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Development of plastic/gelatin bilayer active packaging film with antibacterial and water-absorbing functions for lamb preservation

Abstract

In order to extend the shelf life of refrigerating raw lamb by inhibiting the growth of microorganisms, preventing the oxidation of fat and protein, and absorbing the juice outflow of lamb during storage, an active packaging system based on plastic/gelatin bilayer film with essential oil was developed in this study. Three kinds of petroleum-derived plastic films, oriented polypropylene (OPP), polyethylene terephthalate (PET), and polyethylene (PE), were coated with gelatin to make bilayer films for lamb preservation. The results showed significant improvement in the mechanical properties, oxygen, moisture, and light barriers of the bilayer films compared to the gelatin film. The OPP/gelatin bilayer film was selected for further experiments because of its highest acceptance by panelists. If the amount of juice outflow was less than 350% of the mass of the gelatin layer, it was difficult for the gelatin film to separate from lamb. With the increase in essential oil concentration, the water absorption capacity decreased. The OPP/gelatin bilayer films with 20% mustard or 10% oregano essential oils inhibited the growth of bacteria in lamb and displayed better mechanical properties. Essential oil decreased the brightness and light transmittance of the bilayer films and made the film yellow. In conclusion, our results suggested that the active packaging system based on OPP/gelatin bilayer film was more suitable for raw lamb preservation than single-layer gelatin film or petroleum-derived plastic film, but need further study, including minimizing the amount of essential oil, enhancing the mechanical strength of the gelatin film after water absorption.

Keywords: active packaging system; chilled lamb; gelatin/plastic bilayer film; water

absorption; bacterial inactivation

Introduction

Raw meat is a perishable food, however, due to online shopping and globalization of the market, the transportation radius of raw meat has increased, and consumers have higher requirements for the extension of the shelf life of raw meat (Ahmed et al., 2017; Mills & Brightwell, 2014). Microbial spoilage, color deterioration, a decline in eating quality, fluid outflow, lipid oxidation, and inferior types of meat (in particular, dark, firm, and dry and pale, soft, and exudative meats) are the major problems in refrigerating raw lamb meat (Barbosa-Pereira et al., 2014; Holman et al., 2018). In addition, these problems lead to food waste, substantial economic losses, and a huge carbon footprint, as discussed in some articles (Rood et al., 2022; Shao et al., 2022; Soro et al., 2021). To solve the problems mentioned above, active packaging (AP) has been proposed by meat producers and scientists. AP has extra functions in addition to the characteristics provided by traditional packaging (Ahmed et al., 2017; Barbosa-Pereira et al., 2014; Smaoui et al., 2022). Currently, there are mainly two strategies to make AP. The first strategy includes the use of relatively independent accessories with specific functions that exist in the headspace of the packaging. The second strategy to make AP consists of using active substances with functional characteristics in combination with packaging materials (Ghoshal, 2018).

Biodegradable materials, especially edible film used as a carrier for different types of active substances, have been increasingly studied in recent years to extend the shelf life of meat and meat products. However, the main goal of these studies was to replace plastic films because of the non-degradable characteristics and problems related to the incineration of petroleum-derived plastic polymers commonly used in meat packaging and the migration of toxic and harmful substances such as phthalates into the meat (Harmon & Otter, 2022; Moreira et al., 2015; Soro et al., 2021; Xu et al., 2010). Gelatin is one of the most widely investigated biopolymers in the field of edible films (Falguera et al., 2011; Gupta et al., 2022; Lacroix & Vu, 2014; Paulo et al., 2021; Sun et al., 2022). It is a protein material derived from the collagen of animal bones or skin after physical or chemical degradation or denaturation. Gelatin can be used as a food additive, an edible packaging film material, and an encapsulant (Etxabide et al., 2016; Moorhouse & Grundon, 1994). It is of great interest in the field of edible films because of its abundant sources (Tongnuanchan et al., 2015), superior film-forming properties, better degradability, high biosafety, good nutritional value (de Carvalho & Grosso, 2004), and complete absorption in the body. Edible packaging films made from gelatin have been widely studied for their suitability in various food applications (Nilsuwan et al., 2017). Several of these studies are on the application of monolayer gelatin film in meat and fish products. Some researchers studied the inactivation effect of gelatin-based edible films containing active substances (which were mostly antioxidants and antimicrobials) on spoilage and pathogenic microorganisms of raw meat, including total viable bacterial counts, total mesophilic aerobic bacterial counts, psychrophilic aerobic bacterial counts, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *L. monocytogenes*, and obtained good inactivation effects (Albertos et al., 2017; Ejaz et al., 2018; Lin et al., 2018; Qiu et al., 2022; Wu et al., 2014; Yilmaz et al., 2022). Some studies investigated the effect of gelatin-based edible films incorporated with active substances on the sensory properties, color, textural properties, total volatile basic nitrogen, thiobarbituric acid reactive substances, and moisture loss of raw meat; and found that these films extended meats' shelf life (Kaewprachu et al., 2015; Qiu et al., 2022; Yilmaz et al., 2022). Some researchers found that gelatin-based edible films carrying active substances retarded lipid oxidation and decreased the peroxide values of meat (Hematizad et al., 2021; Kaewprachu et al., 2015; Kaewprachu et al., 2018; Hamann et al., 2022; Syahida et al., 2021). However, few studies have focused on the consumer acceptance of the changes in gelatin films during storage and meat quality changes induced by gelatin film packaging during storage.

Although gelatin has many advantages, it also has disadvantages when used alone. Raw lamb muscle meat contains more than 70% water (Rao et al., 2018), and during storage, juice outflow of raw lamb meat varies from 0.5% to 15% based on storage conditions, premortem management, and so on (Bond & Warner, 2007; Ekiz et al., 2022; Holman et al., 2020; Moore, 1990). After the gelatin film wrapped on the surface of the meat absorbs the juice outflow, the gelatin film disintegrates, and its mechanical strength decreases dramatically. Thus, the use of a monolayer gelatin film in the preservation of fresh meat has many limitations (Ciannamea et al., 2018; Lim et al., 1999; Villasante et al., 2020; Yuan et al., 2021). It is also difficult to develop AP based on petroleum-derived plastic polymers because of the instability of antioxidants and antimicrobials at high temperatures during the production of plastic polymers (Nur Hanani et al., 2018). An approach that combines petroleum-derived plastic polymers with other biopolymers to gain an extra function by mixing antioxidants or antimicrobials has attracted the attention of researchers (Joerger et al., 2009; Nur Hanani et al., 2018). To control the microorganism growth and the sensory quality deterioration of muscles induced by juice outflow, moisture-absorbent sheets, blankets, and pads are usually used for absorbing fluid exudates from raw fresh meat and fish (Ahmed et al., 2017). These existing absorbent sheets on the market can be replaced by gelatin films since they have a strong affinity to moisture and can absorb several times their own weight of water (Kavoosi et al., 2013).

