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Preservative effect of chitosan-gelatine composite incorporated with pomegranate peel polyphenol on fresh meat

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Abstract

The present work aimed to prepare a pomegranate peel polyphenol (PPP)-chitosan (CS) composite membrane solution to preserve fresh beef. Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), viscometer, colorimeter, and pH meter were used to determine the physicochemical properties of the PPP-CS composite membrane solution, and evaluate its antioxidant properties. Additionally, the effect of the PPP-CS composite membrane solution on fresh beef preservation was investigated. Results showed that the *in vitro* antioxidant activity, viscosity, a* value, and b* value of the composite membrane solution increased significantly. In contrast, the pH and L* values decreased significantly after adding PPP ($p < 0.05$). FTIR analysis showed that the interaction between PPP and CS might have been physical. XRD analysis showed that the composite membrane solution had an amorphous structure after the addition of PPP. The pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid reactants (TBARS), hardness, and colour of the 0.2, 0.4, 0.6, and 0.8% composite membrane solutions were better than those of the control group when stored at 4°C for 12 d. The 0.6% PPP-CS treatment group had the best preservation effect, and the shelf life of beef was extended by 2 - 3 d. Therefore, the PPP-CS membrane solution could be a promising method for preserving fresh beef.

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Introduction

Pomegranate (*Punica granatum* L.; Lythraceae) is a fruit with extraordinary medicinal and nutritional values, cultivated widely in Africa, America, and South Asia (Danesi and Ferguson, 2017; Vučić *et al*., 2019; Shahkoomahally *et al*., 2021). Pomegranate peel, the inedible part of pomegranate, accounts for approximately 40 - 50% of the total fruit weight, and is usually discarded and wasted by the pomegranate juice industry every year (Petrotos *et al*., 2021; Kumar *et al*., 2022). The application significance of pomegranate peel is greater than that of other pomegranate parts (Ola *et al*., 2014). Zhu *et al*. (2015) showed that pomegranate peels had better antioxidant properties than pomegranate seeds, and could be used as a source of natural plant antioxidants to avoid damage to the human body by synthetic antioxidants. Furthermore, pomegranate peel is rich in polyphenols, and considered to have various antioxidant and antibacterial activities (Magangana *et al*., 2022). The microbial activity of meat products during storage can be effectively inhibited by pomegranate peel polyphenols, thereby delaying their deterioration (El-Hadary and Taha, 2020).

Beef is one of the meats that people often consume due to its rich nutritional value and pleasant taste (Mahbubi *et al*., 2019). The oxidation of fresh beef after slaughter, and the oxidation of lipids and proteins during storage are serious problems (Gruffat *et al*., 2021). Beef contains polyunsaturated fats, water, and proteins that oxidise easily, resulting in poor meat quality, including changes in sensory quality, flavour, and odour (Antic *et al*., 2021; Júnior *et al*., 2022). The commonly used fresh-keeping methods for beef include vacuum packaging, air-controlled packaging, and the addition of food additives and other methods (McMillin, 2017; Peck *et al*., 2020). Pennacchia *et al*. (2011) showed that vacuum packaging limited the number of *Thermophilus*, *Pseudomonas*, and *Enterobacter* in meat; had significant effects on the number of viable bacteria, and little impact on the diversity of

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microbial populations on beef. Brooks *et al*. (2008) showed that, compared to traditional packaging, the growth and reproduction of pathogenic bacteria such as *Escherichia coli* and *Salmonella* in beef in an air-conditioned packaging group could be effectively inhibited. In a study by Yang *et al.* (2022a; 2022b), low-energy electron beam irradiation at 4 - 8 kGy was a promising innovation that could extend the shelf life of vacuum-packed cold steaks. However, problems such as the high cost, complex operation, and low safety of fresh beef preservation methods need to be urgently addressed. Therefore, cost-effective and safe solutions should be developed to extend the shelf life of beef, which is valuable for addressing the short shelf life of beef and reducing waste.

