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Synergistic effects of EDTA and lysozyme on the properties of hydroxypropyl starch nano antibacterial films

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ABSTRACT

Hydroxypropyl starch (HPS) nano antibacterial films incorporating Ethylene Diamine Tetraacetic Acid (EDTA) and lysozyme (LY) were fabricated via solvent casting method. The synergistic effects of EDTA and LY on the microstructure, component interactions, color, optical, mechanical, barrier and antibacterial properties of HPS nano antibacterial films were evaluated. The results indicated that EDTA and LY were well dispersed in the matrix of the HPS nano antibacterial films, the film-forming substrates have good compatibility, resulting in a dense multi-layer structure of the HPS nano antibacterial films. The addition of EDTA and LY increased the color parameters (L^*, a^*, b^* and $\triangle E^*$) of the HPS nano antibacterial films. The synergistic effects of EDTA and LY significantly decreased the light transmission of the HPS nano antibacterial films. The presence of EDTA and LY increased the tensile strength (TS) and the elongation at break (EAB) of the HPS nano antibacterial films. The TS and EAB of E2.5L1 reached the highest values of 6.329 MPa and 50.24 %, respectively. The incorporation of EDTA and LY had positive effects on the improvement of water vapor permeability (WVP) and oxygen permeability (OP). The WVP and OP of E2.5L1 reached the highest values of 0.9350×10^{-12} g cm/cm^2•s•Pa and 0.297×10^{-2} g m/m^2 •d, respectively. In addition, EDTA and LY had significant synergistic effects on the antibacterial activity against *S. aureus* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria). E2.5L1 exhibited the highest antibacterial activity and the inhibition zone diameters of *S. aureus* and *E. coli* were 3.69 mm and 4.28 mm, respectively. The HPS nano antibacterial films incorporating EDTA and LY are potential functional packaging materials.

1. Introduction

According to statistics, 1.6 billion tons of food are wasted globally every year. Food packaging can effectively protect food from chemical, physical, and biological factors, reduce the loss of food nutrients, extend the shelf life, and reduce food waste. The traditional petroleum-based plastic packaging materials have the advantages of lightweight, low cost, good processing properties and good physical properties (Mania et al., 2020). However, petroleum-based packaging materials are non-degradable, and pollutants accumulated in the environment harm human health and the environment (Chae and An 2018). To overcome these shortcomings, many researchers have developed biodegradable, renewable, environmentally friendly biopolymer-based materials to replace petroleum-based packaging materials, and edible films made of natural biopolymer-based materials have become a research topic.

Starch is common edible films material with a wide range of sources, cost-effective and good compatibility. However, starch films generally have poor mechanical properties, thermal stability, barrier properties and antibacterial properties. Hydroxypropyl starch (HPS) is widely used in the food industry as a modified starch. HPS has attracted considerable attention due to its good film-forming properties, good stability, high transparency, good resistance to acid, alkali and corrosion (Chen et al., 2019b; Yu et al., 2023). However, the chemical modification process of HPS results in poor water barrier properties. Therefore, further research to improve the hydrophobicity and impermeability of HPS based films is imperative (Duan et al., 2023). It is feasible to overcome the restriction of HPS-based films through incorporating methyl cellulose (MC) and nano-titanium dioxide (nano-TiO_2). Nano-TiO_2 has the characteristics of

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small particle size, large specific surface area, thermal stability, good water and ultraviolet barrier properties. Previous research has reported that Nano-TiO_2 enhanced the mechanical properties and barrier properties of the bovine bone collagen/hydroxypropyl methylcellulose films (Chen et al., 2019a).

Microbial contamination is the main factor causing food spoilage and loss of commercial value. Therefore, it seems to be an exciting strategy to incorporate active components into the films, prolong the shelf life of food products and improve the functionality of food packaging materials (Bosch et al., 2000). The antibacterial agents, essential oil, antioxidants and other active components can be effectively released into packaged foods through active films (Qian et al., 2021; Ehsani et al., 2020). Radhalakshmi V et al. (Radhalakshmi et al., 2023) developed active packaging films based on poly (lactic acid) and betel leaf ethanolic extract. The structural, functional, mechanical and antibacterial properties of the active packaging films were investigated. The results showed that the active packaging films had significant film and antibacterial properties and can be used in food packaging. Wang Y et al. (Wang et al., 2023) developed an environmentally friendly active packaging film using polyvinyl alcohol and sesbania gum as the film matrix, tea tree oil as the active components, and hydroxyapatite porous microspheres as the loading system. The addition of tea tree oil increased the thermal stability, antioxidant and antibacterial properties of the active packaging films. Tkaczewska J et al. (Tkaczewska et al., 2023) obtained antioxidant active films from furcellaran and protein hydrolysates, and investigated the possibility of films with antioxidant properties as food packaging materials.

Ethylene diamine tetraacetic acid (EDTA) is an important, safe and low-cost amino polycarboxylic acid metal chelator in the food industry (Shahbazi et al., 2019). EDTA chelates the cations on the cell membrane surface (such as Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}, etc.), destroying cell membrane surface enzymes activity and cell membrane integrity (Chang et al., 2021). Previous studies have shown that EDTA has inhibitory effects on both Gram-positive bacteria (GPB) and Gram-negative bacteria (GNB) (Tsai et al., 2002). Leelaphiwat et al., (2022) prepared EDTA/PBAT/TPS (EDTA/poly butylene adipate terephthalate/thermoplastic starch) biodegradable active packaging films by blown-film extrusion technology, and applied the films in pork fresh-keeping packaging. The results clearly indicated that the films containing EDTA showed lower total viable counts in packaged pork, and EDTA showed great potential in antibacterial. Murdock et al., (2007) proved the combination of EDTA and other antibacterial agents have significant synergistic effects in vitro tests, including *S. typhimurium* and *E. coli O157:H7*. Abarca R L et al. (Abarca et al., 2022) developed gelatin-based films with nisin and EDTA for active packaging. The addition of nisin and EDTA in the polymer matrix generated a significant decrease in the growth of *E. coli* by approximately 3 logarithmic cycles. Previous studies had shown that the combination of nisin and EDTA was effective against other GNB such as *Salmonella typhimurium* (Prudêncio et al., 2016).

Lysozyme (LY) is a natural and safe alkaline enzyme widely distributed in animals tissues and secretions. The mucopolysaccharides in pathogenic bacteria can be hydrolyzed by LY (Ozer et al., 2016). LY breaks down insoluble mucopolysaccharides into soluble glycopeptides by breaking the β-1,4 glycosidic bonds, which causes the cell wall to rupture and the bacteria to lyse (Lesnierowski and Yang 2021). The exposed thiol group of LY had higher surface hydrophobicity, enhanced the binding ability with peptidoglycan, and showed strong antibacterial activity against GPB(Silva et al., 2018). LY has strong inhibitory effects on GPB due to the high content of peptidoglycan in the cell wall of GPB. In contrast, due to the low content of peptidoglycan in GNB, the inhibitory effects of LY on GNB is relatively weaker. (Morsy et al., 2018). Recently, many researchers have suggested adding LY as a preservative to extend the shelf life of food and medicines (Zhang and Rhim 2022). Romruen et al., (2022) developed a smart bilayer film (alginate/agar) containing 0.5% (w/v) catechin-lysozyme, the film possessed exceptional antibacterial and antioxidant activities. The results of the study showed that smart bilayer films containing LY extend the shelf life of food effectively. According to Rawdkuen et al. (Rawdkuen et al., 2012), the combination of catechin and lysozyme enhanced antibacterial activity over either catechin or lysozyme alone. LY in combination with chelating agents (organic acid, EDTA) or emulsifiers (Tween 80) has strong inhibitory effects on GNB. It is related to changes in the protective cell membrane and the formation of pores, resulting in the output of ion export and the loss of the proton matrix force, thus generating cell death (Gong et al., 2017). For the above reasons, EDTA and LY are good candidates for antibacterial agents in food packaging films.

