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Preparation and performance testing of corn starch/pullulan/gallic acid multicomponent composite films for active food packaging

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ABSTRACT

The present study investigated the mechanical characteristics, hydrophobicity, antioxidant and antibacterial properties, FTIR, SEM and XRD of films fabricated with corn starch and pullulan (CS/PUL) by adding different concentrations of Gallic acid (GA) (0%, 0.5%, 1.0%, 1.5% w/v). The mechanical strength and opacity of CS/PUL films were enhanced by the addition of 1.0% GA. The water vapor permeability (WVP) of CS/PUL films was significantly lower in films with GA compared to those without ($P < 0.05$). The addition of GA, especially at concentrations of 1.0% and 1.5%, resulted in considerably better free radical scavenging activities on DPPH than films without GA ($P < 0.05$). Interestingly, the highest water contact angle (WCA) value was observed in films with 0.5% GA, indicating stronger hydrophobicity. Furthermore, the antibacterial capabilities of the films, particularly against *E. coli* and *P. aeruginosa*, improved with an increase in GA concentration. The results of FTIR, SEM and XRD analyses showed that GA was well distributed in the CS/PUL matrix.

1. Introduction

It is challenging to degrade plastic packaging in nature, which has led to increased customer attention towards environmental protection. In recent years, active packaging has gained widespread scholarly interest due to its edible, safe, efficient, and low-cost advantages (Fdg, Fc, Ca, Vg, & Et, 2022). Active packaging typically employs natural polymer compounds as a film-forming matrix with plasticizers or cross-linking agents to cover the interior or surface of the food through spraying, impregnating, coating, or wrapping, thereby preventing the penetration of moisture, oxygen, and other substances (Porta, Mariniello, Pierro, Sorrentino, & Giosafatto, 2011).

Currently, common natural polymer packaging materials include lipids, polysaccharides, and proteins. Among them, starch-based materials have become a research hotspot due to their low cost, good gas barrier, high solubility, and good degradability compared to other natural polymer materials. However, films composed of a single starch component have poor mechanical properties and are brittle. Therefore, the addition of plasticizers and crosslinkers is necessary to improve film performance. Glycerol is a widely used plasticizer in natural polymer packaging. Hazrati, Sapuan, Zuhri, and Jumaidin (2021) discovered that the addition of glycerol to potato starch films improved their flexibility and thermal stability. Additionally, pullulan (PUL), a water-soluble substance, can create colorless, odorless, transparent, flexible, highly oil-resistant, and heat-sealable films with excellent oxygen barrier properties. The films prepared from these materials exhibit good resistance to oil and oxygen, but their mechanical properties are relatively poor. Therefore, it is common practice to enhance their properties by incorporating and blending other substances. Kaewprachu et al. (2017) discovered that the combination of PUL and tapioca starch resulted in the formation of numerous intermolecular hydrogen bonds, leading to an increase in the mechanical strength and hydrophobicity of the composite film. Similarly, Zhao et al. improved the water resistance, mechanical properties, and thermodynamic properties of a film made with rice starch and pullulan.

Plant polyphenols are often used as natural modifiers for preparing polysaccharide protein films. Due to their outstanding antioxidant and antibacterial properties, natural phenolic compounds are considered ideal candidates for active packaging (Sanches-Silva et al., 2014). Some studies have reported that phenols have been added to active packaging

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films to extend the shelf life of food. Gallic acid (GA) (3,4,5- trihydroxy benzoic) is a naturally occurring polyphenolic substance primarily derived from plants (Rajan & Muraleedharan, 2017). Cassava starch/chitosan/GA has special biochemical characteristics and is used in food and medicine due to its antibacterial and antioxidant properties. The bioactive film developed from this combination can effectively enhance the hydrophobic and mechanical properties of the film (Yujia Zhao, Huerta, & Saldaña, 2019). Aydogdu, Yildiz, Aydogdu, Sumnu, Sahin, and Ayhan (2019) discovered that using active packaging material made from lentil powder loaded with GA improved the oxidation stability of walnuts and extended their shelf life.

The purpose of this study was to prepare and characterize a CS/PUL film incorporated with GA, and to investigate the impact of GA on the mechanical and structural properties of the polysaccharide-based films. The study assessed the tensile strength (TS), elongation at break (EAB), water vapor permeability (WVP), scanning electron microscope (SEM), Fourier transforms infrared (FTIR), and hydrophobic properties. Additionally, the study evaluated whether GA improved the antioxidant and antibacterial properties of the polysaccharide films. The results of this study are not only useful for improving the utilization rate and application range of polysaccharide-based films, but also for developing a new film that can be used in food packaging.

2. Materials and methods

2.1. Materials

Corn starch (CAS: 9005–25-8) was purchased from McLean Biochemical Co., Ltd (Shanghai, China). Pullulan (CAS: 9057–02-7), gallic acid (CAS: 149–91-7) and glycerol (CAS: 56–81-5) were purchased from Aladdin Reagent Co., Ltd (Shanghai, China). *E. coli*, *S. aureus* and *P. aeruginosa* were obtained from China Strain Conservation Centre. LB broth and LB agar were obtained McLean Biotechnology Co., Ltd (Shanghai, China) for microbial detection.

2.2. Preparation of the film

First, 3.5 g CS and 1.0 g PUL were mixed in 100 mL of sterile distilled water, heated and stirred at 85° C about 20 min. Then mixed GA (0.0 %, 0.5 %, 1.0 %, 1.5 %, w/v) and stirred for 10 min. 40% glycerol based on CS (as a plasticizer) was added to the prepared mixture and stirred for 10 min. Afterwards, 40 mL of the CS/PUL/GA mixture was poured onto a round plastic plate and dried at room temperature for 72 h.

2.3. Characterization of film

2.3.1. GA cumulative release properties

The Folin-Ciocalteau method was selected to determine the GA content (Kwaw et al., 2018). In release experiments, 95% ethanol is commonly used as the food simulant. To conduct the experiment, 200 mg of film was placed in a conical flask containing 20 mL of 95% ethanol at room temperature. Every 3 h, 1 mL of simulant was taken from the conical flask and supplemented with 1 mL of 95% ethanol. The absorbance was measured using a spectrophotometer PE-Lambda 35 (PE Instrument Co., Ltd) at 760 nm.

2.3.2. Film thickness

The film thickness was measured using the Model ID-5202–25 μm (Zhejiang Electronics Co., Ltd., China). To calculate the average thickness, five different locations were selected on each film.

