Ultrasound-assisted development and characterization of novel polyphenol-loaded pullulan/trehalose composite films for fruit preservation

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ARTICLE INFO

Keywords:
Tea polyphenols
Ultrasound
Antibacterial activity
Pullulan/trehalose composite film
Preservation

ABSTRACT

A novel food packaging film was developed by incorporating a tea polyphenols-loaded pullulan/trehalose (TP@Pul/Tre) into a composite film with ultrasound-assisted treatment of dual-frequency (20/35 kHz, 40 W/L) for 15 min to assess the physicochemical and mechanical properties of a composite film. The optimized ultrasound-assisted significantly increases elongation at break, tensile strength, and improves the composite film’s UV/water/oxygen barrier properties. Structure analysis using attenuated total reflectance-Fourier transform infrared, X-ray diffraction and thermal stability revealed that these improvements were achieved through ultrasound-enhanced H-bonds, more ordered molecular arrangements, and good intermolecular compatibility. Besides, the ultrasound-assisted TP@Pul/Tre film has proven to have good antibacterial performance against Escherichia coli and Staphylococcus aureus, with approximately 100% lethality at 4 h and 8 h, respectively. Moreover, the ultrasound-assisted TP@Pul/Tre film effectively delayed moisture loss, oxidative browning, decay, and deterioration in fresh-cut apples and pears, thereby extending their shelf life. Thus, ultrasound has proved to be an effective tool for improving the quality of food packaging films, with a wide range of applications.

1. Introduction

Tea polyphenols (TP), a polyhydroxy phenolic natural compounds extracted from tea, containing numerous phenolic compounds with broad physiological activities, including antioxidant, anti-cancer, anti-tumor, antibacterial, and anti-inflammatory [1]. Recently, TP has gained much attention in food industry for the production of degradable packaging films. The incorporation of TP in food packaging films could effectively reduce the production of oxygen free radicals and inhibit the growth of nearly 100 different types of bacteria in nature, including Escherichia coli (E. coli), Staphylococcus aureus (S. aureus) [2,3]. Besides, TP could act as a cross-linking agent, interacting with other components to improve the film’s performance [1].

Packaging plays a crucial role in food preservation, thus extending its shelf life, and reducing food waste. The bio-active films are eco-friendly and have gained much attention in recent years as an alternative to petroleum-based polymer films, which cause “white pollution” and potential toxicity to food [4]. Polysaccharides, proteins, and lipids are commonly used biomacromolecules for the development of biodegradable, low-cost, and renewable food packaging films [5]. Pullulan (Pul) is a microbial polysaccharide produced by Aureobasidium pullulans that has a unique linkage pattern with two α-(1, 4) and α-(1, 6) glycosidic bonds. The unique structure and good film-forming ability makes it an ideal raw material for biodegradable film development [4]. So far, limited studies are available on the use of TP-loaded Pul for preparation of active packaging films. For instance, a solution casting method was previously used for the development of bio-active films based on Pul and carboxylated cellulose nanocrystals incorporated with TP [6]. Moreover, TP-loaded Pul-carboxymethylcellulose sodium electrospray nanofibers were prepared, which enhanced the shelf-life of strawberry [7]. More comprehensive and in-depth studies are required to prepare active packaging films with Pul as a substrate-loaded TP. TP is highly sensitive to light, heat and processing environment, its structure and function can be effected if exposed to these factors [1]. The sensitivity of the TP in the...
film should be considered, while using Pul as the substrate to load TP for the preparation of packaging film. Trehalose (Tre), C12H22O11, a non-reducing disaccharide produced by microorganisms and regarded as safe in food consumption. It has the ability to form close molecular aggregates around active substances and is frequently used as an excipient to protect and stabilize active substances [8]. It was hypothesized that the addition of Tre will reduce TP sensitivity, while preparing TP-loaded Pul films. It has been reported that Tre have a poor film-forming ability and crystallize when air-dried, resulting in loss of stability [9]. The existence of Pul in the composite film may contribute to maintain the stability of Tre. Taken together, this study takes the advantage of stabilizing effect of one compound for another to prepare an active packaging film (TP-loaded Pul/Tre composite film) with excellent performance. Interestingly, most polysaccharide-based biodegradable films have distinct molecular structures that are primarily maintained by inter-molecular and intra-molecular hydrophobic interactions, hydrogen bonds, disulfide bonds, electrostatic interactions, which also influence the mechanical and barrier properties of the films [10]. Different modification methods have been applied to improve the inter- and intra-molecular interactions, such as Maillard reaction, chemical cross-linking, ion gel, and physical processing such as pressure and electrospinning [11]. Ultrasound, a green processing physical technology, has been widely used in extracting active substances, homogenizing and modifying macromolecular substances [12-14]. Yan et al. reported that ultrasound treatment with different intensities reduced the intrinsic viscosity, molecular weight, and enhanced the antioxidant activities of polysaccharides PL-N extracted from Phellinus linteus mycelium [15]. The ultrasound treatment of citrus pectin in an aqueous solution reduced the amount of CP’s methoxylation and neutral sugars in side chains, cleaved the inter- and intra-molecular hydrogen bonds, and enabled the CPs’ semi-flexible chains to flexible chains or even flexible coils [16]. The sonicated citrus pectin exhibited weaker rheological and gelling properties but had better emulsifying properties than the native citrus pectin in the oil-in-water emulsion system. It might be due to ultrasound changing the molecular weight, interaction force, and chain conformation of CPs [17].

