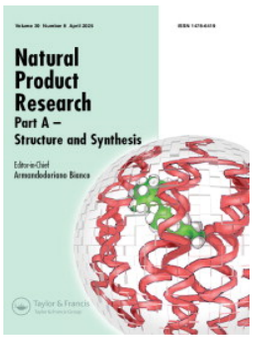

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SHORT COMMUNICATION

Loading esculin can improve the morphology and antibacterial properties of chitosan/whey protein isolate composite film

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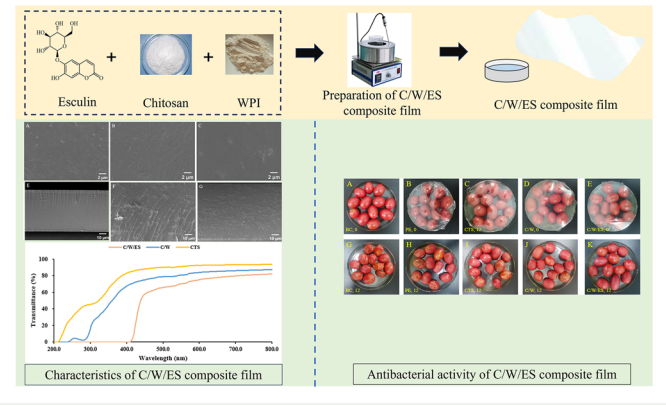
ABSTRACT

In this study, chitosan (CTS), whey protein isolate (WPI) and glycerol were used as biodegradable film-forming matrices to prepare composite films by solution blending. Esculin (ES) as antibacterial active compound, was added during the film modification process. CTS, CTS/WPI (C/W) and CTS/WPI/ES (C/W/ES) films were compared in terms of structure, physical properties and functions. Due to the effect of ES, C/W/ES film has good mechanical properties, barrier properties, and biodegradability. Compared with C/W film, the tensile strength, elongation at break, antioxidant activity, and biodegradability of C/W/ES film increased by 36.4%, 9.6%, 37.0%, and 33.65%, respectively. Its antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* was significantly enhanced. The cherry tomato preservation test showed that C/W/ES film had a good effect on weight loss rate and decay rate of the fruits. In summary, loading ES can improve the morphology and antibacterial properties of the biodegradable composite film.



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1. Introduction

Esculin (ES) is a coumarin-like compound with significant antioxidant and antibacterial activity, which can inhibit the growth and reproduction of bacteria such as *Escherichia coli* and *Staphylococcus aureus* (Ju et al. 2024). Adding ES to packaging film can inhibit the growth and reproduction of microorganisms by using its antimicrobial properties, thereby extending the freshness period of food, reducing microbial contamination and food spoilage (Fink and Filip 2023).

Chitosan (CTS) is the only alkaline polysaccharide in nature, with non-toxic, biodegradable, and biocompatible properties, second only to cellulose in terms of content (Dou et al. 2024). Protein-based films, especially whey protein isolate (WPI) films, are highly effective oxygen barriers with low permeability and good freshness retention. WPI is a globular protein with good nutrient and film-forming ability, forming a transparent and flexible polymer film that blocks oxygen. It can also inhibit *E. coli* and Salmonella (Mohammadi et al. 2024).

In this study, CTS was used as a film forming polymer, and WPI and glycerol were added to improve the film performance. In additon, ES was added to enhance the antioxidant and antibacterial properties. The composite films were evaluated and compared in various aspects, and were used for preservation of cherry tomato. The biodegradable freshness retention films prepared in this study can expand the application of natural active products and have great environmental benefits.

2. Results and discussion

2.1. Microstructure

The structure of the composite film can be observed on a micron scale by scanning electron microscopy (SEM) to determine the surface characteristics and component homogeneity. Supplementary Figure 1 (Fig. S1) showed the SEM scanning results of the composite films. The CTS film has a smooth surface with some cracks, while the C/W film has wrinkles and rough particles. Compared to C/W film, C/W/ES film is smoother and has a more complete and dense structure. The cross-sectional morphology of C/W/ES film is similar to that of the CTS film, which proved the high compatibility between the polymer and the active ingredient (Lavrič et al. 2021).

2.2. Moisture content, water solubility, and swelling degree

According to Supplementary Table 1 (Table S1), compared to CTS film, the moisture content of C/W film decreased from 19.35% to 18.73%. The addition of ES led to a denser film structure, weakened the interaction between the film and water molecules, and further reduced the water content (16.66%). With the addition of WPI, the decrease in water solubility was attributed to the stronger interaction between WPI and CTS functional groups, which reduced the possibility of CTS functional group breaking in aqueous solution. In terms of swelling, the swelling values of CTS films were higher than those of C/W and C/W/ES films ($p < 0.05$). Therefore, the addition of WPI and ES reduced the solubility of the polymer and limits the bonding and cross-linking of the polymer chains.

