Seward Medical, London, UK) with 225 ml sterile peptone water (0.1%). Samples underwent homogenization using a Lab Blender 400 for 60 seconds at ambient temperature, followed by serial ten-fold dilutions in peptone water. Different bacterial populations were quantified using specific culture conditions and media (Heydari-Majd et al., 2019[a]). Total aerobic bacteria were enumerated by plating on PCA medium (Merck) with incubation at 30°C for 48 hours, while psychrophilic bacteria were cultured on the same medium but maintained at 7°C for 10 days. *Pseudomonas* spp. was selectively grown on *Pseudomonas* Agar Base (Oxoid) supplemented with CFC (Cetrimide Fucidine Cephalosporine, Oxoid code CM 559, supplemented with SR 103, Oxoid, Basingstoke, UK) and incubated at 20°C for 48 hours. *Enterobacteria* detection was accomplished through the pour-plate technique using VRBGA medium (Oxoid CM 0485, Basingstoke, UK) incubated at 30°C for 24 hours, where positive results were identified by the presence of characteristic purple-haloed colonies. Microbiological assessments were performed in triplicate at days 0, 3, 6, 9, and 12 of storage. Results were reported as log colony-forming units per gram of meat (log CFU/g).

### 2.8. pH Measurement

The pH levels were assessed by combining 10 g of each sample with 100 ml of distilled water, followed by complete homogenization. After filtration, pH values were recorded using a digital pH-meter (pH 201, Alpha, China) with three replications per sample.

### 2.9. Determination of meat freshness

Meat quality deterioration was evaluated through lipid oxidation and protein degradation measurements using TBARS and TVB-N analyses, respectively. For TBARS determination, meat samples (4 g) were homogenized with 20 mL of chilled 20% trichloroacetic acid (TBA) solution using an Osterizer blender (5000 rpm, 2 min). Following centrifugation at 5000g for 10 minutes, 3 mL of supernatant was combined with an equal volume of 0.1% TBA solution and heated at 90°C for 40 minutes. Malondialdehyde (MDA) concentration was measured spectrophotometrically at 532 nm (Shimadzu CPS-240, Japan) using a standard calibration curve prepared with 1,1,3,3-tetramethoxypropane. Results were expressed as mg MDA/kg sample to quantify lipid peroxidation levels. Total volatile basic nitrogen (TVB-N) content was determined in triplicate following the methodology of Heydari-Majd et al. (2019[a]), with results reported as mg N/100 g meat to assess protein degradation during storage.



### 2.10. Sensory Assessment Protocol

A panel of five trained assessors conducted sensory evaluation of the meat samples. Each treatment was replicated four times, with samples randomly coded and presented on individual plates to ensure blind evaluation. The panelists assessed four key sensory attributes, color, aroma, texture, and overall acceptability, using a nine-point hedonic scale (1 = extremely poor; 9 = excellent).

This double-blind approach eliminated potential bias, allowing evaluators to provide objective ratings based solely on sensory perception rather than treatment knowledge.

### 2.11. Statistical analysis

A completely randomized factorial design was employed with two factors: (1) film formulation (control bilayer and extract-containing bilayer) and (2) storage time (0, 3, 6, 9, and 12 days). Bilayer films were produced in triplicate fabrication batches under identical processing conditions to assess reproducibility. Thickness variability remained below 5%, and variability in mechanical and barrier properties remained below 8%, with statistical analysis indicating no significant batch effect ($p > 0.05$). Data were analyzed using one-way or two-way ANOVA as appropriate, by SPSS (version 16) followed by Tukey's HSD post-hoc test to determine significant differences at $p < 0.05$.



## 3. Results and Discussion

### 3.1. Physical properties of active ESRG/PVA bilayer film

#### 3.1.1. Appearance and film thickness

Edible films with thickness below 0.25-0.3 mm represent a critical parameter that directly governs mechanical properties, barrier effectiveness, and water vapor permeability. This optimal thickness range ensures adequate tensile strength while maintaining visual quality and structural integrity of packaged foods. Achieving appropriate film thickness is essential for maximizing food preservation, extending shelf life, and providing effective protection through enhanced barrier performance during storage and distribution (Niazmand et al., 2025; Díaz-Montes & Castro-Muñoz, 2021).

The findings presented in Table 1 indicate that incorporating *Cuminum cyminum* husk extract into the bilayered ESRG/PVA films notably increased their thickness, ranging from 0.10 to 0.25 mm. The extract itself induced only a modest change in thickness, which aligns with earlier reports by Hoque et al., (2011), who observed similar effects when clove, cinnamon, and star anise extracts were added to gelatin films, and by Altiok et al., (2010) with thyme essential oil in chitosan films. This thickening effect can be attributed to soluble constituents of the extract, particularly phenolic compounds, which interfere with intermolecular interactions between ESRG fructan chains, thereby expanding the film network and leading to thicker layers (Razavi et al., 2015). Consistently, Saberi et al., (2017) suggested that natural phenolics can integrate into film matrices, and also highlighted the role of extract density, which typically exceeds that of water, in increasing thickness.

The thickness of the PVA films obtained here was in agreement with Terzioğlu et al. (2021), who reported comparable outcomes for chitosan/PVA composites. The bilayer configuration also contributed to a higher thickness, simply due to the overlapping of two films. However, contrary results were reported by Ali and Ahmed, (2021), who observed reduced thickness when bilayering PVA with chitosan and *Ocimum tenuiflorum* extract, a phenomenon linked to the formation of compact and crystalline microstructures (Giteru et al., 2019).

In addition to these structural effects, simple and active bilayer films containing *C. cyminum* husk extract exhibited a brown yellowish hue and gold brownish, respectively (Fig. 1). This observation agrees with Ali and Ahmed, (2021), who reported color development in bilayer PVA/chitosan films upon incorporation of *O. tenuiflorum* extract.