Chitosan (CS), a polycationic polysaccharide obtained from chitin after partial or total deacetylation, is widely found in shrimps, crabs, algae, and other substances, and is the second most abundant basic polysaccharide in natural polysaccharides (Santos *et al*., 2020; Kou *et al*., 2021). CS has attracted extensive attention due to its comprehensive advantages such as antimicrobial activity, biocompatibility, safety, and anticancer properties, which are important for maintaining the stability of food systems by covering or coating the CS on food surfaces (Li *et al*., 2021). However, poor antioxidant properties and low mechanical strength of CS coating cannot achieve the desired results when applied as food preservation. To resolve these challenges, an increasing number of scientists have reviewed them from all perspectives. Zhou *et al*. (2023) reported a dynamic cross-linked packaging film with excellent antibacterial and antioxidant properties based on CS/cellulose nanofibers. In a study by Zhou *et al*. (2022b), CS and tannic acid composite coatings effectively prevented oxygen oxidation and water loss, and fruit ripening was delayed. Luo *et al.* (2023) indicated that CS-based packaging films with integrated antimicrobial peptides positively affected fresh pork preservation.

PPP is an active material extracted from pomegranate peel, and its excellent antioxidant and antibacterial activities are widely used in many fields. Preparing CS compounded with PPP in a plastic wrapping fluid enhances its antibacterial activity (Kumar *et al*., 2021). The poor antioxidant capacity and limited bioactivity of CS coating thus could be overcome. Recent studies have shown that PPP could play a crucial role in food preservation. Yu *et al*. (2022) prepared a combination of PPP and CS as a preservation solution for different types of fish; the results indicated that the composite membrane solution could effectively delay spoilage deterioration, and prolong the shelf life of fish meat. Cai *et al*. (2021) reported that nanofibers prepared from PPP with CS and *Pleurotus eryngii* polysaccharides effectively inhibited the growth of *E. coli* O157:H7 on the surface of cucumber and pork. Consequently, the shelf life of meat products can be effectively extended using composite materials prepared using PPP and CS as raw materials.

Currently, several packaging options are available to improve the shelf life of chilled beef. Guo *et al*. (2021) reported that beef quality could be improved by packing starch films containing sea buckthorn fruit residue extract. In a study by Zhang *et al.* (2021b), the shelf life of chilled beef was improved using bio-nanocomposite films of CS and montmorillonite incorporated in ginger essential oil. However, the preservation of chilled beef using PPP-CS composite membrane solution has not been reported. The present work thus aimed to prepare a PPP-CS composite membrane solution, and study its physicochemical properties. In addition, a PPP-CS was developed to evaluate the preservation of beef and provide a foundation for developing safe and natural fresh beef preservative.

Materials and methods

Materials

PPP (purity ≥ 70%), CS (degree of deacetylation, 90.36%), edible gelatine (Karim and Bhart, 2009), vitamin C, trichloroacetic acid, thiobarbituric acid, hydrochloric acid, boric acid, and all other chemical reagents were purchased from Xi'an Jingbo Biotechnology Co., Ltd., Shaanxi, China. All chemical reagents were of analytical grade. Fresh beef was purchased from Xi'an Vanguard Supermarket, Shaanxi, China.

Preparation of PPP-CS composite membrane solution

CS (5 g) was added to 500 mL of distilled water, and stirred at 60°C for 10 min. Glacial acetic acid (10 mL) was added, stirred for 1 h, and left for 30 min to obtain CS membrane solution.

Gelatine (5 g) was accurately weighed, mixed with 500 mL of distilled water, and stirred at 55°C until completely dissolved to obtain a gelatine membrane solution.

The same volume of CS and gelatine membrane solution was mixed into the CS-gelatine composite membrane solution as the control group. The same volume of CS-gelatine composite membrane solution was mixed with 0.2, 0.4, 0.6, and 0.8% PPP solutions to obtain different concentrations of the PPP-CS composite membrane solution.

Determination of pH, viscosity, and colour difference

PPP-CS composite membrane solutions of different concentrations (20 mL) were placed in a 50 mL beaker. The pH and viscosity of the PPP-CS composite membrane solutions with different concentrations were determined using a pH meter (PHS-3C, Shanghai Leici Instrument Factory, China) and viscometer (NDJ-1, Shanghai Lichen Instrument Technology Co., Ltd., China), respectively.

The L* (lightness), a* (redness), and b* (yellowness) values of PPP-CS composite membrane solution of different concentrations were determined using a colorimeter (NR10QC, Shenzhen SAN Enshi Technology Co., Ltd., China).

Determination of antioxidant activity

DPPH (1,1-diphenyl-2-picrylhydrazyl), 2,2′-azino-bis diammonium salt ($ABTS^+$), hydroxyl, and superoxide anion (SA) scavenging rate methods established by Zhou *et al*. (2022a) were referred and improved to evaluate the antioxidant activity of PPP-CS composite membrane solution with different concentrations. Moreover, 0.8% vitamin C was used to compare the antioxidant capacity of different concentrations of PPP-CS.