Therefore, an AP system based on gelatin/plastic bilayer films with different concentrations of oregano or mustard essential oils was developed in this study to absorb juice outflow and inhibit spoilage microorganisms. In this study, we first determined the maximum weight of lamb that could be wrapped in a given size of gelatin film. Next, the thickness of the gelatin film was calculated according to the water absorption capacity of the film that could absorb 5% juice outflow of raw lamb. Food-grade plastic films of different materials, such as oriented polypropylene (OPP), polyethylene terephthalate (PET), and polyethylene (PE), were used for laminating with gelatin to make bilayer films. The type of plastic film in the bilayer film was selected based on the degree of bonding between the plastic and gelatin layers, the mechanical, oxygen barrier, light barrier properties, and sensory evaluation of the bilayer film by consumers after application to lamb. However, no studies are available on consumers acceptability of the packaging film after water absorption and the sticky phenomenon of the meat surface caused by the gelatin film migrating to the meat surface. Finally, the essential oils were added to the gelatin layer of the bilayer films, and the effects of essential oil concentrations on the mechanical properties, bacterial inactivation, color, and light transmittance were studied to determine the suitable concentration of essential oil.

Materials and methods

Preparation of single-layer gelatin film

Gelatin (4 g) was placed in a conical flask containing 300 mL of distilled water and stirred on an intelligent magnetic stirrer (ZNCL-GS, Yike Laboratory Technology Co. Ltd., Guangzhou, Guangdong, China) at a temperature of 60℃ and speed of 800 r/min for 1 h. Subsequently, 1.2 g (30% of the weight of the gelatin) of glycerol was added, and the stirring was continued for 0.5 h. Next, the solution was left to cool at lab temperature (21.9℃). Gelatin and glycerol were purchased from Aladdin (Shanghai, China).

The gelatin solution placed at lab temperature (21.9℃) in the above step was poured into a 28.5 × 28.5 cm acrylic tank, and then the acrylic tank with gelatin solution was placed in a chamber (JYH-66, Yuejin Medical Equipment Co., Ltd, Shanghai, China) and dried at a temperature of 50°C and relative humidity of 30% for 15 h. After drying, the gelatin film was peeled off after its temperature was cooled to room temperature. Films were placed in a thermostatic incubator at 25℃ and 43% relative humidity to further determine the film index.

Preparation of OPP/gelatin, PE/gelatin, and PET/gelatin bilayer films

In a 28.5 × 28.5 cm acrylic tank, the OPP, PE, or PET films were laid flat in the tank,and the same steps were followed as mentioned in the "Preparation of single-layer gelatin film" section. The gelatin solution was poured onto the OPP, PE, or PET film. After drying for 15 h, the OPP/gelatin, PE/gelatin, or PET/gelatin films were taken out and then these double-layer films were placed in a thermostatic incubator at 25℃ and 43% relative humidity until the further determination of the film index. PET plastic, PE plastic, and OPP plastic films were purchased from Hongmei paper and plastic packaging factory (Chaozhou, Guangdong, China).

Preparation of OPP/gelatin bilayer films with essential oil

Gelatin (4 g) was put in a conical flask. Next, 300 mL of distilled water was added to it and stirred with a magnetic stirrer at a temperature of 60°C and a speed of 800 r/min for 1 h. Glycerol (1.2 g) was then added and stirred continuously for 0.5 h. The essential oil (Yuanye, Shanghai, China) and Tween 80 (Aladdin, Shanghai, China) were vortexed at a ratio of 2:1 (v/w) for 1 min and mixed thoroughly before adding them to the gelatin solution. The concentrations of oregano essential oil were 0%, 10%, 20%, 30%, and 40%, and those of mustard essential oil were 20%, 40%, 60%, and 80% of the mass of gelatin. The mixed solution was added to the gelatin solution, stirred at room temperature for 1 h, poured into the acrylic tank covered with OPP film, and dried at 50°C in a vacuum drying oven for 15 h.

Mechanical properties of films

The mechanical properties of films were determined based on the ISO 527-1: 2012 method with some modifications (Jamróz et al., 2021). Film strips (15 × 80 mm) were used to measure tensile strength (TS) and elongation at break (EAB) of films, using a texture analyzer (TMS-PILOT, FTC Co., Ltd., Virginia, USA) with an initial separation of 50 mm and a cross-head speed of 200 mm/min. The measurement was made nine times, and the average value was taken. The TS and EAB were calculated using the following equations:

$$TS = Fm/(d \times b)$$

$$EAB = (L1 - L0)/L0 \times 100\%$$

where Fm was the maximum tensile force (N) of the sample; d was the width of the film (mm); b was the thickness of the film (mm); L0 was the initial length of the sample (mm); and L1 was the maximum fracture length of the sample (mm).

Oxygen resistance properties of films

The oxygen permeability (OP) and oxygen permeability coefficient (OPC) of OPP/gelatin, PE/gelatin, or PET/gelatin bilayer films were determined based on the ISO 2556 method with minor modifications using a gas permeator (VAC-VBS, Languang Electromechanical Technology Co., Ltd., Jinan, Shandong, China). The OP is the volume of oxygen passing through a sample per unit area and per unit time. The OPC is the volume of gas passing through the sample per unit thickness, per unit area, and per unit time at stable transmission. The proportional mode was 10%, the gas replacement time was 60 s, the upper and lower cavity degassing time was 4 h, the temperature was 25°C, relative humidity was 18%, and the gas pressure was 1.01 kgf/cm^2.

Water vapor permeability (WVP) of films

The water vapor permeability of films was determined based on the ASTM E96 method with some modifications (Wang et al., 2018; Liang et al., 2018). A permeable cup containing fully dried $CaCl_2$ particles was sealed with OPP/gelatin, PE/gelatin, or PET/gelatin bilayer films. The permeable cup was then placed in a sealed facility with saturated NaCl solution (osmotic pressure of 1753.55 Pa). The mass of the glass was measured every 1 h until there was no significant change in the mass of the glass. The WVP was calculated as follows:

$$WVP = (\Delta m \times H)/(\Delta t \times S \times P)$$

where Δm was the transit dose of water vapor (g); H was the average thickness of the film (m); S was the test area 0.00196 m^2; Δt was the test time (s); and P was osmotic pressure (Pa) of NaCl saturated solution.

Light transmittance of films

The light transmittance of films was determined according to Liang and Wang's study (2018). The film strips of 1 × 4.5 cm size were pressed close to the inner side of the colorimetric dish at lab temperature, and its light transmission in the wavelength range of 200–800 nm was measured. An empty colorimetric dish was used as the control.

Color analysis of films

The color of bilayer films with mustard essential oil was measured using a colorimeter (CR-400, Konica Minolta, Co., Ltd., Japan). The whiteboard was used as a reference for color difference measurement. Five locations were selected on each film to measure L^*, a^*, and b^*; one at the center and four around the perimeter of the film. The color of bilayer films with oregano essential oil was measured using the colorimeter (NR60CP, Sanenchi, Co., Ltd., Shenzhen, Guangdong, China).

Water absorption and water dissolution properties of gelatin films

The water absorption and water dissolution properties of the films were expressed in terms of the weight change of gelatin films in water. Films were cut into 100 × 100 mm sizes and then completely immersed in distilled water. The weight of the films was measured at a specific time after being immersed in water. The weight change ratio was calculated using the following formula: Weight change ratio = (the weight of film soaked in water for a certain period − the weight of film before being soaked in water)/the weight of film before being soaked in water.

The water dissolution properties of gelatin films were also determined by the protein migration from the film to water when the films were immersed in water. Four portions of the same mass of dried films were put into a conical flask, 50 mL of distilled water with a temperature of 4℃ was added and placed in a 4℃ refrigerator, and the protein content of the water was quantified using the BCA protein assay (Thermo Fisher Scientific Inc., Waltham, USA) at 1, 2, 3, and 4 d.