The sonication is widely used for alteration of polysaccharides functional properties by modifying their structure and stimulate the inter- and intra-molecular interaction forces. Herein, the ultrasound might alter the macromolecular structure of Pul and influence the interaction force between Pul, Tre and TP, enhancing the compactness and mechanical properties of the TP-loaded Pul/Tre composite films. However, based on literature, the use of ultrasound for the development of a novel TP-loaded Pul/Tre composite film is limited. This study aimed to develop the TP-loaded Pul/Tre film and assess the efficacy of multi-frequency power ultrasound-assisted preparation and performance. Furthermore, the structural, mechanical, barrier, physicochemical properties, and antibacterial activity of composite films, as well as their preservation effects on fresh-cut apples and pears, were investigated.

2. Materials and methods

2.1. Materials and reagents

Pul was purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). Glycerol (Gly, 99 % purity). D (-)-Tre, anhydride, agar powder and sodium chloride were obtained from Sigma-Aldrich Chemical Reagent Co. Ltd. (Shanghai, China). Carboxymethyl Cellulose (CMC) was supplied by Sigma (St. Louis, MO, USA). TP, with purity greater than 98 %, was purchased from Nanjing Duly Biotech Co., Ltd (Nanjing, China). Tryptone (LP0042) and yeast extract (LP0021) were purchased from Beijing LABLED Biotech Co., Ltd (Beijing, China). E. coli CICC 10389 and S. aureus CICC 10201 were obtained from the China Center of Industrial Culture Collection (CICC) (Beijing, China).

2.2. Preparation of the TP-loaded Pul/Tre (TP@Pul/Tre) film

The composite films were formulated by a casting method. Pullulan and trehalose powders were dissolved in deionized water to a final concentration of 8 g/100 mL, respectively, to make the Pul/Tre solution, and was combined in various mass ratios (0.4, 1.3, 2.2, 3.1, and 4.0). The Pul/Tre solution was then mixed with different concentrations (1 %, 3 %, 5 %, 7 %, and 9 % w/v) of TP and stirred at 25 °C for 1 h. Furthermore, to obtain a TP@Pul/Tre film-forming solution, CMC and glycercol (both 5 %) were added as plasticizers to the above mixture and stirred for 1 h. Finally, 15 mL of the film-forming solution was poured into a 9 cm diameter petri dish and dried at 60 °C for 6 h. The prepared film samples were stored in a desiccator at 25 °C with relative humidity (RH) of 43 % till further use. The preparation of Pul/Tre films remained unchanged except for not adding TP.

2.3. Ultrasound-assisted preparation of TP@Pul/Tre film

The fresh TP@Pul/Tre film-forming solution was sealed in a plastic bag for multi-frequency power ultrasound treatment. The device was designed by our research team, which was manufactured by Meibo Biotechnology Co., Ltd. (Zhenjiang, Jiangsu, China). The instrument is equipped with three frequency generators that generate ultrasound at an angle of 120° either sequentially or simultaneously. The ultrasonic conditions were as follows: ultrasonic frequency, 20, 35, 50, 20/35, 20/ 50, 35/50 and 20/35/50 kHz; time, 5, 10, 15, 20, 25 and 30 min; ul- trasonic power density, 20, 40, 60, 80 and 100 W/L; pulsed on-time/off-time, 5/5. A circulating water bath was used to control the temperature of the sample at 25 °C during ultrasound treatment.

Determination of physicochemical properties

2.3.1. Mechanical properties

The tensile strength (TS) and elongation at break (EBA) of the film samples (0.2 cm × 0.6 cm) were analyzed using a Texture Analyzer (Vienna Court, Lamas Road, Godalming GU7 1YL, UK) following the method reported previously [10]. The test probe was A/TG, the clamping distance was 30 mm, and the test speed was 2 mm/s.

2.3.2. Optical properties

The film was cut into 4.0 cm × 1.0 cm wide strips to stick to one side of the cuvette. The optical properties including UV-barrier and transparency were measured at 200–800 nm with a Shimadzu UV-3600 plus spectrophotometer (Shimadzu Europa, GmbH, Germany). The empty cuvette was used as a control.

2.3.3. Water vapor permeability (WVP) and moisture content (MC)

The WVP of film samples was assessed using gravimetric analysis according to the previous method with slight modifications. The opening of a weighed vial containing 10 g of CaCl2 pellets (0 % RH) was tightly covered with film sample. The weighed vial was then placed in a sealed container containing saturated KNO3 (93 % RH). A total of 6 measurements were made by weighing vials every 2 h at 25 °C. WVP was calculated as follows:

\[
WVP \ (g \cdot m/m^2 \cdot s \cdot Pa) = \frac{\Delta m \cdot \text{thickness}}{A \cdot \Delta t \cdot \Delta P}
\]

where Δm is the mass increase, g; A is water vapor permeation area, m²; Δt is the time interval, s; and ΔP represents the water vapor pressure difference across the film, which is 2.945 kPa.

The MC was determined by drying the films to a constant weight at 105 °C and calculated as:

\[
MC (%) = \frac{M_1 - M_0}{M_1} \cdot 100 \%
\]
where $M_1$ is the wet weight of the films; and $M_2$ is the dry weight of the films.

2.3.4. Oxygen permeability (OP)

A 50 mL soybean oil sample placed in a 250 mL conical flask was sealed with film samples and placed in a constant temperature oven at 60°C for 7 days to accelerate oil oxidation. Spectral collection of oil samples was performed using a DXR™ 3 Raman spectrometer (Thermo Scientific DXR™, USA) reported previously [18]. The acquisition condition parameters were as follows: excitation wavelength, 532 nm; laser power, 10 mW; temperature, 25°C; scanning time, 10 s; scanning range, 500–2000 cm⁻¹.