2.3.3. Mechanical properties

Mechanical performance was evaluated using an XLW intelligent electric traction tester (Jinan Languang, China). The tensile strength (TS) and elongation at break (EAB) were determined following the method described by Xi et al. (2021).

2.3.4. Water vapor permeability

Water vapor permeability was determined by Marangoni Júnior, Silva, Vieira, and Alves (2021). Place the film in a 50 mL centrifuge tube with saturated sodium chloride solution (25 ° C, 75.5% RH_1) in a dry silica gel (0% RH_2). Then weighed 8 times in 24 h. Water vapour transfer rate (WVTR) is defined as gradient (g/s). The CS/PUL/GA film's WVP was calculated by Eq. (1):

$$WVP = \frac{WVTR \times T}{P(RH_1 - RH_2)} \tag{1}$$

P was the saturated vapor pressure at 25 ° C and T was the thickness of CS/PUL/GA film.

2.3.5. Color, light transmission and opacity

The L^*, a^* and b^* value of the CS/PUL/GA films were calculated with a CR-400 colorimeter (Konica Minolta, Japan) by Zhou et al. (2021). Standard white board calibration ($L^*_0 = 92.91$, $a^*_0 = -0.51$, $b^*_0 = 5.52$). The ΔE of CS/PUL/GA films was by Eq. (2):

$$\Delta E = \sqrt{(L^* - L^*_0)^2 + (a^* - a^*_0)^2 + (b^* - b^*_0)^2} \tag{2}$$

The light transmission was described by Jiang et al. (2023). In briefly, a 40 mm × 10 mm film was inserted into the PE-Lambda 35 spectrophotometer (PE Instrument Co., Ltd), and scanned in the wavelength range of 300–800 nm. Calibrate the instrument with an empty test tube, the opacity of the CS/PUL/GA films was calculated using Eq. (3):

$$\text{Opacity} = \frac{A_{600}}{T} \tag{3}$$

The T was the thickness of the film.

2.3.6. Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD)

The NicoletTMiS^{TM} 50 FTIR Spectrometer (Thermo Scientific, USA) was used to scan the deep structure of the CS/PUL/GA films with a reduced total reflectance (ATR) accessory, and the spectrum was obtained in the range of 400 to 4000 cm^{-1} wavenumbers (Parveen, Chaudhury, Dasmahapatra, & Dasgupta, 2019).

The X-ray diffraction performance of films were assessed by X-ray diffractometer (Brooke D8 advance). The test diffraction angle 2 θ of the film ranged from 10° to 80°, the test rate was 5°/min, and the sample needed to be equilibrated in an RH 50% desiccator for 48 h before testing.

2.3.7. Scanning electron microscope and water contact angle

SEM (Hitachi S-4800, Japan) was used to observe the morphology of the film at 3 kV accelerating voltage. The WCA of the film was measured by a contact Angle tester (Theta Flex, Biolin Scientific).

2.3.8. The free radical scavenging activity of DPPH (1, 1, 1, 2 -Picrylhydrazine)

According to Raspo, Gomez, and Andreatta (2018), the film containing 200 mg was mixed with 25 mL of distilled water and oscillated for 24 h at room temperature using a ZD-85 oscillator (Changzhou Guoyu Instrument Manufacturing Co., Ltd). After centrifugation (Allegra 64 R, Beckmann, Inc) at 5000 g for 10 min, 2 mL of the supernatant was collected and mixed with 2 mL of 0.1 mM DPPH solution (dissolved in anhydrous ethanol). The reaction mixture was then incubated in the dark at ambient temperature for 60 min, and the absorbance was measured at 517 nm. The DPPH-radical scavenging activity was calculated as Eq. (4):

$$\text{Scavenging activity\%} = \frac{OD_B - OD_S}{OD_B} \times 100\% \tag{4}$$

OD_B and OD_S represented the absorbance of the blank and the sample tubes.

2.3.9. Antibacterial activity of films

The antibacterial activity of the CS/PUL/GA films was studied against *S. aureus, E. coli,* and *P. aeruginosa* using the inhibition circle method. The films were cut into 10 mm diameter discs and subjected to UV sterilization for 15–20 min. A bacterial suspension of 10^6 CFU/mL was prepared and incubated on LB plates. After 24 h of incubation at 37。C, the diameter of the inhibition ring was measured using a vernier caliper with a precision of 0.02 mm (Song, Liu, Huang, Zhou, Hong, & Deng, 2022).

2.4. Statistical analysis

All measurements were made in triplicate and the statistical significance was analyzed with the SPSS Statistical Package Program (Windows SPSS 26.0). Variance Analysis (ANOVA) was performed, and the Duncan Multiple Range Test was performed for mean comparison.

3. Results and discussion

3.1. GA cumulative release

A UV–Vis spectrophotometer was utilized to measure the GA content of CS/PUL/GA films in a 95% ethanol solution. The concentration and cumulative release of GA versus immersion time are presented in Fig. 1. The cumulative release of GA in the films exhibited a slow increase during the initial 25 h, which might be attributed to the rapid release of GA from the shallow matrix of the films. The rapid release of GA between 25 and 31 h might be attributed to the migration of GA from the deep matrix to the surface. Gradual stabilization of GA release after 144 h. The results indicated a correlation between the release of GA from CS/PUL/GA films and GA concentration, with a higher GA content resulting in a greater percentage of GA released. Luzi et al. (2019) observed a comparable phenomenon in electrospun fibers that contained GA.

3.2. The thickness of the film

Table 1 presented the thickness of CS/PUL/GA films with 0.5%, 1.0%, and 1.5% GA additions, which were significantly greater than the thickness of CS/PUL films ($P < 0.05$). The increase in film thickness might be attributed to the higher solids content of GA in the CS/PUL film. However, the thickness of CS/PUL/GA films did not significantly differ between 0.5% and 1.0% GA addition ($P > 0.05$), but showed a slight increasing trend. This could be explained by the suitable GA addition interacting with glycerol and CS/PUL, contributing to the film thickness. Additionally, the thickness of CS/PUL/1.5% GA films was significantly greater than that of the CS/PUL/0.5% GA and 1.0% GA films ($P < 0.05$). Similar results were found by Y. Wang et al. (2019) in chitosan films containing GA and caffeic acid.