Adhesiveness of gelatin film after water absorption

A double compression test was performed to evaluate the adhesiveness of gelatin film after water absorption. The films with or without essential oil were cut into sizes of 10× 10 cm. Ten layers were stacked together, placed into distilled water, soaked for a certain period, and taken out, and the double compression test was measured using a texture analyzer (TMS-PILOT, FTC Co., Ltd., Virginia, USA) according to the method described in a previous study (Fiszman et al., 2000). The parameter settings used were as follows: TA5 probe model, an initial force of 0.15 N, test rate of 60 mm/min, test distance of 20 mm, 50% deformation, and interval time of 5 s. Adhesiveness is the negative force during the first bite, representing the force necessary to pull the compressing plunger away from the sample.

Sensory evaluation of bilayer films and gelatin film after packing lamb

The *longissimus dorsi* muscles from both sides of ten small tail Han sheep raised in the same pen and of 7 months of age were selected and slaughtered in Jinhongqingzhen Co. Ltd., Baoding, Hebei, China. After slaughtering, the muscles were taken from both sides of the carcass and frozen in liquid nitrogen. After complete freezing, the meat was stored in a refrigerator at −80℃, and before use, it was thawed to 4℃ for storage experiments. Lamb was divided into meat pieces of about 400 g, wrapped with monolayer gelatin film, OPP/gelatin bilayer film, PE/gelatin bilayer film, or PET/gelatin bilayer film. Packaged muscles stored at 4℃ for 5 days were evaluated by ten trained panelists according to the sensory evaluation criteria (Table 1). Ten pieces of meat were wrapped in each packaging material. When one panelist arrived to assess the film and the lamb, 4 out of the 10 pieces of meat in each packaging material were randomly selected for evaluation. After evaluation, the meat was re-wrapped for the next panelist's assessment test. The higher the score, the higher the acceptability of the film. All procedures described here were undertaken following the principles and guidelines of the Ethics Committee of Hebei Agricultural University, and the approval number is 2021095.

Antibacterial properties of bilayer films

Lamb meat was soaked in saline, and the lamb was slapped to transfer the bacteria to the saline. The saline containing lamb bacteria was diluted to 1×10^5 CFU/mL, and 1 mL was added to the Petri dish. The plate counting agar was poured and shaken up. After the saline was dried, a 4 × 4 cm double-layer film containing essential oil was placed in the center of the Petri dish. The film without essential oil was used as a blank control. The Petri dishes were cultured at 37℃ for 48 h, and the number of colonies in the plate was counted. Bacterial counts were converted to CFU/g before statistical analysis.

Antibacterial properties of gelatin/OPP essential oil film used in vacuum packaging system

The preparation of OPP/gelatin essential oil film used in the vacuum packaging system was carried out according to the method described in the previous section “Preparation of OPP/gelatin bilayer films with essential oil” with some modifications. In this experiment, the essential oil was a mixture of mustard and oregano essential oils. The ratio of mustard and oregano essential oils was 1:4, and the total amount of essential oil was 20% of the weight of gelatin (4 g). Lamb *longissimus dorsi* muscles were divided into meat pieces of about 400 g, packaged in vacuum bags (control group) or vacuum bags + oregano-mustard composite essential oil double layer film (OPP/gelatin essential oil film as the inner film wrapped on the surface of lamb, and then put into the vacuum plastic bag) (treatment group), and stored at 4 ℃. Three packages of these lambs from each treatment group were taken out every 3 days and the total colony count in the samples was measured. The total colony count of bacteria was detemined as follows: 1 g meat was placed in 9 mL saline. The bacteria of the meat was transferred to the saline by beating. One mL of saline was injected into a petri dish. Then AGAR medium was injected to the petri dish. Medium and bacterial saline was mixed and cooled in lab temperature. After the medium has solidified, petri dish were cultured for 2 days at 37℃. The number of colonies in the plate was counted. Bacterial counts were converted to CFU/g before statistical analysis.

Data analysis

Data were analyzed using SPSS software v.22.0 (IBM Corporation Inc., USA), and results were expressed as mean ± standard deviation. One-way analysis of variance was used to compare data differences among multiple groups. Duncan's test and LSD method were used for multiple comparisons of mean values. $P < 0.05$ indicated a statistically significant difference. The graphs were created using the Origin 2019 software (OriginLab, Northampton, MA, USA).

Results and Discussion

Mechanical properties of films

The TS indicates the ability of the material to resist stretching and is a key indicator for evaluating the mechanical properties of the film. As seen in Fig. 1, the order of TSs from largest to smallest was as follows: OPP/gelatin film > PET/gelatin film > PE/gelatin film > gelatin film. The TS of single-layer gelatin film was 2.80 MPa; meanwhile, the TSs of bilayer OPP/gelatin, PE/gelatin, and PET/gelatin films increased by 8.58, 2.94, and 5.71 times, respectively, compared to the gelatin film. As observed for the gelatin film in this work, the TSs of some kinds of gelatin in other studies were also lower than 3.5 MPa, which could be due to the different sources or extraction methods of gelatin, the processing technology of the film, and the addition of plasticizer and other substances (Haghighi et al., 2019; Rather et al., 2022; Said & Sarbon, 2022). However, as a food packaging material, sufficient tensile strength is generally needed; the greater the TS of the film, the better the food is protected. In some countries, the TS of the food packaging film must be greater than 3.5 MPa (Kim et al., 1995). Thus, compared to the single-layer gelatin film, the plastic film present in the bilayer film improved the TS of the bilayer film.

EAB is the deformation of the film before the film breaks during the stretching process, and films with excessive deformation are not suitable for application in packaging. As seen from the data in Fig. 1, the EAB of single-layer gelatin film was 190%, while those of OPP/gelatin, PE/gelatin, and PET/gelatin films decreased by 145%, 175%, and 147%, respectively, compared with the gelatin film.

In summary, the gelatin film laminated with the plastic film was more suitable for application in the packaging of raw meat products. From the point of view of mechanical strength, compared with the other three kinds of films, OPP/gelatin bilayer film was the best.

Oxygen resistance of films

The lower the OP and OPC of the film, the stronger its ability to restrict oxygen entry in the packaging of raw meat, which can reduce the oxidation of meat products and prolong their storage period. As shown in Fig. 2, PET/gelatin and OPP/gelatin bilayer film had better oxygen resistance than PE/gelatin bilayer film. The oxygen permeability and oxygen permeability coefficient of the single-layer gelatin film was not shown in Fig. 2, as the OP of the film was too large for the gas permeator to measure. This indicated that the oxygen permeability of single-layer gelatin film was bigger than that of the plastic/gelatin bilayer films. Many studies have determined that the gelatin film has a good oxygen resistance capacity (Ciannamea et al., 2018; Etxabide et al., 2022). According to the previous studies, the OPC of gelatin film was between 0.01–68492 $cm^3 \cdot \mu m \cdot m^{-2} \cdot d^{-1} \cdot kPa^{-1}$ (Hosseini et al., 2016; Lee & Song, 2017; Nur Hanani et al., 2013; Tyuftin & Kerry, 2021; Wang et al., 2009). This was higher than the OPC of OPP/gelatin, PE/gelatin, and PET/gelatin bilayer films, which showed a value between 0.004–0.0026 $cm^3 \cdot \mu m \cdot m^{-2} \cdot d^{-1} \cdot kPa^{-1}$.