2.4. Determination of structural properties

2.4.1. Scanning electron microscopy (SEM)

The microstructure properties of the surface and cross-section of the films were observed using SEM (JSM-7001F, JEOL Ltd., Japan) at an accelerating voltage of 15 kV. The film samples were fixed on a conductive platform and sputtered with gold for 5 min. The cross-section of the film was obtained by naturally allowing it to rupture in liquid nitrogen.

2.4.2. Attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectroscopy

The FTIR spectra of the films were recorded using an ATR-FTIR spectrometer (Nicolet iS50, Thermo Electron, USA) in the range 4000 to 600 cm⁻¹ with 16 scans at a resolution of 4 cm⁻¹.

2.4.3. X-ray diffraction (XRD)

The XRD patterns of the films were obtained using an X-ray diffractometer (D8 ADVANCE, BRUKER AXS, Karlsruhe, Germany) in the 20 range of 5°–80° at 5°/min.

2.4.4. Thermal stability

The thermal stability of the films was tested on an integrated thermal analyzer STA 449 F3 Jupiter (NETZSCH-Geratebau GmbH, Selb, Germany). The sample was heated from 30°C to 500°C at a rate of 10°C/min under an N₂ atmosphere (flow rate 20 mL/min).

2.5. Measurement of antibacterial activity

The antibacterial properties of film were investigated by the standard colony counting method, as described previously [19]. Fresh E. coli and S. aureus were incubated at 37°C and their concentration was adjusted with sterile 0.9% NaCl solution to ~10⁷ colony forming units per mL (CFU/mL). The 2 g of film samples were then immersed in 50 mL of the fresh bacterial culture, and was incubated at 37°C. The supernatant was collected and diluted to an appropriate concentration with 0.9% NaCl solution after 0, 1 h, 2 h, 3 h, and 4 h of sample addition. Then, 0.1 mL of the above solution was cultured on LB solid medium and was incubated at 37°C for 24 h. The E. coli and S. aureus cultures on LB medium without film samples were used as the control.

Furthermore, the morphology of E. coli and S. aureus was further observed by SEM imaging. The 2 g of composite films was immersed in 50 mL of fresh E. coli and S. aureus culture (10⁷ CFU/mL) for 4 h and 8 h, respectively. The pellet was collected by centrifugation (6000 rpm, 5 min), fixed with 2.5% glutaraldehyde at 4°C for 6 h, and washed twice with 0.9% NaCl solution. Subsequently, the colonies were dehydrated in gradient alcohol (30%, 50%, 70%, 85%, 90%, 95% and 100%) for 15 min. Finally, 0.01 mL bacterial solution was taken out and dried at 37°C. After gold plating, SEM observation was performed.

2.6. Practical application of composite films on fresh fruits

2.6.1. Appearance observation and weightlessness rate of apple

Fresh apples that were not damaged or moldy were cut into 1 cm³ cube and immersed in 0.9% NaCl for 5 min to protect the color. The apple pieces were then removed to air dry surface moisture at 25°C and placed in plastic petri dishes. The composite films prepared as described in Section 2.2 and Section 2.3 were covered on the above plastic petri dishes for 4 days at 25°C. The apple samples were weighed and photographed every 24 h. The uncovered fresh apple pieces were served as controls. The weightlessness rate was calculated as the percentage of apple samples weight lost to the total apple samples weight.

2.6.2. Decay observation of pear

The artificial wound-accelerated fruit decay method was used to study the preservative effect of composite films. Fresh pears were given artificial wounds with a depth of 15 mm and a diameter of 4 mm using sterile needles. The wounded pears were immediately immersed in the film-forming solution prepared as described in Sections 2.2 and 2.3 for 2 min at 25°C. The pear samples were then taken out and dried in a fume hood at 25°C for 3 min to form film-coated pears, then stored at 25°C for subsequent 7-day experiments. Pear samples were photographed on days 1, 3, and 7; tissue sections of the same size were excised on day 7, and cross-sections of the pears were photographed; the diameter of the rot apertures was measured and recorded. Uncoated pears were used as controls.

2.7. Statistical analysis

Each experiment was replicated at least three times. The results are presented as mean ± SD. Duncan’s multiple range test and analysis of variance were performed using SPSS 19.0. Results were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Single factor optimization based on mechanical properties

The mechanical properties used to quantify the strength and flexibility of degradable films against external forces (including TS and EBA) are directly related to the inter-molecular interactions of polymer materials and the preparation conditions of the composite film [10].