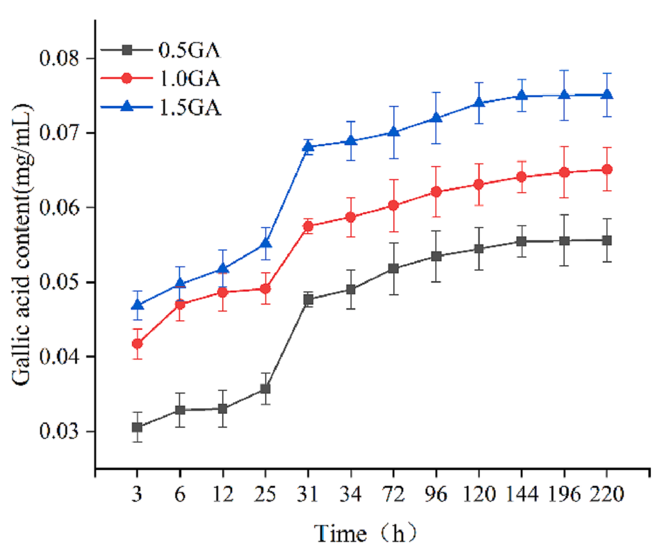


Fig. 1. GA cumulative release properties.

Table 1
Effects of GA concentration on thickness, tensile strength (TS), elongation at break (EAB) and water vapor permeability (WVP) of CS/PUL/GA films.

Film	Thickness/mm	TS/Mpa	EAB/%	WVP/10^{-7}gm^{-1}h^{-1}pa^{-1}
CS/PUL	0.154 ± 0.007^c	18.63 ± 1.50^a	13.29 ± 3.17^c	9.934 ± 0.531^a
CS/PUL/0.5 GA	0.164 ± 0.003^b	7.84 ± 0.76^c	65.75 ± 2.19^a	3.032 ± 0.247^d
CS/PUL/1.0 GA	0.168 ± 0.001^b	15.17 ± 1.05^b	64.04 ± 1.48^a	4.726 ± 0.170^c
CS/PUL/1.5 GA	0.178 ± 0.002^a	5.86 ± 0.09^d	58.24 ± 1.23^b	7.676 ± 0.430^b

The data were show as mean ± standard deviation and superscript letter (a-d) indicate significant difference ($P < 0.05$) within the same column.

3.3. Mechanical properties

In general, edible films must possess sufficient mechanical strength to maintain their integrity and withstand external damage in food packaging. Table 1 presented the tensile strength (TS) and elongation at break (EAB) of CS/PUL/GA films. Compared to the control treatment without GA, the TS of CS/PUL/GA films with 0.5%, 1.0%, and 1.5% GA decreased significantly from 18.63 ± 1.50 MPa to 7.84 ± 0.76 MPa, 15.17 ± 1.05 MPa, and 5.86 ± 0.09 MPa, respectively ($P < 0.05$), while the EAB increased significantly from 13.29 ± 3.17% to 65.75 ± 2.19%, 64.04 ± 1.48%, and 58.24 ± 1.23%, respectively ($P < 0.05$). Among the GA addition treatments, the TS of CS/PUL/GA films initially increased and then decreased, while the EAB gradually decreased with increasing GA addition. The changes in TS might be attributed to the fact that in the early stages of GA addition, more GA was hydrogen-bonded to the CS/PUL molecules, which increased the overall polymer bonding and the pressure-bearing capacity of the film, thereby increasing the TS of the films (Leceta, Urdanpilleta, Zugasti, Guerrero, & de la Caba, 2018). However, excessive hydrogen bonding between GA molecules in the later stages could alter the structure of CS/PUL/GA polymer, resulting in a reduction in the TS of CS/PUL/GA films. The changes in EAB were attributed to the excessive hydrogen bonding of GA in the later stages, which caused the film structure to weaken and reduced the EAB of the films. Qu et al. (2016) also observed that the TS of GA-modified ultrasound film initially increased with the addition of GA and then decreased ($P < 0.05$), while the EAB remained unchanged initially and then decreased with the increase of GA ($P < 0.05$).

3.4. Water vapor permeability

Table 1 displayed the WVP values of different GA concentrations for the film set. The addition of GA resulted in lower WVP values compared to films without GA ($P < 0.05$). This reduction in WVP indicated that the water barrier ability of CS/PUL films was improved by the addition of GA. This improvement could be attributed to the increased cross-linking of hydrogen bonds between GA and CS/PUL, which might have reduced the number of free hydroxyl groups interacting with water molecules, thereby lowering the WVP of the films. This effect is similar to the observed effects of ferulic acid and GA on gelatinous films of horse mackerel scales. According to Yujia Zhao, Teixeira, Gänzle, and Saldaña (2018), the addition of GA to cassava starch and chitosan films resulted in a significant reduction in WVP. This was attributed to the high degree of cross-linking, which led to the formation of ester and electrostatic bonds between cassava starch, GA, and chitosan. However, as the concentration of GA increased from 3.032 × 10–7 to 7.676 × 10-7gm-1 h-1pa-1 (P < 0.05), the WVP of the films also increased significantly. This could be due to the fact that the higher concentration of GA altered the structure of the CS/PUL polymer, causing a disruption of the intermolecular interactions and resulting in an increase in WVP values. Khan, Volpe, Salucci, Sadiq, and Torrieri (2022) observed an increase in water vapor permeability (WVP) in the GA/active caseinate/guar gum film when the concentration of GA was increased to 250 μg/mL.

3.5. Color, opacity and light transmission

The optical characteristics of a packing film are essential properties that relate to its function, particularly in terms of the greasiness on the film's surface. Table 2 presented the color parameters and opacity values of the films based on CS/PUL/GA. As expected, the CS/PUL film was relatively whitish. However, the *L** value of the films significantly decreased when the addition of GA was above 1.0%, compared to the film without GA ($P < 0.05$). This demonstrates that GA had a significant influence on the film's brightness. Additionally, the *a** and *b** values of the CS/PUL/GA films significantly increased as the addition of GA increased ($P < 0.05$). This increase might be due to GA's yellowish color, which intensified the yellow hue of the films. Yujia Zhao et al. (2019) discovered that increasing the GA content in cassava starch/chitosan/GA nanocellulose films resulted in higher b* values. Manuhara, Praseptiangga, Muhammad, and Maimuni (2016) reported that plant-based antioxidants, such as phenolic compounds, absorb short-wavelength light, which could cause an increase in b* values. The increase in a* values might be due to the interaction between GA and the film-forming materials or to oxidation changes in GA during heating (Fabra, Hambleton, Talens, Debeaufort, & Chiralt, 2011).

