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Designing a pH-sensitive smart detector from gelatin-kappacarrageenan *Mirabilis jalapa* and *Berberis vulgaris* anthocyanin to evaluate the freshness/spoilage of lamb meat

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ABSTRACT

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Today, the utilization of smart indicators in food packaging to monitor and detect food quality through analyzing quality data and color changes in packaging films based on the food's condition is on the rise. In this comparative study, halochromic films made of gelatin and k-carrageenan with mirabilis jalapa extract (6, 12, and 24%) and barberry extract (6, 12, and 24%) were developed and examined. Through SEM images and FTIR spectroscopy, it was observed that both types of films incorporating barberry and mirabilis jalapa extracts were uniformly dispersed in the gelatin and k-carrageenan polymer matrix, displaying notable molecular interactions like hydrogen bonding and electrostatic forces. However, films containing mirabilis jalapa extract exhibited more irregular and rough surfaces compared to those with barberry extract. Both types of films displayed good antioxidant properties and responsiveness to changes in pH and ammonia levels. Films with barberry extract demonstrated higher antioxidant activity and greater sensitivity to pH variations. The gelatin and k-carrageenan films with barberry and mirabilis jalapa extracts effectively indicated the freshness of lamb meat stored at 25°C by correlating with the presence of ammonia gases in the storage packages and pH fluctuations. Our results highlight that the structural, physical, and functional attributes of gelatin and k-carrageenan films incorporating pH-responsive extracts are significantly influenced by the extract type and concentration.

1- Introduction

Preservation of food products to increase their shelf life and protect them completely from the risks of internal and external spoilage factors such as microorganisms and oxidative deterioration from production to consumption is a very important and necessary matter that can be achieved through various means such as food packaging (1-3). With changes in people's lifestyles, there has been an increased demand for high-quality, fresh, minimally processed, and long-lasting ready-to-eat products. Consequently, various solutions such as the use of innovative packaging (active and intelligent) have garnered significant attention from researchers (3-5). In fact, these types of packaging have been developed to better meet consumer needs and increase the shelf life of the products. Changes in the safety and quality of food products can occur during production, storage, distribution, transportation, and consumption. Consumers usually assess the freshness and quality of packaged foods by using the printed expiration date on the packaging (6-8). However, the expiration date alone is not sufficient to assess the freshness and quality of some food products such as fresh fruits and vegetables. Color is considered a natural indicator of food quality and also as one of the key factors for identifying and monitoring physicochemical changes in food products. In this regard, the use of sensors and smart color labels is considered as an innovative system for detection, tracking, protection, and assurance in producing safe and quality products (1, 6). This innovation has led to the emergence of new generation packaging called smart and active, which can take a significant step in producing various sensors or smart indicators by producing kits or color-sensitive labels that are responsive to changes in food substances (pH, gases, etc.) and attaching them to packaging (7, 9). Today, due to the concerns about environmental issues related to the disposal and accumulation of non-biodegradable plastics, as well as the reduction of petroleum resources, researchers and consumers have paid special attention to the use of biodegradable and compostable compounds in the environment. Thus, extensive efforts have been made to investigate and utilize biodegradable polymers based on natural compounds from renewable sources (10, 11). For this reason, in recent years, scientists have focused on using natural polymers to produce

packaging systems with high mechanical performance as well as suitable physical and functional properties (12). Proteins and polysaccharides are the main polymers widely used to produce stable films and packages (13, 14).

Gelatin is a protein derived from collagen by water extraction and due to its unique properties such as surface activity, gel formation capability, viscosity control, and film formation, it is one of the most important natural polymers used in various industries including the food industry. Gelatin creates films with good mechanical properties and proper preservation against oxygen and odor in low to medium relative humidity, but due to the hydrophilic nature of this polymer, films made from it are permeable to moisture. On the other hand, studies have shown that edible films produced with a combination of multiple biopolymers have better properties compared to films made from a single component (15, 16). In fact, by using other biodegradable polymers, composites with suitable physicochemical, structural, and functional properties can be created. Based on this, usually indicator and composite films, semi-synthetic with improved technological performance and biological features, are made using two or more natural or synthetic polymers. Among these suitable compounds with high potential for forming such composites, κ -carrageenan can be mentioned (17). κ -carrageenan are a family of linear sulfated natural polysaccharides extracted from red edible seaweeds. Due to their biodegradability, transparency, and high flexibility, κ -carrageenan has wide and acceptable applications in food products. On the other hand, this compound creates a film with adequate strength and the resulting film is transparent, odorless, tasteless, and resistant, but it has high permeability to water vapor (11, 17).

Mirabilis jalapa (MJ) is the most common ornamental species of the Mirabilis plant, available in various color spectrums including yellow, purple, and orange. MJ has been cultivated for medicinal and ornamental purposes in many tropical regions as well as in Europe, the Mediterranean coasts, and Iran. This plant exhibits suitable antimicrobial, antiviral, and antioxidant activities (18-20). Chemical analysis of different species has confirmed the

presence of alkaloids, flavonoids, phenols, steroids, triterpenes, glycosides, tannins, saponins, lignans, and several other compounds in this plant. On the other hand, barberry (BB) (*Berberis vulgaris*) is a common plant in Iran and regions like North America, Central and South Europe, and South Asia, used both as a medicinal plant and its fruits as a food additive (20, 21). Around 22 alkaloid compounds have been identified in the roots, leaves, and fruits of BB, including protoberberine, berberamines, tetrandrine, candicorine, and palmatine. The fruits of this plant contain significant amounts of phenolic compounds (including anthocyanins and carotenoids), pectin, saponins, vitamin C, resin, and tannins (21, 22). The antioxidant activity of BB is significant, reducing the survival of cancer cells, possibly due to the presence of phenolic compounds and flavonols in the plant. Extracted anthocyanins from BB and MJ are unique sources of sensitive color compounds that are suitable antioxidants for use in food packaging industries due to their color sensitivity to changes in food quality (1, 22, 23). Generally, smart films are used as a potential freshness indicator for many perishable foods such as seafood and meat, as the color changes that occur in these films due to food spoilage provide a reliable method for detecting the degree of food deterioration during storage. Studies have been conducted on the use of pigments extracted from MJ and BB. Additionally, research has been focused on comparing the anthocyanins of MJ and BB in smart food packaging. Therefore, the aim of this research is to produce and optimize the functional properties of a gelatin/ κ -carrageenan composite containing smart indicators sensitive to the pH of anthocyanin pigments from MJ and BB in monitoring the freshness/spoilage of fresh lamb meat.

2- Materials and Methods

1.1. Materials and Chemicals

κ -carrageenan powder (molecular weight 560-400 kDa) and gelatin (molecular weight 80 kDa, gel strength 200 Bloom, moisture less than 12g per 100g) were purchased from Sigma company. Ethanol, glycerol, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were acquired from Merck Germany. The water used in the experiment was obtained from the Millipore purification system. All chemical substances were of analytical grade, and the lamb meat was

purchased from the local market in Kermanshah, Iran.

1.2. Extraction of anthocyanin from MJ and BB

The extraction process was conducted using the method proposed by Tavassoli and et al. (2024) (24). In summary, 10 g of dried and crushed petals of MJ flowers and BB were mixed in 200 mL of a solvent mixture of water and alcohol in a 40:60 ratio in a flask. The flask was placed on a magnetic stirrer at 25 °C for 24 h. The flask was covered with aluminum foil to prevent the detrimental effects of light. The next day, the samples were filtered using filter paper and a centrifuge device for 20 min to separate the liquid phase. The upper liquid phase was separated, and the solvent was evaporated using a rotary evaporator. Similarly, for BB anthocyanin, after drying, crushing, and powdering the BB seeds, they were mixed with alcohol and water in a 20:80 ratio in a flask covered with aluminum foil and stirred on a magnetic stirrer for 24 h. Then, similar to the MJ extraction method, the remaining steps were applied to extract the BB pigment as well (11).

1.3. pH-sensitivity of anthocyanin from MJ and BB

Using HCl and NaOH solutions, the pH of the solution was initially adjusted between 2-13. Subsequently, concentrated MJ and BB pigments were immersed in prepared acidic or alkaline solutions (pH 2-13), and the color changes of the extracts were recorded by a digital camera (24).