According to Fig. 1A, the EBA of composite film increased and then decreased significantly ($p < 0.05$), while TS value increased, as the proportion of Tre decreased. According to Haque’s [20] preferential exclusion theory, Tre limited the unfolding of the Pul chain, preventing the cross-linking of Pul and TP, thus reducing film strength. Furthermore, the addition of Tre caused a rearrangement of the original structure between Pul and TP, breaking the existing hydrogen bonds and forming new hydrogen bonds with Tre, resulting in varying degree of change in the EBA and TS of the composite film. Briefly, when TP/Pul/Tre film was fabricated at a ratio of 2: 2 (Pul: Tre), the mechanical properties were reached at their optimum, with EBA and TS being 122.60% and 10.54 N/mm², respectively, and increased by 487.76% and 101.05% compared to 0: 4 (Pul: Tre) group. The impact of TP addition percentage on mechanical properties of TP/Pul/Tre film was examined based on the ratio of 2: 2 (Pul: Tre). As demonstrated in Fig. 1B, as the TP concentration was increased, the EBA and TS of composite film increased and then decreased, respectively. Especially when TP concentration was 5%, the EBA and TS of TP/Pul/Tre film reached its maximum level of 168.54% and 15.03 N/mm², respectively. There was 28.13% and 74.68% increase in EBA and TS relative to Pul/Tre film. It was reported previously, that adding 5–20% TP significantly increase the TS and EBA of chitosan film [21], instead of 1–5% TP, which does not coincide with the results of this study; these differences might be attributed to different film substrates. The polyhydroxy...
TS values of 118.62% and 21.90 N/mm² dramatically decreased the mechanical properties of the TP@Pul/Tre film. Moreover, the contrasting effect of TP could be the accumulation of excessive TP on the long chain Pul, disrupting the original dense and ordered network structure of the film and weakening the secondary force between polymer macromolecules [1], which dramatically decreased the mechanical properties of the TP@Pul/Tre film. Therefore, the optimum concentration of TP was proved to be 5%.

The changes in EBA and TS induced by ultrasonic working mode at the same ultrasonic power density (40 W/L) and ultrasonic time (15 min) was compared with the film without ultrasound treatment (Fig. 1C). The results revealed that the dual-frequency ultrasonic treatment at 20/35 kHz exhibited the most significant effect, with EBA and TS values of 118.62% and 21.90 N/mm², respectively. On comparison with the control group, the above parameters were improved by 32.91% and 73.53%. These findings are consistent with our previous work, because compared with single- and triple-frequency ultrasound, simultaneous dual-frequency ultrasound at a specific frequency could consistently achieve the best processing effect on biomacromolecules [12]. The mutual interference or superposition of ultrasonic waves at 20 kHz and 35 kHz might produce more resonance effects on complex biomacromolecular system, which was beneficial to expanding Pul structure and strengthening inter-molecular interactions, resulting in higher EBA and TS values.

The ultrasonic power directly controls the generation of cavitation, which mediates sonochemical and physical effects that weaken or enhance molecular interactions, leading to different EBA and TS values [23]. Experiments were performed at 5 different power density levels (20, 40, 60, 80, and 100 W/L) to investigate the ultrasonic properties of the composite films. The TS reached a maximum value of 19.60 N/mm² at the ultrasonic power density of 40 W/L, which gained an increase of 47.35% compared with untreated film (Fig. 1D). However, as ultrasonic power intensity continued to increase (greater than 40 W/L), particle breakdown and erosion emerged due to the excessive cavitation effect [24], resulting in a significant decrease in TS. Actually, the energy generated and transmitted by ultrasound was directly correlated to ultrasonic power, because the excessive ultrasonic power will not only cause mechanical damage, but will also contribute to acoustic cavitation, producing many free radicals and a high temperature [25]. This effect of ultrasound may cause irreversible destruction of the molecular structure and weakened the inter-molecular interactions. On the contrary, sonication at 80 and 100 W/L improved the values of EBA significantly (p < 0.05). This phenomenon indicated that, when evaluating the mechanical properties of composite films, EBA and TS are not always correlated, which are in accordance with Wang et al. [26] who found that increase in ultrasound power handling increased EBA values, but decreased TS of rice protein hydrolysates/chitosan film. Collectively, our findings proved that 40 W/L (moderate power density) ultrasound treatment was effective in improving the mechanical strength of the film.

The different sonication times had significant (p < 0.05) effects on the TS of the U-TP@Pul/Tre film, but not on the EBA (Fig. 1E). The TS gradually increased with the prolongation of ultrasound time, and reached a maximum value (19.96 N/mm²) at 15 min; and was then decreased. The differences in TS were attributed to ultrasound-induced dynamic changes in Pul structure and intermolecular forces. Liang et al. reported that relatively short periods of sonication leads to proteins unfolding, while prolonged sonication, leads to unfolded proteins reassembling themselves [27]. The stretching of the macromolecular Pul structure would inevitably lead to the exposure of the interaction bonds, increasing the interaction force between the molecules and resulting in TS. However, the reassembling of macromolecule Pul has the opposite result.

Based on the above single factor optimization experiments, a Pul/Tre ratio of 2:2, TP addition amount of 5%, dual-frequency synchronized ultrasound of 20/35 kHz, an ultrasound time of 15 min, and a power density of 40 W/L were chosen to prepare the U-TP@Pul/Tre composite film. The TS and EBA of the U-TP@Pul/Tre composite film were 19.18 N/mm² and 119.48%, respectively, under the above ultrasound conditions; compared to the control group, the TS and EBA increased by...
66.78% and 24.35% (Fig. 2A). The effects of TP and ultrasound on the composite films’ physicochemical characteristics, structure, and antibacterial capacity were assessed using the composite films prepared under the ideal TP concentration and ultrasonic treatment.

