Effect of Grapefruit Essential Oil Content on Properties of Composite Film and Shelf Life of *Agaricus bisporus*

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Abstract: The effect of various concentrations (0%, 5%, 7.5%, 10%, and 12.5% *V/m*) of grapefruit essential oil (GEO) was studied on the on thermal, structural, mechanical, gas barrier, and antimicrobial properties of polylactic acid (PLA)/GEO composite film. By reducing the intermolecular force of polymer chains, GEO improved the flexibility of the composite film and showed a certain plasticization effect on the films. The presence of GEO decreased the crystallinity of PLA phase. The water vapor barrier properties of the films significantly decreased with increasing GEO content. Additionally, the incorporation of GEO in the PLA polymer matrix improved the antimicrobial activity of packaging films. PLA packaging films with or without GEO were used for preservation of button mushroom (*Agaricus bisporus*). The results indicate that PLA/GEO composite film is more effective in retaining the firmness, reducing the microbial count, and maintaining a high overall acceptability of mushrooms than pure PLA and low-density polyethylene (LDPE) films. Therefore, the results suggest that PLA/GEO composite film is a useful packaging material for extending the shelf life of button mushrooms.

Key words: polyester resins; essential oils; blends; button mushroom; packaging

GEO 添加量对 PLA/GEO 复合膜性能及双孢蘑菇保鲜效果的影响

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摘要：本文研究了不同浓度（0%、5%、7.5%、10%和 12.5%）的葡萄柚精油（GEO）对聚乳酸(PLA)/葡萄柚精油复合膜的热性能，结构性能，机械性能，气体阻隔性能和抗菌性能等方面的影响。GEO 通过减少聚合物链段的分子间作用力，从而改善复合膜的柔韧性，对复合膜起到一定的增塑作用。GEO 的添加降低了 PLA 相的结晶性。随着 GEO 浓度的增加，复合膜的水蒸汽阻隔性能显著降低。但是，复合膜的抗菌活性因 GEO 的加入得以提高。将聚乳酸/葡萄柚精油复合膜应用于双孢蘑菇保鲜。结果表明，聚乳酸/葡萄柚精油复合膜与纯聚乳酸膜、低密度聚乙烯膜相比较，更能有效地保持蘑菇的硬度，阻止微生物生长，维持较好的总体接受度。因此，聚乳酸/葡萄柚精油复合膜可作为一种有效的包装材料，用于延长双孢蘑菇的货架期。

关键词：聚酯；精油；共混；双孢蘑菇；包装
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During the last decades, the increase in fossil energy costs and the environmental concerns result in new opportunities for the industrial production of biodegradable polymers[1]. Poly(lactic acid) (PLA) is a biodegradable, thermoplastic, aliphatic polyester made from 100% renewable resources, such as corn and sugar beets following fermentation of starch and condensation of lactic acid[2,3]. Its relatively low cost, low toxicity, and ease of processing have made PLA an ideal material for many food packaging applications[1]. Comparing with some petro-chemical based polymers, the applications of PLA are limited by several factors such as low glass transition temperature, weak thermal stability, brittleness, and low ductility[4]. PLA has the ability to be tailor-made for the properties needed for special applications[3].

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A large number of investigations have been made to modify PLA properties *via* plasticization. Poly (*ε*-caprolactone) (PCL), poly(ethylene glycol) (PEG), synthetic phenolic antioxidants, limonene, propolis, and cinnamaldehyde have been studied as PLA modifiers[5]. For food packaging applications, only nontoxic substances approved for food contact can be considered as modifying agents. The addition of active compounds in polymer formulations can help to extend the shelf-life of produce by providing protection against microbial growth, enzymatic browning, vitamin losses, and oxidation. Gonçalves, Tomé, Coutinho, & Marrucho[6] reported that the addition of a well-known antioxidant, α-tocopherol, could improve the flexural and barrier properties of PLA films. Liu et al[7]. also reported that a model antimicrobial polypeptide (nisin) was loaded into the PLA/pectin composite by diffusion. The resulted composite films were able to suppress *Lactobacillus plantarum* growth and retain tensile strength, flexibility, and toughness to an extent that satisfied the requirements for packaging materials. Qin, Yang, & Xue[8] observed that incorporation of cinnamaldehyde to the PLA/poly(trimethylene carbonate) polymer matrix improved the antimicrobial activity of active packaging films. Furthermore, cinnamaldehyde acted as plasticizers which reduce the intermolecular forces of polymer chains, thus improving the flexibility and extensibility of the composite films.