**Insert Table 1. about here**

**Insert Fig. 1 about here.**

### 3.1.2. Water vapor permeability (WVP)

As shown in Table 1, bilayer PVA/ESRG films activated with husk extract achieved the lowest WVP (0.095), confirming the effectiveness of active multilayer films. Similar findings were reported by Kanatt et al. (2012), where natural extracts incorporated into chitosan/PVA films reduced WVTR. This effect is attributed to phenolic compounds forming intermolecular interactions within the polymer matrix, strengthening film structure and limiting vapor diffusion. Extract concentration also affects barrier behavior by increasing film thickness and solid density, thereby reducing free volume available for water transport. The addition of *Cuminum cyminum* husk extract disrupted the hydrophilic phase, enhanced matrix rigidity, and consequently lowered WVP (Thakur et al., 2016; Atarés & Chiralt, 2016).

PVA films contain a high density of hydroxyl (-OH) groups, which makes them strongly hydrophilic and therefore less effective as moisture barriers (Limpan et al., 2010).

Barrier performance in the bilayer system arises from complementary mechanisms: the PVA layer contributes oxygen resistance through crystalline domains, the ESRG layer regulates moisture transport and matrix densification, and the extract reduces free volume while increasing diffusion tortuosity. The combined behavior exceeds simple additive effects, indicating synergistic mass-transfer resistance.

Although diffusion coefficients were not directly quantified, indirect indicators such as sustained antimicrobial activity, reduced TBARS and TVB-N values, and improved barrier performance suggest controlled migration of phenolic compounds across the bilayer interface, while the PVA layer restricts excessive moisture and gas transfer.

### 3.1.3. Film solubility

Low water solubility is a desirable characteristic for food packaging films, as it enables them to withstand humid environments and prolong food shelf life during storage. The solubility of a film in water is largely dictated by its composition and structural attributes. In general, water solubility reflects the film's stability and resistance to moisture, which are determined by the type, concentration, and hydrophilic–hydrophobic balance of its constituents. Hydrophilic compounds typically increase solubility, whereas hydrophobic ones reduce it (Niazmand et al., 2022).

As shown in Table 1, pure PVA exhibited higher solubility than pure ESRG-based films. The high solubility of PVA films is attributed to their relatively weak intermolecular forces, particularly hydrogen bonds, which allow the polymer matrix to readily hydrate and dissolve in water (Limpan et al., 2010). By contrast, ESRG films displayed the low solubility (46.5%), likely due to the combined presence of soluble and insoluble fibers in the ESRG extract. PVA films alone showed 50.2% solubility, but when combined with ESRG films in a bilayer structure, solubility decreased to 50.0% for PVA/ESRG-simple and further to 45.3% for PVA/ESRG-active films (Table 1). This reduction highlights their suitability for food packaging, as bilayer films effectively restrict moisture, $O_2$, and $CO_2$ transfer between food and the surrounding environment. The lower solubility of the active bilayer film can be explained by the hydrogen bonding and covalent interactions formed between glycerol, *Cuminum cyminum* husk extract, and ESRG within the film matrix, as confirmed by FTIR analysis. These interactions limit the availability of hydroxyl groups to bind with water molecules, thereby enhancing hydrophobicity and reducing solubility (Sharayei et al., 2025).

This mechanism aligns with earlier findings: Azarifar et al. (2019) observed similar reductions in solubility for gelatin/CMC bilayer films containing *Trachyspermum ammi* essential oil, while Shojaee-Aliabad et al. (2013) and Hosseini et al. (2009) reported comparable effects in κ-carrageenan films with *Satureja* hortensis oil and chitosan films containing thyme, clove, and cinnamon, respectively. In all cases, incorporation of bioactive compounds that hinder water access to hydroxyl groups contributed to reduced solubility and improved film stability.

### 3.1.4. Mechanical properties

In this study, TS and EB values for both pure and active blended films were measured, ranging between 4.35–11.64 MPa and 14.31–19.62%, respectively (Table 1). Incorporation of husk extract led to a reduction in TS accompanied by an increase in EB. This trend is attributed to disruption of the film network, as the extract interferes with structural density and uniformity, thereby weakening chain interactions and creating fracture points (Heydari-Majd et al., 2022; Hernández et al., 2008). These results support the hypothesis that bioactive extracts generally disturb polymer networks, decreasing tensile resistance while improving extensibility.

Among the prepared films, PVA displayed superior TS and EB compared with ESRG. When ESRG was laminated with PVA, the bilayer structure significantly enhanced TS (11.64 MPa) while reducing EB (14.31%). This observation is in line with findings by Azahari et al., (2011) for PVA/corn starch films. Similarly, Mozafarpour et al., (2021) reported that incorporation of fructan improved both TS and extensibility of whey protein films. However, when 50% w/w dry matter *C. cyminum* husk extract was introduced into the bilayer PVA/ESRG film, TS dropped markedly to 4.35 MPa. As suggested by Ebrahimzadeh et al. (2021), such reductions may stem from the replacement of strong polymer–polymer interactions with weaker polymer–extract interactions, leading to a compromised film structure and diminished tensile strength.

No interlayer separation was observed during sample handling or tensile testing, indicating adequate interfacial integrity under static mechanical loading. The plasticized interface and hydrogen bonding network likely contribute to flexibility while maintaining adhesion. Cyclic bending performance is suggested for future investigation.

### 3.2. Structural analysis of active bilayer PVA/ESRG film

#### 3.2.1. FTIR spectrum

The ESRG film plasticized with 40% glycerol displayed a broad O–H stretching band at 3300–3400 $cm^{-1}$, intensified by hydrogen bonding between glycerol and ESRG chains (Fig. 2a). Absorptions at 2850–2950 $cm^{-1}$ (C–H stretching), 1000–1150 $cm^{-1}$ (C–O stretching of glycosidic and hydroxyl groups), and 1400–1500 $cm^{-1}$ (C–H bending) confirmed its polysaccharide structure (Fig. 2a). Comprehensive evidence indicates that ESRG contains fructo-furanose, lignin, cellulose, hemicelluloses, and various monosaccharides, consistent with previous reports of arabinogalactan, galactomannan, and glucofructan structures (Beigi & Jahanbin, 2019).