The difference in OPC can be induced by factors such as differences in film thickness, pH, relative humidity, gelatin source, plasticizer concentration (Ciannamea et al., 2018). The approach used in this study could allow bubble formation in the gelatin film and introduce variation in film thickness; the higher the film thickness, the bigger the OPC (Tyuftin & Kerry, 2021). The OP of gelatin film is also affected by the glycerol concentration; the higher the glycerol concentration, the higher the OP values (Lim et al., 1999). The OP of gelatin films is also influenced by pH; the higher the pH, the lower the oxygen permeability of the gelatin film (Nur Hanani et al., 2014).

Moisture resistance of films

The water vapor transmission rate of a film reflects the water barrier effect of the film (Rhim et al., 2000; Du et al., 2011) and should be low enough to avoid moisture loss on the surface of the meat. As shown in Fig. 3, the water vapor transmission coefficient of gelatin film was 5.1×10^{-8} g/(s·m·Pa). However, the water vapor transmission coefficients of

OPP/gelatin, PET/gelatin, and PE/gelatin bilayer films were significantly lower than that of the gelatin film, and the values were 0.47×10^{-8} g/(s·m·Pa), 0.81×10^{-8} g/(s·m·Pa), and 0.39×10^{-8} g/(s·m·Pa), respectively. The larger the transmission coefficient, the worse the moisture barrier performance; therefore, compared to OPP, PET, and PE films, gelatin film had poorer moisture barrier performance, which was consistent with the results of other studies (Etxabide et al., 2022; Tyuftin & Kerry, 2021; Villasante et al., 2020). In addition, there was no significant difference in moisture barrier performance among the three bilayer films.

Light resistance of films

The light resistance performance is an important indicator of the film, and the transmittance can indirectly represent the magnitude of the film's light resistance. As shown in Fig. 4 (a), all the films had a strong ultraviolet absorption at 200–280 nm, which was consistent with the results obtained by other researchers (Nilsuwan et al., 2018; Wang et al., 2018). The gelatin film had good resistance to ultraviolet radiation at 200-280 nm, due to the rich content of tyrosine and tryptophan that absorbed ultraviolet radiation in protein films such as gelatin (Ejaz et al., 2018). Ultraviolet light can cause changes in nutrients and flavor substances of raw meat and accelerate lipid oxidation, inducing its deterioration. Therefore, the more ultraviolet light absorption of the film, the lower the transmittance, the slower the lipid oxidation rate of food, and the better the quality of raw meat during storage. Fig. 4 (a) shows that the bilayer film improved the light resistance of gelatin film, which could be better for maintaining the stability of the food system. A wavelength of 700 nm was selected to analyze the differences between the four films at visible light transmittance. As shown in Fig. 4 (b), compared with the monolayer gelatin film, the light resistance of the double-layer films improved to a greater extent. However, all the values of the double-layer films were between

88% and 91%, and no significant differences were observed between the three bilayer films.

Sensory evaluation of films and lamb after 5 days storage at 4℃

From the sensory evaluation results in Fig. 5, it can be seen that no drying occurred on the surface of the lamb meat in the bilayer packaging, but the surface of the lamb packed with a monolayer gelatin film had an obvious drying phenomenon. The surface of lamb meat wrapped in four kinds of films was somewhat sticky because the ingredients in gelatin migrated to the meat surface, which was acceptable to panelists; the score of these films was above 6 points. Some juice outflow was seen in the package when it was wrapped with either a bilayer film or a monolayer gelatin film. This might be because the lamb used in this study exhibited a higher purge loss during storage, indicating the need for a thicker gelatin film.

Due to the water-absorbing property, the monolayer gelatin film was more severely cracked and due to the drying of the surface of the lamb wrapped in a monolayer gelatin film, the acceptability of this film was the lowest among others. The score for the acceptability of the monolayer gelatin film was only 2 points, suggesting that the gelatin film was highly unacceptable to panelists. The overall acceptability of the samples wrapped in OPP/gelatin bilayer film was higher as its flexibility was the highest of all bilayer films.

Water absorption and water dissolution properties of films

As seen in Table 2, the weight change rate of the gelatin layer with different concentrations of mustard or oregano essential oils was 3-5 when films were immersed in 4℃ water for 1 day. This implied that after being wrapped in OPP/gelatin bilayer film, the gelatin film absorbed the water of the lamb before the juice outflow occurred. A slight reduction in the weight of meat is usually observed in the early stages of storage (Kaewprachu et al., 2018). However, this study showed that the loss of meat storage in the middle and late stages of storage was more likely due to the loss of water through the gelatin film into the air, inducing the drying of the meat surface. As shown in Fig. 5, the acceptance of the lamb decreased when the surface of the lamb was dry. Some evaluators also indicated that the dried meat surface was unacceptable, thus limiting the application of monolayer pure gelatin film in meat storage. In some studies, there was less storage loss of meat wrapped in gelatin film, which might be due to the differences in the methods used to measure the storage loss of meat. These studies wrapped the meat in gelatin film, placed them in a plastic bag, and then measured the weight loss during storage. The gelatin film could absorb the moisture released by the meat, thus lowering the storage loss (Kaewprachu et al., 2015).

The value of the water absorption rate of the gelatin film was close to 550%, as the gelatin film could absorb more than 5 times its mass of water because of the relatively hydrophilic nature of gelatin (Etxabide et al., 2022; Kaewprachu et al., 2015; Yang et al., 2022). With a juice loss rate of 5% for fresh lamb, the thickness of the film could be calculated by the mass of fresh lamb that needed to be wrapped by the gelatin film and the water absorption rate of gelatin. With the increase in the amount of essential oil added, the mass change rate of the gelatin film gradually decreased. Moreover, the water absorption of the film decreased because the tight film structure could be destroyed by essential oil molecules, making the film easier to dissolve. Meanwhile, Ciannamea et al. (2018) found that the hydrophobic character of essential oil droplets within the matrix could obstruct the migration of water through the film.

When the gelatin film was immersed in 4°C water for more than 2 days, the weight of the film decreased, which was consistent with the findings of a previous study (Ahammed et al., 2020). This was mainly due to glycerin (Liu et al., 2017) and protein in the film dissolving into the water (Table 2). Mustard and oregano essential oil significantly increased the solubility of gelatin films, which was consistent with the results of a previous study (Kavoosi et al., 2013). The dissolution rate of the gelatin film decreased from 1 d to 4 days. It was speculated that the faster growth rate on 1 and 2 days was because most of the soluble protein was dissolved; therefore, the growth rate dropped on 3 days.

