Development of active packaging films based on quaternary ammonium chitosan, polyvinyl alcohol and litchi (*Litchi chinensis* Sonn.) pericarp extract

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ORIGINAL ARTICLE

Abstract

Litchi (*Litchi chinensis* Sonn.) pericarp contains abundant polyphenols that are suitable materials for developing active packaging films. In this study, 1 wt%, 3 wt% and 5 wt% of litchi pericarp extract (LPE) was added into quaternary ammonium chitosan (QAC) and polyvinyl alcohol (PVA) matrix to develop active packaging films. The structural, physical and functional properties of QAC-PVA (QP) films were compared with LPE (QP-LPE films) and without LPE (QP films). Results showed QP film had a heterogenous cross-section whereas QP-LPE films displayed rough and uneven cross-sections. After adding LPE, the N–H, O–H, C–H and C=O stretching bands of QP films shifted due to the formation of intermolecular interactions between LPE and film matrix. LPE made the colorless QP film turned brown. QP-LPE films presented lower ultraviolet–visible light transmittance than QP film. After adding LPE, film thickness increased from 0.091 to 0.103 mm, film water vapor permeability increased from 14.98×10^{-11} to 17.21×10^{-11} g m^{-1} s^{-1} Pa^{-1}, film oxygen permeability increased from 0.16 to 0.22 cm^3 mm m^{-2} day^{-1} atm^{-1}, film tensile strength increased from 14.10 to 17.41 MPa, and film elongation at break decreased from 36.94% to 25.13%. QP-LPE films quickly released polyphenols in distilled water within 4 h and displayed potent antioxidant activity. The antimicrobial ratio of the film against *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes* was elevated from 50.40–68.04% to 58.93–91.38% after adding LPE. Results suggested QP-LPE films could be utilized as antioxidant and antimicrobial packaging materials in food industry.

Keywords: antioxidant activity; antimicrobial activity; litchi pericarp; polyphenols; packaging film

Introduction

The conventionally used polyolefin-based food packaging films are created from non-degradable and unrenewable fossil fuels, resulting in serious environmental pollution. The replacement of petro-based synthetic packaging films by natural and biodegradable substance-based packaging films is of great importance (Khalil *et al.*, 2018). Nowadays, biopolymer-based packaging films are on the upsurge because they are manufactured from sustainable and renewable resources (Díaz-Montes and Castro-Muñoz, 2021). Food grade polysaccharides (e.g. alginate, starch, pectin chitosan, cellulose, plant/microbial gums etc.) and proteins (e.g. whey protein, casein, gelatin, zein etc.) are considered as good sources of biopolymers for manufacturing packaging films (Mohamed *et al.*, 2020; Yuan *et al.*, 2020). Among various biopolymers, chitosan (CS) has been widely used to prepare food packaging films because of its low cost, biodegradability and excellent film-forming property (Mujtaba *et al.*, 2019). The abundant hydroxyl and amino groups in CS make it possible to impart other chemical groups and additional functional properties to CS itself (Bakshi *et al.*, 2020). Therefore, several studies have been conducted

to prepare food packaging films based on CS derivatives (Haghighi *et al.*, 2020; Kumar *et al.*, 2020).

Quaternization, one of the structural modification methods for CS, is an effective means to improve the antimicrobial activity of CS (Luan *et al.*, 2018). In this regard, quaternary ammonium chitosan (QAC) is considered as a suitable material for manufacturing active food packaging films (Cheah *et al.*, 2019). Moreover, several researchers have managed to improve the barrier and mechanical properties of QAC films by blending QAC with other polymers, such as carboxymethyl cellulose, gelatin and polyvinyl alcohol (PVA) (Hu and Wang, 2016; Hu *et al.*, 2020; Min *et al.*, 2020; Wang *et al.*, 2018; Yao *et al.*, 2020). Among different polymers, PVA is a nontoxic and biocompatible substance with good film-forming ability (Al-Tayyar *et al.*, 2020). The films prepared by blending QAC and PVA have excellent mechanical and antimicrobial properties, which is attributed to the strong hydrogen bonding interactions between QAC and PVA (Hu and Wang, 2016; Min *et al.*, 2020; Yao *et al.*, 2020).

Litchi (*Litchi chinensis* Sonn.) is a subtropical/tropical tree of the *Sapindaceae* family and is extensively cultivated in China (Kilari and Putta, 2016). Litchi fruits are delicious and possess nutrition values and health benefits (Zhao *et al.*, 2020). Litchi pericarp, accounting for about 30% dry weight of whole fruit, is often discarded as waste during fruit processing (Punia and Kumar, 2021). It should be noted that litchi pericarp contains a large number of polyphenols (e.g. proanthocyanidins and (–)-epicatechin), which can be extracted from litchi pericarp by maceration, ultrasound-assisted extraction, ultra-high pressure-assisted extraction and enzyme-assisted extraction (Zhu *et al.*, 2019). Till now, litchi pericarp polyphenols have been demonstrated to possess strong antioxidant and antimicrobial activities, indicating that litchi pericarp polyphenols have a potential contribution in manufacturing active packaging films (Sarni-Manchado *et al.*, 2000; Wang *et al.*, 2011). Nonetheless, only one study has focused on the preparation of active packaging films based on litchi pericarp polyphenols up to now (Liu *et al.*, 2021). Liu *et al.* (2021) prepared active packaging films by using CS and 3 wt% litchi pericarp extract (LPE) and found the films had strong antioxidant and antimicrobial activities. However, the impact of LPE content on the structural, physical and functional properties of the films is still unclear. Meanwhile, LPE has not been integrated with other biopolymers to develop active packaging films. Therefore, in this study, we developed active packaging films based on QAC with antimicrobial activity, PVA with reinforcement ability, and polyphenols-rich LPE with antioxidant and antimicrobial activities. The effect of LPE content (1 wt%, 3 wt% and 5 wt%) on the structural, physical and functional properties of QAC–PVA films was evaluated.