Incorporation of *Cuminum cyminum* husk extract produced shifts and broadening of the O–H band, along with new absorptions at ~1600 $cm^{-1}$ (aromatic C=C), 1200–1000 $cm^{-1}$ (C–O of phenolics/ethers), and a minor band near 1700 $cm^{-1}$ (C=O), confirming the integration of phenolic compounds into the ESRG matrix (Fig. 2b). Changes in the vibrational frequencies of the functional groups can be considered as the result of the presence of extracts in the polymer matrix. The results indicate that the presence of extract alters the arrangement of the molecular and intermolecular interactions in the film matrix (Sharayei et al., 2025).

The PVA spectrum exhibited a broad O–H band (3300–3500 $cm^{-1}$), C–H stretching at 2940–2910 $cm^{-1}$, and a weak carbonyl band around 1730–1650 $cm^{-1}$ from residual acetate groups. Bands at

1420–1325 $cm^{-1}$ ($CH_2$ bending), 1240–1085 $cm^{-1}$ (C–O stretching), and 840–920 $cm^{-1}$ ($CH_2$ rocking) supported the semi-crystalline PVA backbone (Fig. 2c) (Ma et al., 2017).

The edible Tutkal glue showed a polysaccharide-based profile, with a broad O–H band near 3400 $cm^{-1}$, C–H stretching at 2800–3000 $cm^{-1}$, and a strong C–O band around 1050 $cm^{-1}$, while weaker absorptions near 1760 $cm^{-1}$ suggested minor carbonyl contributions (Fig. 2d). These results confirm its starch/sugar origin with slight modifications or additives (Arruda et al., 2025).

The simple bilayer film of PVA and ESRG joined with edible glue presented the expected O–H, C–H, and C–O bands of polysaccharides (ESRG) and PVA (Fig. 2e). In contrast, the active bilayer film containing *Cuminum cyminum husk* extract exhibited additional absorptions at ~1600 $cm^{-1}$ (aromatic vibrations) and ~1700 $cm^{-1}$ (C=O), as well as subtle shifts in O–H and C–O regions (Fig. 2f). These spectral variations demonstrate the successful incorporation of bioactive compounds into the bilayer structure and validate the functionalization of the active multilayer edible film.



**Insert Fig. 2 about here.**

### 3.2.2. Morphological analysis

Scanning electron microscopy (SEM) offered critical insights into the surface morphology and microstructural organization of the biopolymer-based bilayer films. As shown in Figure 3, the simple bilayer PVA/ ESRG film displayed a relatively homogeneous and smooth topography, whereas the incorporation of *Cuminum cyminum* husk extract markedly altered the film microstructure. The active bilayer exhibited increased surface roughness and porosity, which can be ascribed to the partial incompatibility of the extract constituents with the polymeric matrix, leading to phase separation, localized aggregation of phytochemicals, and disruption of

intermolecular bonding patterns. These interfacial instabilities gave rise to morphological heterogeneity, including void formation, cavities, and surface protrusions (How et al., 2024). Such microstructural disruptions are consistent with previous observations, where the incorporation of plant-derived extracts into starch-based and furcellaran–gelatin bilayer films induced comparable surface irregularities and heterogeneities, as evidenced by SEM characterization (Aaliya et al., 2022; Nowak et al., 2024).

**Insert Fig. 3 about here.**

### 3.2.3. Thermal Properties

DSC analysis revealed that the pure ESRG film exhibited two endothermic transitions: the first, related to moisture loss, initiated at 61.2 °C and peaked at 78.5 °C ($\Delta H$ = 19.51 J $g^{-1}$), while the second, attributed to structural rearrangements, occurred between 207.6 and 223.4 °C ($\Delta H$ = 0.31 J $g^{-1}$). Edible glue showed a major endothermic peak starting at 79.8 °C with a maximum at 106 °C and a high enthalpy of 748.82 J $g^{-1}$, indicating strong intermolecular interactions (Table 2).



For the bilayer systems, combining ESRG with PVA shifted the onset temperature of the first endothermic event to higher values compared with pure ESRG, while still remaining lower than that of neat PVA (Table 2). This intermediate behavior reflects a synergistic effect between the two polymers, where bilayering enhances thermal stability relative to single ESRG but does not completely reach the stability of PVA alone. In contrast, neat PVA showed structural transitions at lower temperatures than the active bilayer film, confirming that multilayering improved the resistance of the material to thermal rearrangements (Table 2).

The incorporation of *Cuminum cyminum* husk extract into the bilayer film further increased both the onset and melting point temperatures (Table 2). This enhancement can be explained by the reduced chain mobility and stronger intermolecular interactions resulting from the presence of phenolic compounds in the extract, which favor a more compact and organized polymer network. Such stabilization is consistent with the findings of Jouki et al. (2014), who reported improved thermal resistance in mucilage-based films containing oregano and thyme essential oils.

Overall, the results confirm that both multilayer technology and incorporation of bioactive extracts contribute to higher thermal stability of edible films. These modifications raise the melting transition temperature, delay degradation processes, and therefore improve the applicability of such films for food packaging. Similar trends were observed by Nagarajan et al. (2017), who showed that bilayer PLA films exhibited significantly higher gelatinization temperatures compared with single-layer gelatin films.

The absence of additional or split thermal transitions in DSC thermograms indicates that no phase incompatibility or stress concentration occurred. Instead, the observed shifts in transition temperatures reflect restricted polymer chain mobility and enhanced intermolecular interactions within the bilayer system.



**Insert Table 2 about here.**

### 3.3. Microbiological changes of beef sample

Aerobic Plate Count (APC) reflects the total viable load of aerobic mesophilic bacteria and serves as a general indicator of product hygiene, quality, and shelf life rather than the presence of specific

pathogens. The initial APC of beef was ~2.5 log CFU/g, confirming acceptable hygienic status (Fig. 4a). Comparable values have been reported for beef loin (~3.0 log CFU/g; Kim et al., 2018) and retail beef in Ethiopia (~5.0 log CFU/g; Kebede & Getu, 2023). In beef, APC <5 log CFU/g denotes good quality, whereas counts >7 log CFU/g indicate spoilage (ICMSF, 2002). In this study, APC of control beef exceeded 7 log CFU/g after 9 days of refrigerated storage, reaching ~8.35 log CFU/g by the end, thus limiting shelf life to 9 days. Conversely, samples wrapped with bilayer films remained below 7 log CFU/g up to day 12, with active bilayer films showing superior inhibitory effects compared to simple bilayers.