At the later stage of lamb storage, the gelatin film still maintained its film shape and attached to the OPP plastic film after absorbing the juice out of the raw lamb, making this edible film extremely easy to be peeled off from the raw lamb and not dissolve totally into the raw lamb. When the OPP/gelatin bilayer film was incorporated with essential oil, it disintegrated after some days of packaging the lamb (data not shown). Therefore, increasing the intactness of gelatin films containing essential oil after absorbing water will be a hot research topic for the future development of gelatin AP. To date, few studies are available related to this topic. Some scholars have confirmed that tannic acid addition could keep the films prepared using a mixture of dialdehyde glucomannan and gelatin intact after being immersed in water (Yuan et al., 2021). It should be noted that the focus is not on enhancing the hydrophobic characteristics of the gelatin film with essential oil but on improving the intactness, flexibility, and mechanical strength of the gelatin film after water absorption. The results of studies related to enhancing the mechanical properties of gelatin hydrogels or sponges might provide some references that could be useful for strengthening the mechanical strength of gelatin films after absorbing water in this experiment. For example, the Maillard reaction between polysaccharides and gelatin was a useful method to strengthen the links between the molecules of gelatin sponges; however, whether the intactness of gelatin film was maintained after being immersed in water needs further study (Yang et al., 2022). Gelatin modifications, pH adjustment, temperature control, and ion addition might be other effective methods to enhance the strength of gelatin gel (Bao et al., 2019; Farris et al., 2009; Huang et al., 2019) after absorbing water.

Adhesiveness of gelatin films with essential oil after water absorption

Adhesiveness is the energy or a force required to overcome the attraction between the food surface and the surface contact with other substances. As seen in Table 3, the adhesiveness of gelatin film was 1.9 N in 1 min after water absorption, then the adhesiveness started to decrease, and it dropped to 0.15 N after 5 min. Thus, the thickness of the gelatin film should be determined considering both the water absorption and adhesiveness characteristics of the gelatin film. If adhesiveness is very large, the gelatin film can adhere to the meat when unpacking, and the film and meat may not be easy to separate. When the time was between 1–3 min, the water absorption rate of gelatin was close to 4 times the gelatin mass. Therefore, the weight of the gelatin film needs to be less than a quarter of the juice outflow to lower the adhesiveness of the gelatin film. For the gelatin film with essential oil, the maximum adhesiveness of the gelatin film decreased with the addition of essential oil.

Effect of essential oil on mechanical and antibacterial properties of OPP/gelatin bilayer film

As shown in Table 4, when the concentration of essential oil increased, the TS first increased and then decreased. This increase might be attributed to an appropriate amount of essential oil embedded in gelatin molecules, improved interaction between molecules, and increased TS. As the concentration continued to increase, the gelatin molecules could not encapsulate more essential oil molecules, and the agglomeration of the essential oil molecules resulted in the formation of yellow bumps on the surface of the films. An excess of essential oil formed a discontinuous and non-uniform film structure, thus weakening the network structure of the film and reducing the TS of the film. With the increase in essential oil, the EAB of the film decreased, which might be because the addition of essential oil increased the interaction force between gelatin molecules, increased the strength of the film, and decreased the EAB. However, some researchers have found different results. Martucci et al. (2015) found a decrease in TS and a stable value of EAB in gelatin films incorporated with oregano essential oil. Carvacrol is one of the main components of oregano essential oil. Kavoosi et al. (2013) found a decrease in TS and an increase in EAB in gelatin films containing carvacrol, glycerol and glutaraldehyde. The difference between the results in this study and those in the published articles might be caused by the different concentrations of essential oil used in gelatin film. The concentrations of essential oils used in this study were several to ten times that of the concentrations reported in the literature.

The antibacterial properties of OPP/gelatin bilayer films with different essential oil concentrations are shown in Table 4. The present study showed that gelatin films with essential oils could inhibit the growth of bacteria in lamb. Mustard and oregano essential oils were effective at concentrations of more than 10% and 20%, respectively, which was similar to the concentration of antimicrobial effectiveness of protein films containing essential oils (Martucci et al., 2015). But, colony count was not much reduced at high concentration of essential oils group. We have reason to doubt whether this concentration could extend the shelf life of meat. The possible reasons were as follows. The antibacterial activity of plant essential oil depends on its active components. The main components of oregano essential oil are carvacrol and thymol, while that of mustard essential oil is allyl isothiocyanate (Jayasena & Jo, 2013). In the preparation of the bilayer film, the emulsified essential oil was added, and films were dried at 50℃, which could cause the volatilization of allyl isothiocyanate, carvacrol, and thymol. Meanwhile, allyl isothiocyanate could react with the free amino acids in the gelatin. Allyl isothiocyanate also degraded during the mixing and drying of the film. When drying was complete, these effects led to a decrease in mustard and oregano essential oils in the bilayer films. The remaining essential oil after volatilization combined with the components of the gelatin film, which was another reason for the decreased antimicrobial effectiveness of essential oil, preventing the migration of essential oil to agar (Burt, 2004; Jayasena & Jo, 2013; Martucci et al., 2015). The gelatin composition and the emulsification process might also be related to the migration rate of essential oil from gelatin film to agar or food surface.

In this study, the bacterial inactivation of oregano essential oil was stronger than that of mustard essential oil. Moreover, mustard oil is more flavorful than oregano oil, which could cause significant changes in food senses and non-acceptance by consumers. The combination of oregano and mustard essential oils can be an excellent way to add them to the AP system. The changes in the total colony count of refrigerated lamb packaged in a vacuum bag + OPP/gelatin film containing a combination of oregano-mustard essential oil are shown in Fig. 6. The results showed that the OPP/gelatin film with essential oils prolonged the shelf life of vacuum-packaged lamb. Since essential oil migrating from edible films such as gelatin to food is a slow process, the concentration of essential oil on the surface of food can be maintained within a certain range for a long time, thus extending the shelf life of food. In this way, it is more effective to inhibit the growth of microorganisms than to spray essential oil directly on the surface of products (Quintavalla & Vicini, 2002).

Effect of essential oil on color and light transmittance of OPP/gelatin bilayer film

As reported in other articles, gelatin film exhibited good transparency (Table 5) (Etxabide et al., 2022). The light transmittance of the films reduced with increased essential oil concentration, which could be attributed to the astigmatism of essential oil, increasing the light resistance of the film (Ejaz et al., 2018). The more the addition of essential oil, the stronger the astigmatism effect and the lower the light resistance of the film. As shown in Table 6, the light transmittance at 700 nm was significantly influenced by the essential oil concentration. The more the essential oil concentration, the lower the transmittance of the film at 700 nm.

The film's color was affected by the natural color of essential oils (Said & Sarbon, 2022). As seen in Table 6, after adding the oregano or mustard essential oils, the brightness of the gelatin film kept decreasing and the color became increasingly darker. Furthermore, the film tended to turn yellow, and the b^* value became significantly bigger. The consumers prefer the color of the lamb to be red. They do not consider the lamb fresh when it is yellow in color, especially in China. The yellow color decreases consumers' willingness to select meat at the point of purchase (Tomasevic et al., 2021). Therefore, the concentrations of oregano and mustard essential oils used in gelatin films should be less than 20% and 10%, respectively.