PPP-CS composite membrane solutions with different concentrations (1 mL) were placed in a 10 mL test tube, 1 mL of 0.1 mmol/L DPPH ethanol solution was added, shaken well, and allowed to react for 30 min in the dark. Absorbance was measured at 517 nm, and denoted as A_x. These steps were repeated by replacing the sample and DPPH solution with distilled water, and the absorbance was measured as A_k and A_d, respectively. The DPPH scavenging capacity was calculated using Eq. 1:

$$DPPH\ (\%) = [(A_d - A_x)/A_k] \times 100 \quad \text{(Eq. 1)}$$

PPP-CS composite membrane solutions of different concentrations (1.5 mL) were placed in a 10 mL test tube, 1.5 mL of $ABTS^+$ solution was added, and the absorbance was measured at 734 nm, denoted as A_x. These steps were repeated by replacing the sample with an $ABTS^+$ solution and distilled water, and the absorbance was measured as A_k and A_d, respectively. The scavenging capacity of the $ABTS^+$ free radical was calculated using Eq. 2:

$$ABTS^+\ (\%) = [(A_d - A_x)/A_k] \times 100 \quad \text{(Eq. 2)}$$

PPP-CS composite membrane solutions of different concentrations (1 mL) were placed in 10 mL test tubes. After adding 1 mL of 9 mmol/L $FeSO_4$ solution and 1 mL of 9 mmol/L salicylic acid solution, 1 mL of 8.8 mmol/L H_2O_2 solution was allowed to react for 30 min, centrifuged at 6,000 rpm for 10 min, and the absorbance at 510 nm of the supernatant was measured, and denoted as A_x. These steps were repeated by replacing the sample with an H_2O_2 solution and distilled water, and the absorbance was measured as A_k and A_d, respectively. The scavenging capacity of hydroxyl radicals was calculated using Eq. 3:

$$Hydroxyl\ (\%) = [(A_d - A_x)/A_k] \times 100 \quad \text{(Eq. 3)}$$

PPP-CS composite membrane solutions of different concentrations (2 mL) were placed in 10 mL test tubes. Then, 2 mL of nitroblue tetrazolium chloride (NBT) solution and 2 mL of nicotinamide adenine dinucleotide (NADH) solution were successively added, and then, 0.8 mL of phenazine methosulphate (PMS) solution was added. After incubation for 10 min, the absorbance was measured at 560 nm, and denoted as A_x. These steps were repeated by replacing the sample and PMS solution with distilled water, and the absorbance values were measured as A_k and A_d, respectively. The scavenging capacity was calculated using Eq. 4:

$$SA\ (\%) = [(A_d - A_x)/A_k] \times 100 \quad \text{(Eq. 4)}$$

Determination of Fourier-transform infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR; Tensor 27, Bruker, Germany) was used for the scanning determination of powder samples of PPP-CS composite membrane solutions with different concentrations after freeze-drying for 48 h at 4000 - 400 cm^{-1}.

Determination of X-ray diffraction

X-ray diffraction (XRD; D8 Advance, Bruckner, Germany) determined the different

concentrations of freeze-dried PPP-CS samples under the conditions of radiation current 100 mA, radiation voltage 40 kV, and a measurement range of 2θ (5 - 40°).

Preparation of fresh beef

After the fresh beef was washed and dried with ultrapure water, the fascia, fat, and visible connective tissue on the surface were removed on an ultra-clean table. The beef was divided into uniform blocks weighing approximately 100 g each. The beef was evenly divided into five portions and placed in a sterile Petri dish, followed by immersion in the control, 0.2, 0.4, 0.6, and 0.8% composite membrane solution for 5 min, then dried and placed in a sterile Petri dish. Beef treated with composite membrane solution of different concentrations was stored at 4°C for 12 d, and the various indexes of beef during storage were determined.

Determination of preservation effect of beef

The pH of the beef in the different groups was determined using a pH meter (PHS-3C, Shanghai Leici Instrument Factory, China). Beef samples treated with different PPP-CS composite membrane solutions were accurately weighed (5 g), and 50 mL of distilled water was added.