Materials and Methods

Materials and reagents

Fresh litchi (*Litchi chinensis* Sonn.) fruits were commercially available from the local market (Yangzhou, China). QAC (*N*-(2-hydroxy) propyl-3-trimethyl ammonium chloride chitosan, substitution degree of 98% and average molecular weight of 250 kDa), PVA (polymerization degree of 1750 ± 50, alcoholysis degree of 98%), Folin-Ciocalteu reagent, gallic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Macklin Biochemical Co. Ltd. (Shanghai, China). AB-8 macroporous adsorption resin was purchased from Donghong Chemical Co. Ltd. (Zibo, China). Ethanol, glycerol, sodium carbonate, silica gel and methanol were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Lysogeny broth medium was purchased from Land Bridge Technology Co. Ltd. (Beijing, China).

Extraction of polyphenols from litchi pericarp

Polyphenols were extracted from litchi pericarp following the method given by Liu *et al.* (2021) with some modifications. Litchi pericarp was separated from fresh fruits and washed with distilled water. Then, litchi pericarp was frozen immediately in liquid nitrogen and lyophilized. The lyophilized pericarp was crushed by a grinder. The pericarp power (65 g) was immersed in 1,000 mL of 80% ethanol solution at 4°C for 24 h. After that, the mixture was filtered and centrifuged at 10,000 g for 30 min to remove debris, and concentrated at 40°C by a rotary evaporation (RE-52AA, Shanghai Yarong Biochemical Instrument Co., Shanghai, China). The obtained concentrate was purified on AB-8 macroporous resin column (1.6 cm × 60 cm), which was first eluted by distilled water and then by 80% ethanol solution. Only the eluate of 80% ethanol solution was collected and vacuum-dried at 50°C for 48 h to obtain polyphenol-rich LPE power. The yield of LPE was 1.59% based on the fresh weight of litchi pericarp. The total phenol content (TPC) of LPE was determined as 383.85 mg gallic acid equivalent/g dried extract by the Folin–Ciocalteu assay (Liu *et al.*, 2017).

Preparation of films

Films were prepared according to the method given by Yao *et al.* (2020) with some modifications. QAC (3.4 g) was dissolved in 80-mL deionized water and stirred magnetically at 20°C overnight. Meanwhile, PVA (1.7 g) was dissolved in 50-mL deionized water at 100°C for 2 h with constant stirring. After cooling to 80°C, PVA solution was thoroughly blended with QAC solution for 4 h.

Then, LPE (1 wt%, 3 wt% and 5 wt%) and 10 wt% of glycerol on QAC and PVA total weight basis were added into QAC–PVA-blended solution. The obtained homogenous solutions (160 mL each) were degassed in a KQ-200KDE ultrasonic apparatus (Kunshan Ultrasonic Instruments Co. Ltd., Suzhou, China) at an ultrasonic power of 200 W for 30 min, cast onto Plexiglas moulds (24 cm × 24 cm) and dried at 30°C for 48 h. QAC–PVA films containing 0 wt%, 1 wt%, 3 wt% and 5 wt% of LPE were termed as QP, QP-LPE1, QP-LPE3 and QP-LPE5 films, respectively. At the same time, QAC–PVA film without LPE was prepared and named as QP film. All the films were placed in a desiccator with 50% relative humidity (RH) at 20°C for at least 48 h before testing.

Structural characterization of films

The cross-sectional microstructure of the film was characterized by GeminiSEM 300 (Carl Zeiss, Oberkochen, Germany) with accelerating voltage of 5 kV and magnification of 1500×. Before observation, film was fractured in liquid nitrogen and sputter-coated with gold by an ion sputter coater (MC1000, Hitachi High-Technologies Co., Tokyo, Japan). Attenuated total reflectance (ATR) Fourier transform infrared (FT-IR) analysis of the film was carried out by Varian 670 spectrophotometer (Agilent Technologies, CA, USA) in the range of 400–4000 cm^{-1} using 16 scans at the resolution of 2 cm^{-1}. X-ray diffractogram (XRD) analysis of the film was carried out on AXS D8 Advance diffractometer (Bruker Inc., Karlsruhe, Germany) in an angular range of 5–75° (2θ) with Ni-filtered CuKα radiation.

Determination of color and light transmittance of films

Color parameters of the film including L^*, a^*, b^* and ΔE were measured by SC–80C colorimeter (Kangguang Instrument Co. Ltd., Beijing, China). The light transmittance of the film was measured by scanning film sample (1 cm × 4 cm) at 200–800 nm with Lambda 35 ultraviolet–visible (UV-Vis) spectrophotometer (PerkinElmer Inc., MA, USA).