The addition of GA to CS/PUL films resulted in a significant increase in opacity compared to CS/PUL films alone ($P < 0.05$). Notably, CS/PUL/1.5% GA exhibited the highest opacity value ($P < 0.05$). However, no significant difference in opacity was observed between 0.5% and 1.0% GA additions to CS/PUL films. The transparency of CS/PUL films was attributed to their polysaccharide structure, as indicated in Table 2. UV–VIS spectroscopy analysis of the films scanned at 300–800 nm (Fig. 2) revealed that the light transmittance of CS/PUL films was 15% and 45% in the ultraviolet and visible light regions, respectively. In contrast, the addition of GA to CS/PUL films resulted in a significant decrease in light transmittance (UV and visible light regions) compared to CS/PUL films alone ($P < 0.05$), with a decrease in transmittance observed with increasing GA concentration. The aromatic ring in GA contributed to its anti-UV properties in the UV region (280–320 nm) (Luo, Wu, Wang, & Yu, 2021).

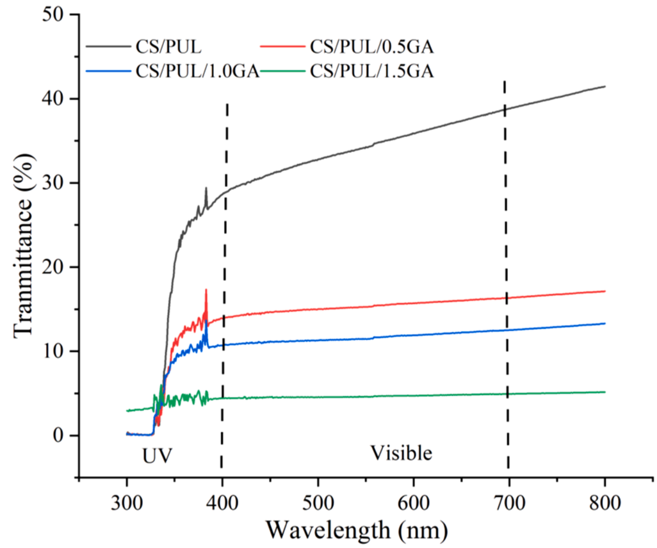
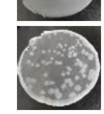


Fig. 2. Uv-vis spectra of CS/PUL/GA films.

3.6. Analysis of Fourier transforms infrared

The FTIR spectra of CS/PUL/GA film samples were showed in Fig. 3. The absorption peak observed at 3350 cm^{-1} in the spectrum of CS/PUL/GA films was attributed to the tensile vibration of –OH. This absorption peak exhibited a decrease in intensity and a slight shift towards a lower wavenumber when compared to the CS/PUL film. This shift might be attributed to the reduction of –OH groups resulting from hydrogen bonding between GA and CS/PUL (Lu, Nie, Belton, Tang, & Zhao, 2006). The absorption peak observed in the spectrum of CS/PUL/GA films at 1685–1612 cm-1 was mainly due to the structure of the GA aromatic ring. The absorption peak increased with the addition of GA, indicating that the interaction between GA and CS/PUL steadily increased with increasing GA concentration. These findings confirm the strong affinity between GA and CS/PUL (X. Wang et al., 2018). The steep peaks at 1538 cm-1, 1452 cm-1 and 1340 cm-1 were mainly the stretching and bending of –COOH. Tapia-Hernandez et al. (2019) identified characteristic peaks in electrospray films of zein and gallic acid. The absorption peaks at 1180–957 cm-1 were attributed to C—C and C—O stretching, as

Table 2
Effects of GA concentration on color characteristics, opacity and appearance of CS/PUL/GA films.

Film	*L**	*a**	*b**	Δ E	Opacity	Appearance
CS/PUL	88.82 ± 0.20^{a}	0.50 ± 0.01^{c}	6.30 ± 0.01^{c}	4.19 ± 0.20^{c}	2.89 ± 0.32^{c}	
CS/PUL/0.5GA	88.40 ± 0.23^{ab}	0.62 ± 0.01^{b}	7.42 ± 0.16^{b}	4.58 ± 0.13^{bc}	4.90 ± 0.28^{b}	
CS/PUL/1.0GA	87.89 ± 0.49^{bc}	0.64 ± 0.03^{ab}	7.60 ± 0.11^{b}	5.12 ± 0.49^{b}	5.50 ± 0.46^{b}	
CS/PUL/1.5GA	87.68 ± 0.32^{c}	0.67 ± 0.02^{a}	8.38 ± 0.19^{a}	5.96 ± 0.36^{a}	7.44 ± 0.30^{a}	

The data were show as mean ± standard deviation and superscript letter (a-d) indicate significant difference ($P < 0.05$) within the same column.

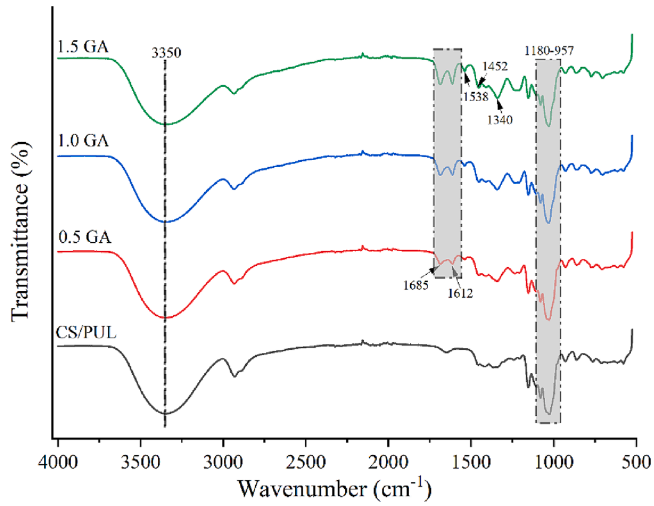


Fig. 3. FTIR analysis of CS/PUL/GA films.

well as C—H bond bending in the polysaccharide structure. In the CS/PUL/GA film, the absorption peak was observed between 707 and 773 cm-1, which was mainly due to the flexural vibration outside the phenol O—H plane. The presence of phenol –OH was found to be crucial for the DPPH radical scavenging efficiency. Similar characteristic peaks were observed in the CS/PUL and CSPUL/GA films, indicating that no new chemical bonds were formed in the CS/PUL and CSPUL/GA network structures. The above findings suggest that the electrospray films of zein and gallic acid have potential applications in the food industry due to their antioxidant properties.