Despite the availability of many antimicrobial agents, essential oils are particularly attractive in food packaging industry due to their promising antibacterial, antifungal, and antioxidant properties[9]. Grapefruit essential oil (GEO), whose major chemical volatile compounds are limonene (91.5~88.6%), is a bioactive substance derived from grapefruit plant. The antimicrobial efficacy of limonene has been proved to be especially effective in inhibiting the proliferation of a variety of microorganisms that cause crop damage or food spoilage, including *Aspergillus niger*, *Listeria monocytogenes*, *Bacillus subtilis*, *Salmonella enterica*, *Staphylococcus aureus,* and molds[10,11].

The incorporation of active compounds may affect the structure, physical, and mechanical properties important to the technological and functional aspects of packaging materials, so an active packaging without the desired properties will be useless. Therefore, the objective of the present study was to prepare a GEO incorporated PLA film as a new active packaging material and apply the film in the packing of button mushroom (*Agaricus bisporus*).

1 Materials and methods

1.1 Materials and chemicals

Poly (lactic acid) (PLA, M_w=280 ku, M_w/M_n=1.98) was obtained from Natureworks LLC (Nebraska, USA). Grapefruit essential oil (GEO) was purchased from doTERRA Co., Ltd (New York, USA) and used as received. Chloroform was purchased from Chengdu kelong chemical Co., Ltd (Sichuan, China). All other reagents and chemicals used in the study were of analytical grade.

1.2 Preparation and characterization of films

Prior to preparation, PLA resins were dried in a vaccum oven at 60 ℃ for 24 h. PLA/GEO composite films were prepared by a solvent casting method. Briefly, 2 g PLA was dissolved in 50 mL chloroform. 0%, 5%, 7.5%, 10%, and 12.5% (*V*/*m*) GEO were added to PLA chloroform solution by vigorous mixing. Then, the solutions were cast on glass plates (20 cm×20 cm) and dried to form films. All of the films were dried in vacuum at 25 ℃ to remove the solvent. GEO was incorporated into PLA matrix as 0%, 5%, 7.5%, 10%, and 12.5% (*V*/*m*) loading named as PLA, PLA/GEO-5, PLA/GEO-7.5, PLA/GEO-10, and PLA/GEO-12.5.

Tensile tests were performed on a Universal tensile machine (CMT 4104, MTS Systems Co., Ltd, China). Films were cut into 1.5 cm×10 cm strips. The initial grip separation was set at 100 mm and the crosshead speed was set at 50 mm/min. Average values of tensile modulus, tensile strength, and elongation at break of the various composite films were calculated according to ASTM Method D882-88 standard procedure. Five samples were tested and average values were reported.

Water vapor permeability (WVP) of film samples was studied gravimetrically in accordance with the ASTM E96-95 standard method at 20 ℃ and 50% relative humidity (RH), using silica gel as the desiccant material[3].

Opacity of the films was determined by measuring the percent transmittance of light at a wavelength of 600 nm using a UV-visible spectrophotometer (T 90, Beijing Purkinje general instrument Co., Ltd, Beijing, China). The method used was adapted from Siripatrawan and Harte.[12] The sample was cut into rectangular strips and directly placed in the spectrophotometer test cell. Air was used as reference. Opacity of the film samples was expressed as absorbance units per thickness unit (mm^{-1}).

Differential scanning calorimetry (DSC) was conducted on a TA Instrument (STA449, Netzsch, Germany) under a dry nitrogen gas flow rate of 50 mL/min. First, about 10 mg of samples was sealed in aluminium pans and heated from 20 ℃ to 210 ℃ at 10 ℃/min to identify possible change in crystallization and melting transitions. Subsequently, the sample was cooled to room temperature at a cooling rate of 10 ℃ /min and further heated to 210 ℃ at 10 ℃/min. The thermal properties of PLA/GEO composite films were examined using DSC in order to evaluate the effect of GEO on the thermal properties of the films compared to pure PLA polymer. The glass transition temperature (T_g), peak crystallization temperature (T_c), melting temperatures (T_m), and melting enthalpy (ΔH_m) were evaluated from the DSC thermogram obtained from the second scanning.

Scanning electron microscopy (SEM) was conducted on an S-4800 Hitachi microscope applying an acceleration voltage of 5.0 kV. Samples were cryo-fractured in liquid nitrogen. The surface and cross-sectional surfaces of samples were studied. Prior to the SEM examination, the samples were sputter-coated with a thin conductive gold layer 20 nm thick.