In this context, incorporating nanofillers and antibacterial agents into biopolymers-based films is a promising way to modify the properties of food packaging materials. Consequently, adding MC, nano-TiO_2, EDTA and LY together to HPS-based nano antibacterial films will enhance the functionality of edible packaging materials through interaction between polymer and antibacterial agents. Nevertheless, the synergistic effects of EDTA and LY on the properties of HPS nano antibacterial films have been scarcely reported, not to mention the antibacterial activity in vitro. The research aimed to develop a novel HPS nano antibacterial film containing MC, nano-TiO_2, EDTA and LY via casting method, investigate the synergistic effects of EDTA and LY on the microstructure, component interactions, color, optical, mechanical, barrier and antibacterial properties of HPS nano antibacterial films. The preparation of biodegradable HPS nano antibacterial films incorporating EDTA and LY have potential as a new active packaging material. The research findings can lay the groundwork for the commercialization of food active packaging materials.

2. Experimental

2.1. Materials

HPS (molar substitution: 011, amylose content: 23 %) was supplied by Henan Wanbang Chemical Technology Co., Ltd. (Zhengzhou, China). MC was obtained from Liaoning Quanrui ReagentCo., Ltd. (Shenyang, China). Nano-TiO_2 (diameter of 50–100 nm, purity of 99.9 %) were supplied by Xusheng Biotechnology Co., Ltd. (Shandong, China). EDTA was purchased from Tianjin Zhiyuan Chemical Reagent Co., Ltd. (Tianjin, China). LY was purchased from Guangdong Hengyuan Food Ingredients Co., Ltd. (Guangzhou, China). Glycerol (Gly, purity≥99 %) was supplied by Yongda Reagent. (Tianjin, China). Nutrient agar, lysogeny broth and tryptic soy broth were supplied by Aoboxing Biotech Co., Ltd. (Beijing, China). *S. aureus* (ATCC25923) and *E. coli* (ATCC25922) were used as indicators of antibacterial activity.

2.2. Fabrication of the films

The HPS nano antibacterial films were fabricated via solvent casting method (Sun et al., 2019). HPS (3 wt %) was dissolved and gelatinized in distilled water at 85 °C for 25 min. MC (1.2 wt %) was dissolved in distilled water at 45 °C for 25 min. Nano-TiO_2 (0.04 wt %) was dispersed in Gly (1.5 wt %) with ultrasound assistance. EDTA (1.5 wt %, 2.5 wt %) and LY (1 wt %) were dissolved in distilled water, respectively. All solutions were mixed accordthing to e ingredients in Table 1. The mixture was homogenized at 12 000 rpm for 30 min, and the mixture was defoamed for 20 min under vacuum (−0.07 MPa). Subsequently, the mixture was poured into the film former (20 cm × 20 cm) and dried at 55 °C for 6 h. Finally, the HPS nano antibacterial films were detached and placed at 25 °C (60 % relative humidity) for 24 h. The HPS nano antibacterial films prepared with different formulations are coded as E0L0, E0L1, E1.5L0, E1.5L1, E2.5L1, respectively.

2.3. Characterization of the films

2.3.1. Morphology

The samples were trimmed into 5 × 5 mm and 5 × 1 mm squares. The

Table 1
Color parameters and antibacterial parameters of the HPS nano antibacterial films.

Film sample	Color parameters				Antibacterial parameters	
	L*	a*	b*	△E*	S. aureus	E.coli
E0L0	91.25 ± 0.24^{b}	−1.08 ± 0.07^{b}	1.40 ± 0.09^{e}	3.69 ± 0.14^{b}	0	0
E0L1	91.98 ± 0.08^{ab}	−0.85 ± 0.09^{a}	2.45 ± 0.18^{c}	4.01 ± 0.17^{b}	0	0
E1.5L0	91.77 ± 0.33^{ab}	−1.07 ± 0.10^{b}	1.98 ± 0.11^{d}	3.73 ± 0.12^{b}	0	0
E1.5L1	92.38 ± 1.18^{a}	−0.78 ± 0.11^{a}	3.20 ± 0.18^{b}	4.60 ± 0.28^{a}	0.29 ± 0.06^{b}	1.03 ± 0.15^{b}
E2.5L1	92.46 ± 0.19^{a}	−0.71 ± 0.08^{a}	3.63 ± 0.14^{a}	4.88 ± 0.08^{a}	3.69 ± 0.37^{a}	4.28 ± 0.27^{a}

Data reported are mean ± standard deviation. Within a column, different superscript letters indicate significant differences ($P < 0.05$).

samples were fixed and sputter coated with gold. The surface and cross-section morphology characteristics of the HPS nano antibacterial films were investigated by scanning electron microscopy (Hitachi S-4800, Japan) (Ahmad et al., 2016).

2.3.2. Fourier transform infrared spectroscopy (FTIR)

Infrared absorption spectra of the HPS nano antibacterial films were collected by attenuated total reflectance Fourier transform infrared spectroscopy (Thermo Nicolet IS50, USA) with the scanning range of 4000 cm^{-1}-550 cm^{-1} at the resolution of 4 cm^{-1} and the average of 16 times (Qin et al., 2020).

2.4. Properties of the films

2.4.1. Color properties

The color parameters of the HPS nano antibacterial films were recorded using RT-300 portable surface colorimeter (Tintometer-Lovibond, UK). The L* (light/dark), a^* (red/green) and b^* (yellow/blue) values were collected by placing the samples on the white standard plate. The total color difference ($\triangle E^*$) was calculated according to the following formula (Adilah et al., 2018):

$$\Delta E* = \left[(L*-L_0*)^2 + (a*-a_0*)^2 + (b*-b_0*)^2\right]^{1/2}$$

2.4.2. Optical properties

The samples were trimmed into 40 × 10 mm rectangles and attached to the inner wall of the cuvette. The light transmission of samples was measured using UV-2600 ultraviolet spectrophotometer (Shimadzu, JPN) at the wavelength range from 200 nm to 800 nm (Zhang et al., 2020).

2.4.3. Mechanical properties

Mechanical properties of the HPS nanobacterial films include tensile strength (TS) and elongation at break (EAB). The mechanical properties were evaluated using a CT-3 Texture Analyzer (Brookfield Engineers Laboratories, USA) according to ASTM D882 standard method. The texture analysis-dual grip assembly (TA-DGA) stainless fixture was used for measurement. The samples were cut in the size of 70 × 20 mm and clamped between two tensile clamps with an initial distance of 20 mm and cross-head speed of 1 mm/s (Davoodi et al., 2017).