The weight of beef in each group before wrapping with a cling film was measured and denoted as m_1. Before the next measurement of each beef indicator, the beef was removed from the sterile Petri dish, dried with sterile filter paper, and weighed, denoted as m_2. The juice loss rate was calculated using Eq. 5:

$$\text{Juice loss rate (\%)} = (m_1 - m_2)/m_1 \times 100 \qquad \text{(Eq. 5)}$$

The thiobarbituric acid reactant (TBARS) levels were measured using colorimetric determination (Zhang *et al*., 2020). Briefly, 5 g of ground meat samples were weighed in a 100 mL corked conical flask, 50 mL of 10% trichloroacetic acid was added, oscillated in a thermostatic oscillator (DK-98, Tianjin Tester Instrument Co., Ltd., China) at 50°C for 30 min, and filtered two times. The supernatant (5 mL) was placed into a 25 mL colorimetric tube, and 5 mL of 0.02 mol/L thiobarbituric acid solution was added. The mixture was heated in a constant-temperature water bath at 80°C for 30 min, and then cooled to room temperature. Absorbance was measured at 532 nm.

Total volatile basic nitrogen (TVB-N) was measured using the trace-diffusion method (Del Blanco *et al*., 2017). Briefly, 5 g of ground beef was weighed, and 25 mL of distilled water was added. Water-soluble glue was smeared on the edge of the diffusion plate, one drop of mixed indicator and 1 mL of boric acid solution were added to the centre of the diffusion plate, and 1 mL of beef impregnation solution was added to the outside. A saturated potassium carbonate solution (1 mL) was added to the gap between the diffusion plate and the lid, which was quickly sealed and shaken well, allowed to stand for 2 h, and titrated with 0.01 mol/L hydrochloric acid.

The hardness of each group of beef samples was measured using a texture analyser (TA. XT. Plus, Stable Micro Systems, Germany). The beef treated with different concentrations of the PPP-CS composite membrane solution was cut into cubes of the same size. A P/36R stainless-steel cylindrical probe was used. The pre-test, test, and post-test rates were set to 2, 1, and 4 mm/s, respectively, and the compression ratio was set to 40%. Both compression intervals were set to 5 s.

The surface L*, a*, and b* values of the beef were determined using a colorimeter (NR10QC, Shenzhen SAN Enshi Technology Co., Ltd., China).

Statistical analysis

All results were expressed as mean ± SD (standard deviation), and all analyses were performed using three parallel tests. SPSS software (version 25.0) was used to analyse the variance (ANOVA). Statistical significance was set at $p < 0.05$.

Results

pH, viscosity, and colour difference analysis

The pH values of the PPP-CS composite membrane solutions at different concentrations are shown in Figure 1A. The results showed that the pH of the control group was significantly higher than that of the PPP-CS composite membrane solution ($p < 0.05$). No significant difference was observed in the pH among the 0.2, 0.4, and 0.6% composite membrane solutions ($p > 0.05$).

As shown in Figure 1B, the viscosity was positively correlated with the concentration of the PPP-CS composite membrane, and there were significant differences between the composite membrane solutions with different concentrations ($p < 0.05$). As the PPP concentration increased, the

internal frictional and differential pressure resistances of the fluid in the solution also increased. Therefore, viscosity increased as the composite membrane solution's concentration increased.

As shown in Figure 1C, as the concentration of the composite membrane solution increased, the L* value decreased. No significant difference was observed in the L* values between the control and 0.2% groups ($p > 0.05$), whereas the L* values in the 0.4, 0.6, and 0.8% groups were significantly lower than those in the control group ($p < 0.05$). As shown in Figure 1D, the a* value showed an obvious upward trend with an increase in the concentration of the composite membrane solution, and there was no significant difference in the a* value between the control group and the 0.2% composite membrane solution ($p > 0.05$), which was similar to the L* value. As shown in Figure 1E, the b* value exhibited an obvious upward trend with increasing concentrations of the composite membrane solution. The b* values of the 0.2, 0.4, 0.6, and 0.8% groups were significantly higher than those of the control group ($p < 0.05$).