The liquid culture test was used to determine the ability of GEO (both incorporated and in the free form) in inhibiting the growth of two food pathogenic bacteria: Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. All bacterial strains were obtained from Laboratory of Microbiology, Faculty of Life Science and Technology, Kunming University of Science and Technology, Yunnan, China. The antimicrobial test was determined by method of Liu et al. with slight modification[7,13]. Bacteria constant temperature cultivation condition was 37 ℃ and 18~24 h. A glass test tube containing testing specimens (0.18~0.20 g for each) was filled with 10 mL of broth (5 g/L beef extract, 10 g/L peptone, and 5 g/L NaCl). The medium was inoculated with 0.1 mL of an overnight culture of bacteria. The bacterial cultures were adjusted to 10^5 colony forming units per mL (CFU/mL). The test tubes were transferred to a shaker at 20 ℃ and shaken at 200 r/min. The culture was sampled at 24 h. Bacteria in the culture was serially diluted and then pourplated onto agar plates (60 mm×15 mm). All plates were incubated at 37 ℃ for 24 h and the colony-forming units (CFU) were counted[14].

1.3 Application of packaging film to button mushrooms

Fresh button mushroom (*Agaricus bisporus*) samples were obtained from a local farm in Kunming, Yunnan Province, China. Each sample weighing 500 g was packed into a rectangular bag (150 mm×200 mm) that was constructed from the PLA, PLA/GEO-5, PLA/GEO-7.5, PLA/GEO-10, PLA/GEO-12.5 films, and low density polyethylene (LDPE) (control treatment), respectively, stored at 4±1 ℃ for 16 d. The stability tests (firmness, microbial counts, and overall acceptability) were carried out at 4 d intervals (0, 4, 8, 12, and 16 d).

The firmness was measured as an indicator of texture. Firmness of mushrooms was measured using a Texture Analyzer (Texture Exponent 32, Stable MicroSystem Ltd., London, UK). The puncture diameter was 2 mm with 2 mm/s test speed. From the force versus time curves, firmness was defined as the maximum force (N).

All samples were analyzed for *mesophilic* and *psychrophilic* bacteria counts. 25 g of fresh mushroom was removed aseptically from the package and homogenized in a sterile stomacher bag for 2 min with 225 mL of 0.1% peptone water. The plates were incubated at 37 ℃ for 2 d for *mesophilic* bacteria, and at 4 ℃ for 7 d for *psychrophilic* bacteria[15].

The overall acceptability was determined by a ten member trained panel from Institute of Yunnan Food Safety, Kunming University of Science and Technology, using a 10 points scale (0=very poor, 2=poor, 4=fair, 6=good and limit of marketability, 8=very good, and 10=excellent) [16].

1.4 Statistical analysis

A completely randomized design was used. SPSS statistical computer software package (SPSS version 13.0) was employed in this study. Duncan's test was used to compare treatment means when significant differences were found with the ANOVA. The significance level was always set to 0.05.

2 Results and discussion

2.1 Effect of GEO content on mechanical properties of PLA/GEO composite film

Mechanical properties of films are very important in selecting diverse applications for polymer films[17]. When the PLA films are applied for food applications, especially for fruits and vegetables packaging, the films should maintain integrity under the stress occurring during storage.

It could be seen that tensile modulus of PLA/GEO composites was lowered by 18.1, 15.8, 26.1, and 28.3% upon incorporation of 5%, 7.5%, 10%, and 12.5% (V/m) of GEO content, respectively (Table 1). Adding GEO to the PLA film significantly ($p<0.05$) decreased tensile strength of the PLA/GEO composite films. A certain increase in elongation at break for these samples was also observed. The pure PLA film had the lowest elongation at break (5.39%) while PLA/GEO-10 had the highest elongation at break (220%). Addition of GEO into the PLA film increased elongation and reduced brittleness by decreasing T_g[18]. The addition of a plasticizer causes a decrease in the T_g of PLA. This indicated that GEO could act as a plasticizer to enhance plastic elongation and reduce brittleness of PLA films. This behavior has been also reported for bio-based samples with antimicrobial compounds[13]. Persico et al.[19] observed that the mechanical performance of LDPE film was affected by the presence of carvacrol, which acts as a plasticizer for the matrix as well as a dispersing agent for the inorganic filler. However, there was no significant ($p>0.05$) difference in mechanical properties between PLA/GEO-10 and PLA/GEO-12.5 film. When GEO was used in the appropriate amount, it led to obvious improvements in the mechanical properties of the composites, but additional increase in the amount of the additive did not improve the mechanical properties anymore[20].