Determination of thickness of films

Film thickness was determined by a micrometer (Mitutoyo No. 293–766, Tester Sangyo Co., Ltd., Tokyo, Japan) with precision of 0.001 mm.

Determination of water vapor permeability of films

Water vapor permeability (WVP) of the film was determined by the method given by Yong *et al.* (2019). Briefly, film sample was cut into squares (6 cm × 6 cm) and sealed over a centrifuge tube with 40-g fully dried silica gel. The tube was stored in a desiccator at 20°C and 100% RH and was weighted every 24 h for 6 days. WVP of the film was calculated as follows:

$$WVP = \frac{W \times x}{t \times A \times \Delta P}, \quad (1)$$

where W is the weight gain of sealed centrifuge tube (g), x is the film thickness (m), t is the time (s), A is the permeation area of film sample (m^2) and ΔP is the saturated vapor pressure at 20°C.

Determination of oxygen permeability of films

Oxygen permeability (OP) of films was determined by a gas permeability tester (Basic 201, Labthink Instruments Co. Ltd., Jinan, China) at 23°C and 50% RH (Bi *et al.*, 2019). The film disc with diameter of 10 cm divided the gas permeation chamber into two compartments. Air in upper and lower compartments was evacuated continuously for at least 12 h prior to test. After that, oxygen was injected into the upper compartment and pressure variations in the downstream compartment were recorded as a function of time. OP of the film was calculated as follows:

$$OP = \frac{OTR \times x}{\Delta P}, \quad (2)$$

where OTR is the oxygen transmission rate ($cm^3\ m^{-2}\ day^{-1}$), x is the film thickness (mm) and ΔP is the pressure difference (atm) between two compartments.

Determination of mechanical properties of films

The mechanical properties of the film, including tensile strength (TS) and elongation-at-break (EB), were determined according to the method given by Yong *et al.* (2019). Briefly, rectangular film sample (6 cm × 1 cm) was placed in TMS-Pro texture analyzer (Food Technology Corp., VA, USA) with an initial distance of 4 cm and a testing speed of 1 mm/s.

Determination of antioxidant activity of films

Film sample (8 cm × 8 cm) was gently stirred in 150-mL distilled water at 50 rpm and 20°C for 24 h. At different time intervals (0.5, 1, 2, 4, 8, 12 and 24 h), 2-mL film sample solution was withdrawn and the TPC and DPPH radical scavenging activity of the solution were determined (Liu *et al.*, 2017). TPC released from the film was determined by reacting 1-mL film sample solution with 1-mL

Folin–Ciocalteau reagent at 20°C for 5 min in the dark. Afterwards, 5-mL saturated sodium carbonate solution was added and reacted at 20°C for 2 h. The absorbance of the reaction solution was recorded at 760 nm and TPC was calculated based on the calibration curve of gallic acid. The antioxidant activity of the film was determined by reacting 1-mL film sample solution with 3-mL 75-μM DPPH methanol solution at 20°C for 30 min in the dark. The absorbance of the reaction solution was recorded at 517 nm. DPPH radical scavenging activity of the film was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} \times 100, \quad (3)$$

where A_0 is the absorbance of the control (water instead of film sample solution), A_1 is the absorbance of film sample reaction solution, and A_2 is the absorbance of film sample solution only (water instead of DPPH).

Determination of antimicrobial activity of films

The antimicrobial activity of the film against four foodborne pathogens (*Escherichia coli* ATCC 43895, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 6538 and *Listeria monocytogenes* ATCC 19115) was evaluated based on the method given by Wu *et al.* (2021). Briefly, UV-sterilized film sample (1 cm × 1 cm) was placed in bacterial liquid suspension (10^7 CFU/mL) at 37°C for 24 h. After that, film sample was taken out and the rest bacterial suspension was diluted and spread uniformly on lysogeny broth agar plate. After incubation at 37°C for 24 h, bacteria colonies formed on the plate were counted, and the colony forming unit (CFU) was calculated. The bacterial suspension without film sample was used as the control group. Antimicrobial rate of the film was calculated as follows:

$$\text{Antimicrobial rate (\%)} = \frac{CFU_s}{CFU_c} \times 100 \quad (4)$$

where CFU_s and CFU_c are the CFUs with and without film sample, respectively.

Statistical analysis

Results were analyzed by one-way analysis of variance and Duncan's test using SPSS13.0 software (SPSS Inc., IL, USA) at a significant level of $P < 0.05$.

Results and discussion

Micro-structural analysis

The cross-sections of QP films with and without LPE are presented in Figure 1. The cross-section of QP film was relatively compact and uniform, indicating QAC and PVA were homogeneously blended in the film. A similar phenomenon of QP film was reported by Min *et al.* (2020). However, Yao *et al.* (2020) found that QP film presented a heterogenous cross-section, which was because QAC and PVA were somewhat incompatible. In