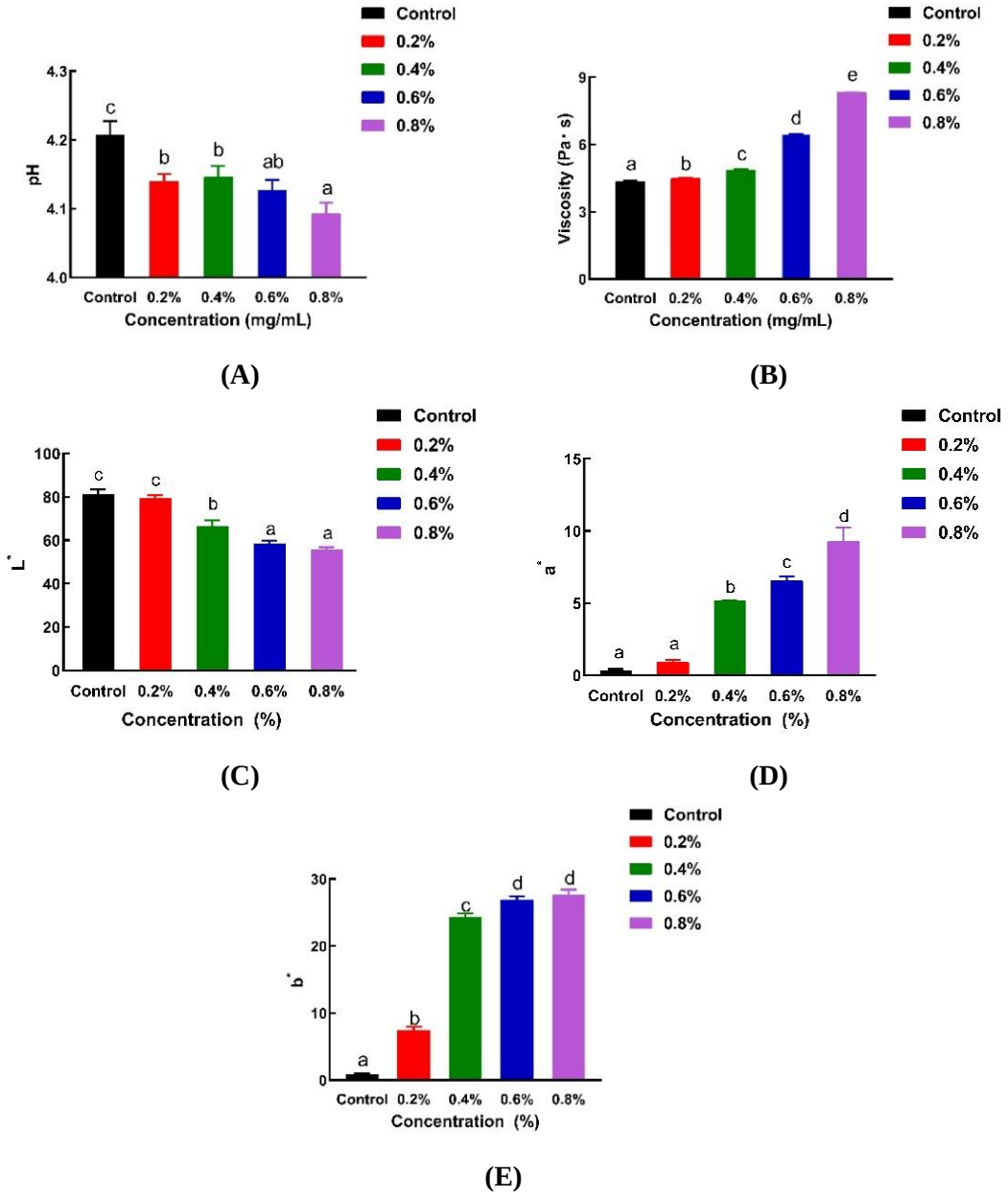

Figure 1. PH, viscosity, and colour difference of PPP-CS composite membrane solution at different concentrations. **(A)** pH, **(B)** viscosity, **(C)** L*, **(D)** a*, and **(E)** b*. Different lowercase letters indicated statistically significant difference between groups ($p < 0.05$).

Analysis of antioxidant capacity

The DPPH, $ABTS^+$, hydroxyl, and SA free radical scavenging abilities of the PPP-CS composite membrane solutions at different concentrations are shown in Figure 2.

The DPPH-scavenging ability of the PPP-CS composite membrane solution increased with increasing PPP-CS content (Figure 2A). The DPPH scavenging rates of the PPP-CS composite membrane solutions at different concentrations were significantly higher than those of the control group ($p < 0.05$). The DPPH scavenging rates of the 0.6 and 0.8% groups reached more than 99%.

Compared with the control group, the addition of different concentrations of the PPP-CS composite membrane solution significantly improved the scavenging rate of $ABTS^+$, and the $ABTS^+$ scavenging rates of the 0.4, 0.6, and 0.8% groups were significantly higher than those of the 0.2% group ($p < 0.05$) (Figure 2B).