1.4. Film Preparation

To produce intelligent films containing MJ and BB extract, the film-forming bases were first prepared with a casting technique according to Tavassoli et al. (2024) with some modifications (24). Films were prepared from 2% w/w κ -carrageenan and 3% w/w gelatin, each separately dispersed in distilled water containing 30% w/w glycerol and vigorously stirred at 100 °C for 30 min. Then, two solutions were combined in a 50:50 ratio and homogenized for 2 h to achieve a uniform mixture. Separate solutions of MJ and BB anthocyanins at concentrations of 6, 12, and 24% v/v polymer were added separately to the solutions by stirring at room temperature for 10 min. The solutions were sonicated for the first 3 min to remove bubbles and then the final solutions were spread on 8 cm diameter glass

plates and dried at room temperature for 72 h to form films. The dried films were separated from the plates and stored in airtight plastic containers with lids in the dark until further experiments were conducted (25).

1.5. Physical and Chemical Properties of film samples

1.5.1. Thickness

The thickness of the films was evaluated using a digital micrometer (Mitutoyo Co, Tokyo, Japan) at six random points with an accuracy of 0.001 mm (25).

1.5.2. Moisture Content

To evaluate the moisture content (MC), samples were cut into small pieces (20mm x 20mm) and weighed before drying at 25 °C, then dried for 6 h in an oven at 105 °C, reweighed, and finally, the percentage weight loss of the sample based on the initial weight was calculated using the following formula (11):

$$MC (\%) = (M1 - M2/M1) \times 100$$

1.5.3. Water Solubility

Water solubility (WS) was evaluated based on the weight loss of samples after dissolving in distilled water. In this regard, the samples were first dried in an oven at 105 °C for 24 h and then weighed. After that, the samples were immersed in 100 mL of distilled water at room temperature for 24 h. This system was filtered with filter paper (Whatman No. 1) to collect the undissolved sample and the residue was filtered. Finally, the remaining samples on the filter paper were dried at 105 °C for 24 h and weighed again. Ultimately, the WS of the samples in water was determined as follows (11):

$$WS (\%) = (W1 - W2/W1) \times 100$$

1.5.4. Water Vapor Permeability

The water vapor permeability (WVP) of the samples was evaluated using the ASTM E96-00 standard method with slight modifications (11). Film samples were prepared precisely on top of a glass diffusion cup containing 3 g of CaCl₂ granules as a moisture absorbent material, and then placed in a desiccator containing distilled water. The weight changes of the cups after predetermined intervals of time (daily; every 1 hour for 6 h, and intermittently every 24 h for 5 days) were recorded. By multiplying the vapor transmission rate in the film thickness and

dividing it by the difference in relative humidity between the inside of the cell and the desiccator, the WVP is calculated (11):

$$WVP = \frac{WVTR \times L}{A \times \Delta P}$$

1.5.5. Mechanical Properties

The mechanical strength of the samples was determined using a texture analyzer (M350-10CT, Testometric Co., England). The samples were cut into rectangular pieces (80 mm × 25 mm) and placed between the grips of the testing machine. The gauge length was set at 50 mm and the speed at 50 mm/min. Tensile strength (TS) and Young modulus (YM) in MPa and elongation at break (EAB) as a percentage were examined as follows (26):

$$TS \text{ (MPa)} = \frac{\text{Stress at break (N)}}{\text{Thickness (mm)} \times \text{Width (mm)}}$$

$$EAB (\%) = \frac{\text{Increase in length}}{\text{Initial length}} \times 100$$

$$YM \text{ (MPa)} = \frac{\text{Stress at initial straight stress} - \text{strain curve}}{\text{strain at initial straight stress} - \text{strain curve}}$$

1.6. Films transparency

Film strips (30 mm × 12 mm) were directly placed in a test cell, and the light transmittance (T%) of the samples was recorded using a visible-UV spectrophotometer (UNICO 2100, USA) with wavelengths ranging from 800 to 200 nm at room temperature. For each sample, the thickness before scanning was calculated, and the transparency values of the samples were determined by examining the light transmittance (%) at a wavelength of 600 nm (T₆₀₀) as follows (27):

$$\text{Transparency value} = \frac{\text{Log } T_{600}}{\text{Thickness}}$$

1.7. Morphological and structural characteristics of film samples

To identify the microstructure of the film samples prepared, a scanning electron microscope (SEM) (Quanta 450, USA) was utilized to observe the surface and cross-sectional morphology of samples containing various concentrations of BB and MJ extract (7). Before scanning, the samples were coated with a

thin layer of gold at an accelerating voltage of 10 kV for 120 s. The specific chemical groups' status and the intermolecular interactions among different compounds of the samples after adding varying percentages of dyes to the polymer samples relative to the control sample were analyzed using Fourier-transform infrared spectroscopy (FTIR) in the frequency range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and an average of 32 scans per sample. The crystalline characteristics of the prepared samples were examined using X-ray diffraction patterns (XRD) in the range of $2\theta = 80-5^\circ$ at a scanning rate of $4^\circ \cdot \text{min}^{-1}$ using a Rigaku intelligent diffractometer in the laboratory (28).

1.8. Freshness/spoilage Indicator for Lamb Meat Monitoring

In this study, the method described by Tavassoli et al. (2024) was used to monitor the freshness of lamb meat (24). Initially, lamb meat was cut into 1-cm^3 dimensions. A film sample (10 mm \times 10 mm) was placed on the inner surface of a container holding 30 g of lamb meat at a constant temperature of 25 $^\circ\text{C}$. The samples were incubated at a temperature of 25 $^\circ\text{C}$. The total volatile basic nitrogen (TVB-N) content and pH values of lamb meat were measured every 6 h with three repetitions. The Kjeldahl method was used to determine the TVB-N content in the meat. Initially, 10 g of meat sample and 2 g of magnesium oxide were evenly dispersed in 300 mL of distilled water. Then the solution was diluted in 50 mL of boric acid and methyl red indicator was added to it, and after that, the volume reached 150 mL. The solution was titrated with hydrochloric acid (HCl). Finally, the TVB-N amount of the sample was calculated using the following formula and reported as mg/100g of meat, where V represents the volume of added HCl and C is its concentration (29):

$$\text{TVBN} = \frac{V \times C \times 14 \times 100}{10}$$

1.9. Functional properties of films

1.9.1. Halochromic Behavior

Film samples containing 24% BB and MJ extracts were cut into 30 mm² squares and soaked for 5 min in a series of buffer solutions (pH 13-2) and their color changes in response to different pH levels were recorded by a digital camera. Furthermore, samples containing different concentrations of extract were placed on top of

an ammonia solution (0.2 mol/L) for 60 min in a glass cup. The color changes of the films were recorded using a digital camera at 10-min intervals (29, 30).

1.9.2. Antioxidant Activity

The antioxidant activities of the films prepared using the method described by Wang et al. (2017) were evaluated by inhibiting the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). A diluted film solution (1 mL) was mixed with 3 mL of ethanol DPPH solution (50 mg/L; Ruitaibio Company, Beijing, China). The prepared solution was incubated at room temperature for 30 min in darkness, and its absorption at a wavelength of 517 nm was measured. Finally, the percentage of DPPH inhibition activity was determined as follows (31, 32):

$$\text{AA} = \frac{\text{AB} - \text{As}}{\text{AB}} \times 100$$

1.9.3. Antimicrobial Properties

To investigate the effect of the produced films against *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *B. cereus* (ATCC 11778), the agar diffusion test method was used. The turbidity of the bacterial suspension was determined using a visible-UV spectrophotometer (UNICO 2100, USA) at a wavelength of 625 nm (bacterial cell density CFU/mL 10^8). Standardized bacterial suspension was inoculated on Muller-Hinton agar plates, then pieces of the produced and dried films cut into 14.3 cm² were placed on the agar for inoculation. The prepared samples were then incubated at 37 $^\circ\text{C}$ for 24 h, and finally, the bacterial growth inhibition zone was examined (11, 32).

1.10. Biodegradability

The biodegradability of the prepared films qualitatively was evaluated using the method presented by Riyaz et al. (2020) (33). Initially, plant compost (tested soil) was poured into a plastic tray (30 \times 10 \times 8 cm), and then the prepared films (25 \times 25 mm) were buried at a depth of about 2 cm for 21 days. To simulate environmental conditions, the treatments were sprayed twice a day with a hose with tap water at ambient temperature, and their appearance changes were recorded using a digital camera at different time intervals (0, 7, 14, and 21 days).