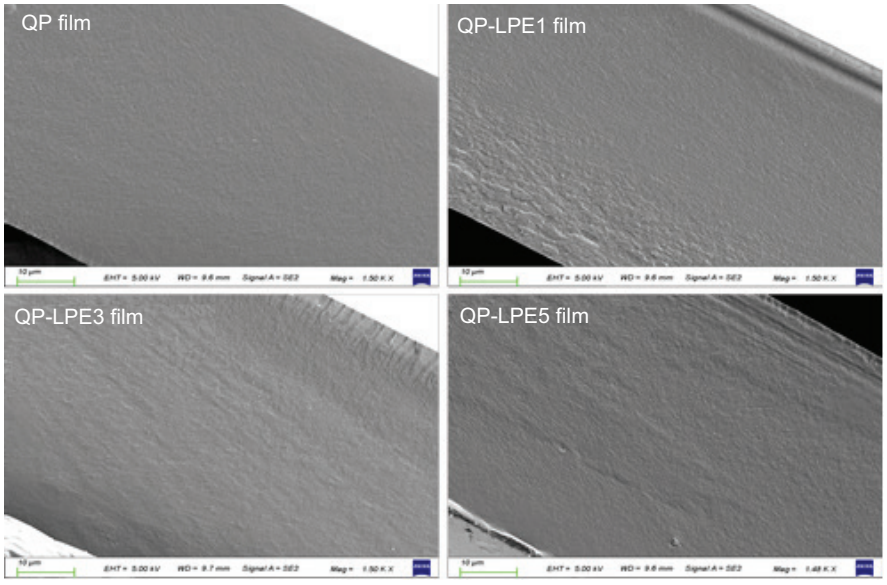

Figure 1. Scanning electron microscope (SEM) micrographs on the cross-section of QP, QP-LPE1, QP-LPE3 and QP-LPE5 films.

this study, QAC and PVA were blended at higher temperature for longer time as compared with the study conducted by Yao *et al.* (2020). Thus, QP film prepared in this study showed a homogeneous cross-section. After LPE was added into the films, the films displayed rough and uneven cross-sections, indicating that addition of LPE decreased the compatibility of QAC and PVA. This was because the main polyphenols (e.g. proanthocyanidins and (–)-epicatechin) in LPE were more hydrophobic than QAC and PVA. As a result, LPE had a limited compatibility with QAC and PVA. Similar phenomena were observed by Bi *et al.* (2019), who found that proanthocyanidins increased the unevenness of CS films. Recently, Yao *et al.* (2020) found that the cross-section of QP film became uniform when 1 wt% of cactus pear extract was added but turned rough when 2 wt% and 3 wt% of the extract were incorporated into the film, which was because cactus pear extract was rich in hydrophilic betalains that had good compatibility with QAC and PVA.

FT-IR spectra

Fourier transform infrared analysis was carried out to determine the typical functional groups and intermolecular interactions in the films (Figure 2). QP film exhibited the characteristic bands of QAC at 3294 cm^{-1} (N–H/O–H stretching), 2927 cm^{-1} (C–H stretching), 1653 cm^{-1} (C=O stretching of amide I), 1563 cm^{-1} (C=O stretching of amide II) and 1475 cm^{-1} (C–H bending of trimethylamine group). Meanwhile, QP film showed the characteristic bands of PVA at 3294 cm^{-1} (O–H stretching), 2927 cm^{-1} (C–H stretching) and 1416 cm^{-1} (C–H bending of CH_2 group). The similar FT-IR spectra of QP film were also observed by Yao *et al.* (2020) and Min *et al.* (2020). These researchers also demonstrated that QAC and PVA formed hydrogen bonds with each other (Min *et al.*, 2020; Yao *et al.*, 2020). QP-LPE films had similar FT-IR spectra with QP film. However, some bands of QP film shifted after LPE was added. The N–H/O–H stretching band shifted to 3296–3297 cm^{-1}, while the C–H stretching band shifted to 2925–2933 cm^{-1}. In addition, the C=O stretching band of amide I shifted to 1649–1651 cm^{-1}. The band shifts were caused by the intermolecular interactions between polyphenols in LPE and film matrix. Since LPE was mainly composed of proanthocyanidins and (–)-epicatechin, it could interact with film matrix through hydrogen bonds. Recently, Liu *et al.* (2021) observed that the N–H/O–H stretching and C=O stretching bands shifted after LPE was added