2.4.4. Barrier properties

Barrier properties of HPS nano antibacterial films include water vapor permeability (WVP) and oxygen permeability (OP). WVP was determined by the modified ASTME96 standard cup method. 20 mL distilled water was added to the test dish, and the cropped samples were sealed at the mouth of the test dish. The test dish was placed in a dry container containing silica gel at 25 C. Later on, the change in weight of the test dish was recorded after 48 h (You et al., 2022). WVP of HPS nano antibacterial films was calculated using the following formula.

$$\mathrm{WVP} = \frac{\Delta m \times \mathrm{x}}{S \times \Delta P \times t}$$

where *WVP* is water vapor permeability (g•cm/cm^2•s•Pa), △m is the weight change of the test dish (g), *x* is the thickness of the sample (cm), *S* is the mouth area of the test dish (cm^2), $\triangle P$ is the water vapor pressure difference between both sides of the sample, respectively (Pa), t is the test time (s).

The oxygen permeability (OP) of the HPS nano antibacterial films was determined and modified using the method of Sun et al., (2019). OP of the samples is calculated as the product of weight change and film thickness, divided by the test area and test time.

2.4.5. Antibacterial properties

The inhibitory effect of HPS nano antibacterial films on GPB and GNB in vitro was evaluated by the disk diffusion method (Singh et al., 2018). Firstly, *S. aureus* and *E. coli* cultures were grown in the shaker incubator at 37 °C for 18–24 h. The bacterial suspension was diluted to the concentration of 10^5 CFU/mL (Shao et al., 2020). Subsequently, 100 μL of the bacterial suspension was coated on nutrient agar Petri dish. The samples were cut into circular slices (diameter 6 mm) by the hole punch, and spread on the Petri dish individually. The Petri dish was placed in the SPX-250B incubator (Boxun Medical Biological Instrument Co., Ltd., China) at 37 °C for 24 h. Finally, the diameter of inhibition zone was measured by the vernier caliper.

2.5. Statistical analysis

All the measurements were performed in triplicate for each treatment. Experimental data was statistically analyzed using one-way analysis of variance (ANOVA), and multiple comparisons between treatment means were completed by Duncan's tests. The statistical significance level of $p < 0.05$ was assessed using SPSS statistics software 19.0.

3. Results and discussion

3.1. Characterization of the films

3.1.1. Morphology

The micrstructure of the HPS nano antibacterial films is investigated by SEM, and the morphology of the films are shown in Fig. 1 (surface morphology) and Fig. 2 (cross-section morphology). The surface morphology of E0L1 and E1.5L0 was similar to E0L0, showing smooth, homogeneous, uniform, without cracks and holes. It showed that the film-forming substrates have good compatibility. It indicated that EDTA and LY were well dispersed in the matrix of the HPS nano antibacterial films, and the film-forming substrates had good compatibility. The surface morphology of E1.5L1 exhibited relatively smooth, uniform with slightly cracked. It indicated that EDTA and LY were well dispersed in the film matrix. However, E2.5L1 showed roughness and visible irregular cracks. It may be attributed to the uneven dispersion and local enrichment of the high content of EDTA and LY in the matrix during film preparation. These results were consistent with the research conclusions of Wang et al., (2021), who reported the immobilization of lysozyme and sodium alginate on layer-by-layer self-assembled cellulose acetate films. Ahmad et al., (2012) reported the excessive addition was likely to result in discontinuities or irregularities in the film structure.

Fig. 2 depicts that the cross-section morphology of the HPS nano antibacterial films is dense. Compared to E0L0 with a more regular cross-section, E0L1, E1.5L0, E1.5L1 and E2.5L1 became more compact,

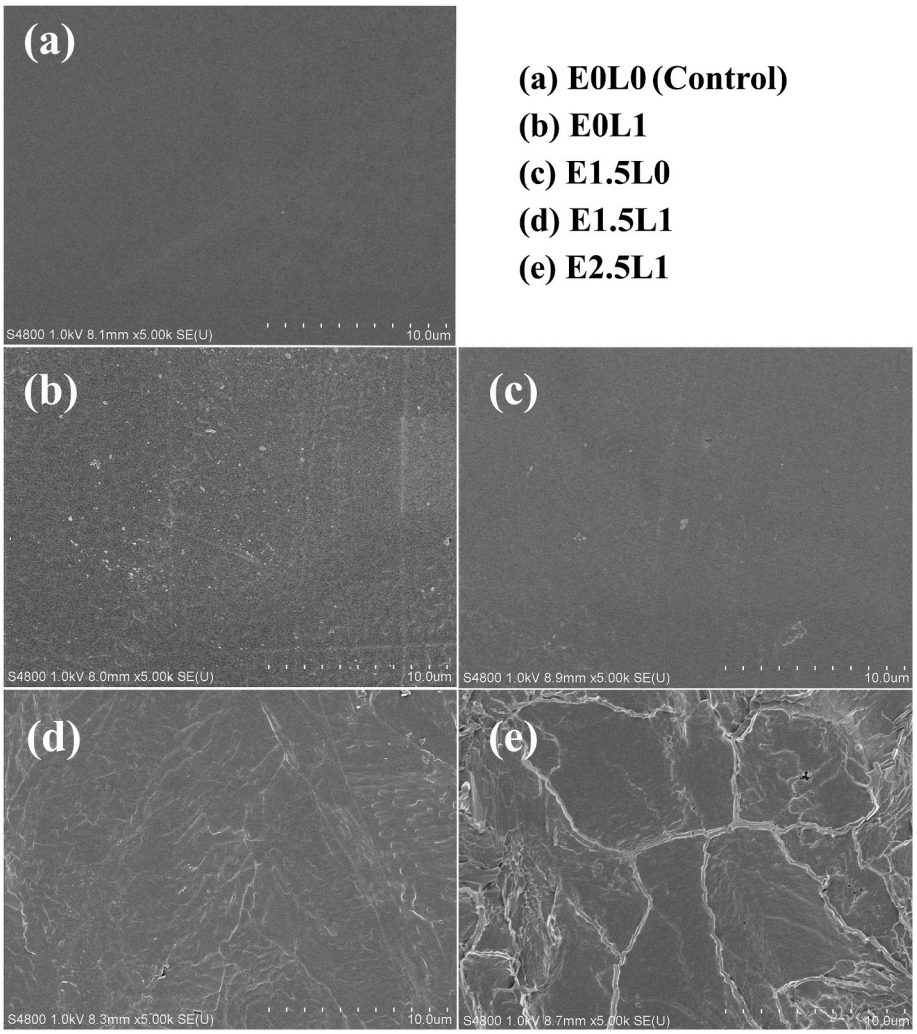

Fig. 1. Surface morphology of the HPS nano antibacterial films.

rough with a higher degree of shriveling and obvious multi-layer structure. The cross-sectional morphology of the HPS nano antibacterial films with different formulations was not completely consistent due to the EDTA and LY increased interaction among the film matrix networks. The results indicated that the synergistic effects of EDTA and LY had significant effects on microstructures of the HPS nano antibacterial films. These experimental results were predictable and similar results were reported by Leelaphiwat et al., (2022) the PBAT/TPS biodegradable films were rough due to the plasticizing effect of Nisin and EDTA.