1.11. Statistical Analysis

The data were analyzed using SPSS statistical software (version 23.0, SPSS INC., Chicago, IL, USA). All tests were performed with 3 replicates, and the results were presented as mean ± standard deviation. The significance of the means was determined using one-way analysis of variance (ANOVA) with a significance level of $p < 0.05$.

2. Results and discussion

2.1. pH Sensitivity

Changes in the properties of food products, such as changes in their quality, visual characteristics, safety, and food spoilage, often lead to changes in their pH levels (34). Therefore, analyzing changes in the pH of food products can serve as a useful method for evaluating changes in these important quality characteristics. As a result, we investigated the effect of pH changes on the color of BB and MJ extract solutions. The color changes dependent on the pH of BB and MJ extract solutions at different pH levels (2-13)

reported in Figure 1. Accordingly, BB extract exhibited a red color (related to the cationic form of flavonium) at pH 2 and gradually changed color in different pH levels, such that at pH 13, the extract color shifted to bluish-green (related to chalcone form) (35, 36). Similarly, MJ extract at pH 2 showed a pink color (related to betalamic acid and cyclo-dopa residues) and gradually changed color with increasing pH, such that at pH 13, it turned yellow (related to betalamic acid, neo-betacyanin/neo-betainin, and cyclo-dopa glucoside) (35). In general, MJ extract, rich in betacyanins compared to BB rich in anthocyanins, showed greater color stability in various pH levels. Thus, it can be concluded that color changes in extracts rich in anthocyanins and betacyanins are related to changes in molecular structure and are pH-dependent. Other studies have also investigated color changes in various extracts, such as rose extract (36) and saffron petal extract (37), in different buffers with a similar trend.

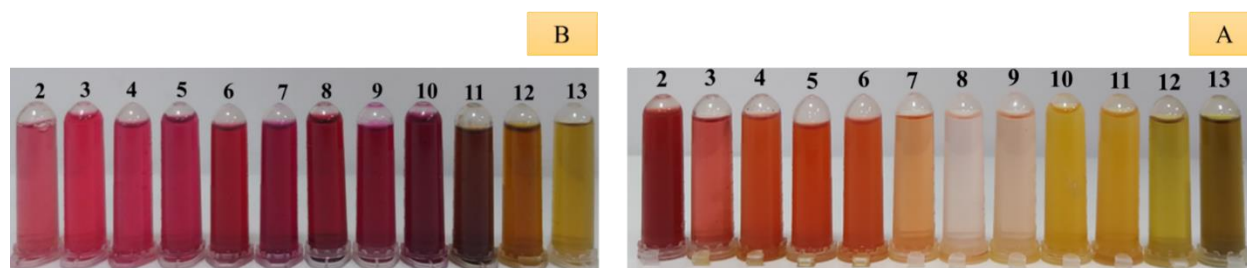


Fig. 1 Color changes of (a) BB extract and (b) MJ flower extract in different buffer solutions (2-13 pH).

2.2. Physicochemical Properties of Gelatin and K-Carrageenan Films

Table 1 displays the measurement of physicochemical properties (thickness, WVP, MC, and WS) of films prepared from gelatin and k-carrageenan along with MJ and BB extracts.

Table 1. Physical and mechanical properties of gelatin and capacarrageenan films with different concentration of BB extract and MJ flower.

Characteristics	Films type						
	Blank	MJ 6%	MJ 12%	MJ 24%	BB 6%	BB 12%	BB 24%
Physical properties							
Thickness (μ m)	92±2 ^c	101±1 ^b	107±0 ^a	109±2 ^a	102±1 ^b	107±0 ^a	108±2 ^a
WVP ($\times 10^{-11}$ g. m/m ² . s. Pa)	1.91±0.01 ^a	1.28±0.05 ^e	1.35±0.05 ^d	1.38±0.05 ^d	1.45±0.03 ^c	1.46±0.02 ^c	1.59±0.01 ^b
Water solubility (%)	80.19±0.02 ^b	92.73±0.03 ^a	77.12±0.01 ^c	73.21±0.06 ^d	43.11±0.05 ^g	49.03±0.03 ^f	68.36±0.02 ^e

Moisture content (%)	16.03±0.05 ^f	19.23±0.03 ^c	23.12±0.04 ^b	28.16±0.02 ^a	17.91±0.04 ^d	16.04±0.03 ^e	8.67±0.01 ^g
Visual feature							
Transparency	15.68±0.05 ^a	10.5±0.03 ^c	10.04±0.04 ^e	9.46±0.03 ^f	10.33±0.01 ^d	10.66±0 ^b	8.69±0 ^g
Mechanical properties							
TS (MPa)	1.01±0 ^{de}	2.89±0 ^a	2.2±0.03 ^b	1.76±0.01 ^c	1.84±0.01 ^{bc}	1.17±0.01 ^d	0.73±0.55 ^e
YM (MPa)	1.70±0.01 ^a	3.85±0.01 ^b	5.11±0.02 ^c	4.71±0 ^d	2.02±0.01 ^e	5.26±0 ^f	2.19±0.03 ^g
EAB (%)	16.72±0 ^f	55.47±0 ^a	33.69±0.03 ^c	21.28±0.02 ^e	39.52±0.03 ^b	22.19±0.04 ^d	16.21±0.01 ^g
Functional properties							
DPPH radical quenching (%)	4.2±0.01 ^g	6.3±0.02 ^f	10.38±0.03 ^e	31.39±0.02 ^d	87.97±0.03 ^c	89.18±0.04 ^b	90.2±0.03 ^a

TS: tensile strength, YM: young modulus, EAB: elongation at break, DPPH: 2,2-diphenyl-1-picrylhydrazyl. The data are presented as mean ± standard deviation. Any two means in the same row followed by the same letter are not significantly ($P > 0.05$) different from Duncan's multiple range tests.

2.2.1. Thickness

Since the thickness of films plays a crucial role in determining the mechanical and protective properties of the final produced films, we initially measured the thickness of gelatin and K-carrageenan-based films (34). According to our results in Table 1, the thickness of the films significantly increased by adding extracts to the prepared film samples, due to the filling of the free volume of the prepared films and subsequently compacting them after adding the extracts. A similar trend was observed in thickness increasing with the enhance in anthocyanin extract concentration from saffron petal and red berry (37), grapefruit seed extract (38) and curcumin (39), and red cabbage anthocyanins (40) in smart packaging films.

Water Solubility

WS refers to a physical property that indicates the extent to which prepared films are sensitive to highly moist food materials (38). Ideal packaging films for storing food products with moderate or high moisture are those that have sufficient resistance to water dissolution even when directly in contact with water (26). The dissolution percentages of the prepared samples are summarized in Table 1. Accordingly, the control sample without dye showed high WS, likely due to the hydrophilic nature of the polar peptide bonds of gelatin and K-carrageenan (37, 41). Despite the hydrophilic nature of the extracts, the WS of the prepared films decreased

with the addition of BB and MJ extracts. However, the decrease in WS was less in samples prepared with MJ extract compared to those prepared with BB extract. Since the WS of films is influenced by the number of hydroxyl groups and hydrogen bonds between polymers in the film structure, adding BB and MJ extracts reduced the solubility of the films, likely due to increased intermolecular attraction forces between biopolymer molecules and reduced water absorption. Other studies, such as the production of gelatin-based films enhanced by covalent interaction with oxidized guar gum containing green tea extract by Yadav et al. (2020) (28), and a study by Roy et al. (2020) (39) on the preparation of color indicator films based on gelatin/carrageenan combined with chitosan, also obtained similar solubility trends (6). However, our results showed that the solubility of the samples produced decreased with the addition of MJ extract. Among all samples, films produced with 6% MJ extract showed the highest WS due to the hydrophilic nature of the MJ extract and the reduced stability of gelatin and K-carrageenan films after adding the MJ extract. Nevertheless, with further increases in the percentages of MJ extract added to the samples (12 and 24%), solubility slightly decreased, likely due to the reduced hydrophilicity of the films as a result of the filling effects of the added extracts (42).