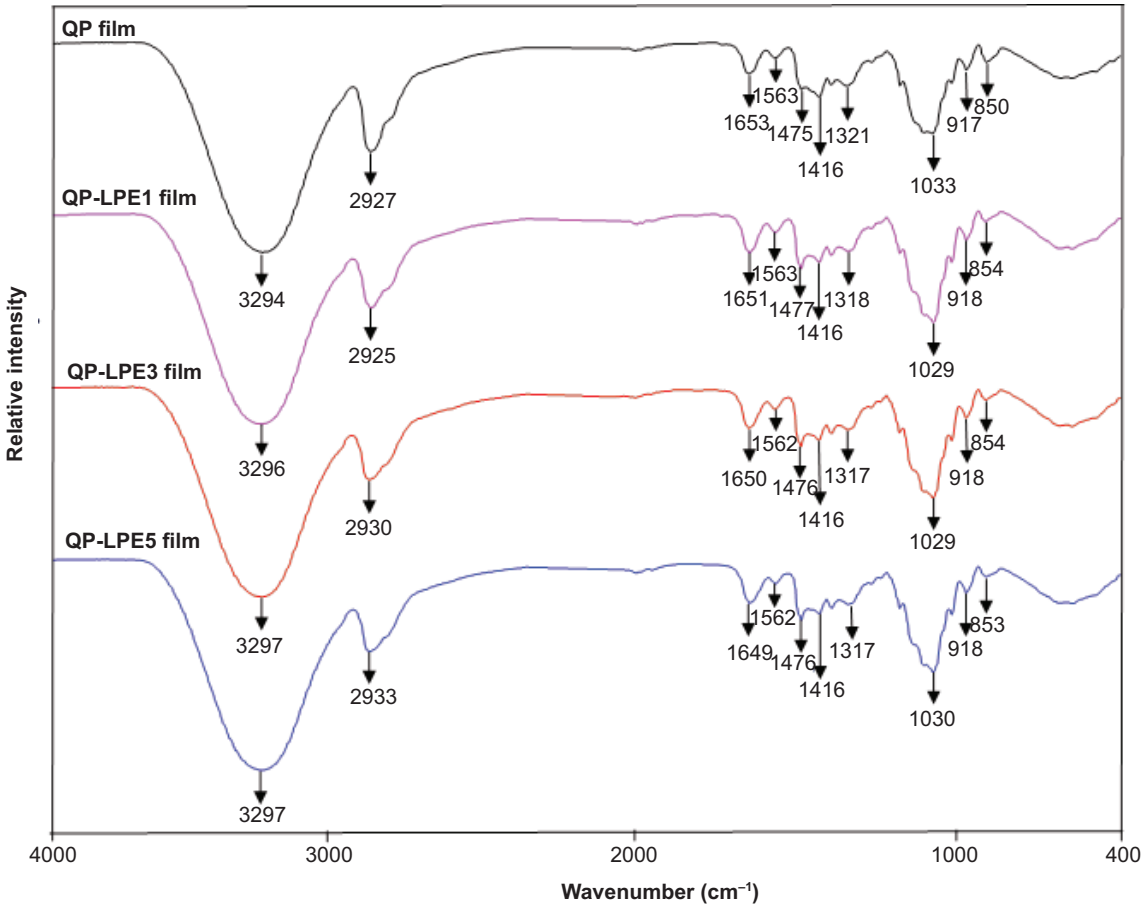

Figure 2. Normalized FT-IR spectra of QP, QP-LPE1, QP-LPE3 and QP-LPE5 films.

into CS film. Similarly, Yao *et al.* (2020) also found that the O–H/N–H stretching band shifted after cactus pear extract was added into QP film, which was because the extract formed hydrogen bonds with film matrix.

X-ray diffractogram patterns

X-ray diffractogram (XRD) was used to analyze the crystalline character of the films (Figure 3). QP film displayed a major diffraction peak at 20.1°. According to Hu and Wang (2016) and Yao *et al.* (2020), QAC displayed a single diffraction peak at 20.8° whereas PVA had two diffraction peaks at 19.9° and 41.0°. Thus, the diffraction peak of QP film was attributed to the semi-crystalline character of QAC and PVA that formed hydrogen bonds during film-forming process. QP-LPE films showed similar XRD patterns with QP film, presenting a main diffraction peak at 19.9°. The relative crystalline degree of QP, QP-LPE1, QP-LPE3 and QP-LPE5 films was 16.9%, 18.4%, 17.6% and 18.9%, respectively. This indicated that LPE increased the crystallinity of QP film, which was because polyphenols in LPE had limited compatibility with film matrix. As a result, LPE formed agglomerations in film matrix (Figure 1) and increased the crystallinity of QP film.

Color

The appearance of films is an important factor affecting their applications in food packaging. As shown in Figure 4A and Table 1, there were significant color differences between QP and QP-LPE films. QP film displayed transparent and colorless appearance with the highest L^* value but the lowest total color difference (ΔE) value, which was consistent with previous reports (Min *et al.*, 2020; Yao *et al.*, 2020). QP-LPE films presented brown color, which was consistent with the color of polyphenols (e.g. proanthocyanidins and (–)-epicatechin) in LPE. As compared with QP film, QP-LPE films had lower L^* value but higher a*, b* and ΔE values. In addition, the a, b and ΔE values of QP-LPE films increased gradually if LPE content increased from 1 wt% to 5 wt%. These results confirmed that the brown color of QP-LPE films was attributed to the presence of LPE in the films. Liu *et al.* (2021) also observed that the CS film containing LPE had a similar brown color. However, Yao *et al.* (2020) found that QP film turned purple after cactus pear extract was added, which was because the extract was rich in betacyanins with purple color.

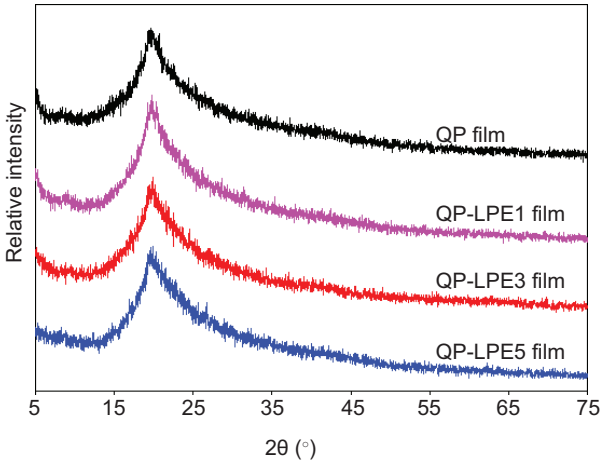

Figure 3. XRD patterns of QP, QP-LPE1, QP-LPE3 and QP-LPE5 films.