Table 1 The mechanical properties of PLA blend films with different GEO contents

Sample	Tensile modulus/MPa	Tensile strength/MPa	Elongation at break/%
PLA	1702 ± 54.43^{c}	45.83 ± 1.73^{d}	5.39 ± 0.02^{a}
PLA/GEO-5	1393 ± 87.83^{b}	25.76 ± 3.10^{bc}	191 ± 26.95^{b}
PLA/GEO-7.5	1433 ± 69.90^{b}	23.54 ± 1.20^{b}	197 ± 21.78^{b}
PLA/GEO-10	1257 ± 91.68^{a}	21.37 ± 0.93^{a}	220 ± 20.97^{c}
PLA/GEO-12.5	1220 ± 14.56^{a}	20.51 ± 0.28^{a}	217 ± 11.59^{c}

Note: $^{a\sim d}$ Values followed by different letters in the same column were significantly different ($p<0.05$), where "a" is the lowest value.

2.2 Effect of GEO content on WVP of PLA/GEO composite film

WVP is one of the most important properties in food packaging mainly due to the significant role of water in deteriorative reactions and microbial growth[21]. WVP of pure PLA film [2.39×10^{-11} g·m/(m^2·s·Pa)]was different from that previously reported by other researchers.[3,4] The reason for the disagreement could be related to the method of film preparation (lab scale solvent casting method vs. industrial scale extruding or blowing production), molecular weight, the solvent used for the casting, and the differences in relative humidity used for testing[22]. GEO content on WVP values of PLA/GEO composites films were shown in Table 2. The WVP value of PLA/GEO composite films was much higher than that of commercial low density polyethylene (LDPE) film [0.85×10^{-11} g·m/(m^2·s·Pa)] . Although the film samples were of the same thickness, the commercial LDPE film was prepared by industrial scale extruding and PLA/GEO composite films were prepared by solvent casting method.

GEO addition significantly ($p<0.05$) increased the WVP of PLA/GEO composites films. The highest WVP value was obtained with PLA/GEO-12.5 film, which increased by 24.6% compared to pure PLA film. GEO acted as a plasticizer to lower the glass transition temperature (T_g) of polymer and increase the free volume of the polymer (Rhim, 2013)[3]. As a result, WVP of PLA/GEO composites films increased. Similar trend in WVP values of polymer films have been well reported in the literature. Tsuji, Okino, Daimon, & Fujie[21] reported that the water vapor transition rate of PLA films decreased gradually with increasing the crystallinity from 0 to 20%. This was might be because of the higher resistance of "restricted" amorphous regions to water vapor permeation compared with that of the "free" amorphous regions[4]. Jo, Song, Lee, & Song[23] also reported that grapefruit seed extract increased the WVP value of persimmon peel/red algae composite film. The increase in WVP value could also be verified by SEM images Fig. 1 that many voids existed in PLA/GEO composite films and permitted more water vapor transfer[5].

Table 2 Effect of GEO content on water vapor permeability and opacity of composite films

Sample	WVP×10^{-11} /[g·m/(m^2·s·Pa)]	Opacity /mm^{-1}
PLA	2.39±0.19^{a}	17.71±1.51^{a}
PLA/GEO-5	2.34±0.32^{a}	23.66±0.52^{b}
PLA/GEO-7.5	2.45±0.14^{a}	24.38±2.35^{b}
PLA/GEO-10	2.63±0.56^{ab}	25.92±1.12^{b}
PLA/GEO-12.5	2.98±0.19^{b}	29.44±1.20^{c}

Note: $^{a\text{-}b}$ Values followed by different letters in the same column were significantly different ($p<0.05$), where "a" is the lowest value.

Water relations of produce are an important quality feature of fresh fruit and vegetables that should be maintained during storage. The consequences of water loss are wilting, shrivelling, and loss of firmness, crispness, and succulence[24]. However, comparatively high humidity inside the film packaging would lead to water condensation on the surface of fruit and vegetables. This would promote the growth of micro-organisms, accelerate the forming of spores, and thus accelerate deterioration. So, a suitable WVP value for the PLA/GEO composite films was very important. Although the WVP value of PLA/GEO composite films was much higher than that of commercial LDPE film, the PLA/GEO composite films were still suitable for fresh fruit and vegetables preservation.