Conclusion and Prospect

After comprehensively considering the mechanical properties, oxygen barrier, light barrier, and moisture barrier and sensory evaluation of OPP/gelatin, PET/gelatin, and PE/gelatin bilayer films, OPP was selected as the binding layer to the gelatin surface. Compared with food-grade plastic films, the OPP/gelatin bilayer film with mustard or oregano essential oils could absorb the juice outflow of raw lamb and inhibit the growth of the bacteria of lamb. Compared with monolayer gelatin film, the mechanical properties, oxygen barrier, moisture barrier, and light barrier properties and consumer acceptance of the OPP/gelatin bilayer film were improved. Thus, OPP/gelatin bilayer film incorporated with 10% oregano essential oil was more suitable for application to the packaging of raw lamb meat. Bilayer films improved the degree of gelatin fracture after water absorption, but increasing the intactness of gelatin films containing oregano essential oil after absorbing water, and reducing the loss of essential oils during the drying of the bilayer film are still urgent research topics for the future development of gelatin AP.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Shijing Wang: Methodology, Investigation, Data curation, Formal analysis, Writing original draft. Weili Rao: Conceptualization, Methodology, Investigation, Supervision, Writing review & editing. Chenli Hou: Validation, Data curation, Funding acquisition. Raheel Suleman: Methodology, Writing review & editing. Zhisheng Zhang: Resources, Supervision. Xiaoyu Chai: Investigation, Resources. Hanxue Tian: Investigation, Resources.

Ethics Approval

This article were undertaken following the principles and guidelines of the Ethics Committee of Hebei Agricultural University, and the approval number is 2021095.

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Table 1. Sensory evaluation criteria of bilayer films and single layer gelatin films after packing lamb

	8-10 points	6-7 points	4-5 points	1-3 points
Drying of lamb surface	No drying	Slight drying	Some drying	Severe drying
Slipperiness of lamb surface	Not slippery	Slight slippery	Some slippery	Severe slippery
Degree of film damage	No damage	Slight damage	Some damage	Severe damage
Juices running out of lamb	No juices	Slight juices	Some juices	Severe juices
Flexibility of film	Fully acceptable	Acceptable	Unacceptable	Highly unacceptable
Overall acceptability (lamb and film)	Fully acceptable	Acceptable	Unacceptable	Highly unacceptable

Table 2. Weight change ratio and protein solubility of single layer gelatin films with different concentrations of mustard or oregano essential oils at 4°C in water.

	Time	Mustard Essential Oil					Oregano Essential Oil				
		0%	20%	40%	60%	80%	0%	10%	20%	30%	40%
Weigh change ratio	1 day	5.50±0.31^{a}	4.48±0.55^{b}	4.01±0.63^{bc}	3.26±0.26^{cd}	3.11±0.28^{d}	5.50±0.32^{a}	4.21±0.59^{b}	3.50±0.83^{bc}	3.11±0.40^{c}	2.95±0.24^{c}
	2 day	5.40±0.21^{a}	4.39±0.38^{b}	4.00±0.20^{b}	3.22±0.58^{c}	3.05±0.41^{c}	5.40±0.21^{a}	4.10±0.16^{ab}	2.96±0.26^{b}	3.13±0.21^{b}	2.86±0.11^{b}
	3 day	5.39±0.45^{a}	4.32±0.35^{b}	3.96±0.31^{b}	3.23±0.34^{c}	3.12±0.62^{c}	5.39±0.45^{a}	4.10±0.80^{b}	2.90±0.24^{c}	3.10±0.14^{c}	2.80±0.45^{c}
	4 day	5.23±0.23^{a}	4.12±0.23^{b}	3.83±0.35^{b}	3.12±0.20^{c}	3.10±0.42^{c}	5.23±0.23^{a}	3.91±0.12^{ab}	2.69±0.63^{b}	2.85±0.29^{b}	2.71±0.13^{b}
P-value	Time		0.539						0.550		
	Essential Oil		0.000						0.000		
	Time×Essential Oil		1.000						1.000		
Protein solubility (mg/mL)	1 day	0.34±0.01^{dD}	2.14±0.01^{cC}	3.02±0.02^{bC}	3.23±0.03^{aC}	3.39±0.06^{aC}	0.34±0.01^{eD}	1.24±0.02^{dD}	1.98±0.04^{cD}	3.02±0.16^{bB}	3.23±0.04^{aC}
	2 day	0.41±0.01^{dC}	2.56±0.07^{cB}	3.23±0.09^{bB}	3.31±0.08^{abC}	3.43±0.06^{aC}	0.41±0.01^{dC}	1.64±0.06^{cC}	2.44±0.08^{bB}	3.23±0.09^{aA}	3.31±0.08^{aC}
	3 day	0.43±0.08^{eB}	2.84±0.09^{dA}	3.31±0.09^{cB}	3.43±0.06^{bB}	3.57±0.02^{aB}	0.43±0.08^{eB}	1.91±0.04^{dB}	2.33±0.02^{cC}	3.31±0.18^{bA}	3.43±0.06^{aB}
	4 day	0.46±0.01^{dA}	2.97±0.08^{cA}	3.55±0.08^{bA}	3.66±0.07^{abA}	3.72±0.08^{aA}	0.46±0.01^{eA}	2.04±0.03^{dA}	2.69±0.05^{cA}	3.33±0.09^{bA}	3.60±0.06^{aA}
P-value	Time		0.000						0.000		
	Essential Oil		0.000						0.000		
	Time×Essential Oil		0.000						0.000		

For each essential oil, lowercase letters in each row represent significant differences ($P < 0.05$). For each essential oil, capital letters in each column represent significant differences ($P < 0.05$).

Table 3. Adhesiveness of gelatin films with 10% oregano or 20% mustard essential oils after absorbing water for different times at 4°C.

Time	Gelatin film	Gelatin film with 10% oregano oil	Gelatin film with 20% mustard oil
1 min	-1.91±0.30^{bC}	-0.35±0.09^{aC}	-0.32±0.04^{aB}
2 min	-1.32±0.28^{bB}	-0.27±0.05^{aB}	-1.35±0.13^{bC}
3 min	-0.40±0.16^{A}	-0.30±0.00^{BC}	-0.30±0.05^{B}
4 min	-0.20±0.04^{aA}	-0.27±0.05^{bB}	-0.30±0.02^{bB}
5 min	-0.17±0.04^{A}	-0.15±0.02^{A}	-0.15±0.00^{A}
10 min	-0.15±0.00^{A}	-0.15±0.01^{A}	-0.15±0.01^{A}
60 min	-0.15±0.01^{A}	-0.15±0.00^{A}	-0.15±0.00^{A}
3 days	-0.15±0.00^{A}	-0.15±0.00^{A}	-0.15±0.00^{A}
P-value	Time	0.000	
	Film	0.000	
	Time × Film	0.000	

Lowercase letters in each row represent significant differences ($P < 0.05$). Capital letters in each column represent significant differences ($P < 0.05$).

Table 4. Mechanical and antibacterial propertiesof OPP/gelatin bilayer films with different concentrations of oregano or mustard essential oils

Essential Oil	Concentration (%)	Tensile strength (MPa)	Elongation at break (%)	Colony count (log CFU/mL)
Oregano Essential Oil	0	26.81±0.52 ^{c}	158.35±28.84 ^{a}	6.48±0.01 ^{a}
	10	61.23±5.22 ^{a}	60.66±2.63 ^{b}	6.31±0.02 ^{b}
	20	43.20±2.31 ^{b}	37.39±6.32 ^{b}	6.17±0.05 ^{c}
	30	31.54±2.45 ^{c}	30.28±7.08 ^{b}	6.14±0.07 ^{c}
	40	28.79±3.69 ^{c}	29.37±5.08 ^{b}	6.05±0.08 ^{c}
Mustard Essential Oil	0	26.81±0.52 ^{b}	158.35±28.84 ^{a}	6.48±0.01 ^{a}
	20	29.81±3.37 ^{b}	56.36±12.33 ^{b}	6.35±0.03 ^{b}
	40	32.12±2.53 ^{b}	36.33±6.98 ^{bc}	6.29±0.05 ^{b}
	60	74.92±8.21 ^{a}	32.47±8.85 ^{bc}	6.14±0.07 ^{c}
	80	38.02±5.10 ^{b}	15.85±4.77 ^{c}	6.02±0.03 ^{d}

For each essential oil, lowercase letters in each column represent significant differences ($P < 0.05$). Antibacterial properties was done as follow: bilayer film containing essential oil was placed in the center of the Petri dish containing bacteria. The stronger the inhibition of bacteria, the fewer colonies could grow in the Petri dish.