Light transmittance

The light transmittance of the films was measured in the UV-Vis light range between 200 nm and 800 nm (Figure 4B). QP film displayed the highest light transmittance, indicating the film had little UV-Vis light barrier ability. By contrast, QP-LPE films showed lower light transmission than QP film. In addition, the light transmission of QP-LPE films gradually decreased when LPE content increased. This revealed that the UV-Vis light barrier ability of QP film was significantly elevated by LPE, which was because LPE contained abundant polyphenols that could adsorb light radiation (Riaz *et al.*, 2018). Meanwhile, LPE particles in the film matrix could scatter and block light transmission. Bi *et al.* (2019) also observed that the light transmittance of CS film was remarkably decreased by proanthocyanidins. Similarly, Liu *et al.* (2021) found the opacity of QP film decreased after LPE was added into the film. Yao *et al.* (2020) observed that the light transmission of QP film decreased after adding cactus pear extract, which was because betacyanins contained in the extract could adsorb light radiation. However, QP-LPE films prepared in this study showed stronger UV light barrier ability than QP films containing cactus pear extract, suggesting polyphenols had stronger UV radiation-adsorbing ability than betacyanins.

Water vapor permeability

Film thickness is an important variable affecting the WVP, OP and TS of packaging films. As shown in Table 2, the thickness of QP film increased after adding LPE. Moreover, the thickness of QP-LPE films increased with increase of LPE content. Similarly, Liu *et al.* (2021) found that CS-LPE film showed higher thickness than CS film. Yao *et al.* (2020) documented that the thickness of QP film gradually increased if the content of cactus pear extract increased from 1 wt% to 3 wt%. WVP was measured to evaluate the ability of the film to prevent water vapor transfer. As shown in Table 2, the incorporation of

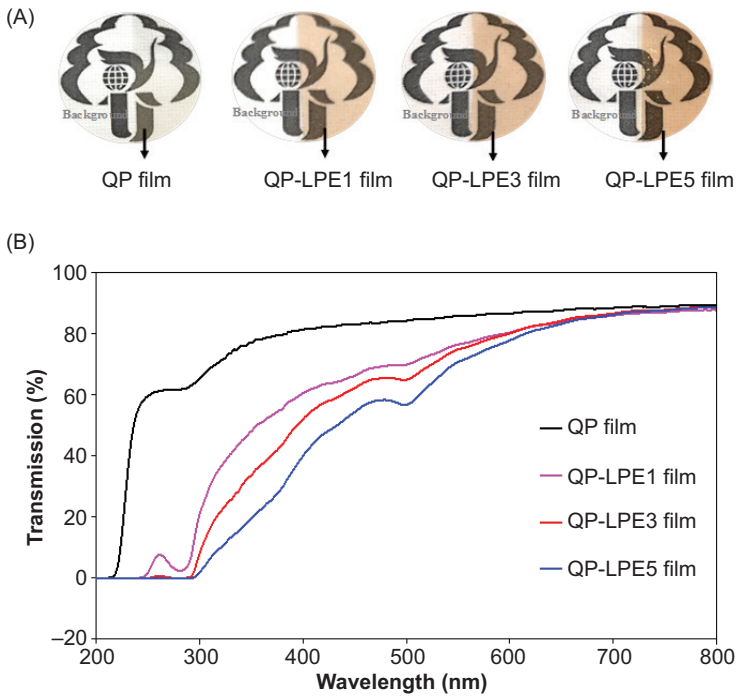

Figure 4. (A) Color and (B) UV-Vis light transmittance of QP, QP-LPE1, QP-LPE3 and QP-LPE5 films.

Table 1. Color values including *L, *a**, *b** and Δ*E* of QP films with and without LPE.**

Films	*L**	*a**	*b**	Δ*E*
QP film	91.29 ± 0.03^{a}	–0.16 ± 0.04^{d}	−0.37 ± 0.13^{d}	1.62 ± 0.01^{d}
QP-LPE1 film	83.51 ± 0.22^{b}	3.65 ± 0.06^{c}	7.39 ± 0.14^{c}	12.48 ± 0.26^{c}
QP-LPE3 film	82.03 ± 0.21^{c}	4.74 ± 0.11^{b}	10.59 ± 0.25^{b}	15.91 ± 0.34^{b}
QP-LPE5 film	79.02 ± 0.58^{d}	7.16 ± 0.43^{a}	15.49 ± 0.33^{a}	22.05 ± 0.74^{a}

Values are given as mean ± SD (n = 3). Different lower case superscript letters in the same column indicate significant difference ($P < 0.05$).

Table 2. Thickness, WVP, OP, TS and EB of QP films with and without LPE.