2.3 Effect of GEO content on optical properties of PLA/GEO composite film

Because the aspect of packaging could influence the decision of consumers, high transparency of packaging film for fruits and vegetables is required. The PLA film, with the intrinsic color of PLA, could have an advantage as a food packaging material. The results of opacity of composite films determined by absorbance units per thickness unit showed that opacity of the PLA/GEO composite films increased significantly ($p<0.05$) compared to that of pure PLA film Table 2. Incorporation of GEO into the PLA film resulted in more opaque of the film (higher opacity value). Such effect of active compounds on the optical property had been reported by other researchers. Byun, Kim, & Whiteside[18] observed that the PLA/buthylated hydroxytoluene (BHT) composite film had higher haze value than the pure PLA film. Wu et al[5] reported that opacity of the PLA/PCL/thymol composite films was a little higher than that of PLA/PCL film. However, the differences among samples were not perceptible to the human eyes. The results indicated that the PLA/GEO composite films had good transparency even at high GEO content and this material was suitable for food packaging application.

2.4 Effect of GEO content on thermal characteristics of PLA/GEO composite films

Table 3 Thermal characteristics of PLA/GEO composite films

Sample	T_g/℃	T_c/℃	T_m/℃	X_c/%
PLA	57.27	110.68	169.15	5.66
PLA/GEO-5	55.44	106.39	167.94	5.04
PLA/GEO-7.5	52.39	103.02	164.64	4.36
PLA/GEO-10	49.90	99.40	162.03	3.49
PLA/GEO-12.5	46.16	97.69	158.88	3.00

The results of thermal characteristics of PLA/GEO composite films were listed in Table 3. With the increase of GEO content, T_g decreased by 1.8~11.1 ℃, T_c decreased by 4.3~13.0 ℃, and T_m decreased by 1.2~10.3 ℃. Such decrease in T_g value for plasticized PLA systems was very common and attributed by segmental mobility of PLA chains due to plasticization[25]. This confirmed that GEO exerted a plasticizer effect. A shift in T_c and T_m value was observed for the composite materials. The more plasticized the material, the larger the shift to a lower temperature.

In table 3, the degree of crystallinity (X_c) of the PLA/GEO composites was determined by subtracting the enthalpy for cold crystallization from the melting enthalpy[26]. It was found that the degree of crystallinity in the films was lower in the more plasticized materials. This observation indicated a higher crystallinity of PLA with no additives, confirming the observed changes in the mechanical properties where a decrease in the tensile modulus and tensile strength was noticed[27]. The decrease in X_c of samples also meant that less energy was needed for breaking the crystalline arrangement.[28] Additionally, the inhibition of the crystallization process occured for samples with GEO, as evidenced by the decrease in T_m. A similar effect was reported for bio-based polymer with the addition of essential oil. Yang and Paulson[29] showed that the lower T_m values of plasticized gellan films could be attributed to their inherent structural characteristics (high chain mobility) and to their relatively high hydrophilicity. Jouki, Yazdi, Mortazavi, & Koocheki[30] also reported that addition of oregano essential oil decreased T_g values of quince seed mucilage-based films.

2.5 SEM analysis of PLA/GEO composite film

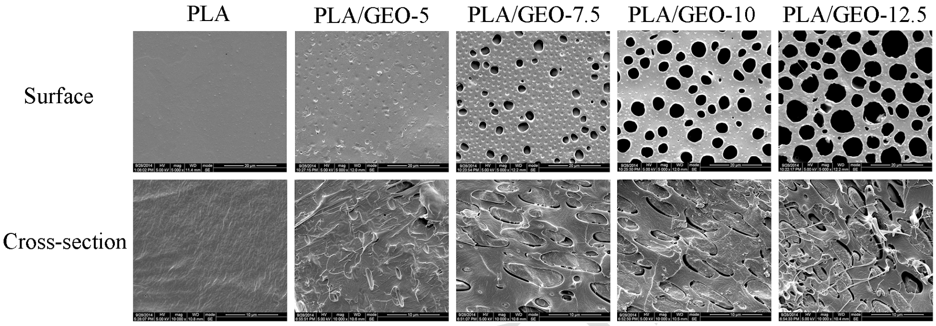

Fig.1 Typical canning electron microscopy images from surface and cross-section of PLA/GEO composite films