Table 5. Light transmittance of OPP/gelatin bilayer films with different concentrations of mustard essential oil or oregano essential oil at 4°C.

Wavelength (nm)	Mustard Essential Oil					Oregano Essential Oil				
	0%	20%	40%	60%	80%	0%	10%	20%	30%	40%
200	1.93±0.12^{bF}	1.57±0.21^{cD}	2.17±0.05^{bE}	2.63±0.12^{aD}	2.04±0.13^{bE}	3.63±0.31^{bE}	3.67±0.05^{bC}	3.40±0.08^{bD}	4.37±0.09^{aD}	4.23±0.05^{aF}
300	66.23±2.85^{aD}	36.43±3.53^{bC}	14.63±2.60^{cD}	12.73±2.42^{cC}	10.23±0.76^{cD}	66.23±2.85^{aD}	63.33±4.86^{aB}	50.07±4.13^{bC}	47.53±7.69^{bcC}	38.43±2.73^{cE}
400	86.53±0.54^{aC}	77.87±1.39^{bB}	68.00±1.96^{cC}	66.13±3.97^{cdB}	62.77±1.28^{dC}	86.53±0.54^{aC}	85.77±2.35^{aA}	75.40±2.05^{bB}	71.03±1.98^{bB}	66.60±0.86^{cD}
500	88.63±1.91^{aBC}	83.27±1.62^{aA}	74.97±3.24^{bB}	73.67±2.84^{bA}	73.20±4.12^{bB}	88.63±1.91^{aBC}	88.33±1.76^{aA}	81.30±1.24^{bA}	76.63±1.16^{cAB}	73.17±0.71^{dC}
600	89.30±2.33^{aBC}	83.67±1.48^{bA}	77.73±1.80^{cAB}	75.30±1.43^{cA}	74.50±2.35^{cB}	89.30±2.33^{aBC}	88.03±2.55^{aA}	82.31±0.94^{bA}	79.30±1.88^{bcA}	76.37±1.09^{cBC}
700	92.03±1.32^{aAB}	83.63±2.80^{bA}	79.47±1.32^{bcAB}	75.23±3.77^{cA}	76.97±2.15^{cAB}	92.03±1.32^{aAB}	91.27±0.17^{aA}	83.57±0.82^{bA}	80.53±1.35^{cA}	78.71±1.64^{cAB}
800	94.33±3.22^{aA}	84.97±2.27^{bA}	80.80±1.48^{bA}	80.67±5.07^{bA}	80.17±1.70^{bA}	94.33±3.22^{aA}	91.30±0.22^{aA}	84.13±1.02^{bA}	82.00±1.42^{bA}	80.07±1.87^{bA}
P-value	Wavelength			0.000				0.000		
	Essential Oil			0.000				0.000		
	Wavelength ×Essential Oil			0.000				0.000		

For each essential oil, lowercase letters in each row represent significant differences ($P < 0.05$). For each essential oil, capital letters in each column represent significant differences ($P < 0.05$).

Table 6. Color difference and light transmittance of OPP/gelatin bilayer films with different concentrations of oregano or mustard essential oils at 700 nm.

Essential Oil	Concentration (%)	CIE L*	CIE a*	CIE b*	Transmittance at 700 nm （%）
Oregano Essential Oil	0	55.42±0.80 ^{a}	2.81±0.27 ^{a}	3.36±0.34 ^{c}	92.03±1.32 ^{a}
	10	53.43±0.53 ^{b}	2.31±0.14 ^{b}	4.29±0.24 ^{ab}	91.27±0.17 ^{a}
	20	51.23±0.55 ^{c}	1.82±0.22 ^{c}	3.94±0.12 ^{b}	83.57±0.82 ^{b}
	30	51.81±1.15 ^{cd}	1.79±0.19 ^{c}	3.90±0.50 ^{b}	80.53±1.35 ^{c}
	40	50.79±0.93 ^{d}	2.14±0.16 ^{b}	4.56±0.16 ^{a}	78.70±1.64 ^{c}
Mustard Essential Oil	0	67.81±0.29 ^{a}	11.01±0.39 ^{a}	9.76±0.63 ^{d}	92.03±1.32 ^{a}
	20	63.38±0.86 ^{b}	8.70±1.21 ^{b}	9.93±0.68 ^{cd}	83.63±2.80 ^{b}
	40	59.66±1.11 ^{c}	8.54±1.03 ^{b}	10.67±0.5 ^{bc}	79.47±1.32 ^{bc}
	60	58.79±1.31 ^{cd}	8.13±0.66 ^{b}	10.83±0.45 ^{b}	75.23±3.77 ^{c}

	80	57.2±1.53 ^{d}	6.79±0.57 ^{c}	13.18±0.38 ^{a}	76.97±2.15 ^{c}

For each essential oil, lowercase letters in each column represent significant differences ($P < 0.05$).

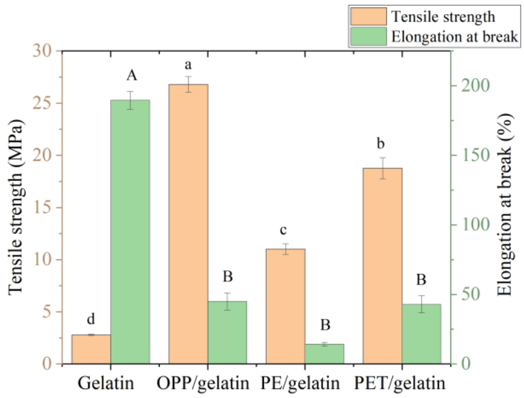

Fig. 1. Mechanical properties of single-layer gelatin film and bilayer plastic/gelatin film which were made as follow: gelatin solution without essential oil was poured onto the OPP, PE, or PET film. After drying for 15 h, the gelatin film was formed and bonded together with the plastic film to form a bilayer film. Different lowercase letters indicate significant differences between tensile strength of films ($P < 0.05$). Different capital letters indicate significant differences between elongation at break of films ($P < 0.05$).

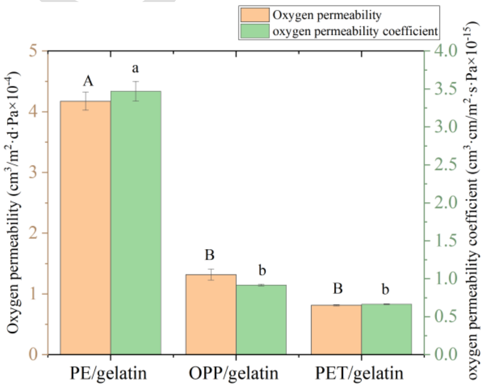

Fig. 2. Oxygen permeability and oxygen permeability coefficient of plastic/gelatin bilayer films which were made as follow: gelatin solution without essential oil was poured onto the OPP, PE, or PET film. After drying for 15 h, the gelatin film was formed and bonded together with the plastic film to form a bilayer film. Different lowercase letters indicate significant differences between oxygen permeability coefficient of films ($P < 0.05$). Different capital letters indicate significant differences between oxygen permeability of films ($P < 0.05$).