*Enterobacteriaceae* are a group of bacteria associated with meat spoilage and are considered hygiene indicator microflora (Heydari-Majd et al., 2019[a]). Therefore, they were monitored in beef fillet samples during storage (Fig. 4b). The *Enterobacteriaceae* population started at ~2.5 log CFU/g and increased to 6.18 log CFU/g in control beef after 12 days of refrigeration (Fig. 4b). In contrast, counts were reduced to 5.49 log CFU/g with the simple bilayer film and further suppressed to 4.14 log CFU/g by the active bilayer film at the end of storage, demonstrating the superior inhibitory effect of the latter on bacterial proliferation (Fig. 4b).

*Pseudomonads*, gram-negative and psychrotrophic aerobes, are among the main spoilage bacteria of meat at low temperatures. As shown in Fig. 4c, their initial count of *Pseudomonads* in beef was 3.59 log CFU/g, which increased to 8.33 log CFU/g in the control after 12 days at 4 °C. In contrast, samples wrapped in bilayer films showed lower counts (6.83 log CFU/g), with the active bilayer containing *Cuminum cyminum* husk extract exerting stronger inhibitory effects. The reduction is likely associated with the low oxygen permeability of bilayer films combined with the antimicrobial activity of the extract. Previous studies also confirmed that modified atmosphere

packaging (Karabagias et al., 2011) and incorporation of essential oils such as oregano and thyme (Emiroğlu et al., 2010) can effectively suppress *Pseudomonas* spp. during refrigerated storage. The initial *Psychrotrophic* bacterial load was 2.49 log CFU/g, which progressively increased in the control samples, reaching 5.82 log CFU/g by the end of storage (Fig. 4d). In contrast, the active bilayer film showed the greatest inhibitory effect, limiting bacterial growth to 4.17 log CFU/g. The effects of the bilayer film on the aforementioned bacteria can be attributed to the antimicrobial properties of *Cuminum cyminum* husk extract, PVA, and the ESRG film, as well as the reduction of oxygen permeability by these films. *Cuminum cyminum* husk extract is rich in antimicrobial and antioxidant constituents, including quinoline, benzoic acid, para-xylene, toluene, *β*-selinene, and quercetin (Srinivasan, 2018; Fatima et al., 2018; Li & Jiang, 2004). Jafari et al. (2023) reported that films incorporating ESRG exhibited antimicrobial effects against both Gram-positive and Gram-negative bacteria. Similarly, Tuzcu et al. (2017) demonstrated its inhibitory activity against *Escherichia coli* and *Bacillus subtilis*, while Kanaani and Mohamadi Sani, (2015) confirmed that ESRG can suppress the growth of various Gram-positive and Gram-negative strains.



**Insert Fig. 4 about here.**

### 3.4. pH value

The initial pH was 5.85, which increased over time due to bacterial activity. At the end of the storage period (12$^{th}$ day of the storage), the pH of the control treatment reached 7.4, while the simple and active bilayer films showed values of 7.0 and 6.5, respectively (Fig. 5a). This rise in pH is mainly attributed to the production of volatile bases such as ammonia and trimethylamine, as well as the action of microbial enzymes. Over time, enzymatic activity of microorganisms leads

to the degradation of meat tissue, accompanied by the breakdown of protein compounds and the release of nitrogenous compounds, ultimately resulting in an increased pH (Bekhit et al., 2021; Lee & Shin, 2019). Such pH changes negatively affect the quality characteristics of the coated meat. The pH test results were consistent with the microbial analysis, showing the greatest increase in the control group, which lacked any protective effect. In contrast, the active bilayer film showed the best performance, as the incorporation of *Cuminum cyminum* husk extract significantly inhibited microbial growth and, consequently, limited the increase in pH.

**Insert Fig. 5 about here.**



### 3.5. Determination of meat freshness

The TBARS assay is a common method for evaluating lipid oxidation, with malondialdehyde (MDA) serving as a key indicator due to its close association with meat sensory attributes such as flavor, odor, and texture. As shown in Fig. 5b, the initial TBA value was 0.1 mg MDA/kg, within the normal range, and increased in all treatments during storage. By the end of the period ($12^{th}$ day of the storage), TBA levels reached 1.0 mg MDA/kg in the control, while samples wrapped with the active bilayer film showed the lowest value (0.5 mg MDA/kg). The superior antioxidative effect of the active bilayer is likely attributed to the dual film barrier and the presence of *Cuminum cyminum* husk extract. Essential oils and plant extracts are known to suppress TBA formation through radical scavenging, peroxide decomposition, and metal chelation (Perumalla & Hettiarachchy, 2011). Since the reported threshold for off-flavor development is >0.5 mg MDA/kg, the relatively low final MDA values across treatments indicate minimal lipid oxidation in the beef samples, further aided by storage conditions (dark refrigeration, protection from light).

Low TVB-N indicates limited bacterial activity and reduced spoilage, as microbial growth and metabolism generate ammonia and amines. The initial TVB-N value of beef was 14.5 mg N/100 g, with lower levels observed in packaged treatments compared to the control, consistent with microbial results (Fig. 5c). By day 12, TVB-N reached 33.9 mg N/100 g in the control but only 19.5 mg N/100 g in samples wrapped with the active bilayer film containing *Cuminum cyminum* husk extract, demonstrating its superior inhibitory effect. Minor decrease was observed on days 3 and 6, followed by a steady increase as microbial activity intensified. The protective effect of the active bilayer likely resulted from both the antimicrobial compounds of the extract and the oxygen barrier properties of PVA. Similar reductions in TVB-N accumulation with biopolymer films have been reported for starch–PVA–anthocyanin composites (Qin et al., 2021). According to the European Commission (CEC, 1995), the maximum acceptable limit for TVB-N is 35 mg/100 g; in this study, all treatments remained below this threshold throughout storage.