Films	Thickness (mm)	WVP (×10^{-11} g m^{-1} s^{-1} Pa^{-1})	OP (cm^{3} mm m^{-2} day^{-1} atm^{-1})	TS (MPa)	EB (%)
QP film	0.091 ± 0.004^{c}	14.98 ± 0.86^{b}	0.16 ± 0.03^{c}	14.10 ± 1.01^{c}	36.94 ± 0.96^{a}
QP-LPE1 film	0.093 ± 0.004^{c}	15.51 ± 0.77^{b}	0.17 ± 0.02^{c}	14.54 ± 0.84^{c}	31.21 ± 1.28^{b}
QP-LPE3 film	0.099 ± 0.003^{b}	15.90 ± 0.99^{b}	0.19 ± 0.02^{b}	15.64 ± 1.07^{b}	28.89 ± 1.58^{c}
QP-LPE5 film	0.103 ± 0.005^{a}	17.21 ± 0.33^{a}	0.22 ± 0.01^{a}	17.41 ± 1.33^{a}	25.13 ± 1.15^{d}

Values are given as mean ± SD (n = 10 for film thickness, 3 for WVP and OP, and 6 for TS and EB). Different lower case superscript letters in the same column indicate significant difference ($P < 0.05$). WVP: water vapor permeability; TS: tensile strength; EB: elongation at break.

1 wt% and 3 wt% of LPE did not significantly change the WVP of QP film. However, the incorporation of 5 wt% of LPE remarkably increased the WVP of QP film. This was because high contents of LPE formed aggregates in the film matrix and destroyed the dense and compact network of film, producing more free volumes in the film to facilitate moisture transfer. This result was supported by the micro-structures of QP-LPE films observed in Figure 1. Similarly, Bi *et al.* (2019) observed that the WVP of QP film was increased by proanthocyanidins. Recently, Yao *et al.* (2020) have found that the WVP of QP film increased after 3 wt% of cactus pear extract was added into the film. However, Liu *et al.* (2021) observed that CS-LPE film had lower WVP than CS film, which was because LPE addition made the film matrix uniform and compact.

Oxygen permeability

Oxygen permeability reflects the oxygen barrier ability of packaging films. The OP values of QP and QP-LPE films are shown in Table 2. The OP value of QP film was 0.16 cm^3 mm m^{-2} day^{-1} atm^{-1}, which agreed with the results of Hu and Wang (2016). Researchers found that the OP value of QP film decreased when the proportion of PVA increased in the film (Hu and Wang, 2016), which was because the compactness of the film decreased with increase of QAC proportion. QP-LPE films exhibited OP values of 017–0.22 cm^3 mm m^{-2} day^{-1} atm^{-1}, which were higher than the OP value of QP film. Meanwhile, the OP value of QP-LPE films increased with the increase of LPE content in the films, suggesting that the oxygen barrier ability of QP film was somewhat decreased by LPE. This was mainly because LPE had limited compatibility with film matrix and decreased the uniformity of films, making penetration of oxygen easier in films.

Mechanical properties

Mechanical properties, including TS and EB, reflect the ability of packaging films to protect the physical integrity of food. The TS and EB of different films are listed in Table 2. The TS of QP film was elevated from 14.10 to 17.41 MPa after adding LPE. Moreover, the TS of QP-LPE films significantly increased when LPE content increased from 1 wt% to 5 wt%. Improvement in the TS of QP-LPE films was attributed to the interactions between polyphenols in LPE and film matrix, which resulted in strong interfacial adhesion and made the films more resistant to mechanical stress. Several researchers also reported polyphenol-rich extracts performed as reinforcing fillers to increase the mechanical strength of films (Bi *et al.*, 2019; Siripatrawan and Vitchayakitti, 2016). Yao *et al.* (2021) also found the incorporation of 1 wt% and 3 wt% of cactus pear extract increased the TS of QP film, which was because betacyanins in the extract formed strong hydrogen bonds with film matrix. However, the EB of QP film was reduced from 36.94% to 25.13% after adding LPE. Meanwhile, the EB of QP-LPE films remarkably decreased when LPE content increased from 1 wt% to 5 wt%. The decreased EB of the films was due to low compatibility of hydrophobic polyphenols with film matrix and reduced chain-to-chain interactions between QAC and PVA, leading to a reduction in film flexibility. Recently, Liu *et al.* (2021) have documented that the TS of CS film decreased whereas the EB of CS film increased after adding LPE, which was due to good compatibility of LPE with CS film matrix.

Total phenol content and antioxidant activity

Antioxidant activity is a key property of active packaging films (Zhang *et al.*, 2020). The antioxidant activity of

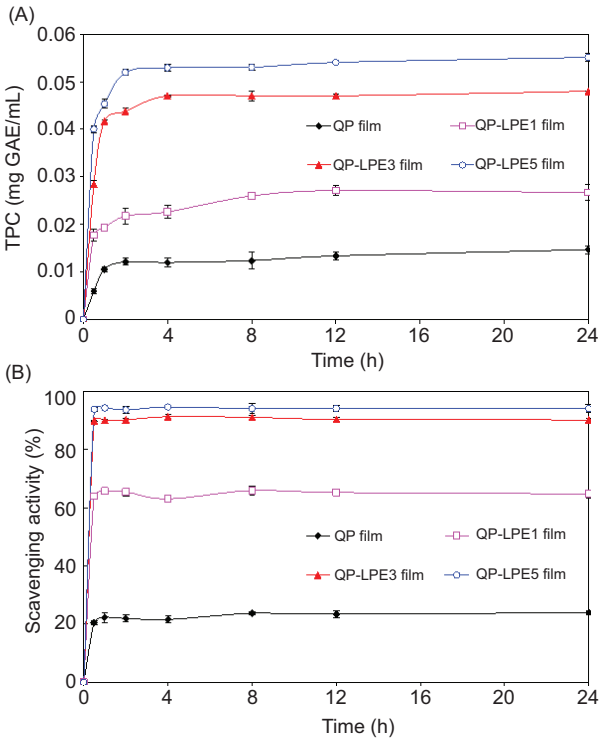

Figure 5. (A) TPC and (B) DPPH radical scavenging activity of QP, QP-LPE1, QP-LPE3 and QP-LPE5 films within 24 h. Each value represents mean ± SD of triplicate.