Typical SEM images from surface of PLA/GEO composite films were shown in Fig.1. Surface of pure PLA film was uniform and smooth. However, there were some voids on the surface of PLA films incorporated with GEO additives. These voids might be induced by evaporation of solvent and GEO additives when PLA/GEO composite films were prepared by solvent casting method. With the increasing content of GEO additives, the voids on the surface of PLA/GEO composite films became larger. SEM images from fractured surface of PLA/GEO composite films were shown in Fig. 1. The fractured surface of pure PLA film was regular without thread. This was because of the brittleness of PLA polymer. When GEO was incorporated into PLA matrix, the fractured surface of PLA/GEO composite films became rough with voids and threads. This was might be due to the changes in the degree of crystallinity of the PLA matrix, as shown from DSC analysis. SEM images also revealed that GEO was efficiently dispersed within the PLA matrix without the need for compatibilizing agents, which confirmed the effectiveness of the processing route employed for the preparation of PLA/GEO composite films.[14] Although the voids on the surface of PLA/GEO-12.5 film was larger than other films, the threads on the fractured surface of PLA/GEO-12.5 film verified that PLA film incorporated with 12.5% GEO was flexible enough to maintain its integrity under the stress. The increase in the extent of porosity also contributed to increase in WVP values of food packaging material. The voids in the PLA/GEO composite films facilitated water vapour to pass through packaging films, as verified by WVP analysis.

2.6 Effect of GEO content on antimicrobial activity of PLA/GEO composite film

The results of the antimicrobial tests performed against *E. coli* and *S. aureus* were presented in Fig.2. There have been many studies on antimicrobial activity of GEO or limonene. Aloui et al[10]. reported that biodegradable coatings based on sodium alginate with GEO showed antifungal effects, with an effective control of water and firmness losses of table grapes. Limonene has been considered to be a safer alternative compared to synthetic antimicrobial food. Zahi, Liang, & Yuan[11] reported that a novel antimicrobial delivery system by encapsulating limonene into an organogel-based nanoemulsion could improve its antimicrobial activity against *E. coli, B. subtilis*, and *S. aureus*. *E. coli* and *S. aureus* were common food-born bacterial pathogen occurring in various kinds of food products. So, they were selected in this study. PLA polymer itself was not harmful to *E. coli* and *S. aureus*. Pure PLA film did not show any antimicrobial activity. At a content of 5% or 7.5% GEO, the growth of *E. coli* and *S. aureus* was suppressed. Films containing 10% or 12.5% GEO significantly ($p<0.05$) inhibited the growth of *E. coli* and *S. aureus*, when compared with PLA/GEO-5 and PLA/GEO-7.5 films. After 24 h of incubation, PLA/GEO-10 reduced *E. coli* from 5.0 logs to 4.1 logs and *S. aureus* from 5.0 logs to 4.5 logs, and PLA/GEO-12.5 reduced *E. coli* from 5.0 logs to 3.9 logs and *S. aureus* from 5.0 logs to 4.3 logs. The results indicated that PLA/GEO-10 and PLA/GEO-12.5 composite films showed a good antimicrobial activity against *E. coli* and *S. aureus*.

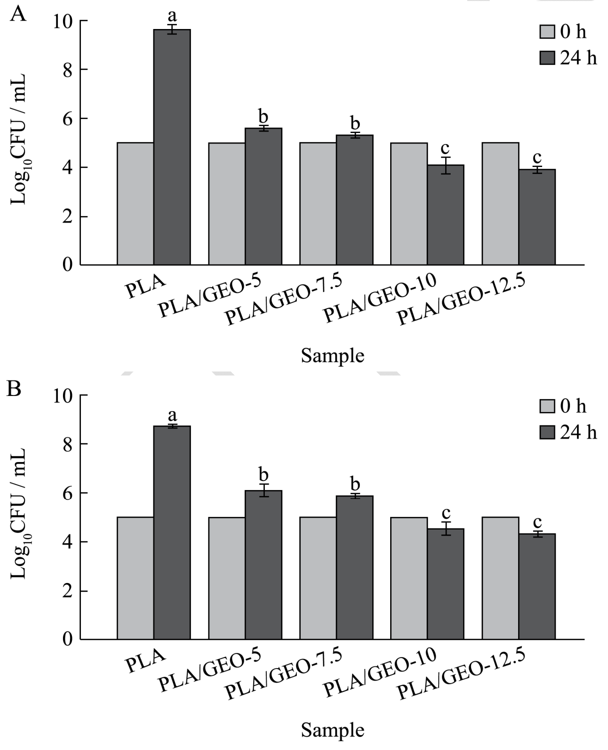

Fig.2 Antimicrobial abilities of PLA/GEO composite films: (A) *Escherichia coli* and (B) *Staphylococcus aureus*

Note: Values followed by different letters in the figure were significantly different ($p<0.05$), where “a” is the highest value.