### 3.6. Sensory evaluation

Sensory evaluation is a key parameter in assessing product quality, as microbial spoilage and lipid oxidation lead to undesirable changes in odor, color, and texture, thereby shortening shelf life. As shown in Fig. 6, sensory scores declined over time, with the control samples showing the greatest reduction due to microbial and enzymatic activity, which caused off-odors and texture softening. In contrast, treatments with bilayer films, particularly those containing *Cuminum cyminum* husk extract, better preserved odor and texture attributes by limiting microbial growth and oxidation. Color was the least affected attribute, showing only minor changes during storage, with the lowest values again observed in the control. Overall acceptability followed the same trend, remaining significantly higher in coated samples compared to the control throughout storage.

**Insert Fig. 6 about here.**

Although accelerated aging or long-term environmental durability tests were not conducted, the absence of interlayer separation during handling and testing, along with stable barrier performance and sustained antimicrobial activity during the 12-day refrigerated storage trial, suggests that interfacial integrity was maintained under intended application conditions. Future studies should include humidity and thermal cycling to evaluate long-term structural stability.

### 3.7. Limitations and future perspectives

Despite promising performance, the proposed bilayer system has limitations. The solvent-casting technique used in this study is primarily suited for laboratory-scale production, and industrial scalability would require adaptation to extrusion or roll-to-roll coating technologies. Due to the hydrophilic biopolymer composition, compatibility with conventional plastic recycling streams may be limited; however, the system is more compatible with biodegradation or composting pathways. Future studies should address scalable processing, life-cycle assessment, and end-of-life behavior to fully evaluate environmental sustainability.



## 4. Conclusion

The findings of this study demonstrate that active bilayer films prepared from ESRG and PVA, activated with cumin husk extract, exhibit excellent potential as biodegradable alternatives to conventional synthetic packaging. The films showed improved barrier and thermal properties, alongside antimicrobial and antioxidant activities that effectively enhanced the microbial safety, oxidative stability, and sensory acceptability of beef fillets during refrigerated storage.

Incorporation of cumin husk extract not only improved the functionality of the bilayer system but also valorized an agricultural by-product, supporting both food preservation and sustainability goals. Therefore, the proposed bilayer packaging can serve as a novel, eco-friendly approach for extending shelf-life of perishable food products, with potential applications in meat and other protein-rich foods. Although petrochemical multilayer films may provide higher absolute barrier values, the developed bilayer system demonstrates competitive performance within the context of biodegradable active packaging, offering natural antimicrobial functionality and effective meat shelf-life extension.

## CRediT authorship contribution statement

All authors meet the criteria for authorship as outlined by the International Committee of Medical Journal Editors (ICMJE). Specifically:

**Elham Merrikhi Ardebili** was responsible for writting original draft, Software, Methodology, Investigation, Formal analysis. **Abdollah Jamshidi** conducted the literature review and organized the data. **Razieh Niazmand** administrated project, review & editing, supervision, , investigation, data curation and conceptualization. **Mojtaba Heydari-Majd** drafted the manuscript and revised it critically for important intellectual content.



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The authors have no financial conflicts of interest.

**Data availability statement**

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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**Figure captions**

**Fig. 1.** Visual appearance of (a) simple bilayer and (b) active bilayer of PVA/ESRG film.

**Fig. 2.** FTIR spectrum of active bilayer film: (a) ESRG films containing 40% of glycerol, (b) active ESRG films containing *Cuminum* husk extract, (c) pure PVA film, (d) Tutkal edible glue, (e) simple bilayer ESRG/PVA film and (f) active bilayer PVA/ESRG film

**Fig. 3.** SEM micrographs of (a) simple bilayer ESRG/PVA film and (b) active bilayer ESRG/PVA film.

**Fig. 4.** Temporal evolution of microbial populations in beef fillet samples packaged with various bilayer PVA/ ESRG film formulations during storage period. Aerobic plate count (APC) (a), *Enterobacteriaceae* family, (b) *Pseudomonas* spp. (c) and *Psychrotrophic* bacteria (d) populations represented by distinct colored trend lines for each treatment. Unpackaged samples serve as the reference control group. Data are shown as mean ± SD of three independent measurement.

**Fig. 5.** Changes in (a), pH (b), TBA and (c), TVB-N values of packaged-beef fillets during storage in refrigerator. Data are shown as mean ± SD of three independent measurements.

**Fig. 6.** Sensory evaluation of packaged meat samples over time. (a) odor (b), colour (c), texture and (d) overall acceptance. Data are shown as mean ± SD of three independent measurements.

**Table 1.** Physical properties of active PVA/ESRG bilayer films containing *Cuminum husk* extract

| Film | Thickness (mm) | WVP ($g/m^2$ s Pa) | Solubility (%) | TS (*M*Pa) | EB (%) |
|---|---|---|---|---|---|
| **ESRG** | $0.15 \pm 0.002^{b}$ | $1.58 \pm 0.024^{d}$ | $46.5 \pm 1.45^{b}$ | $4.65 \pm 0.12^{a}$ | $15.4 \pm 0.89^{a}$ |
| **PVA** | $0.10 \pm 0.008^{a}$ | $1.12 \pm 1.034^{c}$ | $50.2 \pm 1.54^{cd}$ | $9.36 \pm 0.42^{a}$ | $19.62 \pm 0.34^{a}$ |
| **PVA/ESRG** | $0.22 \pm 0.021^{c}$ | $0.98 \pm 0.084^{ab}$ | $50.0 \pm 1.25^{c}$ | $11.64 \pm 0.24^{b}$ | $14.31 \pm 0.32^{b}$ |
| **Active PVA/ESRG** | $0.25 \pm 0.012^{d}$ | $0.95 \pm 0.012^{a}$ | $45.3 \pm 1.37^{a}$ | $4.35 \pm 0.24^{c}$ | $16.34 \pm 0.99^{c}$ |

*Values are given as mean ± standard deviation. Values within each column with different letters are significantly different ($p < 0.05$

**Table 2.** Thermal properties (starting and peak) and enthalpy simple and active films

| Film | Peak 1 | | | Peak 2 | | |
|---|---|---|---|---|---|---|
| | Teo (˚C) | Tm (°C) | H (J/g) | Teo (˚C) | Tm (°C) | H (J/g) |
| ESRG | 61.2 | 78.5 | 19.51 | 207.6 | 223.4 | 0.31 |
| Glue | 79.8 | 106.0 | 748.8 | - | - | - |
| PVA | 61.2 | 81.6 | 19.5 | 260.7 | 279.7 | 14.7 |
| PVA/ESRG | 33.5 | 84.2 | 72.8 | - | - | - |
| Active PVA/ESRG | 40.1 | 71.2 | 35.7 | 361.3 | 376.7 | 1.9 |