3.2. WVP and MC

Water vapor can be divided into three stages: adsorption, diffusion, and decomposition as it passes through a composite film. A composite film can effectively stop the moisture loss from the food, if the film has a good water barrier property. So, it is crucial to analyze the WVP and MC of the composite film. The addition of TP increased the WVP of the composite film from 7.151 × 10⁻¹² g cm⁻¹ s⁻¹ Pa⁻¹ to 7.85 × 10⁻¹² g cm⁻¹ s⁻¹ Pa⁻¹ (Fig. 2C). Similar results were obtained by Gao et al. [28], while adding 2.5–15% TP to pectin-chitosan composite films, significantly increased the WVP, and the water repellent properties were decreased. This might be due to the addition of TP, which disrupted the original molecular structure of Tre/Pul, causing molecular rearrangement. The newly formed molecular structure was not much compact as the original film matrix. Furthermore, the unequal distribution of TP might form agglomerates and generate voids in the film matrix, which allows water molecules to pass through, and enhanced the WVP. The WVP value of the composite film was significantly (p < 0.05) reduced after ultrasound treatment for 3.49 × 10⁻¹² g cm⁻¹ s⁻¹ Pa⁻¹. It demonstrated that moderate sonication was useful for the uniform dispersion of small molecules, promoted the formation of hydrogen bonds between polyphenols and polysaccharides, reduced the hydrophilicity and diffusion coefficient of the film. Besides, increased the degree of cross-linking, and enabled U-TP@Pul/Tre film compact and complex, forming a “curved path”, limiting the penetration of water vapor and reducing the WVP [29,30].

The MC of the TP@Pul/Tre composite film was substantially reduced from 8.21% to 6.55% by the addition of TP (Fig. 2B), which might be due to the presence of phenolic compounds in the TP. The presence of carboxyl and hydroxyl groups in the phenolic compounds increased the binding affinity of Pul and Tre molecules, thus inhibited the binding of polysaccharides to water molecules. Recently it was reported that the addition of plant polyphenols reduced the water content of the polysaccharide film matrix [30]. However, the ultrasonic treatment has no significant impact on the MC of the composite film, and the ultrasound’s altered the inter-molecular forces and network structure were insufficient to change its affinity for water molecules.

3.3. Optical properties and OP

Determination optical properties such as transparency and UV-barrier for food packaging films are crucial. The packaging film’s UV barrier qualities protect the food from UV-mediated oxidation, while transparency attracts the consumers to see packed foods. So, the UV–vis absorption and transmittance (%) spectra of the films were analyzed (Fig. 2D). Lowest transmittance was observed in the film with TP, and have a significant absorption value at 280 nm then the film without TP. Our results demonstrated that TP endowed excellent UV light screening properties to the TP@Pul/Tre film. It was attributed to distinct phenolic hydroxyl structure of TP, with an absorption peak at 280 nm, which could improve the n–π* transition in the UV range, thereby improving the light barrier performance of the biodegradable film. Generally, the packaging film with good light barrier properties could effectively reduce the oxidation and inhibit the degradation of certain substances in food matrix, thereby prolonging its shelf life. The transmittance of TP@Pul/Tre film is lower than that of Pul/Tre film in the range of 250–800 nm (Fig. 2D). Moreover, Dou et al. [31] reported that the addition of polyphenols reduced the transmittance of gelatin sodium alginate films at 200–800 nm. The U-TP@Pul/Tre composite film exhibited a higher absorbance value at 280 nm and better transmittance than TP@Pul/Tre film. This result indicates that ultrasound not only
improves the ability of film to absorb UV but also increases other wavelengths transmittance. It might be due to the effect of ultrasound, which contributes to the uniform dispersion of small molecules, making a compact and well-organized network structure with high transparency.

The Raman spectra of oils under various film sample covering are displayed in Fig. 2E. The Raman spectra of oils containing spectral peaks caused by the vibration of hydrocarbon chains, which provides the information about the molecular group structure of its components, for example, 970 cm\(^{-1}\) (trans- C=H bending vibration), 1080 cm\(^{-1}\) (+CH\(_3\) stretching vibration), 1264 cm\(^{-1}\) (cis- C=H bending vibration), 1300 cm\(^{-1}\) (in-phase -CH\(_2\) bending vibration), 1438 cm\(^{-1}\) (CH\(_2\) shear bending vibration), 1565 cm\(^{-1}\) (cis=C=C stretching vibration), 1746 cm\(^{-1}\) (C=O stretching vibration), etc. Significant differences were observed in the intensities of Raman spectra characteristic peaks of the oil covered by the three films, which indicates that oil oxidation inhibition degree was different; indirectly inferred that the OPs of the three films were different. Upon oxidation of lipids, the cis double bond is isomerized and rearranged into a stable trans double bond configuration. In addition, the ratios \(I_{1470}/I_{1650}\) and \(I_{1650}/I_{1746}\) can be used to describe changes in oil unsaturation and trans double bonds, respectively. The composite film’s ratio \(I_{1470}/I_{1650}\) occurred in decreasing order, with Pul/Tre > TP@Pul/Tre > U-TP@Pul/Tre, whereas it’s \(I_{1650}/I_{1746}\) exhibited the opposite trend (Fig. 2E). These results demonstrated that TP and ultrasound treatment successfully prevent O\(_2\) infiltration and delay oil oxidation. The addition of TP reduced the OP of the composite film, one of the critical reasons that TP have excellent antioxidant activity, reduced the oxygen permeability of the film through self-oxidization. In addition, the ultrasonic treatment has a positive impact on the oxygen barrier properties of the film, because the ultrasonic treatment makes a uniform network structure and compact micro-structure of the film matrix, as well as vertical layers to prevent gas diffusion through the film.