The antimicrobial activity of GEO is mainly due to the fact that GEO can disrupt bacterial membrane and release cytoplasmic content[23]. Films containing GEO have been previously studied in the antimicrobial packaging of table grapes[10]. When GEO content was high up to 10%, many voids existed in composite films and the interactions between the components became weak. This would facilitate GEO to diffuse out of the films and achieve higher antimicrobial activity[28]. To investigate the suitability of the films for food packaging, and how the continuous contact of a food matrix may affect their efficacy, a challenge test must be performed[19]. The present study evinced a high antimicrobial effect of the PLA/GEO composite films on a food matrix, which represented a step forward in the implementation of GEO in active packaging of fruits and vegetables.

2.7 Effect of different packages on firmness of mushrooms during storage

Texture is an important quality parameter for fresh mushrooms. As shown in Fig.3, a decrease in firmness of button mushrooms was observed for all the packaging films (LDPE, PLA, PLA/GEO-5, PLA/GEO-7.5, PLA/GEO-10, and PLA/GEO-12.5 films). Firmness decreased from 2.23 N to a range of 0.82~1.44 N, after 16 d of storage. Similar results had been reported by Guillaume, Schwab, Gastaldi & Gontard[31] for button mushrooms packed by biobased packaging and Xing, Wang, Feng & Tan[32] for *Hypsizygus marmoreus* mushrooms packed by a biaxially oriented polypropylene film. On the other hand, no significant difference ($p>0.05$) in terms of firmness value was found among all the samples on day 8, while PLA/GEO-10 and PLA/GEO-12.5 samples maintained significantly ($p<0.05$) higher firmness than other samples from day 12. Storage of mushrooms under atmospheres saturated in water vapor was responsible for the acceleration of mushroom softening[33]. Packaging films with a higher WVP value would result in a lower relative humidity inside packages. No condensation was observed on the surface of button mushroom when using PLA/GEO-10 and PLA/GEO-12.5 films. This might be because that many micro-pores existed in PLA/GEO composite films and permitted more water vapor transfer[34]. Guillaume, Schwab, Gastaldi & Gontard[31] also reported a high water vapour permeability for biodegradable materials made from crops proteins that appeared well adapted for fresh fruits and vegetables preservation.

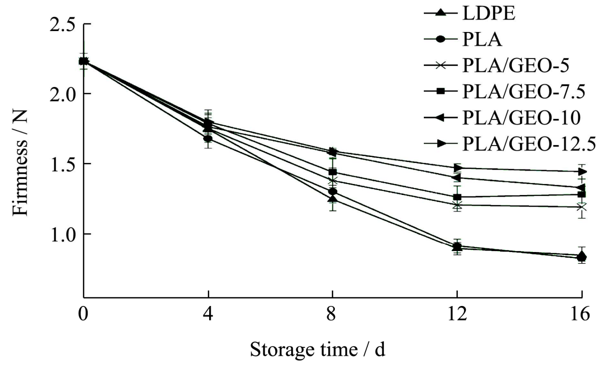

Fig.3 Effect of different packaging materials on the firmness of mushrooms stored at 4 ℃±1 ℃for 16 d

Note: Data are presented as mean ± standard deviation.

2.8 Effect of different packages on microbial counts of mushrooms during storage

The populations of mesophilic and psychrophilic bacteria in button mushrooms packed with films were determined during storage at 4±1 ℃ (Fig.4). The initial populations of mesophilic and psychrophilic bacteria in button mushrooms were 4.45 $\log_{10}$CFU/g and 1.59 $\log_{10}$CFU/g, respectively. In all of the groups, the mesophilic and psychrophilic bacteria counts gradually increased with storage time, independent of the packaging materials.

After 16 d of storage, the population of mesophilic bacteria on button mushroom packed by LDPE and PLA film was 7.75 $\log_{10}$CFU/g and 7.21 $\log_{10}$CFU/g, respectively, while button mushroom packed by PLA/GEO-12.5 film was only 5.86 $\log_{10}$CFU/g. In the case of psychrophilic bacteria, the total bacteria counts on mushroom packed by LDPE and PLA film were 3.15 $\log_{10}$CFU/g and 3.08 $\log_{10}$CFU/g, respectively, while mushroom packed by PLA/GEO-12.5 film was no more than 2.24 $\log_{10}$CFU/g. The results suggested that button mushroom packed with PLA film containing GEO might inhibit bacterial growth during storage. However, it should be noted that the degree of microbial reduction was not greater than expected. This was probably due to the limited antimicrobial activity of the film[35]. To effectively inhibit bacterial growth on button mushroom, PLA film containing high GEO content was preferred. However, the migration of GEO from the packaging film to mushroom must be studied before the commercial use of the antimicrobial packaging.