films is closely related to TPC released from the films. In this study, the TPC and antioxidant activity of the films were tested by using distilled water as a medium. As shown in Figure 5, TPC released from the films quickly increased within 4 h and tended to be constant afterwards. Moreover, QP-LPE films showed higher TPC than QP film. TPC released from QP-LPE films decreased in the order of QP-LPE5 film, QP-LPE3 film and QP-LPE1 film. Similarly, Liu *et al.* (2021) also found that CS-LPE film had higher TPC than CS film. The antioxidant activity of the films was evaluated by DPPH radical scavenging assay. The DPPH radical scavenging activity of the films increased sharply within 1 h and remained almost unchanged afterwards. Addition of LPE remarkably improved the DPPH radical scavenging activity of the films. Meanwhile, the DPPH radical scavenging activity of QP-LPE films increased with the increase of LPE content. Notably, the trend of TPC was positively correlated to that of DPPH radical scavenging activity. The correlation coefficient between TPC and DPPH radical scavenging activity was 0.84. This revealed that the strong DPPH radical scavenging ability of QP-LPE films was attributed to the polyphenols released from the films. Liu *et al.* (2021) also found that the incorporation of LPE significantly increased the DPPH radical scavenging activity of CS film. Our results indicated that QP-LPE films could be used as antioxidant packaging to extend the shelf life of food.

Table 3. Antimicrobial activity of QP films with and without LPE.

Films	Antimicrobial rate (%)			
	S. aureus	*L. monocytogenes*	*E. coli*	*S. typhimurium*
QP film	68.04 ± 0.56^{d}	60.91 ± 1.29^{d}	53.40 ± 6.48^{c}	50.40 ± 0.22^{d}
QP-LPE1 film	75.46 ± 1.69^{c}	67.27 ± 2.57^{c}	58.93 ± 2.68^{c}	59.75 ± 0.45^{c}
QP-LPE3 film	84.88 ± 3.00^{b}	74.70 ± 1.93^{b}	66.03 ± 1.12^{b}	70.84 ± 3.14^{b}
QP-LPE5 film	91.38 ± 1.69^{a}	86.82 ± 3.21^{a}	74.09 ± 4.47^{a}	78.45 ± 3.14^{a}

Values are expressed as mean ± SD (n= 3). Different lower case superscript letters in the same column indicate significant difference ($P < 0.05$).

Antimicrobial activity

Foodborne pathogens can seriously affect the quality of food. Therefore, antimicrobial activity is very important for active packaging films (Madanayake *et al.*, 2021; Yong and Liu, 2021). In this study, the antimicrobial activity of the films against four foodborne pathogens (*S. aureus*, *L. monocytogenes*, *E. coli* and *S. typhimurium*) was tested. As shown in Table 3, QP film showed good antimicrobial activity against four foodborne pathogens, presenting antimicrobial ratio between 50.40% and 68.04%. The antimicrobial activity of QP film was mainly attributed to the bactericidal effect of QAC, which was confirmed by Min *et al.* (2020) and Yao *et al.* (2020). QAC possessed positively charged trimethylammonium groups that could interact with microbial cell membrane carrying negative charges (Hu and Wang, 2016). As compared with QP film, QP-LPE films showed significantly higher antimicrobial ratios. The antimicrobial ratio of QP-LPE films against four foodborne pathogens varied between 58.93% and 91.38%. In addition, the antimicrobial ratio of QP-LPE films increased with the increase of LPE content in the films. The increased antimicrobial activity of QP-LPE films was mainly because polyphenols in LPE could increase the permeability of cell membranes and inhibit the synthesis of DNA/RNA. Yao *et al.* (2020) also found that the antimicrobial activity of QP film was elevated by cactus pear extract, which was because the extract was rich in betacyanins that had bactericidal effect. Our results suggested that the antimicrobial activity of QP-LPE films was attributed to the action of QAC and polyphenols in LPE. The developed QP-LPE films could be used as antimicrobial packaging in food industry.

Conclusions

Active packaging films were successfully developed based on QAC, PVA and polyphenols-rich LPE. LPE decreased the uniformity of QP film, thereby increasing the WVP and OP of the film. Meanwhile, LPE formed strong hydrogen bonds with film matrix and thus increased the TS of QP film. Since LPE contained a high content of polyphenols, it significantly increased the UV-Vis light barrier ability and antioxidant and antimicrobial activities of QP film. Among different QP-LPE films, QP-LPE5 film presented the highest UV-Vis light barrier ability, TS and antioxidant and antimicrobial activities. In the future, use of QP-LPE5 film must be encouraged in the active packaging of foods.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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