3.4 ATR-FTIR

Infrared spectroscopy is widely used for analyzing the interactions between polymer molecules. It can effectively reflect the cross-linking of Pul, Tre, and TP, as well as the impact of ultrasound on the structural properties of composite film. The ATR-FTIR spectra (4000–600 cm\(^{-1}\)) of the Pul/Tre, TP@Pul/Tre, and U-TP@Pul/Tre films is displayed in Fig. 2F. The characteristic peaks of the Pul/Tre film could be seen at 3348 cm\(^{-1}\), 2932 cm\(^{-1}\), 1596 cm\(^{-1}\) and 1031 cm\(^{-1}\) which represented O–H stretching vibration, C–H bending vibration, and C=O stretching vibration (amide I), respectively [32]. Furthermore, the peak at 1106 cm\(^{-1}\) was reported to represent a typical saccharide structure [33]. In contrast the absorption peak at 994 cm\(^{-1}\) was attributed to the hydrogen bond formed by the oxygen atom on the glycosidic bond and the O–H in the glycerol molecule [34]; and Pul/Tre, TP@Pul/Tre and U-TP@Pul/Tre film exhibited these two peaks. After the addition of TP, the –OH absorption peak in the Pul/Tre film shifted from 3348 cm\(^{-1}\) to 3343 cm\(^{-1}\) compared with the Pul/Tre film, indicating that TP contributes to the formation of new hydrogen bonds between the molecules, restricted the mobility of polysaccharides and enhanced the interaction between them, resulting in improved EBA and TS (Section 3.1). Previous studies have also reported that TP can break the original interaction force between molecules, and a new hydrogen bond was formed between C=O in TP and –OH in polysaccharides [35,36]. Apart from this, no significant wavelength alterations or additional peaks were observed, indicating that no covalent bond was formed between TP and Pul/Tre. The TP@Pul/Tre film’s absorption peak, initially at 1596 cm\(^{-1}\) after ultrasonic treatment, shifted to 1600 cm\(^{-1}\), showing that sonication improved the film-forming matrix’s capacity for non-covalent bonding. This was beneficial to improve the cross-linking of the polymer and make the polymer structure compact, which could justify the significant improvements in the mechanical and barrier properties of the film after ultrasonic treatment (Section 3.1, 3.2 and 3.3).

3.5 XRD

The XRD pattern of the Pul/Tre, TP@Pul/Tre, and U-TP@Pul/Tre films which defines the effect of TP and ultrasound on the crystallinity of the films (Fig. 2G). The Pul/Tre film showed a broad diffraction peak at 20 = 18.66°, indicating the amorphous structure of the film, which attributes primarily to the existence of significant covalent linkages between Pul and Tre. Moreover, the TP@Pul/Tre film exhibited similar peak positions and intensities compared to Pul/Tre film, suggesting that TP was encapsulated in the network structure formed by trehalose and pullulan as active filler material. The diffraction peak of U-TP@Pul/Tre composite film shifted from 19.04° to 19.74°, and the peak became wider and flatter after ultrasonic treatment. The changes in the XRD patterns of the U-TP@Pul/Tre film indicated that the ultrasonic treatment makes the macromolecules in the films more compatible, which was attributed to the more ordered molecular arrangement of the composite films and the enhanced intermolecular forces shown by the ATR-FTIR (Section 3.4). Besides, the cavitation effect of ultrasound could change the macromolecular structure and improve the order of the film structure [37]. Meanwhile, the physical impact of the ultrasound causes mechanical agitation, which increases the inter-molecular collision frequency, thus enhancing the inter-molecular interaction force [38]. These structural changes further explain that ultrasound enhanced the mechanical properties and moisture resistance of U-TP@Pul/Tre films.

3.6 Thermal stability

The TGA was used to investigate and present the thermal stability and kinetic characteristics associated with the degradation process of biocompatible films, such as weight loss and maximum decomposition temperature, and displayed in the TGA/DTG thermal curves form 30–500 °C, respectively (Fig. 2H and 2I). The first stage of thermal degradation occurred at 30–170 °C, which was caused by moisture evaporation from the film. The TGA curve shows that the weight loss of Pul/Tre film was more than that of TP@Pul/Tre and U-TP@Pul/Tre film, indicating that the Pul/Tre film has a higher moisture content than the other two films, which was consistent with that of MC (Fig. 2B). The DTG curves showed that the water loss temperatures of TP@Pul/Tre and U-TP@Pul/Tre films were 130.26 °C and 134.64 °C, respectively, which were significantly higher than that of Pul/Tre (95.75 °C). Our results demonstrated that the TP inhibited the loss of moisture by the formation of a compact network structure, and enhanced the inter-molecular interaction force, particularly in the form of H-bonds among water, glycerol, and polymer depict by ATR-FTIR (Fig. 2F). In second stage, the thermal degradation occurs at 170–350 °C, primarily attributed to the breakdown of covalent bonds, electrostatic interactions, and inter-/intra-molecular hydrogen bonds in TP and polysaccharide molecules [39]. Furthermore, previous studies documented that the thermal decomposition of Pul and Tre mainly refers to the cleavage and degradation of glycosidic bonds [40]. The thermal decomposition temperature (Td) of the U-TP@Pul/Tre film was 307.13 °C higher than that of the TP@Pul/Tre film (304.41 °C) (Fig. 2I), demonstrated that the ultrasound treated composite film had a higher Td. Similar results were obtained by Qu et al. [37] that ultrasound treatment increased the Td of the tuna skin collagen-chitosan composite film. Furthermore, in the second stage of the thermal degradation, a minor weight loss was observed in U-TP@Pul/Tre film with value of 59.13 % compared with TP@Pul/Tre film (Fig. 2H), is nice evidence that ultrasound treatment enhanced inter-molecular interactions. It increased the cross-linking degree of the film, which facilitated the formation of more ordered and stable structures, and thus improved thermal stability. Based on the results of ATR-FTIR, XRD, and thermal stability, schematic diagrams of Pul/Tre, TP@Pul/Tre and U-TP@Pul/Tre films were presented (Fig. 3).
Fig. 3. Schematic diagram of composite films.