2.2.2. Moisture Content

The network structure of polymers in packaging materials mainly contains pores that trap water molecules and other substances (39). Therefore, the MC of films, especially in hydrophilic biopolymer films like gelatin and K-carrageenan, is important. This property, through measuring the density and water resistance of polymer layers, indicates that water absorption and activity can significantly impact the structure and performance of films (11). According to the summarized results in Table 1, by adding BB extract, films prepared with 24% BB showed a significant decrease in MC from about 16.03% to around 8.67%. The reduction in interaction with water molecules and consequently reduced water absorption may be due to the formation of hydrogen bonds between the hydrophilic film components and anthocyanins (43). However, films containing 6% and 12% BB extract showed no significant change in MC, likely due to lower BB extract content. On the other hand, our results showed that the MC of films prepared with MJ extract increased significantly with the addition of extract. As reported in Table 1, gelatin and K-carrageenan films containing 24% MJ extract had the maximum MC at 28.16%. This increase in MC may be due to the integration of MJ extract into the polymer film solution, leading to decreased cohesion and intermolecular interactions in the gelatin and K-carrageenan polymer networks due to the presence of phenolic compounds in the structure of betacyanins.

2.2.3. Water Vapor Permeability

WVP in antimicrobial and smart packaging made from biopolymers is a very important feature (43). This parameter indicates the effectiveness of the film in preventing the passage of water or other hydrophilic escape compounds. Production films should have low permeability to effectively enhance the durability of food compounds by delaying physicochemical and microbiological spoilage. As reported in Table 1, among all produced samples, the pure gelatin and K-carrageenan films showed the highest WVP due to the presence of hydrophilic factors in the film components, which was consistent with the results of He et al. (2020) (44). After adding extracts to the film samples, the WVP decreased. The decrease in WVP in the samples after incorporating extracts may be associated with increased thickness, various aspects of internal microstructures (e.g., crystallinity or amorphous

regions), and the formation of hydrogen bonds between film components and anthocyanin or betacyanin functional groups (45). Adding 6% BB extract to the prepared film samples significantly reduced WVP due to the formation of a uniform and compact film. Gradually, the WVP increased in film samples with the addition of 12% and 24% BB extract, likely related to decreased homogeneity (46). However, this decrease was also observed after adding extracts to film solutions in samples prepared with MJ extract. The results showed that the WVP in samples prepared with MJ extract was lower than in samples prepared with BB extract. This could be attributed to the denser internal structure of the samples prepared with MJ extract, resulting from strong hydrogen bonds formed between betacyanins and the film solution, which significantly reduced the film's hydrophilicity. Similar results were observed in films containing beetroot extract (46) and anthocyanin-rich saffron and red berberry extracts (37).

2.2.4. Mechanical Properties

One of the characteristics of food packaging is the ability to withstand pressures during transport and storage without significant changes (44, 45). Research has shown that the mechanical properties of prepared films depend on the structure of their components and the interactions between them (37). Table 1 reports TS, EAB, and YM of the produced films. Based on this, gelatin and K-carrageenan pure films had relatively low flexibility (16.72%), as well as low TS and YM (1.01 and 1.70 MPa, respectively). In fact, the flexibility and EAB in the prepared samples were consistent. While the TS and YM values increased compared to the control sample, a decreasing trend was observed with the addition of higher percentages of extracts to the film solutions. As the TS and YM values increased compared to the control sample, the mechanical properties of the produced films improved with the addition of BB and MJ extracts, which was more evident in the samples prepared with MJ extract. This enhancement in TS and YM in the produced films can be attributed to relatively better internal microstructures of the films prepared with BB and MJ extracts and strong hydrogen bonds between anthocyanins or betacyanins and the gelatin and K-carrageenan polymer network. The reason for the increase and then decrease in TS and YM in samples prepared with different concentrations of extract is that

initially, with low extract content, the TS increased, and the chains had strong bonding. However, after adding higher percentages of BB and MJ extracts, the extracts situated between the polymer chains, causing the polymer chains to slide over each other. Therefore, although the TS and YM gradually decreased in films produced with extracts, since their values increased compared to the control sample, overall, we observe an improvement in the mechanical properties of films produced with extracts. Similar results were observed when adding *Oxalis triangularis* extract to the solution of films prepared with K-carrageenan and potato starch (47). By adding BB and MJ extracts to the gelatin and K-carrageenan pure solutions, the flexibility of the films increased. This is because anthocyanins or betacyanins can act as a fluid that enhances the mobility of polymer molecules. Similar results have also been recorded for increased flexibility when extracts rich in anthocyanins or betacyanins are added to the film matrix (36, 48).

2.2.5. Transparency

Food packaging should have desirable optical properties, effectively protecting food against harmful ultraviolet radiation effects while maintaining adequate transparency for consumers to see the packaged foods (36, 48, 49). The transparency of the prepared films is reported in Table 1. The results showed that the film prepared with gelatin and pure K-carrageenan is relatively transparent, with the highest transparency (15.68 %) among the film samples due to the components present in the gelatin and pure K-carrageenan film, which had low chromophores, limiting light absorption and aiding in coloration. However, since betacyanins contain chromophores such as C=C, C=O, and C=N bonds, and anthocyanins contain numerous chromophores such as C=C and C=O, the transparency of the prepared films noticeably decreased with the addition of BB and MJ extracts to the film solution (45). Among all samples, films prepared with 24% BB extract showed the lowest transparency (8.7 %), likely due to the density of various anthocyanins such as cyanidin, pelargonidin, and delphinidin in BB extract and subsequently a large number of C=C and C=O bonds (50). Hence the passage of harmful light through food packaging is closely related to their transparency, and our findings show that films prepared with BB extract have

the ability to prevent light passage compared to samples prepared with MJ extract, even though they may not be aesthetically attractive due to their dark color, they remain a priority when selecting packaging materials. As expected, a similar trend of reduced transparency was observed when adding saffron petal and barberry extracts (37).

2.3. Structural and Morphological Characteristics of Samples

2.3.1. Surface Morphology and Composition

In order to evaluate the surface morphology of gelatin and K-carrageenan-based films, SEM imaging method was used (50). As seen in Figure 2, the pure film prepared with gelatin and K-carrageenan had a smooth and uniform surface. After adding 6% BB extract to the film solution, it showed a uniform distribution with very few pores. However, adding concentrations of 12 and 24% BB extract to the prepared films gradually roughened the surfaces of the films, with distinct clusters of particles clearly visible on the film surface, attributed to the high concentration of BB extract and the non-uniform mixing of film components causing densification [48]. Sani et al. (2022) reported that films prepared with gelatin and K-carrageenan along with TiO₂ showed more uniform and smoother surfaces after adding anthocyanins (37). The film prepared with 6% MJ extract demonstrated a predictably more uniform and significantly fewer pores, while the surfaces of gelatin and K-carrageenan films became rougher when the MJ extract concentration increased to 12 and 24%. As shown in Figure 2, the accumulation of MJ extract at high concentrations in the gelatin and K-carrageenan films exhibited rougher and rougher surfaces compared to films prepared with BB extract, likely due to the more non-uniform mixing of MJ extract compared to BB extract. Furthermore, it is worth noting that the formation of cracks was very evident and easily visible in the 24% concentration of both extracts. In a similar study conducted by Singh et al. (2022), the film based on starch mixed with 30 and 40% beetroot extract showed an irregular and rough surface with small pores and some cracks due to the accumulation of extract materials in the film matrix (46). Zhang et al. also confirmed that adding bitter tea anthocyanins to chitosan and polyvinyl alcohol films mixed with

incompatible starch results in densification (51). Therefore, our results indicate that the type and concentration of extracts, along with film

preparation conditions, have significant effects on the morphology of the films (52).