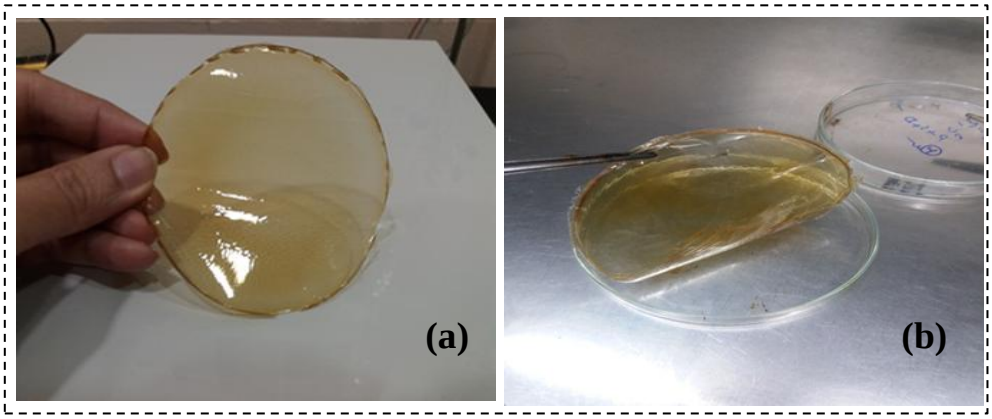


**Fig. 1.**

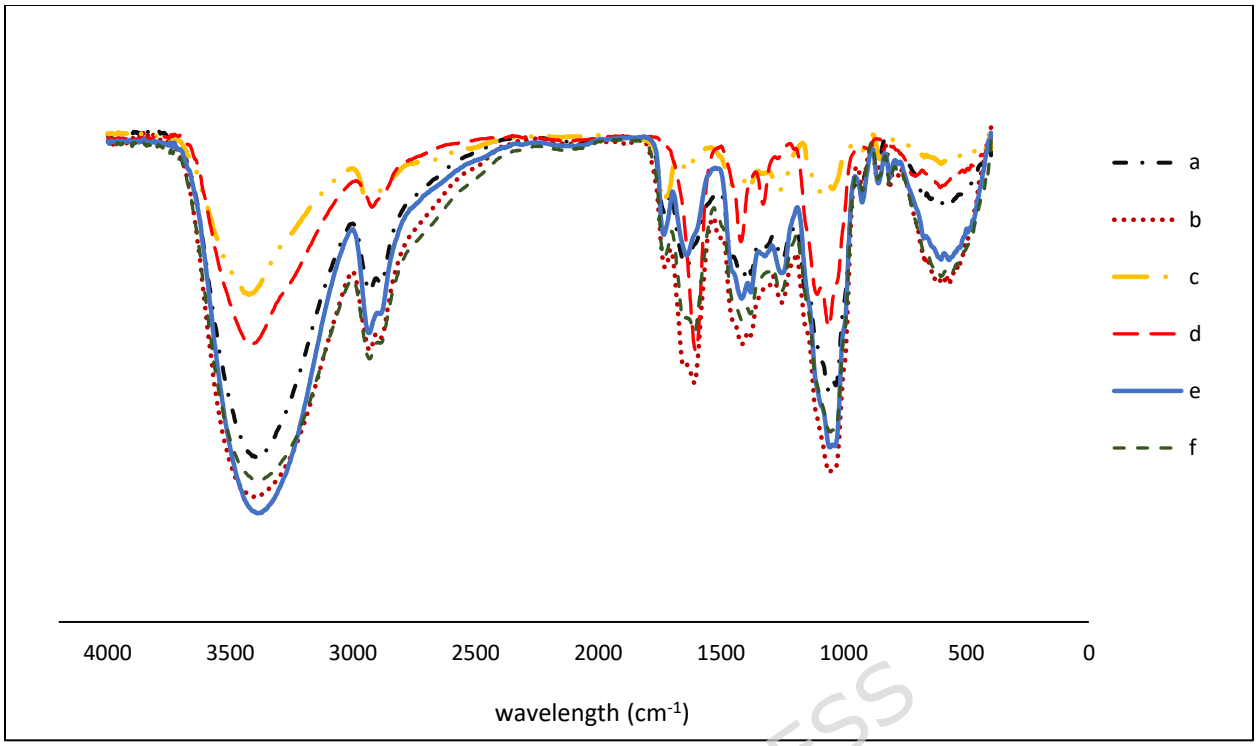


**Fig. 2.**

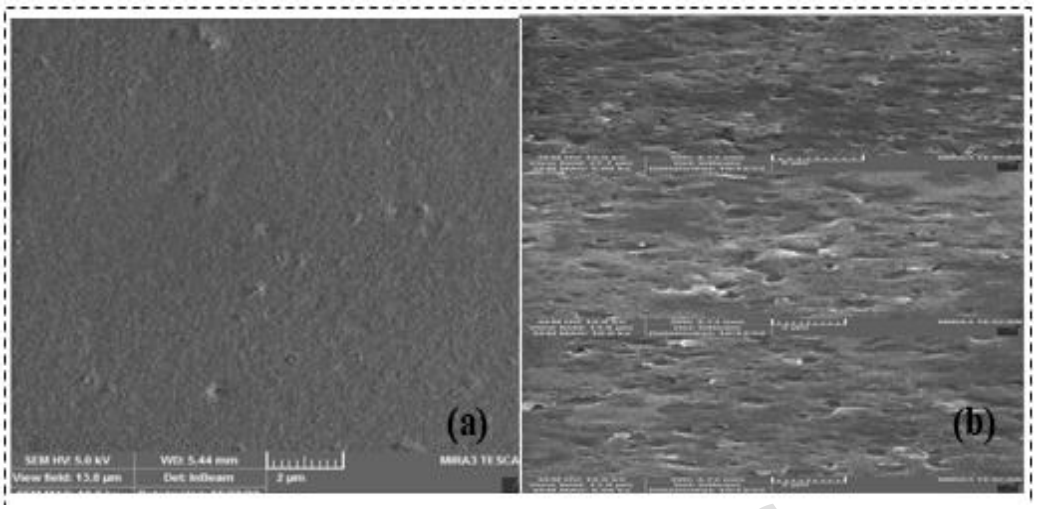


**Fig. 3.**

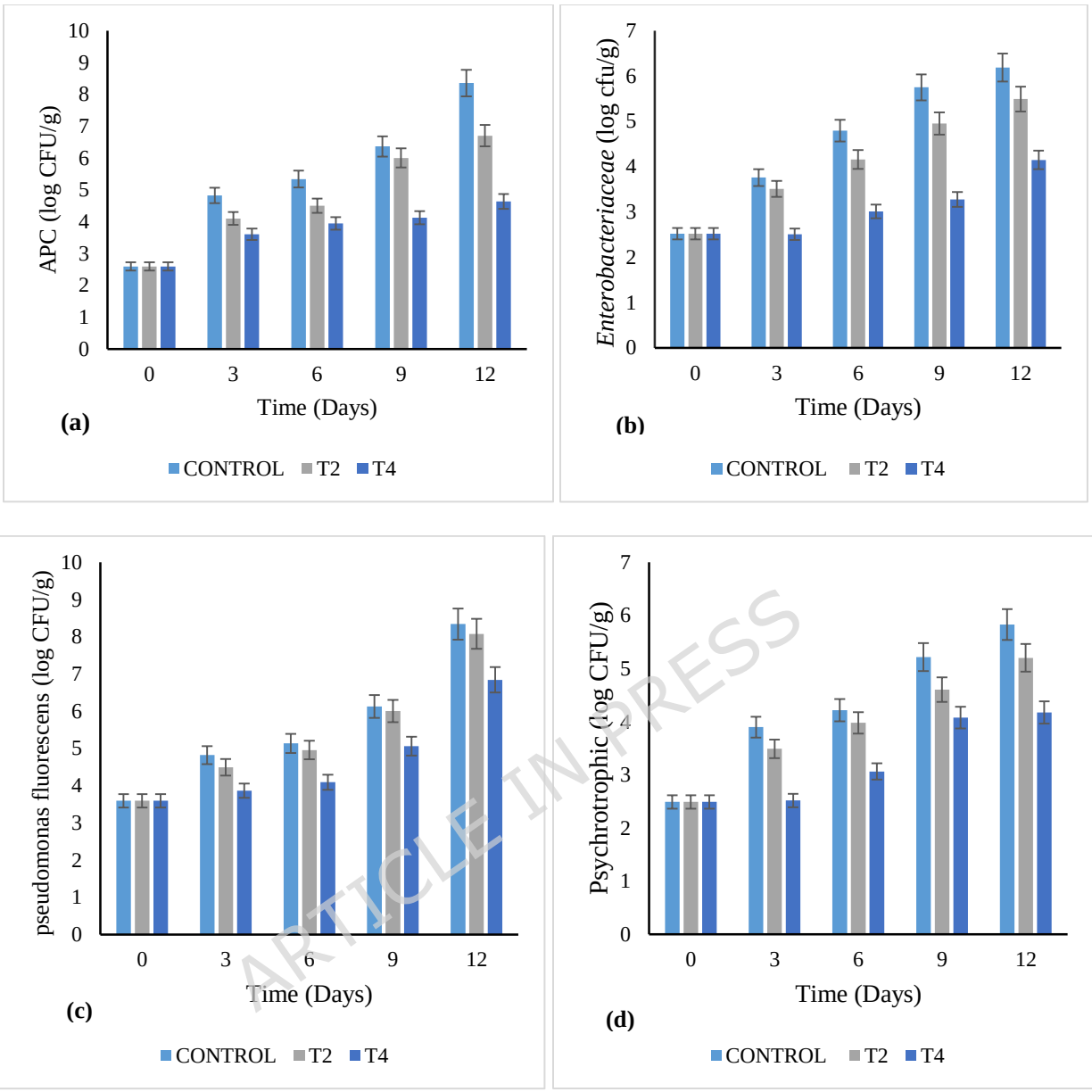
APC (log CFU/g)
Time (Days)
(a)
CONTROL T2 T4
Enterobacteriaceae (log cfu/g)
Time (Days)
(b)
CONTROL T2 T4
pseudomonas fluorescens (log CFU/g)
Time (Days)
(c)
CONTROL T2 T4
Psychrotrophic (log CFU/g)
Time (Days)
(d)
CONTROL T2 T4