2.9 Effect of different packages on overall acceptability of mushrooms during storage

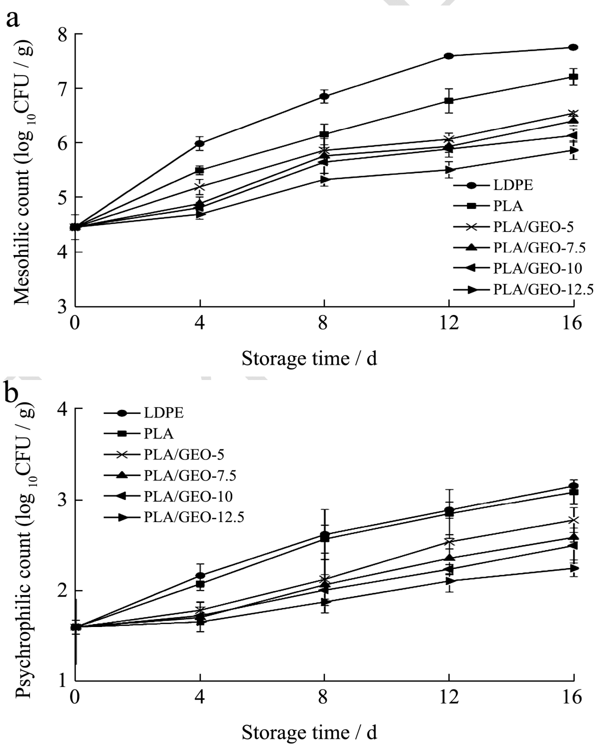

Fig.4 Effect of different packaging materials on the microbial levels of mushrooms stored at 4 ℃ ±1 ℃ for 16 d (a) mesophilic counts; (b) psychrophilic counts

Note: Data are presented as mean ± standard deviation.

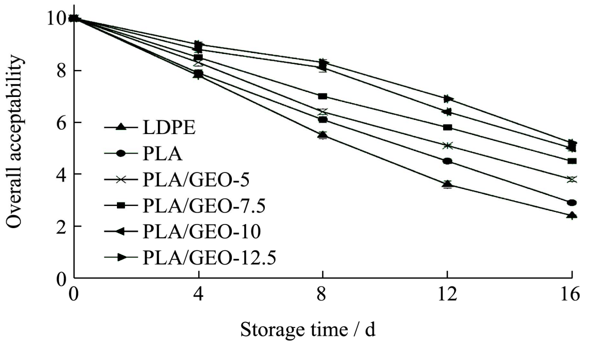

Fig.5 Effect of different packaging materials on the overall acceptability of mushrooms stored at 4 ℃±1 ℃ for 16 d

Note: Data are presented as mean ± standard deviation.

The overall acceptability values recorded were shown in Fig.5. Since overall acceptability test involved comparisons of all treatment samples, results were shown in terms of combined packaging material/storage time. The overall acceptability of mushroom decreased as the storage period proceeded in all of the groups. The antifungal efficacy of GEO has been proved against many moulds commonly associated with food spoilage[10]. No visual mould growth was detected in mushroom packed by PLA film incorporated with GEO. Overall acceptability of the LDPE film packed mushroom fell below the limit of marketability at day 8. Mushrooms packed by the PLA film became unacceptable after day 12 of storage. However, mushrooms packed by the PLA/GEO-10 and PLA/GEO-12.5 film still remained good and at the limit of marketability after 12 d of storage. This suggested that the PLA/GEO film might improve the quality of button mushrooms during storage. Similarly, Aloui et al.[10] reported that grapes wrapped with alginate coatings containing GEO had higher scores than those of control.

3 Conclusion

In this study, physical, mechanical, and antimicrobial properties of PLA films with grapefruit essential oil (GEO) were studied. The addition of GEO to films produced a PLA matrix less rigid and more flexible, the typical behavior of chain mobility that caused plasticization. WVP values and the antimicrobial activity of films were increased by the addition of GEO. The best characteristics of a packaging film would depend on the requirements for each application. Mushrooms packed in the PLA/GEO composite films were significantly (p<0.05) firmer than those packed in the control and pure PLA film. The PLA/GEO composite films were more effective in reducing microbial counts and preserving the overall acceptability of mushrooms than the control and pure PLA film. The results proved the effectiveness of GEO in the PLA polymer matrix after processing, making them able to be used as antimicrobial packaging films to maintain the quality of fresh button mushroom and extend its postharvest life.

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