3.1.2. FTIR

FTIR spectra is used to reveal the interfacial interactions of different components. Fig. 3 clearly shows the FTIR spectra of the HPS nano antibacterial films and displays the fingerprint region and unique absorption bands between 500 and 4000 cm^{-1} attributed to different constitutive materials of the films. All HPS nano antibacterial films had a broad absorption peak from 3000 to 3700 cm^{-1} belonged to the inter- and intra-molecular hydrogen bonding in hydroxyl groups (-OH) and imino group (-NH) (Brandelero et al., 2011). E0L0 showed the maximum absorption peak at 3282 cm^{-1}, which was attributed to the stretching vibration of –OH. Incorporating LY, the maximum absorption peak shifted to lower wavenumbers, indicating the existence of hydrogen bonding between film matrix and LY (Movasaghi et al., 2008). Moreover, the addition of EDTA resulted in a broadening band with a stronger intensity. The characteristic peaks of HPS nano antibacterial films appeared at 2922 cm^{-1} was due to the asymmetric stretching vibration of = C–H and –NH_3(Shahbazi et al., 2019). The difference in intensity and frequency of this region was related to the concentration of LY and EDTA. Similar results were studied by Gussoni et al. (Gussoni and Castiglioni 2000), who revealed that the carbon atoms in C–H bond might be hybridized with the negatively charged carboxyl group in EDTA, and the different hybridization states were related to bond length, bond energy and force constant.

The absorption bands of the HPS nano antibacterial films appeared at 1720-1590 cm^{-1} (amide I), 1480-1590 cm^{-1} (amide-II) and 1335-1200 cm^{-1} (amide-III) were related to C═O stretching vibration and COO- asymmetrical stretching, N–H bending vibration and C–N stretching vibration, C–N vibration and CH_2 groups vibration, respectively (Wang et al., 2018). As expected, the intensity and frequency of the bands from 1720 to 1200 cm^{-1} were changed with the addition of LY. The absorption between 1200 and 1100 cm^{-1} was attributed to asymmetric and symmetric stretching of C–O–C. The typical peaks of HPS nano antibacterial films at 1022 cm^{-1} were assigned to C–O stretching vibration. It was worth noting that the typical peak of HPS nano antibacterial films was enhanced as the EDTA concentration increased from 1.5 % to 2.5 %. The results indicated that the film matrix interacts with EDTA via alkylation, which was corresponded with the C–O stretching vibration of ether and ester bonds in the molecule (Nobrega et al., 2012).

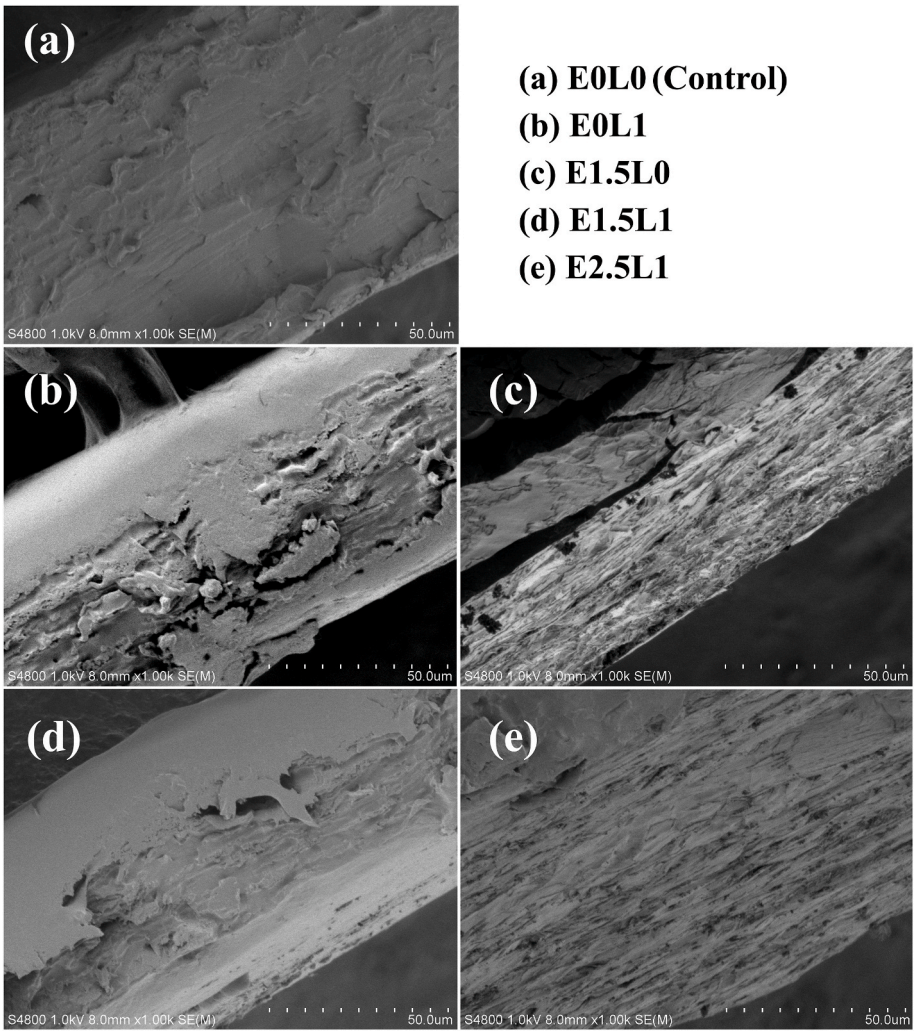

Fig. 2. Cross-section morpholog of the HPS nano antibacterial films.

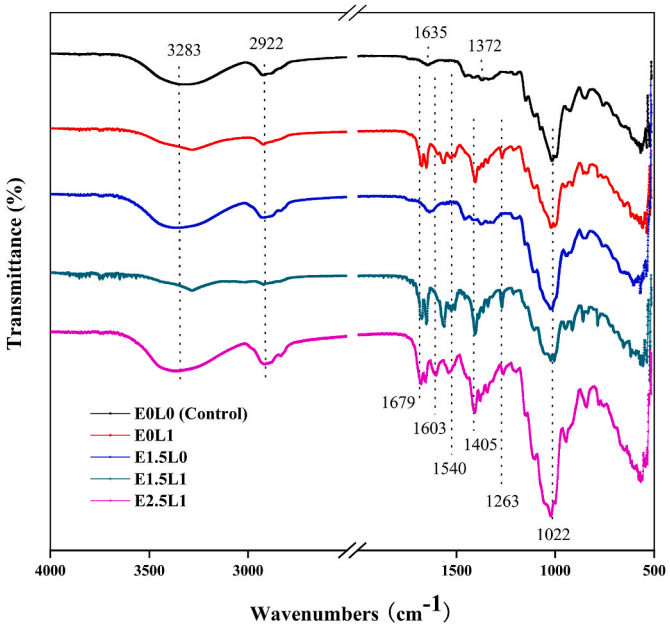

Fig. 3. FTIR spectra of the HPS nano antibacterial films.

3.2. Properties of the films

3.2.1. Color properties

The appearance and color properties of the films are important factors affecting consumer purchasing decision when they are applied to food products. The visual appearance of the HPS nano antibacterial films was white and translucent which exhibited smooth and homogenous surface without any holes or cracks, and easy to peel off. The L*, a^*, b^* and $\triangle E^*$ values of the HPS nano antibacterial films are exhibited in Table 1. The L*, a^*, b^* and $\triangle E^*$ of E0L0 were 91.25, −1.08, +1.40 and 3.69, respectively. Compared with E0L0, the L^*, a^*, b^* and $\triangle E^*$ of the HPS nano antibacterial films were increased with the incorporation of EDTA and LY. E2.5L1 had the highest L^*, a^*, b^* and $\triangle E^*$, which were 92.46, −0.71, 3.63, and 4.88, respectively. It was attributed to the native color of EDTA and LY is white. Furthermore, the increase of color parameters might be due to the light-scattering effect of the additives (EDTA and LY) and the film matrix. Khodanazary (Khodanazary 2019) had similar results in the study of gelatin polycaprolactone/lysozyme/pomegranate peel extract composite film.