3.7. Scanning electron microscope and water contact angle

Fig. 4 displayed the surface and cross-sectional views of the CS/PUL/GA films. The surface of the CS/PUL film appeared smooth and homogeneous, without any visible cracks. However, after the addition of GA, the roughness of the CS/PUL/GA films slightly increased, indicating the formation of hydrogen bonds among hydrophilic compounds (Zarandona, Puertas, Dueñas, Guerrero, & de la Caba, 2020). Furthermore, as reported by Parveen et al. (2019), the roughness increase might be attributed to surface agglomeration resulting from covalent and non-covalent interactions between GA and CS/PUL. The CS/PUL/1.5% GA film exhibited a significant degree of heterogeneity, primarily because the high concentration of GA restricted the movement of the polymer chain within the polymer matrix (Limpisophon & Schleining, 2017). Additionally, the cross-section of CS/PUL/GA films corresponded to changes in the film surface, which became rough with an increase in GA concentration. This finding was consistent with XRD and FTIR analyses, indicating that GA was successfully embedded in the CS/PUL matrix.

The water contact angle (WCA) was utilized to assess the material's affinity for water. A WCA of $\theta < 90°$ indicates a hydrophilic nature, while $\theta > 90°$ indicates a hydrophobic nature of the film. The CS/PUL film exhibited a WCA of $72.51 \pm 2.11°$. However, the addition of 0.5% GA resulted in a significant increase in the WCA ($P < 0.05$) (Fig. 4). This increase might be attributed to the absence of free –OH groups available to interact with water molecules (Yildiz, Emir, Sumnu, & Kahyaoglu, 2022). However, as the concentration of GA increased in the CS/PUL/GA films, the WCA decreased significantly ($P < 0.05$). This could be attributed to the abundance of hydroxyl groups in GA, which interacted with the –OH group in water and enhanced the film's wettability (Ng et al., 2020). Previous studies have reported that the addition of GA improved the hydrophobicity of cassava starch film, but excessive amounts of GA decreased its hydrophobicity (Masamba, Li, Rizwan, Sharif, Ma, & Zhong, 2016).

3.8. Analysis of X-ray diffractometer

Crystallinity is an important criterion for characterizing composite film materials, as it is closely related to their properties and structures. X-ray diffraction analysis was used to observe how GA affected the structural behavior of CS/PUL systems. The diffraction pattern of CS/PUL/GA films was showed in Fig. 5. The CS/PUL film exhibited diffraction peaks at $2\theta = 17.1971°$ and $2\theta = 20.3959°$, indicating a fairly high crystallinity. However, the addition of GA to the CS/PUL system resulted in a broad and low peak at $2\theta = 20.3959°$ in the CS/PUL/GA films. This might be due to the strong hydrogen bonding between CS/PUL and GA, which eliminated the original structure and resulted in a low crystallinity corresponding to its position when the crystalline and amorphous polymers emerged good compatibility without a very pronounced strong diffraction peak (Goudar et al., 2020). The study of Yanzhen Zhao et al. (2022) demonstrated that the incorporation of GA into the modified chitosan/polyethylene (vinyl alcohol) composite film

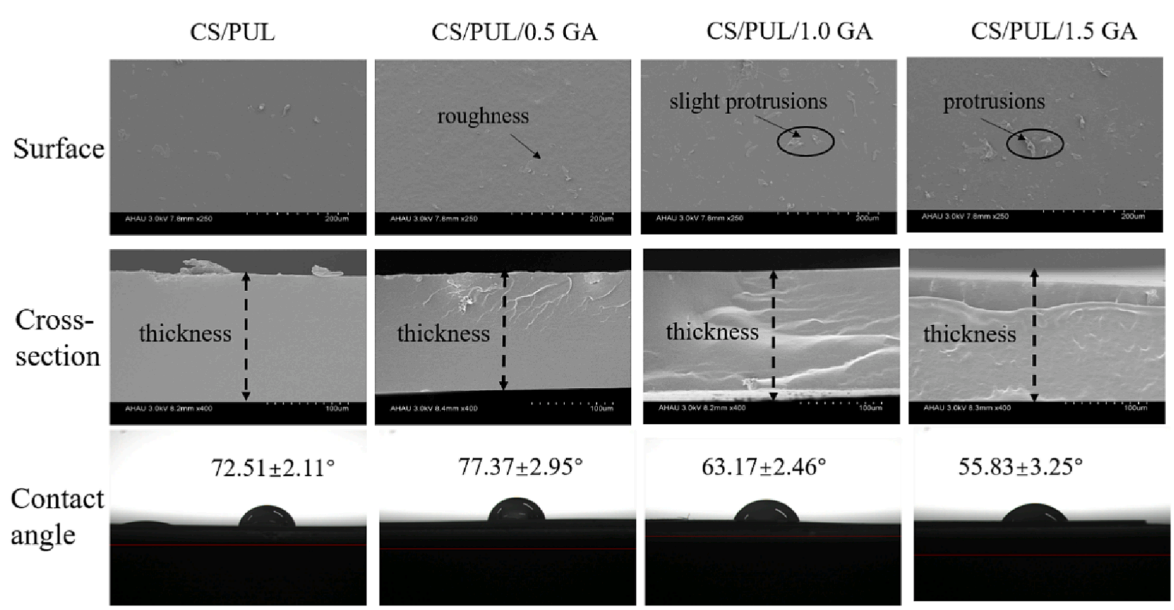


Fig. 4. SEM cross-section, surface and contact angle (WCA) of CS/PUL/GA films.

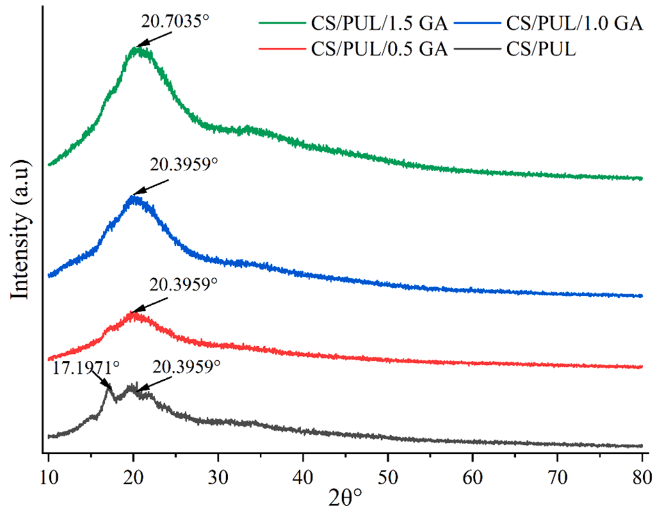


Fig. 5. XRD analysis of CS/PUL/GA films.