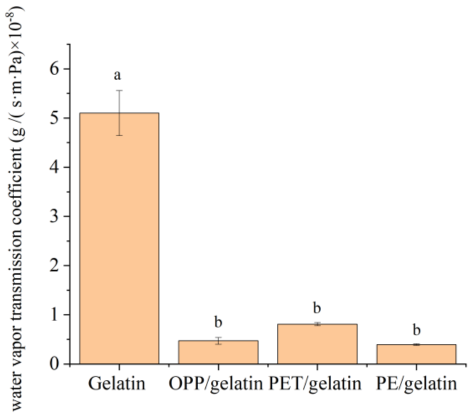

Fig. 3. Water vapor transmission coefficient of single-layer gelatin film and bilayer plastic/gelatin film which were made as follow: gelatin solution without essential oil was poured onto the OPP, PE, or PET film. After drying for 15 h, the gelatin film was formed and bonded together with the plastic film to form a bilayer film. Different lowercase letters indicate significant differences between the water vapor transmission coefficient of films ($P < 0.05$).

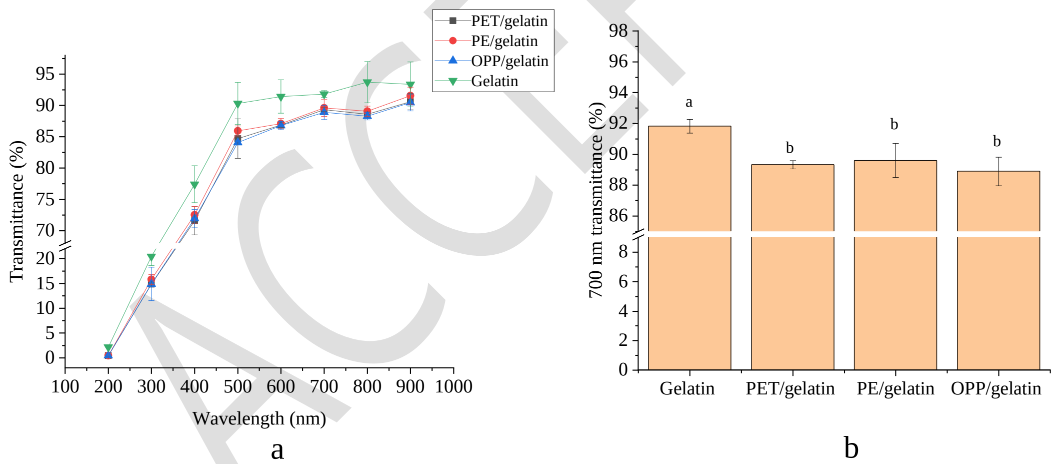

Fig. 4. Transmittance from 100–1000 nm (a) and transmittance at 700 nm (b) of gelatin, PET/gelatin, PE/gelatin, and OPP/gelatin bilayer films which were made as follow: gelatin solution without essential oil was poured onto the OPP, PE, or PET film. After drying for 15 h, the gelatin film was formed and bonded together with the plastic film to form a bilayer film. Different lowercase letters in (b) indicated significant differences between films ($P < 0.05$)

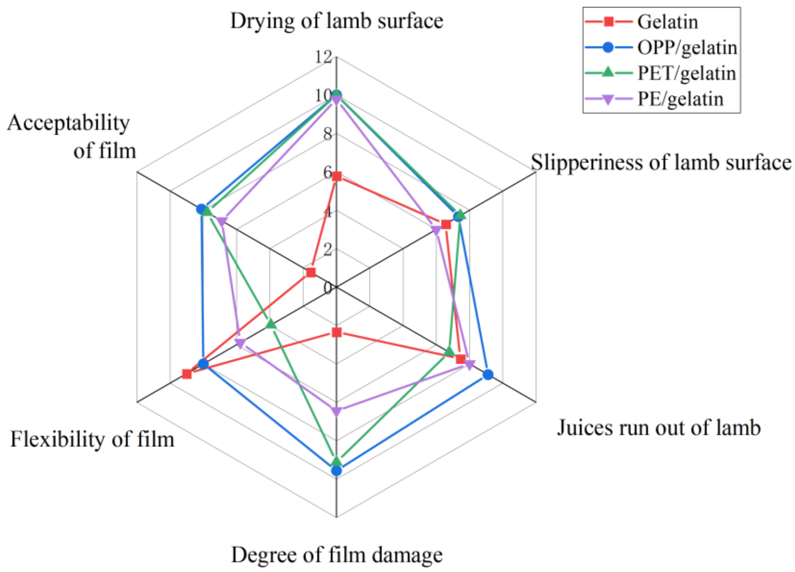

Fig. 5. Sensory evaluation of single layer gelatin film, bilayer films, and lamb wrapped in different films. Bilayer plastic/gelatin films were made as follow: gelatin solution without essential oil was poured onto the OPP, PE, or PET film. After drying for 15 h, the gelatin film was formed and bonded together with the plastic film to form a bilayer film

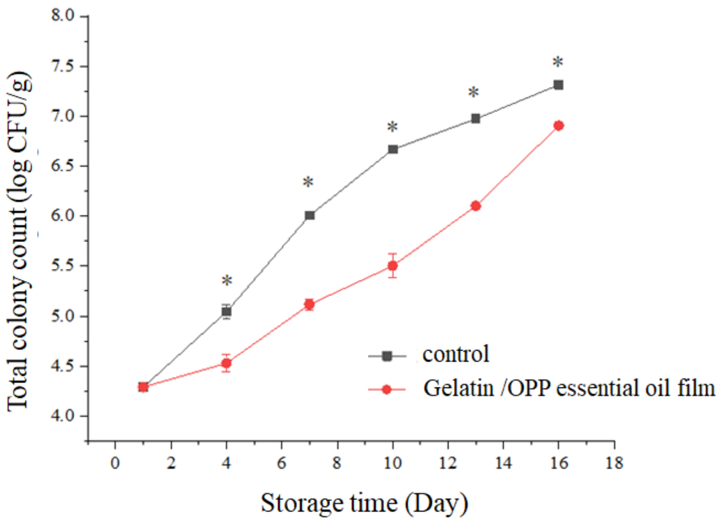

Fig. 6. Changes in the total colony count of refrigerated lamb (4°C) packaged in vacuum bag or vacuum bag + OPP/gelatin film containing a combination of essential oils. The compound ratio of mustard and oregano essential oil was 1:4, and the total amount of essential oil was 20% of the quantity of gelatin. Treatment group: Packaging with vacuum bag + oregano-mustard composite essential oil in double layer film (OPP/gelatin film with essential oils as the inner film wrapped on the surface of lamb, and then put into the vacuum plastic bag). Control group: Lamb packaged in vacuum bag. Asterisk indicate significant differences between control and treatment group ($P < 0.05$).