3.2.2. Optical properties

The ultraviolet spectrophotometer is a simple and direct method of measuring the optical properties of films. The effect of EDTA and LY on the optical properties in the wavelength range between 200 nm and 800 nm of the HPS nano antibacterial films is exhibited in Fig. 4. Fig. 4 showed that the HPS nano antibacterial films were opaque, and the light

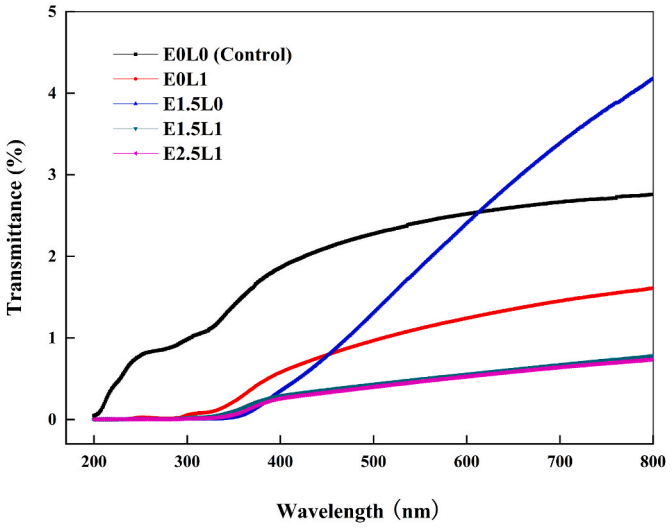

Fig. 4. Optical properties of the HPS nano antibacterial films.

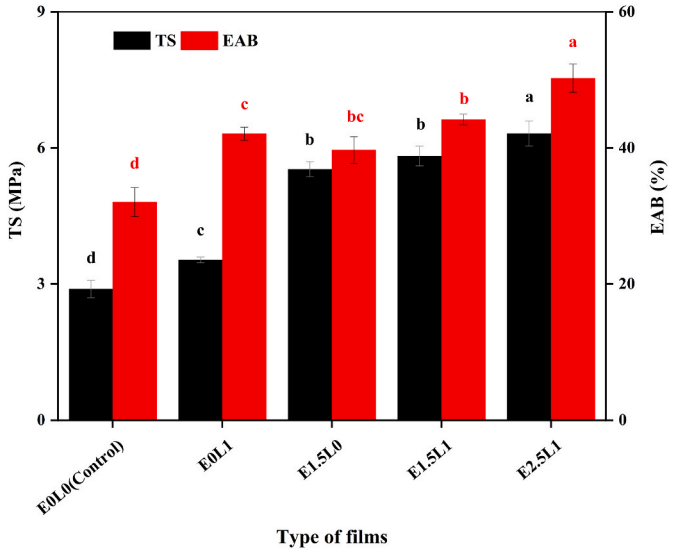

Fig. 5. Mechanical properties of the HPS nano antibacterial films.

transmission in both UV and visible region was less than 5 %. The results suggested that the HPS nano antibacterial films had excellent barrier properties, which was due to as a white pigment TiO_2 limit the penetration of UV and visible light energy. The incorporation of additives (EDTA or LY) reduced the light transmission of the HPS nano antibacterial films in the UVC region (200–280 nm), UVB region (280–315 nm) and UVA region (315–400 nm). The light transmission of the HPS nano antibacterial films in the visible region was reduced by LY. Meanwhile, with the increase of EDTA from 0 to 1.5 %, the light transmission of the HPS nano antibacterial films decreased significantly. The synergistic effects of EDTA and LY significantly decreased the light transmission of the HPS nano antibacterial films. With the increase of EDTA from 1.5 % to 2.5 %, the light transmission of the HPS nano antibacterial films had no obvious change and reached a plateau in UV and visible regions. It indicated that E1.5L1 and E2.5L1 had UV and visible light absorption properties, and showed better UV and visible light barrier properties. UV and visible light accelerate lipid oxidation in food, resulting in nutrient loss, discoloration, and off-flavor. Therefore, the optical properties of films are an important factor affecting its appearance, marketability and applicability. The optical properties of HPS nano antibacterial films containing EDTA and LY exhibited potential in food packaging, which will broaden the application of HPS nano antibacterial films.

3.2.3. Mechanical properties

It is very important that the polymer film has sufficient mechanical properties to maintain food integrity. Therefore, TS and EAB of the HPS nano antibacterial films containing different amounts of EDTA and LY are investigated, and the results are shown in Fig. 5. The incorporation of EDTA and LY in the HPS nano antibacterial films caused a marked increase in TS. Meanwhile, TS was significantly increased with the synergistic effects of EDTA and LY, especially the TS of E2.5L1 reaches the highest value of 6.329 MPa. It indicated E2.5L1 enhanced the TS via alkylation, which was consistent with the FTIR. Kristo, E. et al. (Kristo and Biliaderis 2007) proposed that the highest crystallinity of PBAT/TPS-nisin 9% formed crystallite reinforced fillers that enhanced mechanical properties of TS. Leelaphiwat et al., (2022) reported that the films containing Nisin-EDTA had higher TS than the films containing the same concentration of Nisin. It suggested that EDTA improved the TS, possibly due to enhanced compatibility and plasticisation of the films matrix polymer. Similar trends were found in the study of Pérez-Arauz, Á. O. (Pérez-Arauz et al., 2021), the TS of the base film was increased by the addition of EDTA. Pattarasiriroj et al., (2020) reported an increased TS in rice-flour-gelatine-based films reinforced with LY.

The effects of EDTA and LY on the EAB of HPS nano antibacterial films were similar to the trend of TS. Compared to E0L0, EAB of E0L1 and E1.5L0 were increased 31.3 %, 23.9 %, respectively. It was consistent with the study of Rawdkuen et al., (2012) who observed EAB of the gelatin-based film was increased about 5 times with catechin-lysozyme at the levels of 0.5 %. Pattarasiriroj et al., (2020) observed an increase in EAB from 84.17 % to 97.35 % incorporating catechin-lysozyme into rice-flour-gelatine-based films. Qin et al., (2020) concluded that the increase in TS of chitosan–NC– pomegranate rind extract was due to the dispersion of NC-CL in the polymer matrix and the strong interaction between the polymers. The synergistic effects of EDTA and LY significantly enhanced the EAB of the HPS nano antibacterial films, and the most significant increase in EAB up to 50.24 % was found in E2.5L1. It was attributed to EDTA and LY were used as plasticizers to enhance the stretch ability of the films, and the EAB of the HPS nano antibacterial films were increased. From these findings, it can be concluded that the addition of EDTA and LY significantly effects the EAB of the HPS nano antibacterial films.