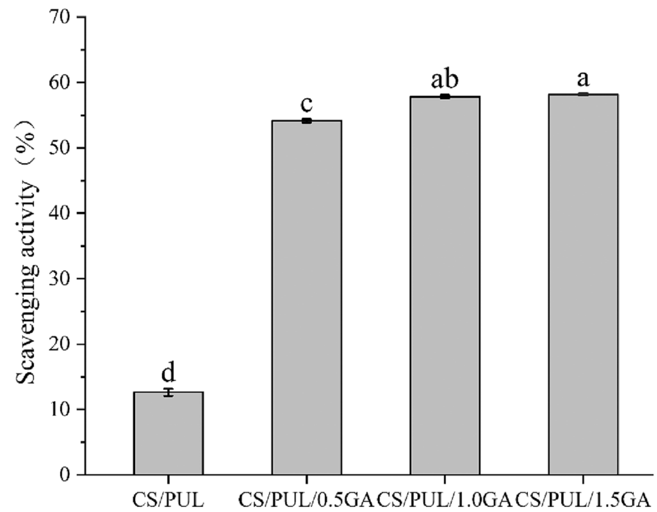


Fig. 6. DPPH radical scavenging activity of CS/PUL/GA films.

resulted in a broader amorphous peak. However, as the concentration of GA increased, the peak started to narrow and become sharper, and the diffraction angle gradually decreased. These findings suggested that GA promotes the crystal structure of CS/PUL/GA films to some extent (Almasi, Azizi, & Amjadi, 2020), indicating a strong interaction between GA, CS, and PUL.

3.9. The free radical scavenging activity of DPPH (1, 1, 1, 2 - Picrylhydrazine)

Due to the susceptibility of food to oxidation during storage, it is imperative that the packaging film possesses a strong antioxidant capacity. Generally, the scavenging activity of DPPH is positively correlated with the antioxidant capacity. Fig. 6 illustrates the scavenging activity of CS/PUL/GA films with varying concentrations of GA. The scavenging activity of the CS/PUL film was 12.62 ± 0.54%, while the scavenging activity of the CS/PUL/GA films was significantly higher at 54.13 ± 0.54%, 57.85 ± 0.25%, and 58.16 ± 0.17%, respectively ($P < 0.05$). This increase in scavenging activity could be attributed to the strong ability of GA to provide hydrogen atoms or electrons to the DPPH radical (Jiang et al., 2023). The scavenging activity of films with varying concentrations of GA increased from 54.13 ± 0.54% to 58.16 ± 0.17%. However, there was no significant difference observed between the addition of 1.5% and 1.0% GA ($P > 0.05$). This could be attributed to the saturation of hydroxyl exposed to the polymer surface, which occurred later due to the stabilization of the polymer structure. As a result, the scavenging ability of the film for DPPH radicals did not improve any further (Song et al., 2022). Yadav, Mehrotra, and Dutta (2021) observed that the antioxidant capacity of potato starch-based ZnO nanoparticle-loaded GA films did not increase beyond a certain level of GA addition. The study results suggest that the addition of GA could enhance the antioxidant activity of the films, and it was recommended that 1.0% GA was the optimal concentration. The findings of this study have important implications for the development of biodegradable food packaging materials with improved antioxidant properties.

3.10. Antibacterial activity of films

The inhibition zone method was used to evaluate the antimicrobial activity of CS/PUL/GA films. Table 3 showed that no inhibitory zone was observed in the CS/PUL films. This finding is consistent with the study by Lun'kov, Shagdarova, Zhuikova, Il'ina, and Varlamov (2018), who reported no significant inhibition zone against E. coli and S. aureus in cassava starch-based films. The antibacterial effect of CS/PUL/GA films on E. coli, S. aureus, and P. aeruginosa was significantly promoted

Table 3
Antibacterial activity of CS/PUL/GA films.

Inhibition zone (mm)	*E. coli*	*S. aureus*	*P. aeruginosa*
CS/PUL	0.00	0.00	0.00
CS/PUL/0.5 GA	21.00 ± 0.25^{c}	20.83 ± 0.28^{c}	20.20 ± 0.28^{c}
CS/PUL/1.0 GA	22.67 ± 0.36^{b}	22.33 ± 0.66^{b}	21.30 ± 0.58^{b}
CS/PUL/1.5 GA	24.33 ± 1.02^{a}	23.87 ± 0.79^{a}	23.34 ± 1.26^{a}

The data were show as mean ± standard deviation and superscript letter (a-d) indicate significant difference ($P < 0.05$) within the same column.

with increasing GA concentration ($P < 0.05$), as indicated in Table 3. This effect might be attributed to the antibacterial properties of GA, which disrupt the cell membrane system of bacteria, leading to the dissolution of the cell membrane and leakage of contents (Yoon, Kim, Kim, & Je, 2017). Yujia Zhao et al. (2018) also reported a high antibacterial rate against *E. coli*, *S. aureus*, and *P. aeruginosa* in a composite coating of GA, cassava starch, and chitosan.

4. Conclusion

The incorporation of GA into CS/PUL films significantly enhanced their mechanical, anti-UV, and antibacterial properties, with increasing GA concentration leading to further improvements. FTIR, SEM, and XRD analyses confirmed successful embedding of GA in the CS/PUL matrix, and the roughness of the resulting films increased with GA concentration. The CS/PUL/1.0% GA film exhibited the highest antioxidant activity (57.85 ± 0.25%) and mechanical properties, while the CS/PUL/0.5% GA film showed the highest hydrophobicity (63.17 ± 2.46 °C) and water vapor barrier (3.032 ± 0.247). Overall, the addition of GA significantly improved the properties of CS/PUL-based films, and the resulting CS/PUL/GA films have potential for use in ensuring food quality and extending the shelf life of food.

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.

Data availability

The data that has been used is confidential.