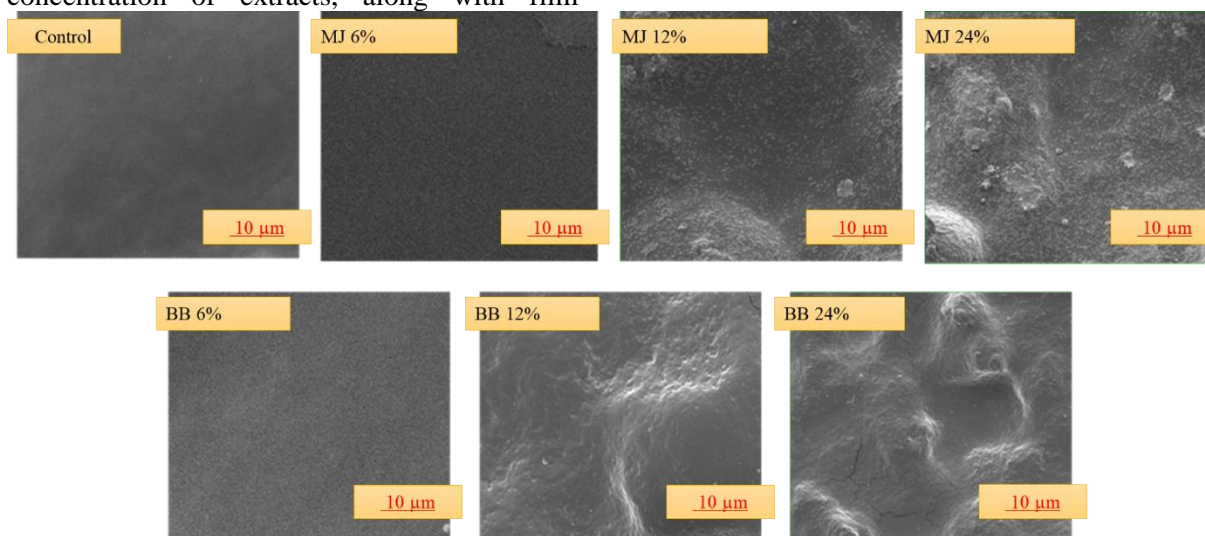


Fig 2. The impact of incorporating BB extract and MJ flower at varying concentrations on gelatin and k-carrageenan films.

2.3.2. X-ray Diffraction (XRD)

The physical state of the prepared films, which includes a crystalline state versus an amorphous structure, affects their optical, mechanical, and barrier properties (53). Therefore, we performed XRD analysis of the prepared films to provide insights into the physical state of various films. As shown in Figures 3a and 3b, the XRD pattern of gelatin and k-carrageenan film exhibited a small peak at 2θ : 28.4 and a broad peak at 2θ : 20 degrees, indicating a high level of amorphous structure in the prepared films. In fact, the broader the band width, the more indicative it is of the amorphous structure of biopolymers (37, 53). As depicted in Figure 3, the peak at 2θ : 28.4 is attributed to potassium chloride present in k-carrageenan. The gelatin and k-carrageenan control samples had narrower band widths compared to samples prepared with BB and MJ extract, indicating the crystalline structure of the control samples. By adding different concentrations of extracts, the band width increased further, making the biopolymer structure more amorphous. Increasing the

concentration of MJ extract widened the band width, with the highest band width associated with the sample containing 12% MJ extract. In samples prepared with BB extract, the widest band width was observed in the sample prepared with 6% BB extract, indicating that adding the extract increases impurities in the control samples and changes the structure from crystalline to amorphous. However, by adding 12% and 24% BB extract to the polymer structure, the band width shifts back toward a crystalline form. Since the concentration of positively charged anthocyanins is high in the 12% and 24% BB extract and since positively charged anthocyanins tend to replace potassium ions in the crystalline structure of potassium chloride present in k-carrageenan, this decrease in peak width after adding high extract concentrations can be attributed to the displacement reaction and the reformation of crystalline anthocyanin with positive charge and chloride ion. A similar trend was observed when adding grape peel anthocyanins to a k-carrageenan-based film (29).

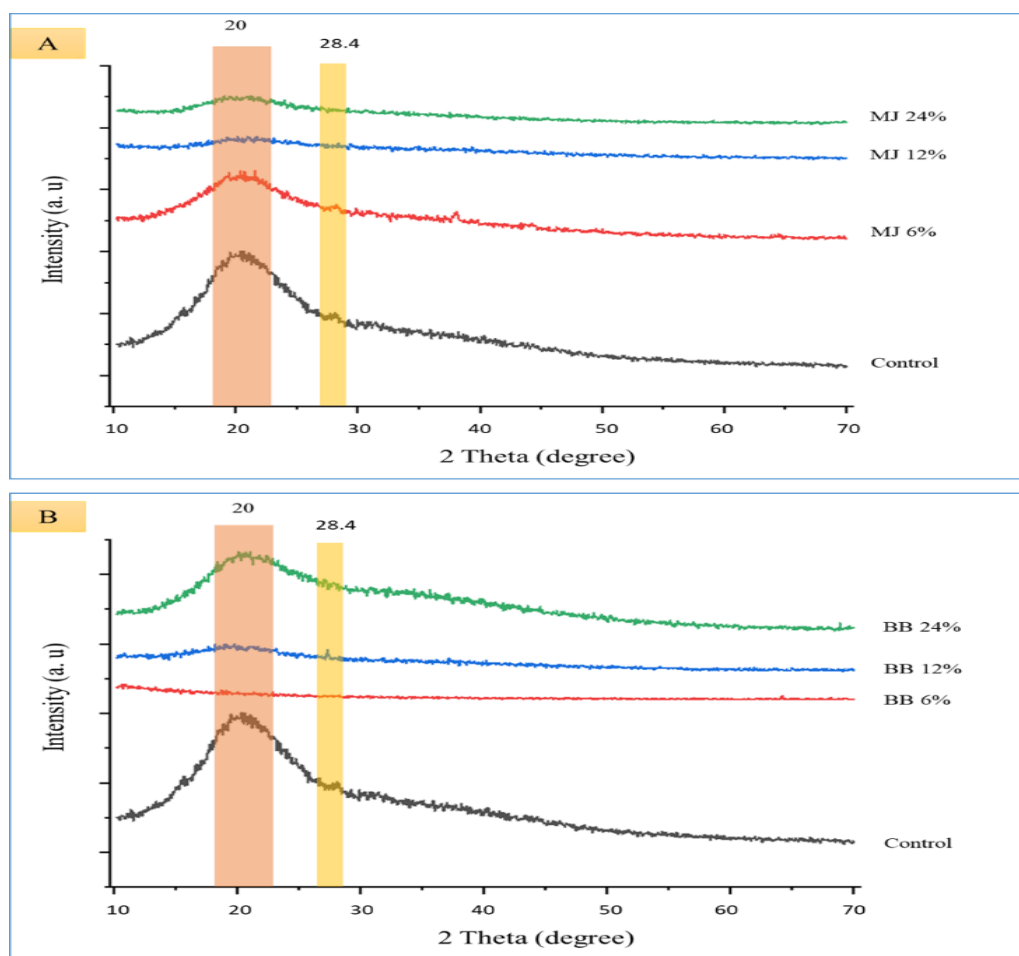


Fig 3. X-ray diffraction of gelatin and K-carrageenan films with different concentrations of MJ flower extract (a) and BB (b).

2.3.3. FTIR Spectroscopy

FTIR spectroscopy is used to provide information about the presence or absence of specific functional groups, as well as the chemical structure of polymer materials and ultimately the chemical changes induced in films prepared due to chemical or physical alterations (26). The main functional groups identified in the structure of polymers include hydroxyl groups (anthocyanins, gelatin, and K-carrageenans) and amine groups (gelatin). Vibrations in these functional groups may change due to their interaction with other substances (54). As shown in Figures 4A and B, the band width at around 3200 cm^{-1} can be attributed to the stretching related to the hydrogen-oxygen and hydrogen-nitrogen groups of the amide A group (29). The stretching of the hydrogen-nitrogen group at about 3200 cm^{-1} increased after adding MJ extract to the gelatin and carrageenan film solution, indicating that the hydrogen-nitrogen groups in gelatin molecules may interact through hydrogen bonding with MJ extract (26, 55).

However, by adding BB extract to the film solutions, the peak intensity decreased, possibly due to the incorporation of BB extract into the film solution and the opening of existing bonds in the polymer. Peaks associated with aromatic ring stretching ($1600\text{-}1585$; $1500\text{-}1400\text{ cm}^{-1}$) are related to the presence of anthocyanins in the BB and MJ extracts in the prepared films. The amide type 1 band observed in all samples at 1629 cm^{-1} is related to the stretching of the carbonyl groups in gelatin (41). The amide type 2 band at 1540 cm^{-1} is related to the bending of hydrogen-nitrogen and the stretching vibrations of nitrogen-carbon amine groups in gelatin. The specified peaks at 1030 cm^{-1} are related to the carbon-oxygen bonds present in gelatin. As seen in Figure 4A, a sample prepared with BB extract shows a peak at 1113 cm^{-1} , indicating the presence of carbon-oxygen-carbon stretching vibrations in the phenolic chemical compounds found in BB extract. Peaks below 1000 cm^{-1} are related to the bonding vibrations present in the BB and MJ extracts. Similar studies on the

functional groups present in coating films using FTIR spectroscopy were conducted by Sani et al. (2020) on gelatin and carrageenan films along with TiO₂ nanoparticles and anthocyanins (37),

and also by Maroufi et al. (2021) for the preparation of gelatin film along with a modified combination of carrageenan nanoparticles and zein (56).