3.2.4. Barrier properties

Water and oxygen play important roles in food spoilage. WVP and OP can be reduced by suitable food packaging films, and the food shelf life can be extended. The WVP of the HPS nano antibacterial films

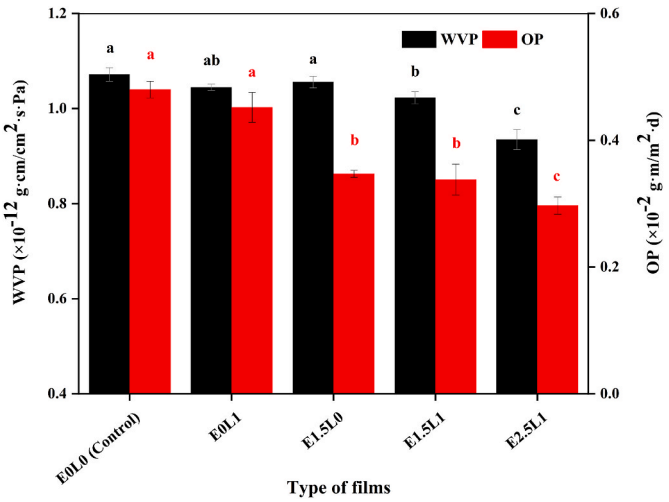

Fig. 6. Barrier properties of the HPS nano antibacterial films.

incorporated with EDTA and LY at different concentrations is presented in Fig. 6. Compared with the control film (E0L0), WVP was reduced slightly containing an additive (EDTA or LY). The synergy of EDTA and LY resulted in the highest and lowest WVP of 1.0718×10^{-12} g cm/cm^2•s•Pa (E0L0), 0.9350×10^{-12} g cm/cm^2•s•Pa (E2.5L1), respectively. It may be due to the structure modification of the HPS network by the synergy of EDTA and LY. It was consistent with the above-mentioned SEM results. Rawdkuen et al., (2012) reported catechin-lysozyme functional groups chemically react with the gelatin film matrix components to form stable bonds and complex structures, resulting in restricted water vapor transfer and reduced WVP of the HPS nano antibacterial films. Moreover, Leelaphiwat et al., (2022) found that films containing EDTA had lower WVP than nisin, which may be due to EDTA can effectively improve the compatibility of film matri.

As shown in Fig. 6, the OP of the HPS nano antibacterial films decreased from 0.480×10^{-2} g m/m^2•d (E0L0) to 0.297×10^{-2} g m/m^2•d (E2.5L1). It indicated that the dense structure formed by the synergistic effects of EDTA and LY effectively inhibited the diffusion of oxygen and improved the oxygen barrier properties of the HPS nano antibacterial films. Similar results were described by previous research, the OP of the PBAT/TPS biodegradable films decreased by 1.4 times with the addition of EDTA (Leelaphiwat et al., 2022).

3.2.5. Antibacterial properties

The growth of microorganisms is an important factor leading to food spoilage and foodborne illnesses, and films with excellent antibacterial properties are promising in food packaging. Antibacterial properties of the HPS nano antibacterial films incorporated with EDTA and LY at various concentrations against *S. aureus* and *E. coli* are presented in Table 1. The control film (E0L0) and the presence of one additive alone (E0L1 and E1.5L0) had no inhibitory effect on the test microorganism. It indicated that incorporating a single, low-concentration antibacterial agent into the HPS nano antibacterial films did not have any antibacterial effect. The films containing EDTA and LY (E1.5L1) showed antibacterial activity against both tested microorganisms, and the inhibition zone diameters of *S. aureus* and *E. coli* were 0.29 and 1.03, respectively. The synergy of EDTA and LY had a strong inhibitory effect on GNB and a weaker inhibitory effect on GPB. Simultaneously, *E. coli*. was more sensitive to the synergistic inhibitory effect of EDTA and LY, while *S. aureus* was less susceptible to the synergistic inhibition. According to literature, EDTA has a strong antibacterial activity on GNB and a weaker antibacterial activity on GPB(Hua et al., 2016). Simultaneously, as an attractive antibacterial agent in the food and medical care industry, the antibacterial activity of LY was related to its muramidase activity, cationic and hydrophobicity (Masschalck and Michiels 2003).

In addition, the antibacterial activity of the combination of EDTA and LY significantly increased as the EDTA concentration increased. The incorporation of EDTA increased from 1.5 % to 2.5 %, the inhibition zone diameters of *S. aureus* and *E. coli* increased from 0.29 mm to 3.69 mm and 1.03 mm–4.28 mm, respectively. E2.5L1 exhibited the highest antibacterial activity. Compared with E1.5L1, the inhibition zone diameters of *S. aureus* and *E. coli* increased by 12.7 times and 4.2 times. It demonstrated that as a metal chelator, EDTA showed a strong inhibitory effect on bacterial growth. The synergistic inhibition of EDTA and LY on GPB and GNB can be significantly improved by increasing the amount of EDTA. It might be due to the chelation of EDTA with cations such as Mg^{2+}, Ca^{2+}, Na^{+}, K^{+} on the cell surface, which destroyed the integrity of the cell membrane and facilitated the invasion of LY (Belfiore et al., 2007). Simultaneously, Murdock et al., (2007) showed EDTA enhanced the penetration of LY into the outer membrane of GNB. The small molecular size of EDTA provided the fluidity of the polymer, increased antibacterial activity of PBAT/TPS biodegradable films containing nisin, and effectively delayed spoilage of pork (Leelaphiwat et al., 2022).

4. Conclusions

In this study, the HPS nano antibacterial films were fabricated via a low-cost solvent casting method, and the incorporation of EDTA and LY improved mechanical properties, barrier properties and antibacterial properties of the HPS nano antibacterial films. The incorporation of EDTA and LY effectively increased interaction among the films matrix networks, giving more compact film microstructures. The results of SEM and FTIR showed that the synergy of EDTA and LY had significant effects on the HPS nano antibacterial films. The color parameters of the HPS nano antibacterial films were increased with the addition of EDTA and LY. The synergistic effect of EDTA and LY had significant effects on the optical properties and mechanical properties of the HPS nano antibacterial films. The addition of EDTA and LY improved the barrier properties, which was the same as the observation of SEM above-mentioned. Furthermore, the films containing EDTA and LY had significant synergistic effects on the antibacterial activity against GPB (*S. aureus*) and GNB (*E.coli*). The results demonstrated that as a biodegradable active packaging material, the synergistic addition of EDTA and LY in the HPS nano antibacterial films is promising, especially in the food packaging industry, and its commercialization requires further research in actual food systems.

CRediT authorship contribution statement

Xinru Shao: conceived of the study and wrote the manuscript (equal). **Haitao Sun:** conceived of the study and wrote the manuscript (equal), participated in its design and coordination. **Ximing Wang:** performed the, Formal analysis, and reviewed the manuscript. **Ran Zhou:** participated in its design and coordination, All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

Abarca, R.L., Medina, J., Alvarado, N., Ortiz, P.A., López, B.C., 2022. Biodegradable gelatin-based films with nisin and edta that inhibit escherichia coli. PLoS One 17 (3). https://doi.org/10.1371/journal.pone.0264851.

Adilah, Z.A.M., Jamilah, B., Hanani, Z.A.N., 2018. Functional and antioxidant properties of protein-based films incorporated with mango kernel extract for active packaging. Food Hydrocolloids 74, 207–218. https://doi.org/10.1016/j.foodhyd.2017.08.017.

Ahmad, M., Benjakul, S., Prodpran, T., Agustini, T.W., 2012. Physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils. Food Hydrocolloids 28 (1), 189–199. https://doi.org/10.1016/j.foodhyd.2011.12.003.

Ahmad, M., Nirmal, N.P., Danish, M., Chuprom, J., Jafarzedeh, S., 2016. Characterisation of composite films fabricated from collagen/chitosan and collagen/soy protein isolate for food packaging applications. RSC Adv. 6 (85), 82191–82204. https://doi.org/10.1039/c6ra13043g.