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References

Almasi, H., Azizi, S., & Amjadi, S. (2020). Development and characterization of pectin films activated by nanoemulsion and Pickering emulsion stabilized marjoram (Origanum majorana L.) essential oil. *Food Hydrocolloids, 99*(2). https://doi.org/10.1016/j.foodhyd.2019.105338

Aydogdu, A., Yildiz, E., Aydogdu, Y., Sumnu, G., Sahin, S., & Ayhan, Z. (2019). Enhancing oxidative stability of walnuts by using gallic acid loaded lentil flour based electrospun nanofibers as active packaging material. *Food Hydrocolloids, 95*, 245–255. https://doi.org/10.1016/j.foodhyd.2019.04.020

Fabra, M. J., Hambleton, A., Talens, P., Debeaufort, F., & Chiralt, A. (2011). Effect of ferulic acid and α-tocopherol antioxidants on properties of sodium caseinate edible films. *Food Hydrocolloids, 25*(6), 1441–1447. https://doi.org/10.1016/j.foodhyd.2011.01.012

Fdg, A., Fc, B., Ca, B., Vg, B., & Et, A. (2022). Activated gallic acid as radical and oxygen scavenger in biodegradable packaging film. *Food packaging and shelf life, 24*(2), 1–17. https://doi.org/10.1016/j.fpsl.2022.100811

Goudar, N., Vanjeri, V. N., Dixit, S., Hiremani, V., Sataraddi, S., Gasti, T., & Chougale, R. B. (2020). Evaluation of multifunctional properties of gallic acid crosslinked Poly (vinyl alcohol)/Tragacanth Gum blend films for food packaging applications. *International Journal of Biological Macromolecules, 158*, 139–149. https://doi.org/10.1016/j.ijbiomac.2020.04.223

Hazrati, K. Z., Sapuan, S. M., Zuhri, M., & Jumaidin, R. (2021). Effect of plasticizers on physical, thermal, and tensile properties of thermoplastic films based on Dioscorea hispida starch. *International Journal of Biological Macromolecules: Structure, Function and Interactions, 18*(5), 171–185. https://doi.org/10.1016/j.ijbiomac.2021.06.099

Jiang, C., Liu, T., Wang, S., Zou, Y., Cao, J., Wang, C., & Jin, L. (2023). Antioxidant and ammonia-sensitive films based on starch, κ-carrageenan and Oxalis triangularis extract as visual indicator of beef meat spoilage. *International Journal Of Biological Macromolecules, 235*, Article 123698. https://doi.org/10.1016/j.ijbiomac.2023.123698

Kaewprachu, P., Osako, K., Tongdeesoontorn, W., & Saroat.. (2017). The effects of microbial transglutaminase on the properties of fish myofibrillar protein film. *Food Packaging and Shelf Life*. https://doi.org/10.1016/j.fpsl.2017.04.002

Khan, M. R., Volpe, S., Salucci, E., Sadiq, M. B., & Torrieri, E. (2022). Active caseinate/guar gum films incorporated with gallic acid: Physicochemical properties and release kinetics. *Journal of Food Engineering, 335*, Article 111190. https://doi.org/10.1016/j.jfoodeng.2022.111190

Kwaw, E., Ma, Y., Tchabo, W., Apaliya, M. T., Wu, M., Sackey, A. S., & Tahir, H. E. (2018). Effect of lactobacillus strains on phenolic profile, color attributes and antioxidant activities of lactic-acid-fermented mulberry juice. *Food Chemistry, 250*, 148–154. https://doi.org/10.1016/j.foodchem.2018.01.009

Leceta, I., Urdanpilleta, M., Zugasti, I., Guerrero, P., & de la Caba, K. (2018). Assessment of gallic acid-modified fish gelatin formulations to optimize the mechanical performance of films. *International Journal Of Biological Macromolecules, 120*, 2131–2136. https://doi.org/10.1016/j.ijbiomac.2018.09.081

Limpisophon, K., & Schleining, G. (2017). Use of Gallic Acid to Enhance the Antioxidant and Mechanical Properties of Active Fish Gelatin Film. *Journal Of Food Science*. https://doi.org/10.1111/1750-3841.13578

Lu, Z., Nie, G., Belton, P. S., Tang, H., & Zhao, B. (2006). Structure–activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. *Neurochemistry International, 48*(4), 263–274. https://doi.org/10.1016/j.neuint.2005.10.010

Lun'kov, A. P., Shagdarova, B. T., Zhuikova, Y. V., Il'ina, A. V., & Varlamov, V. P. (2018). Properties of Functional Films Based on Chitosan Derivative with Gallic Acid. *Applied Biochemistry And Microbiology, 54*(5), 484-490. 10.1134/S0003683818050137.

Luo, Y., Wu, Y., Wang, Y., & Yu, L. (2021). Active and Robust Composite Films Based on Gelatin and Gallic Acid Integrated with Microfibrillated Cellulose. *Foods, 10*(11), 2831. https://doi.org/10.3390/foods10112831

Luzi, F., Pannucci, E., Santi, L., Kenny, J. M., Torre, L., Bernini, R., & Puglia, D. (2019). Gallic Acid and Quercetin as Intelligent and Active Ingredients in Poly(vinyl alcohol) Films for Food Packaging. *Polymers, 11*(12). https://doi.org/10.3390/polym11121999

Manuhara, G. J., Praseptiangga, D., Muhammad, D., & Maimuni, B. H. (2016). Preparation and characterization of semi-refined kappa carrageenan-based edible film for nano coating application on minimally processed food. *Nanoscience & Nanotechnology Symposium*, 030043.

Marangoni Júnior, L., Silva, R. G., & d., Vieira, R. P., & Alves, R. M. V.. (2021). Water vapor sorption and permeability of sustainable alginate/collagen/SiO2 composite films. *LWT, 152*, Article 112261. https://doi.org/10.1016/j.lwt.2021.112261

Masamba, K., Li, Y., Rizwan, H., Sharif, M.a., & J., & Zhong, F.. (2016). mechanical and water barrier properties of zein-corn starch composite films as affected by gallic acid treatment. *International Journal of Food Engineering, 12*(8), 773–781. https://doi.org/10.1515/ijfe-2016-0112

Ng, A., Vnv, A., Sd, B., Vh, A., Ss, A., Tg, A., & Rbc, A. (2020). Evaluation of multifunctional properties of gallic acid crosslinked Poly (vinyl alcohol)/Tragacanth Gum blend films for food packaging applications. *International Journal Of Biological Macromolecules, 158*, 139–149. https://doi.org/10.1016/j.ijbiomac.2020.04.223

Parveen, S., Chaudhury, P., Dasmahapatra, U., & Dasgupta, S. (2019). Biodegradable protein films from gallic acid and the cataractous eye protein isolate. *International Journal Of Biological Macromolecules, 139*, 12–20. https://doi.org/10.1016/j.ijbiomac.2019.07.143

Porta, R., Mariniello, L., Pierro, P. D., Sorrentino, A., & Giosafatto, C. (2011). Transglutaminase Crosslinked Pectin- and Chitosan-based Edible Films: A Review. *Critical Reviews in Food Science and Nutrition, 51*(3), 223-238. https://doi.org/http://10.1080/10408390903548891.