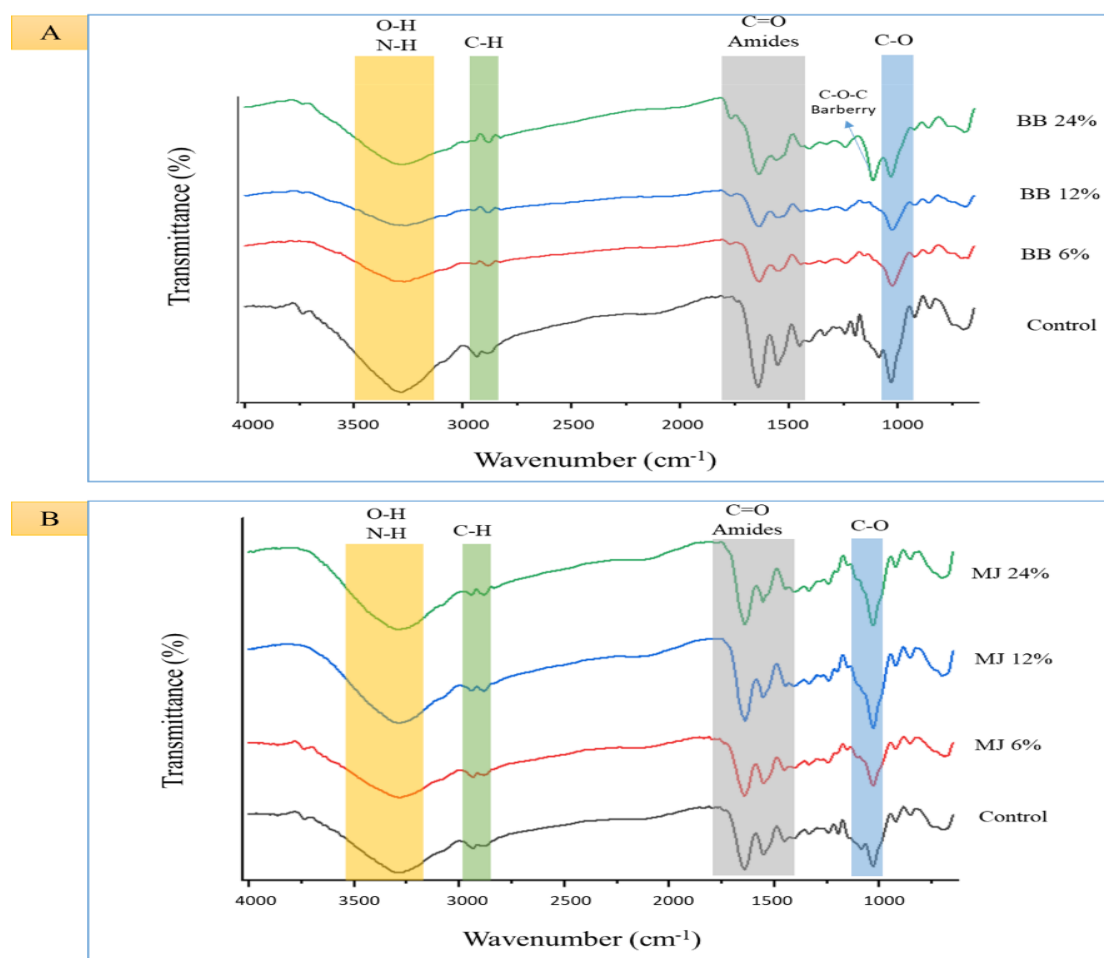


Fig 4. FTIR spectroscopy of gelatin and K-carrageenan films with BB extract (a) and MJ flower (b).

2.4. Freshness Monitoring of Lamb Meat

Meat, as a protein-rich product, becomes spoiled due to bacterial decay, lipid and protein oxidation, and biochemical changes under ambient and refrigerated conditions. When proteins present in raw meat degrade, initial nitrogen compounds such as ammonia and amines are produced (11, 41). Over time, volatile ammonia and biogenic amines accumulate in the closed space and increase the pH level. This accumulation can be detected using pH-sensitive films. As shown in Figure 5, samples of meat were stored for 72 h at 25 °C, and gelatin and K-carrageenan films prepared with BB and MJ extracts at various concentrations were used to monitor their freshness. These conditions are similar to those during meat packaging, storage, and transportation (57). In this study, initial amounts of TVB-N and pH of the meat samples

were reported to be 8.6 and 4.5 mg/100 g, respectively. The pH and TVB-N values of spoiled meat samples are reported to be over 7 and 25 mg/100 g, respectively. Similarly, the pH and TVB-N values of meat samples after 48 h of storage were reported to be 23.7 and 29 mg/100 g, exceeding the standard limit for spoilage. At this point, the color of the films prepared with BB extract turned brown, and the films prepared with MJ extract turned green. Spoilage leads to a pH change from 3.6 to 8, which is associated with the release of vaporized ammonia. This vapor dissolves in the water within the layers, leading to the production of hydroxyl ions and consequently increases the pH, initiating the degradation of anthocyanins and betacyanins by the escaping gas (14, 57). As a result, the films produced with BB and MJ extracts showed suitable functional properties and color changes during the 48-h meat storage period. After 72 h,

the films prepared with BB extract turned colorless, and the films prepared with MJ extract turned dark green. Previous research has also shown that films containing extracts rich in

anthocyanins and betacyanins, such as MJ petal extract, sweet potato extract, red beet extract, and beet extract, were effective in determining the freshness of protein-rich food samples.









Time (h)	pH	TVB-N (mg/100 g)	Gelatin-Kappacarrageenan-BB	Gelatin-Kappacarrageenan-MJ
0	6/8	5/4		
24	6/9	16		
48	7/23	29		
72	7/49	45/25		

Fig 5. TVB-N, pH and color changes during storage of lamb meat at 25°C for 72 h.

2.5. Functional properties of films

2.5.1. Halochromic Behavior

As shown in Figure 6, the film prepared with 24% BB extract and 24% MJ extract exhibited suitable color reactions at different pH levels, which is consistent with the results of extracts rich in anthocyanins and betacyanins after immersion in various buffer solutions (Figure 6a and b). The film prepared with 24% BB extract retained its color as a red halo in acidic and nearly neutral conditions (pH 6.2), while the film prepared with 24% MJ extract remained pink-red within a wider pH range (pH 8-2), showing stability in a broader pH range. This difference can be attributed to the greater stability of the chemical structure of betacyanins compared to anthocyanins in acidic and neutral buffer solutions (58). Protein-rich food products release nitrogen compounds during spoilage, leading to changes in the color of the coating films. Ammonia release can react with the moisture present in the film structure and create an alkaline microenvironment. Similar to the pH sensitivity test, both colored film samples showed significant color changes after exposure to ammonia (Figure 6a). In fact, the reaction of colored films to ammonia is due to the dynamic changes of phenolic substances in the structure of

anthocyanins and betacyanins, leading to the formation of phenolic oxygen anions (11, 14). Research has shown that ammonia (NH_3) infiltrates the prepared films and converts to ammonium ions (NH_4^+). These ammonium ions then interact between phenolic hydroxyl groups and phenolic oxygen anions in the structure of anthocyanins or betacyanins, creating an alkaline environment in the film structure (59). The films prepared with BB extract showed rapid and distinct color changes within 15 min, followed by alternating and gradual color changes from minute 15 to 90. The color changes observed in the film prepared with 6% BB extract over 90 min were more significant than in other films. The sample prepared turned from red to light brown (0-5 min), then to light brown (10-25 min), light green (30 min), and finally dark green (50-60 min) due to the changes in the structure of anthocyanins. Similar findings were found in films containing MJ petal extract and BB extract. In contrast to the films prepared with BB extract, films prepared with MJ extract did not show significant color changes in response to ammonia exposure. Based on this, the sample prepared with 12% MJ extract showed different color reactions compared to films prepared with BB extract: from pinkish-red (0-25 min) to brown (25-90 min). It is worth mentioning that

betacyanins can only be broken down into betalamic acid yellow and colorless cyclo-dopa residues when exposed to alkaline conditions.