Fig. 4. The digital photos and scanning electron microscopy images of Pul/Tre, TP@Pul/Tre and U-TP@Pul/Tre films.
3.7. SEM

The SEM was used to observed the physical features, apparent morphology, and cross-sectional morphology of the films (Fig. 4). The Pul/Tre, TP@Pul/Tre, and U-TP@Pul/Tre films showed a similar microstructure, smooth surface, homogeneous cross-section, without the presence of pores or microfractures, which were not changed with the addition of TP and ultrasonic treatment. These observations were expected, because of low loadings of TP (5 wt%), notable film-forming ability of Pul and the excellent light transmittance (Section 3.3) of the films. However, these results were inconsistent with XRD, ATR-FTIR, and thermal stability. The reason might be that the Pul and Tre polysaccharides films had good compatibility and smoothness; and the effect of TP addition and ultrasonic treatment on the inter-molecular interaction force and the structure of the composite films were not reflected in the morphologies observed under SEM. Previous studies supported this phenomenon such as, Yun et al. [30] reported that addition of polyphenols into chitosan film improved both physical and functional properties, but no difference was observed in SEM. Similar result was reported for Pul film with lysozyme nanofibers, that displayed a uniform surface and a cross-section with crack-free [41]. Work done by Vera et al. [42] showed that there was no significant difference observed in SEM after ultrasonic treatment, which were consistent with our findings.

3.8. Antibacterial activities

One of the leading causes of food spoilage is microbial contamination, and an active film with antibacterial capabilities can significantly extend the shelf life of food. Degradable packaging films incorporating TP have been studied and developed recently. However, the antibacterial activity of TP in the composite film may be partially lost due to oxidative degradation in the film formation process [43]. Therefore, evaluating the antibacterial activity of the novel active packaging films prepared in this study will shed light on the actual antibacterial effect of the active film. The antibacterial activity of the composite films was investigated using colony counting and SEM with \( \text{S. aureus} \) and \( \text{E. coli} \) were chosen as test bacteria (Fig. 5). Both TP@Pul/Tre and U-TP@Pul/Tre films exhibited excellent antibacterial properties, showing time-dependent resistance to \( \text{E. coli} \) and \( \text{S. aureus} \) within 0–8 h. When \( \text{E. coli} \) and \( \text{S. aureus} \) were incubated with TP@Pul/Tre and U-TP@Pul/Tre films for 4 h and 6 h, respectively, the bacterial lethality reached 100 % (Fig. 5A, 5B) and 99.47 % (Fig. 5C, 5D), due to the antibacterial activity of TP. From this result, the adequate protection of Tre on the biological activity of TP could be well confirmed, as hypothesized in the introduction. In addition, with the extension of the incubation time, the colonies of the above two bacteria in the petri dish became smaller, indicating that the composite films inhibited bacterial growth. The antibacterial activities of TP@Pul/Tre film and U-TP@Pul/Tre film were compared, and it was found that there was no apparent difference between the two films, which indicated that ultrasonic treatment had no effect on the composite film’s antibacterial function. The effects of U-TP@Pul/Tre film treatment on the SEM of \( \text{E. coli} \) and \( \text{S. aureus} \) were further observed. The bacteria in the control group had a plump, smooth surface, and complete structure, without deformation or defect (Fig. 5E and 5F). However, \( \text{E. coli} \) and \( \text{S. aureus} \) were deformed and damaged after being treated with U-TP@Pul/Tre film. Specifically, the cell surface morphology of \( \text{E. coli} \) became wrinkled with damage and holes, and even the collapse of the cell structure and the occurrence of cytoplasmic leakage. In contrast, \( \text{S. aureus} \) was less damaged than \( \text{E. coli} \), although its cell surface morphology was severely distorted. Shimamura et al. reported that EGCG in TP could directly bind to bacterial peptidoglycan in the cell wall and precipitate, causing the cell structure to be destroyed and the cell contents to leak out, resulting in bacterial death [44]. In addition to the damage to cell structure, TP might alter the membrane potential caused by changes in H\(^+\) ion flux and interfere with the metabolism of intermediates by binding to enzymes [45]. Furthermore, TP has also been reported to combine with reactive oxygen species in bacteria under the action of superoxide dismutase to generate hydrogen peroxide, induce cytoplasmic acidification and coagulation of cytoplasmic components, and inhibit energy metabolism and DNA/RNA/protein synthesis/function [46]. The above possible antibacterial mechanisms for TP were summarized (Fig. 5G). Our results proved that the prepared TP@Pul/Tre and U-TP@Pul/Tre films had excellent bacteriostatic and killing effects on \( \text{E. coli} \) and \( \text{S. aureus} \).