Qu, W., Guo, T., Zhang, X., Jin, Y., Wang, B., Wahia, H., & Ma, H. (2016). Preparation of Tuna Skin Collagen-Chitosan Composite Film Improved by Sweep Frequency Pulsed Ultrasound Technology. *Social Science Electronic Publishing*. https://doi.org/10.2139/ssrn.3968041

Rajan, V. K., & Muraleedharan, K. (2017). A computational investigation on the structure, global parameters and antioxidant capacity of a polyphenol, Gallic acid. *Food Chemistry, 220*, 93–99. https://doi.org/10.1016/j.foodchem.2016.09.178

Raspo, M. A., Gomez, C. G., & Andreatta, A. E. (2018). Optimization of antioxidant, mechanical and chemical physical properties of chitosan-sorbitol-gallic acid films by response surface methodology. *Polymer Testing, 70*, 180–187. https://doi.org/10.1016/j.polymertesting.2018.07.003

Sanches-Silva, A., Costa, D., Albuquerque, T. G., Buonocore, G. G., Ramos, F., Castilho, M. C., & Costa, H. S. (2014). Trends in the use of natural antioxidants in active food packaging: A review. *Food Additives & Contaminants, 31*(3), 374–395. https://doi.org/10.1080/19440049.2013.879215

Song, Z., Liu, H., Huang, A., Zhou, C., Hong, P., & Deng, C. (2022). Collagen/zein electrospun films incorporated with gallic acid for tilapia (Oreochromis niloticus) muscle preservation. *Journal Of Food Engineering, 317*, Article 110860. https://doi.org/10.1016/j.jfoodeng.2021.110860

Tapia-Hernandez, J. A., Del-Toro-Sanchez, C. L., Cinco-Moroyoqui, F. J., Ruiz-Cruz, S., Juarez, J., Castro-Enriquez, D. D., & Rodriguez-Felix, F. (2019). Gallic Acid-Loaded Zein Nanoparticles by Electrospraying Process. *Journal Of Food Science, 84*(4), 818–831. https://doi.org/10.1111/1750-3841.14486

Wang, X., Xie, Y., Ge, H., Chen, L., Wang, J., Zhang, S., & Feng, X. (2018). Physical properties and antioxidant capacity of chitosan/epigallocatechin-3-gallate films reinforced with nano-bacterial cellulose. *Carbohydrate Polymers, 179*, 207–220. https://doi.org/10.1016/j.carbpol.2017.09.087

Wang, Y., Du, H., Xie, M., Ma, G., Yang, W., Hu, Q., & Pei, F. (2019). Characterization of the physical properties and biological activity of chitosan films grafted with gallic acid and caffeic acid: A comparison study. *Food Packaging and Shelf Life, 22*, Article 100401. https://doi.org/10.1016/j.fpsl.2019.100401

Xi, Z. A., Xz, A., Min, Z. A., Qg, B., Jq, A., Jin, L. A., & Gx, A. (2021). Effect of konjac glucomannan/carrageenan-based edible emulsion coatings with camellia oil on quality and shelf-life of chicken meat. *International Journal Of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2021.04.165

Yadav, S., Mehrotra, G. K., & Dutta, P. K. (2021). Chitosan based ZnO nanoparticles loaded gallic-acid films for active food packaging. *Food Chemistry, 334*, Article 127605. https://doi.org/10.1016/j.foodchem.2020.127605

Yildiz, E., Emir, A. A., Sumnu, G., & Kahyaoglu, L. N. (2022). Citric acid cross-linked curcumin/chitosan/chickpea flour film: An active packaging for chicken breast storage. *Food Bioscience, 50*, Article 102121. https://doi.org/10.1016/j.fbio.2022.102121

Yoon, S.-D., Kim, Y.-M., Kim, B. I., & Je, J.-Y. (2017). Preparation and antibacterial activities of chitosan-gallic acid/polyvinyl alcohol blend film by LED-UV irradiation. *Journal of Photochemistry and Photobiology B: Biology, 176*, 145–149. https://doi.org/10.1016/j.jphotobiol.2017.09.024

Zarandona, I., Puertas, A. I., Dueñas, M. T., Guerrero, P., & de la Caba, K. (2020). Assessment of active chitosan films incorporated with gallic acid. *Food Hydrocolloids, 101*, Article 105486. https://doi.org/10.1016/j.foodhyd.2019.105486

Zhao, Y., Huerta, R. R., & Saldaña, M. D. A. (2019). Use of subcritical water technology to develop cassava starch/chitosan/gallic acid bioactive films reinforced with cellulose nanofibers from canola straw. *The Journal of Supercritical Fluids, 148*, 55–65. https://doi.org/10.1016/j.supflu.2019.02.022

Zhao, Y., Teixeira, J. S., Gänzle, M. M., & Saldaña, M. D. A. (2018). Development of antimicrobial films based on cassava starch, chitosan and gallic acid using subcritical water technology. *The Journal of Supercritical Fluids, 137*, 101–110. https://doi.org/10.1016/j.supflu.2018.03.010

Zhao, Y., Yang, L., Xu, M., Wang, H., Gao, X., Niu, B., & Li, W. (2022). Gallic acid functionalized chitosan immobilized nanosilver for modified chitosan/Poly (vinyl alcohol) composite film. *International Journal of Biological, Macromolecules(7)*, 1–10. https://doi.org/10.1016/j.ijbiomac.2022.10.074

Zhou, X., Yu, X., Xie, F., Fan, Y., Xu, X., Qi, J., & Zhang, F. (2021). pH-responsive double-layer indicator films based on konjac glucomannan/camellia oil and carrageenan/anthocyanin/curcumin for monitoring meat freshness. *Food Hydrocolloids, 118*, Article 106695. https://doi.org/10.1016/j.foodhyd.2021.106695