Our findings confirmed that anthocyanin-rich extracts such as BB can influence the responsiveness of samples to ammonia (11).

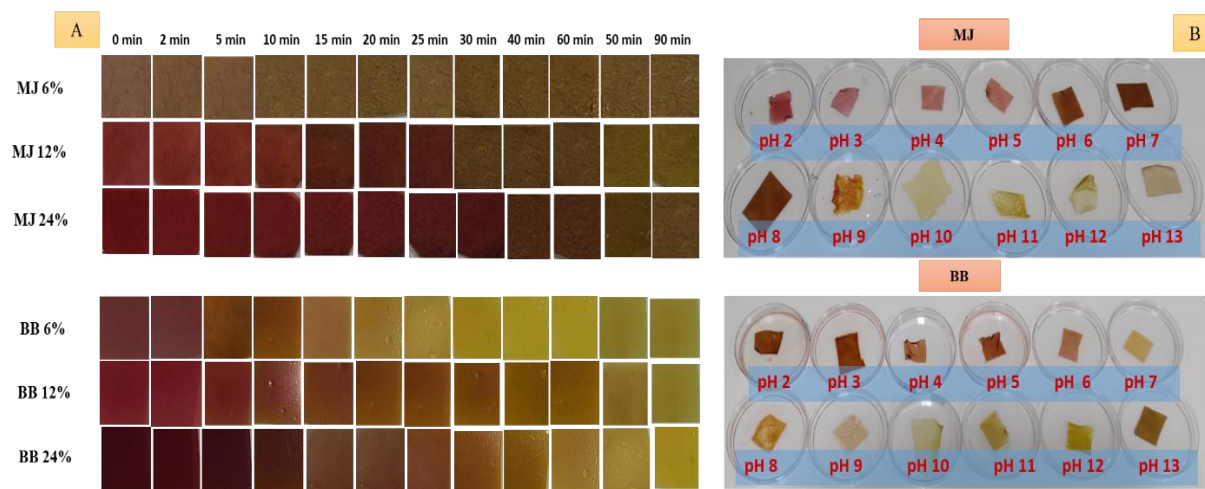


Fig 6. (a) Color changes of BB extract and MJ flower films with different concentrations after being exposed to ammonia (b) Color changes of BB extract and MJ flower films with different concentrations after immersion in buffer solutions (pH = 2-13).

2.5.2. Antioxidant Activity

The compounds present in food packaging play a significant role in preventing food spoilage resulting from oxidative reactions (11, 59). Therefore, evaluating the antioxidant activities of these compounds is crucial for preventing oxidative chemical degradation of food materials. The DPPH radical scavenging method is a rapid and easy technique for evaluating the antioxidant capacity of films; hence, we utilized this technique to measure the antioxidant activity of the samples. Samples containing antioxidant compounds can convert purple DPPH radicals to yellow diphenylhydrazine. Among all samples, control films showed the lowest ability to scavenge free DPPH radicals (Table 1). Previous studies have demonstrated that carrageenans along with their oligomers and derivatives possess antioxidant properties (37). The antioxidant activity of gelatin is also attributed to some of its amino acids acting as electron donors in reactions with free radicals to produce more stable compounds. As reported in Table 1, the addition of MJ petal and BB extracts significantly improved their ability to scavenge DPPH radicals in a concentration-dependent manner (58). The favorable antioxidant properties of samples prepared with different extract percentages are related to the presence of multiple phenolic groups, a high content of

amino groups, and the electron-donating ability of -OH groups in the phenolic network of anthocyanins present in MJ petal and BB extracts (14). It is essential to note that films prepared with BB extract demonstrate much higher antioxidant activity compared to films prepared with MJ petal extract, possibly due to the distinct structure of anthocyanins and the diverse compositions and higher levels of various functional compounds in BB extract. A similar method was conducted by He et al. (2020) for producing edible films based on carrageenans and gelatin for visual freshness detection of carp fish fillets (44), and also by Yaveri et al. (2021) for preparing gelatin films along with a modified combination of carrageenan nanoparticles and zein (56).

2.5.3. Antimicrobial Properties

The antibacterial effects of the prepared samples on an agar matrix containing *Staphylococcus*, *E. coli*, and *B. cereus* were investigated using the disk diffusion method (13). Our results showed that the control samples without dye have the least inhibitory effect on bacteria, especially on *Bacillus*, with the smallest halo diameters related to the dye-free prepared samples (Table 2). The results indicated that all samples prepared with MJ petal and BB extracts prevent the growth of the targeted bacteria. Previous studies have attributed the antibacterial activity of these

extracts to their terpenes, flavonoid compounds, and alkaloids (60). As seen in Table 2, with an increase in the concentration of MJ petal and BB extracts in the prepared samples, their antibacterial activity increased, forming the largest antibacterial halos against *E. coli* and then *S. aureus* and *B. cereus*. Among the prepared samples, the highest observed antibacterial effect was related to samples containing BB extract, while samples containing MJ petal extract had a

lesser impact on the tested bacteria, possibly due to the structure and high concentration of alkaloids present in BB (60). A similar method was conducted by Abdollahzadeh et al. (2018) on the antibacterial activity of agar-based films containing nisin, cinnamon essential oil, and ZnO nanoparticles (60), and also by Motolaakshmi et al. (2021) on a silver-carrageenan hybrid hydrogel nanocomposite along with gelatin (13).

Table 2. Antimicrobial properties of gelatin and k-carrageenan films with different concentrations of BB extract and MJ flower

Antimicrobial properties of films	Films type						
	Blank	MJ 6%	MJ 12%	MJ 24%	BB 6%	BB 12%	BB 24%
	Inhibition zone (mm)						
<i>E.Coli</i>	15±0 ^d	15±1 ^d	16±2 ^d	20±0 ^c	19±1 ^c	23±1 ^b	26±0 ^a
<i>Staphylococcus aureus</i>	12.33±0.57 ^d	10±0 ^f	18±1 ^b	20.67±0.57 ^a	11±1 ^{ef}	12±00 ^{de}	14±1 ^c
<i>Bacillus Cereus</i>	9±1 ^e	11±1 ^{cd}	13±1 ^b	15±0 ^a	9±0 ^e	10±1 ^d	12±1 ^{bc}

The data are presented as mean ± standard deviation. Any two means in the same row followed by the same letter are not significantly ($P > 0.05$) different from Duncan's multiple range tests.

2.6. Biodegradability

Given that reducing environmental damage caused by plastics made from petroleum derivatives is a crucial issue, the widespread use of biopolymer-based films has a significant impact on mitigating these damages (12, 37). Therefore, we conducted a simulated test on the biodegradability of films produced within a 21-day period (refer to Figure 7). After 21 days, the gelatin and K-carrageenan film samples were notably decomposed. In the initial 7 days, minimal changes in the visual aspects of the films prepared with BB and MJ petal extracts were observed. However, over time, the prepared

films underwent changes in shape and color and began to degrade. Consequently, after a 14-day burial period, significant changes in the structure of all film samples were observed. The samples prepared with BB extract showed a 24% and MJ petal extract showed a 24% higher rate of degradation within the 21-day period, likely due to the high concentration of the extract in them. The degradation of the produced films is due to the presence of moisture, enzymes, and microorganisms in the soil. A similar biodegradation trend was observed in biopolymer films prepared with saffron petal or BB extracts (37) and green tea (12) and basil extracts (11).