Belfiore, C., Castellano, P., Vignolo, G., 2007. Reduction of Escherichia coli population following treatment with bacteriocins from lactic acid bacteria and chelators. Food Microbiol. 24 (3), 223–229. https://doi.org/10.1016/j.fm.2006.05.006.

Bosch, E.H., Van Doorne, H., De Vries, S., 2000. The lactoperoxidase system: the influence of iodide and the chemical and antimicrobial stability over the period of

about 18 months. J. Appl. Microbiol. 89 (2), 215–224. https://doi.org/10.1046/j.1365-2672.2000.01098.x.
Brandelero, R.P.H., Grossmann, M.V.E., Yamashita, F., 2011. Effect of the method of production of the blends on mechanical and structural properties of biodegradable starch films produced by blown extrusion. Carbohydr. Polym. 86 (3), 1344–1350. https://doi.org/10.1016/j.carbpol.2011.06.045.
Chae, Y., An, Y.J., 2018. Current research trends on plastic pollution and ecological impacts on the soil ecosystem: a review. Environ. Pollut. 240, 387–395. https://doi.org/10.1016/j.envpol.2018.05.008.
Chang, S.H., Chen, Y.J., Tseng, H.J., Hsiao, H.I., Chai, H.J., Shang, K.C., Tsai, G.J., 2021. Applications of nisin and edta in food packaging for improving fabricated chitosan-polylactate plastic film performance and fish fillet preservation. Membranes 11 (11). https://doi.org/10.3390/membranes11110852.
Chen, J., Chen, F.S., Long, Z., Dai, L., Wang, S.F., Zhang, D., 2019. Hydroxypropyl starch-based films reinforced by incorporation of alkalized microcrystalline cellulose. Polym. Compos. 40, E856–E864. https://doi.org/10.1002/pc.25048.
Chen, X., Cui, F.H., Zi, H., Zhou, Y.C., Liu, H.S., Xiao, J., 2019. Development and characterization of a hydroxypropyl starch/zein bilayer edible film. Int. J. Biol. Macromol. 141, 1175–1182. https://doi.org/10.1016/j.ijbiomac.2019.08.240.
Davoodi, M., Kavoosi, G., Shakeri, R., 2017. Preparation and characterization of potato starch-thymol dispersion and film as potential antioxidant and antibacterial materials. Int. J. Biol. Macromol. 104, 173–179. https://doi.org/10.1016/j.ijbiomac.2017.05.145.
Duan, Q.F., Bao, X.Y., Yu, L., Cui, F.H., Zahid, N., Liu, F.S., Liu, H.S., 2023. Study on hydroxypropyl corn starch/alkyl ketene dimer composite film with enhanced water resistance and mechanical properties. Int. J. Biol. Macromol. 253 https://doi.org/10.1016/j.ijbiomac.2023.126613.
Ehsani, A., Hashemi, M., Afshari, A., Aminzare, M., Raeisi, M., Zeinali, T., 2020. Effect of different types of active biodegradable films containing lactoperoxidase system or sage essential oil on the shelf life of fish burger during refrigerated storage. LWT-Food Sci. 117 https://doi.org/10.1016/j.lwt.2019.108633.
Gong, M., Anderson, D., Rathgeber, B., MacIsaac, J., 2017. The effect of dietary lysozyme with edta on growth performance and intestinal microbiota of broiler chickens in each period of the growth cycle. J. Appl. Poultry Res. 26 (1), 1–8. https://doi.org/10.3382/japr/pfw041.
Gussoni, M., Castiglioni, C., 2000. Infrared intensities. Use of the ch-stretching band intensity as a tool for evaluating the acidity of hydrogen atoms in hydrocarbons. J. Molecul. Struct. 521, 1–18. https://doi.org/10.1016/s0022-2860(99)00421-4.
Hua, W.W., Lou, Y.M., Xu, W.Y., Cheng, Z.X., Gong, X.W., Huang, J.Y., 2016. Batch affinity adsorption of his-tagged proteins with edta-based chitosan. Appl. Microbiol. Biotechnol. 100 (2), 879–891. https://doi.org/10.1007/s00253-015-7137-5.
Khodanazary, A., 2019. Quality characteristics of refrigerated mackerel scomberomorus commerson using gelatin-polycaprolactone composite film incorporated with lysozyme and pomegranate peel extract. Int. J. Food Prop. 22 (1), 2056–2070. https://doi.org/10.1080/10942912.2019.1702997.
Kristo, E., Biliaderis, C.G., 2007. Physical properties of starch nanocrystal-reinforced pullulan films. Carbohydr. Polym. 68 (1), 146–158. https://doi.org/10.1016/j.carbpol.2006.07.021.
Leelaphiwat, P., Pechprankan, C., Siripho, P., Bumbudsanpharoke, N., Harnkarnsujarit, N., 2022. Effects of nisin and edta on morphology and properties of thermoplastic starch and pbat biodegradable films for meat packaging. Food Chem. 369 https://doi.org/10.1016/j.foodchem.2021.130956.
Lesnierowski, G., Yang, T.Y., 2021. Lysozyme and its modified forms: a critical appraisal of selected properties and potential. Trends Food Sci. Technol. 107, 333–342. https://doi.org/10.1016/j.tifs.2020.11.004.
Mania, S., Cieslik, M., Konzorski, M., Swiecikowski, P., Nelson, A., Banach, A., Tylingo, R., 2020. The synergistic microbiological effects of industrial produced packaging polyethylene films incorporated with zinc nanoparticles. Polym. Bull. (Heidelberg, Ger.) 12 (5). https://doi.org/10.3390/polym12051198.
Masschalck, B., Michiels, C.W., 2003. Antimicrobial properties of lysozyme in relation to foodborne vegetative bacteria. Crit. Rev. Microbiol. 29 (3), 191–214. https://doi.org/10.1080/713610448.
Morsy, M.K., Elsabagh, R., Trinetta, V., 2018. Evaluation of novel synergistic antimicrobial activity of nisin, lysozyme, edta nanoparticles, and/or zno nanoparticles to control foodborne pathogens on minced beef. Food Control 92, 249–254. https://doi.org/10.1016/j.foodcont.2018.04.061.
Movasaghi, Z., Rehman, S., Rehman, I.U., 2008. Fourier transform infrared (ftir) spectroscopy of biological tissues. Appl. Spectrosc. Rev. 43 (2), 134–179. https://doi.org/10.1080/05704920701829043.
Murdock, C.A., Cleveland, J., Matthews, K.R., Chikindas, M.L., 2007. The synergistic effect of nisin and lactoferrin on the inhibition of listeria monocytogenes and escherichia coli o157 : H7. Lett. Appl. Microbiol. 44 (3), 255–261. https://doi.org/10.1111/j.1472-765X.2006.02076.x.
Nobrega, M.M., Olivato, J.B., Muller, C.M.O., Yamashita, F., 2012. Biodegradable starch-based films containing saturated fatty acids: thermal, infrared and Raman spectroscopic characterization. Polimeros 22 (5), 475–480. https://doi.org/10.1590/s0104-14282012005000068.
Ozer, B.B.P., Uz, M., Oymaci, P., Altinkaya, S.A., 2016. Development of a novel strategy for controlled release of lysozyme from whey protein isolate based active food packaging films. Food Hydrocolloids 61, 877–886. https://doi.org/10.1016/j.foodhyd.2016.07.001.