3.9. Practical application of composite films on fresh fruits

The post-harvest preservation effect of composite films on fruits was investigated using fresh apples and pears. From the digital photos (Fig. 6A), compared with control (CON), the apple slices in the composite film group were plumper, with lower degree of browning and less weight loss, especially the apple slices in the U-TP@Pul/Tre group were best protected. It could be possible to conclude, that the composite films prepared in this study could effectively prevent moisture loss from apple slices and inhibit the oxygen penetration to different degrees, which was consistent with the results of Fig. 2C and Fig. 2E. Similarly, the order of wound decay diameter (whether in cross-section or apparent) of pear samples was control > Pul/Ptre > TP@Pul/Tre (Fig. 6B), that of further confirmed composite film’s antibacterial efficacy (Fig. 5). In addition, combining the results (Fig. 6A and Fig. 6B), it could be concluded that U-TP@Pul/Tre has the best fresh-keeping effect, followed by TP@Pul/Tre, and finally Pul/Ptre, whether for fresh-cut fruit or whole fruit. This result indicated that U-TP@Pul/Tre film was an ideal fruit preservation material with excellent advantages such as high mechanical strength, high UV resistance, high oxygen and water vapor barrier effects, and strong antibacterial activity.

4. Conclusions

This study revealed the promising use of ultrasound treatment to improve the structural, mechanical, physicochemical properties and resistance activities of a novel, active and degradable packaging film TP@Pul/Tre. We found a compact, smoother, continuous, and more stable composite film of U-TP@Pul/Tre after sonication, which was also confirmed in TGA and DTG. The TP@Pul/Tre and U-TP@Pul/Tre films exhibited about a 100 % bactericidal effect on \( \text{E. coli} \) and \( \text{S. aureus} \) at 4 h and 8 h, respectively, with excellent antibacterial activity. In comparison with the TP@Pul/Tre film, the U-TP@Pul/Tre film showed a better EBA, TS, stronger UV-barrier, lower WS, lower transparency, and lower OP. Moreover, ART-FTIR and XRD results indicated that ultrasound enhanced the hydrogen bonding among Pul, Tre, and TP and improved the compatibility of the macromolecules in the films, and TP was successfully encapsulated in the ordered network structure formed by trehalose and pullulan, and exhibited a high degree of browning and less weight loss compared to the control group, with better protection. It could be possible to conclude, that the composite films prepared in this study could effectively prevent moisture loss from apple slices and inhibit the oxygen penetration to different degrees, which was consistent with the results of Fig. 2C and Fig. 2E. Similarly, the order of wound decay diameter (whether in cross-section or apparent) of pear samples was control > Pul/Ptre > TP@Pul/Tre (Fig. 6B), that of further confirmed composite film’s antibacterial efficacy (Fig. 5). In addition, combining the results (Fig. 6A and Fig. 6B), it could be concluded that U-TP@Pul/Tre has the best fresh-keeping effect, followed by TP@Pul/Tre, and finally Pul/Ptre, whether for fresh-cut fruit or whole fruit. This result indicated that U-TP@Pul/Tre film was an ideal fruit preservation material with excellent advantages such as high mechanical strength, high UV resistance, high oxygen and water vapor barrier effects, and strong antibacterial activity.

CRediT authorship contribution statement

Lixin Kang: Formal analysis. Qiuwang Liang: Writing – review & editing, Funding acquisition. Arif Rashid: Writing – review & editing. Abdul Qayum: Formal analysis. Zhuzhong Chi: Writing – review & editing. Xiaofeng Ren: Funding acquisition. Haile Ma: Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Fig. 5. Antimicrobial ability (A, C), inhibition rates (B, D) of TP@Pul/Tre and U-TP@Pul/Tre films against *E. coli* (A, B) and *S. aureus* (C, D). And the morphological characterization of *E. coli* (E) and *S. aureus* (F) were immersion-treated with TP@Pul/Tre and U-TP@Pul/Tre films. Possible sites of action of polyphenols to exert antibacterial activity at the cellular level (G).
Data availability

Data will be made available on request.

Acknowledgements

The authors wish to express their appreciation for the support obtained from the National Natural Science Foundation of China (Grants No. 32072355); The Primary Research & Development Plan of Zhenjiang (Grants No. NY2021006); The Key Project for Science and Technology Innovation (modern agriculture) of Danyang (Grants No. SNY202201); Sponsered by Qing Lan Project (2020); Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD). There are no conflicts of interest to declare. This article does not contain any studies involving human or animal subjects.

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Fig. 6. Morphology photographs of controls (CON), Pul/Tre-packed, TP@Pul/Tre-packed, and U-TP@Pul/Tre-packed fresh apples at 1–4 d (A) and pears at 1–7 d (B).

| A | CON | Pul/Tre | TP @Pul/Tre | U-TP @Pul/Tre |
|---|-----|--------|-------------|---------------|
| 1 d | 4.0 g | 4.2 g | 4.0 g | 4.3 g |
| 2 d | 3.6 g | 3.8 g | 3.6 g | 3.9 g |
| 3 d | 3.2 g | 3.5 g | 3.2 g | 3.6 g |
| 4 d | 2.6 g | 2.9 g | 2.6 g | 2.9 g |

| B | CON | Pul/Tre | TP @Pul/Tre | U-TP @Pul/Tre |
|---|-----|--------|-------------|---------------|
| 1 d | 13.9 mm | 10.7 mm | 7.0 mm | 5.6 mm |
| 7 d | 13.9 mm | 10.7 mm | 7.0 mm | 5.6 mm |

| Rot diameter | 13.9 mm | 10.7 mm | 7.0 mm | 5.6 mm |
|---|--------|--------|-------|-------|
| Final weight | 38.99% | 54.12% | 84.95% | 106.24% |
| Initial weight | 25.00% | 30.00% | 35.00% | 40.00% |

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