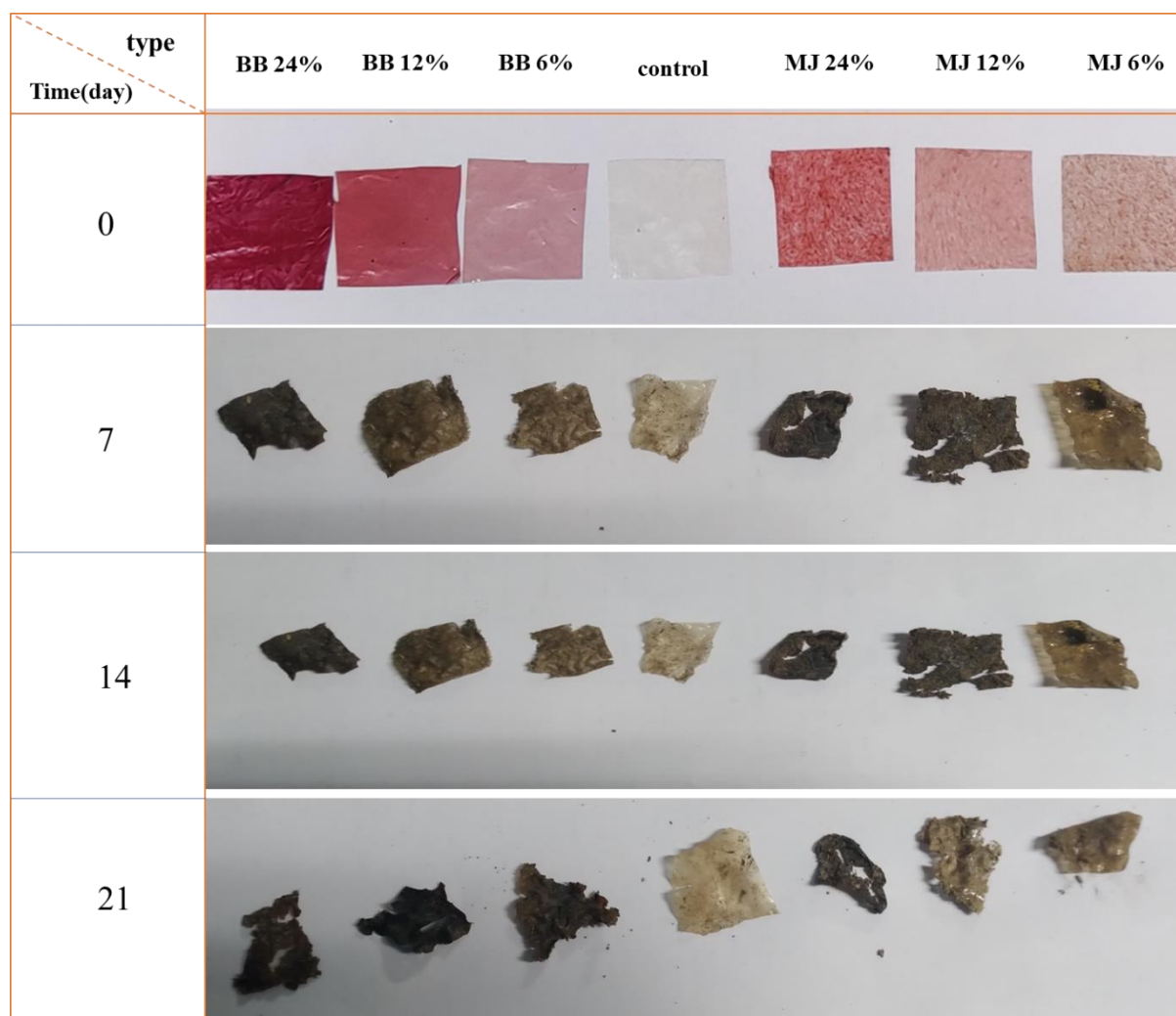


Fig 7. Investigating the biodegradability of gelatin and K-carrageenan films with BB and MJ flower extracts under simulated environmental conditions in a period of 21 days.

4-Conclusion

Overall, the colored films prepared can be used as a simple and practical tool for assessing the quality and safety of perishable food items. In this study, pH- and NH₃-sensitive films based on gelatin and k-carrageenan successfully designed and prepared by adding BB and MJ petal extracts. Based on data analysis, the physical, mechanical, structural, and functional properties of gelatin and K-carrageenan films were significantly influenced by the BB and MJ petal extracts rich in anthocyanins and betacyanins. Since the BB and MJ petal extracts had strong hydrogen bonding with the gelatin and k-carrageenan film structure, films prepared with these extracts improved WVP and mechanical properties compared to the control sample. Additionally, films prepared with MJ petal extract exhibited higher solubility and moisture content than films prepared with BB extract and

control samples. Gelatin and k-carrageenan films containing BB and MJ petal extracts showed visible color changes after exposure to pH and ammonia variations. All films demonstrated suitable antioxidant and biodegradation properties, as they significantly degraded after 21 days. However, films prepared with BB extract exhibited very strong antioxidant effects and appropriate pH sensitivity. Moreover, noticeable color changes, as a freshness indicator, were observed in films prepared with extracts during the storage of meat samples at 25 °C. In summary, our findings indicate that the structure, physical properties, and performance of films prepared with extracts are highly influenced by the type and content of materials present in the colored extracts. However, all samples are suitable for quality assessment and monitoring the freshness of lamb meat, ultimately enhancing continuous quality control and waste reduction. In the continuation of this work, the researchers

can conduct a comprehensive investigation about antimicrobial properties of the films against key microbes in meat, including *Pseudomonas*, as well as investigate the combined effect of anthocyanin, two substances of the Abbasid tulip and barberry.

5-Conflict of Interest

The authors of the article declare that there is no conflict of interest.

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6-Acknowledgements

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مقاله علمی-پژوهشی

طراحی شناساگر هوشمند حساس به پی‌اچ تهیه شده از ژلاتین-کاپاکاراگینان-آنتوسیانین لاله عباسی و زرشک به منظور بررسی تازگی/فساد گوشت بره

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اطلاعات مقاله	چکیده
<p>تاریخ های مقاله : تاریخ دریافت: ۱۴۰۳/۲/۱۶ تاریخ پذیرش: ۱۴۰۳/۴/۲</p>	<p>امروزه استفاده از شناساگرهای هوشمند در بسته بندی مواد غذایی به منظور نظارت و تشخیص کیفیت غذا با تجزیه و تحلیل داده‌های کیفی و تغییر رنگ فیلم‌های بسته بندی با توجه به وضعیت غذا، در حال افزایش است. در این تحقیق مقایسه ای، فیلم های هالوکرومیک بسته‌بندی هوشمند ژلاتین و کاپاکاراگینان با افزودن درصد های مختلف عصاره گل لاله‌عباسی (۶، ۱۲ و ۲۴ درصد) و عصاره زرشک (۶، ۱۲ و ۲۴ درصد) طراحی و تهیه شد. با استفاده از تصاویر SEM و طیف‌سنجی FTIR، مشخص شد که هر دو فیلم تهیه شده با عصاره زرشک و گل لاله‌عباسی به‌طور کامل در ماتریس پلیمری ژلاتین و کاپاکاراگینان، با برهمکنش‌های مولکولی قابل‌توجهی مانند اتصالات هیدروژن و الکترواستاتیک توزیع شده‌اند. با این حال، فیلم های تهیه شده با عصاره گل لاله‌عباسی سطوح غیرمنظم و خشن‌تری نسبت به فیلم‌های تهیه شده با عصاره زرشک نشان دادند. هر دو فیلم تهیه شده با عصاره زرشک و گل لاله‌عباسی دارای فعالیت آنتی‌اکسیدانی و حساسیت به پی‌اچ و آمونیاک مناسبی هستند. فیلم های تهیه شده با عصاره زرشک فعالیت آنتی‌اکسیدانی بالاتر و حساسیت بیشتری نسبت به تغییرات پی‌اچ داشتند. فیلم‌های تهیه شده ژلاتین و کاپاکاراگینان همراه با عصاره زرشک و گل لاله‌عباسی به‌طور موثری تازگی گوشت بره ذخیره شده در دمای ۲۵ درجه سانتی‌گراد را به موازات تجمع گازهای آمونیاک در فضای بالای بسته‌های نگهداری و تغییرات پی‌اچ نشان دادند. یافته‌های ما نشان می‌دهد که ویژگی‌های ساختاری، فیزیکی و عملکردی فیلم‌های ژلاتین و کاپاکاراگینان حاوی عصاره‌های حساس به پی‌اچ بسیار تحت‌تاثیر نوع و غلظت عصاره‌ها قرار دارند.</p>
<p>کلمات کلیدی: بسته بندی هوشمند، فیلم‌های حساس به پی‌اچ، آنتوسیانین، نشانگر رنگ سنجی، شاخص تازگی</p>	
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