Pérez-Arauz, A.O., Rodríguez-Hernández, A.I., López-Cuellar, M.D., Martínez-Juárez, V. M., Chavarría-Hernández, N., 2021. Films based on pectin, gellan, edta, and bacteriocin-like compounds produced by *Streptococcus infantarius* for the bacterial control in fish packaging. J. Food Process. Preserv. 45 (1) https://doi.org/10.1111/jfpp.15006.
Pattarasiriroj, K., Kaewprachu, P., Rawdkuen, S., 2020. Properties of rice flour-gelatine-nanoclay film with catechin-lysozyme and its use for pork belly wrapping. Food Hydrocolloids 107. https://doi.org/10.1016/j.foodhyd.2020.105951.
Prudêncio, C.V., Mantovani, H.C., Cecon, P.R., Prieto, M., Vanetti, M.C.D., 2016. Temperature and pH influence the susceptibility of Salmonella Typhimurium to nisin combined with EDTA. Food Control 61, 248–253. https://doi.org/10.1016/j.foodcont.2015.09.042.
Qian, M.Y., Liu, D.H., Zhang, X.H., Yin, Z.P., Ismail, B.B., Ye, X.Q., Guo, M.M., 2021. A review of active packaging in bakery products: applications and future trends. Trends Food Sci. Technol. 114, 459–471. https://doi.org/10.1016/j.tifs.2021.06.009.
Qin, Y., Xu, F.F., Yuan, L.M., Hu, H.X., Yao, X.Y., Liu, J., 2020. Comparison of the physical and functional properties of starch/polyvinyl alcohol films containing anthocyanins and/or betacyanins. Int. J. Biol. Macromol. 163, 898–909. https://doi.org/10.1016/j.ijbiomac.2020.07.065.
Radhalakshmi, V., Raman, M., Joy, M.R., 2023. Development of active packaging film based on poly (lactic acid) incorporated with Piper betel leaf ethanolic extract and its application in the shelf-life extension of tuna meat. Int. J. Biol. Macromol. 246 https://doi.org/10.1016/j.ijbiomac.2023.125751.
Rawdkuen, S., Suthiluk, P., Kamhangwong, D., Benjakul, S., 2012. Mechanical, physico-chemical, and antimicrobial properties of gelatin-based film incorporated with catechin-lysozyme. Chem. Cent. J. 6 https://doi.org/10.1186/1752-153x-6-131.
Romruen, O., Kaewprachu, P., Karbowiak, T., Rawdkuen, S., 2022. Development of smart bilayer alginate/agar film containing anthocyanin and catechin-lysozyme. Polym. Bull. (Heidelberg, Ger.) 14 (22). https://doi.org/10.3390/polym14225042.
Shahbazi, A., Marnani, N.N., Salahshoor, Z., 2019. Synergistic and antagonistic effects in simultaneous adsorption of pb(ii) and cd(ii) from aqueous solutions onto chitosan functionalized edta-silane/mgo. Biocatal. Agric. Biotechnol. 22 https://doi.org/10.1016/j.bcab.2019.101398.
Shao, X.R., Sun, H.T., Jiang, R.P., Yu, Y.X., 2020. Physical and antibacterial properties of corn distarch phosphate/carboxymethyl cellulose composite films containing tea polyphenol. J. Food Process. Preserv. 44 (4) https://doi.org/10.1111/jfpp.14401.
Silva, Nhcs, Vilela, C., Almeida, A., Marrucho, I.M., Freire, C.S.R., 2018. Pullulan-based nanocomposite films for functional food packaging: exploiting lysozyme nanofibers as antibacterial and antioxidant reinforcing additives. Food Hydrocolloids 77, 921–930. https://doi.org/10.1016/j.foodhyd.2017.11.039.
Singh, S., Gaikwad, K.K., Lee, Y.S., 2018. Antimicrobial and antioxidant properties of polyvinyl alcohol bio composite films containing seaweed extracted cellulose nanocrystal and basil leaves extract. Int. J. Biol. Macromol. 107, 1879–1887. https://doi.org/10.1016/j.ijbiomac.2017.10.057.
Sun, H.T., Shao, X.R., Zhang, M.F., Wang, Z.Y., Dong, J.T., Yu, D., 2019. Mechanical, barrier and antimicrobial properties of corn distarch phosphate/nanocrystalline cellulose films incorporated with nisin and epsilon-polylysine. Int. J. Biol. Macromol. 136, 839–846. https://doi.org/10.1016/j.ijbiomac.2019.06.134.
Tkaczewska, J., Jamróz, E., Zajac, M., Guzik, P., Gedif, H.D., Turek, K., Kopec, M., 2023. Antioxidant edible double-layered film based on waste from soybean production as a vegan active packaging for perishable food products. Food Chem. 400 https://doi.org/10.1016/j.foodchem.2022.134009.
Tsai, G.J., Su, W.H., Chen, H.C., Pan, C.L., 2002. Antimicrobial activity of shrimp chitin and chitosan from different treatments and applications of fish preservation. Fisheries Science 68 (1), 170–177. https://doi.org/10.1046/j.1444-2906.2002.00404.x.
Wang, P., Zhang, C., Zou, Y.C., Li, Y., Zhang, H., 2021. Immobilization of lysozyme on layer-by-layer self-assembled electrospun films: characterization and antibacterial activity in milk. Food Hydrocolloids 113. https://doi.org/10.1016/j.foodhyd.2020.106468.
Wang, W.B., Xiao, J., Chen, X., Luo, M.N., Liu, H.S., Shao, P., 2018. Fabrication and characterization of multilayered kafirin/gelatin film with one-way water barrier property. Food Hydrocolloids 81, 159–168. https://doi.org/10.1016/j.foodhyd.2018.02.044.
Wang, Y.R., Cheng, M., Yan, X.R., Zhao, P.X., Wang, K.Y., Wang, Y.F., Wang, J., 2023. Preparation and characterization of an active packaging film loaded with tea tree oil-hydroxyapatite porous microspheres. Ind. Crop. Prod. 199 https://doi.org/10.1016/j.indcrop.2023.116783.
You, P.Q., Wang, L., Zhou, N., Yang, Y., Pang, J., 2022. A ph-intelligent response fish packaging film: konjac glucomannan/carboxymethyl cellulose/blackcurrant anthocyanin antibacterial composite film. Int. J. Biol. Macromol. 204, 386–396. https://doi.org/10.1016/j.ijbiomac.2022.02.027.
Yu, X.P., Yang, Y.Y., Liu, Q., Jin, Z.Y., Jiao, A.Q., 2023. A hydroxypropyl methylcellulose/hydroxypropyl starch nanocomposite film reinforced with chitosan nanoparticles encapsulating cinnamon essential oil: preparation and characterization. Int. J. Biol. Macromol. 242 https://doi.org/10.1016/j.ijbiomac.2023.124605.
Zhang, K.L., Huang, T.S., Yan, H., Hu, X.Z., Ren, T., 2020. Novel ph-sensitive films based on starch/polyvinyl alcohol and food anthocyanins as a visual indicator of shrimp deterioration. Int. J. Biol. Macromol. 145, 768–776. https://doi.org/10.1016/j.ijbiomac.2019.12.159.
Zhang, W.L., Rhim, J.W., 2022. Functional edible films/coatings integrated with lactoperoxidase and lysozyme and their application in food preservation. Food Control 133. https://doi.org/10.1016/j.foodcont.2021.108670.