2.3. Water vapour permeability (WVP)

Moisture transfer is a key challenge affecting the quality of food products and a factor to be considered in the production of food packaging materials. The results of WVP of the films were shown in Table S2. The incorporation of ES significantly reduced the WVP of the polymer films ($p < 0.05$). With the addition of ES, the crystallinity of the film increased, which is attributed to the reaction between the compounds contained in ES and the free hydrophilic groups in CTS, forming bonds (Gao et al. 2021).

2.4. Mechanical properties

In order to withstand the typical pressure of food transportation and storage, food packaging films must have high tensile strength and elongation at break. Table S2 showed the tensile strength and elongation at break of the composite films. With the addition of WPI into CTS, the mechanical properties of the CTS films were improved due to the interaction between the functional groups of CTS and WPI compounds, which enhanced the strength and flexibility of the film substrate. In addition, the addition of ES can further improve the tensile strength and elongation at break of C/W films ($p < 0.05$).

2.5. Light transmittance

Food products deteriorate when exposed to UV light, so the UV resistance of food packaging films is critical. As shown in Figure S2, C/W film had the highest UV transmittance. Compared with C/W film, the UV transmittance of ES-containing films was significantly reduced, almost zero in the wavelength range of 300 ~ 400 nm. Due to the UV-resistant properties of ES, the addition of it to pure or mixed biopolymer films can significantly improve their UV-resistant properties (Sun et al. 2019).

2.6. Antioxidant activity

Oxidation resistance is an important indicator of food packaging films. Table S2 listed the DPPH radical scavenging ability of the films. CTS film has the weakest DPPH radical scavenging property. The addition of WPI increased the DPPH radical scavenging ability of the composite film by 31.2%. After adding ES, the antioxidant capacity of the composite film was significantly increased by 79.8% ($p < 0.05$). This is mainly due to the excellent antioxidant capacity of ES (Sun et al. 2020).

2.7. Biodegradability test

The films become fragile and degrade in highly humid environments. The degradation rates of C/W and C/W/ES films were 54.27% and 72.48%, respectively. With the addition of ES, the degradation rate accelerates and the films are more susceptible to degradation (Silva et al. 2012). On the other hand, the addition of ES may affect the crystallisation of the composite film, making it more susceptible to microbial attack and degradation upon contact with water and soil, thereby increasing the degradation rate and making it more environmentally friendly. This is consistent with previous studies showing similar degradation rates for biodegradable films (Han et al. 2023).

2.8. Bacteriostatic test

The antibacterial activity of packaging materials creates a suitable environment for extending shelf life. The antibacterial zones of the composite films were evaluated using *E. coli and S. aureus* (Fig. S3). Compared with CTS and C/W films, C/W/ES film has a larger antibacterial zone and exhibits significant antibacterial activity. Therefore, C/W/ES films can achieve food preservation by inhibiting bacterial growth. This is consistent with previous studies that esculin can inhibit bacterial growth by increasing the permeability of the bacterial cell membrane, regulating bacterial resistance, and interfering with bacterial metabolic pathways (Mokdad-Bzeouich et al. 2015).

2.9. Cherry tomato preservation test

The changes in the appearance of the fruit and decay rate under different types of packaging film were shown in Figure S4 and Table S3. The fruits in the blank control group were directly exposed to the air, which makes it easier for water to evaporate and dissipate, and microorganisms to attach and infest the fruits, leading to mould and decay. The weight loss of the plastic wrap group decreased to 19.73%, mainly due to the presence of the packaging film slowing down the moisture loss of packaged fruits.

The reduction in weight loss and decay rate (17.65% and 47.22%) of cherry tomato wrapped in CTS film is mainly attributed to the antibacterial properties of CTS. The addition of WPI to CTS films helps to improve the barrier properties against gases, moisture, and other substances. WPI inhibits various bacteria such as *E. coli* and *S. aureus*, which can synergize with the antibacterial properties of CTS to enhance the overall antibacterial effect.

After 12 days of C/W/ES film coverage, the weight loss rate of cherry tomato was 14.67%, and the decay rate was 13.89%, significantly lower than other groups ($p < 0.05$). ES has certain antibacterial activity, which can inhibit the growth of bacteria, help to maintain the hygiene and quality of the packaging items, and to some extent prevent oxidation and deterioration of packaging items, extending their shelf life. In addition, the addition of ES makes the membrane structure more compact and reduces WVP, which can maintain a certain water-vapour balance inside and outside the membrane and inhibit the propagation of bacteria, and help to preserve the freshness of the cherry tomato.

3. Conclusions

In this study, the natural active compound esculin was added to the CS/WPI polymer matrix to prepare composite films. Compared with CTS and C/W films, the addition of ES greatly improves the tensile, oxidation, antibacterial, UV and water vapour barrier properties of the composite film, as well as the cherry tomato preservation performance. Therefore, C/W/ES composite film has better morphology properties and antibacterial preservation ability. C/W/ES films are made from commercially available and environmentally friendly materials, and their preparation process is compatible with industrial techniques. Although their initial cost may be higher than that of

polyethylene films, their environmental benefits and long-term waste management savings justify the investment. In summary, composite film loading with ES as a potential packaging material for food preservation has broad application prospects, further expanding the application of natural active products.

Disclosure statement

No potential conflict of interest was reported by the authors.

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