**Fig. 4.**

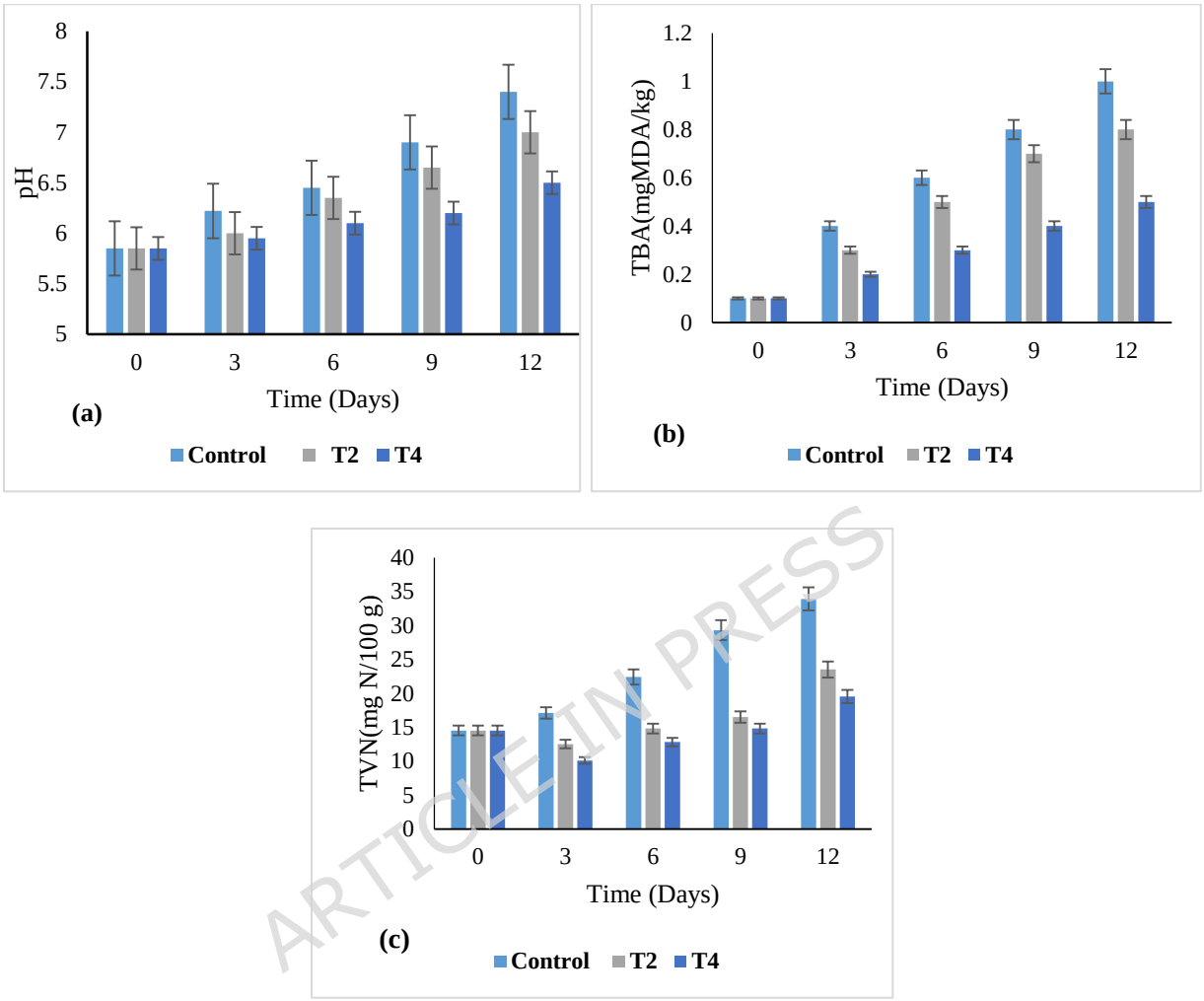




**Fig. 5.**

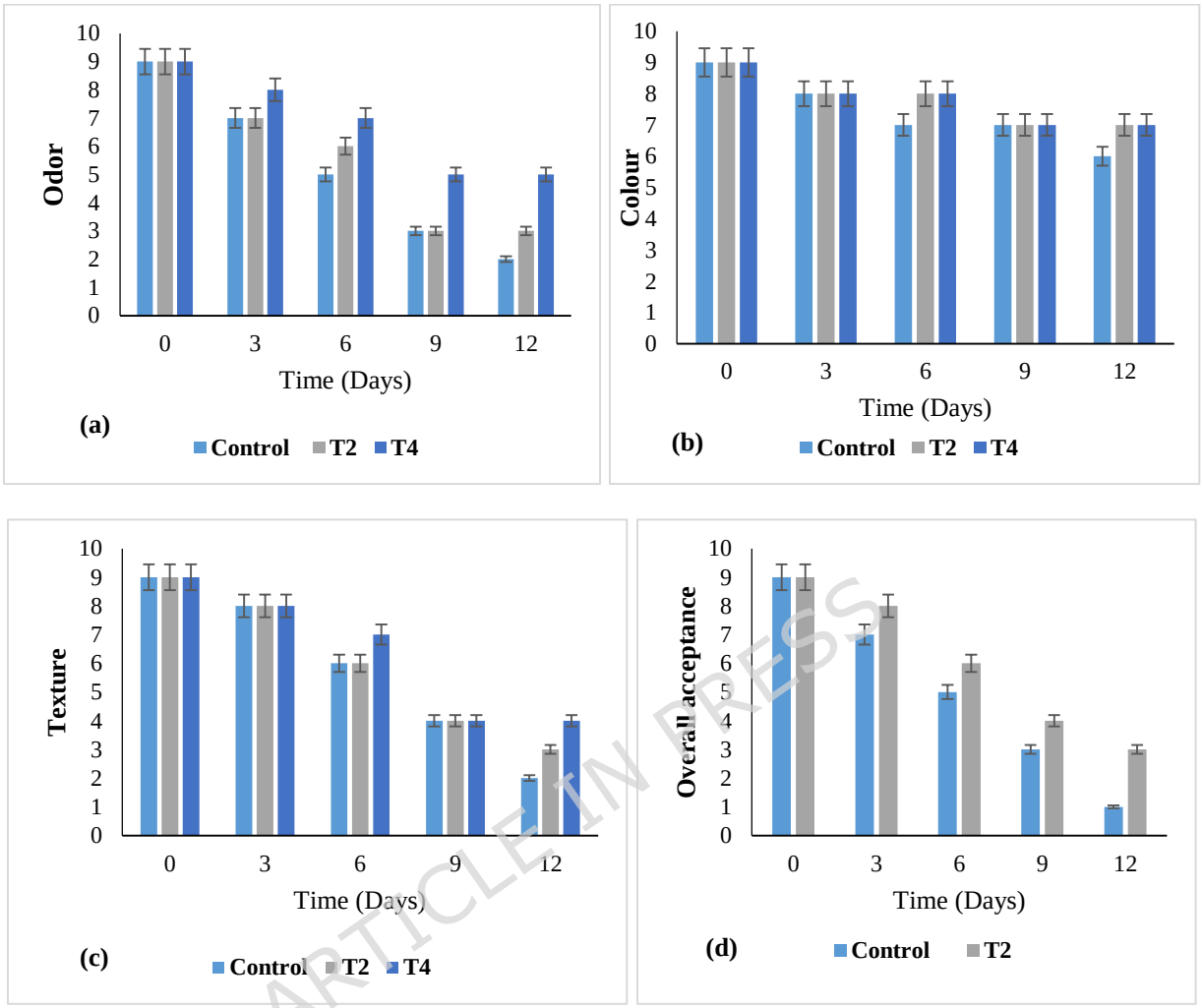




**Fig. 6.**