With an increase in the PPP supplementation level, the hydroxyl radical scavenging ability of the composite membrane solution increased, indicating that the concentration of the PPP-CS composite membrane solution was positively correlated with the hydroxyl radical scavenging ability (Figure 2C). The hydroxyl radical scavenging ability of the PPP-CS composite membrane solution was significantly higher than that of the control ($p < 0.05$). When the PPP content was 0.8%, the hydroxyl radical scavenging rate of the composite membrane solution was 98.30%.

Compared to the control group, the SA scavenging ability of PPP-CS composite membrane solution significantly increased ($p < 0.05$), and the scavenging rate was more than 93% (Figure 2D).

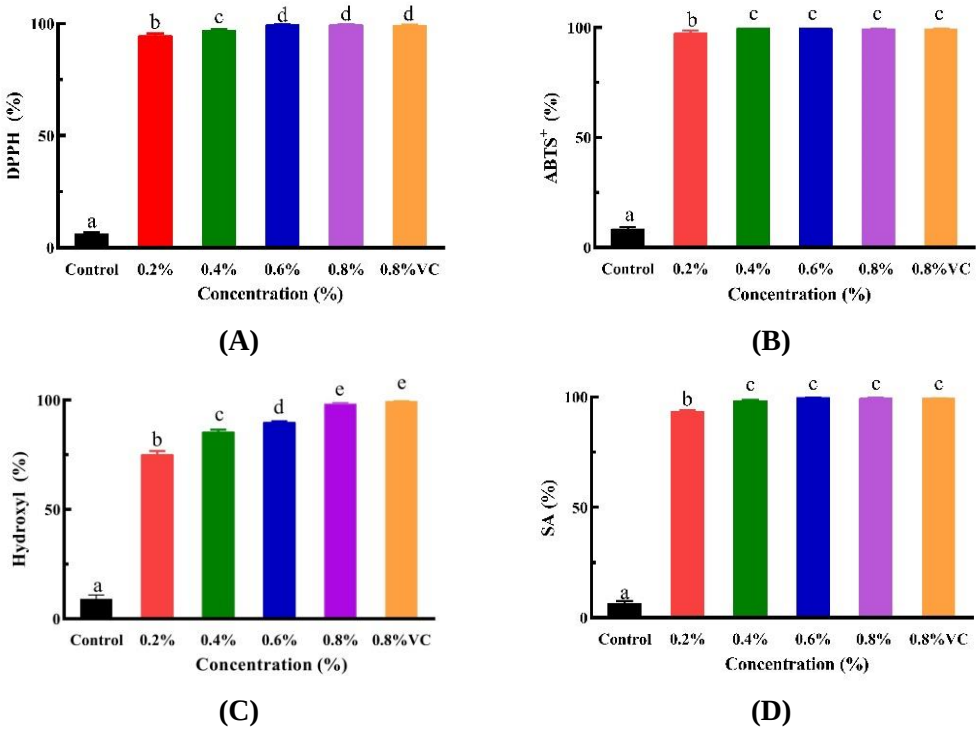

Figure 2. Antioxidant properties of PPP-CS composite membrane solutions at different concentrations. **(A)** DPPH, **(B)** $ABTS^+$, **(C)** hydroxyl, and **(D)** SA. Different lowercase letters indicated statistically significant difference between groups ($p < 0.05$).

FTIR and XRD analysis

The FTIR spectra of the PPP-CS composite membrane solutions at different concentrations are shown in Figure 3A. The results showed that there was a strong and wide absorption peak at 3441 cm^{-1} for different concentrations of the composite membrane solution, which was formed by the superposition of the characteristic peak of the O-H stretching vibration in PPP and the characteristic peak of the amide band in gelatine (Asiamah *et al.*, 2022). The peaks at 2910 and 2880 cm^{-1} are attributed to the symmetric and asymmetric stretching vibrations of -CH (Kadam *et al.*, 2019). An absorption peak was observed at 1626 cm^{-1}, indicating a C=O stretching

vibration absorption peak (amide I) in the interaction between CS and gelatine, and the stretching vibration peak at 1489 cm^{-1} was the absorption peak generated by the overlapping of the bending vibration (amide II) of the -NH of CS and gelatine (Varma and Vasudevan, 2020). The stretching vibration absorption peak at 1008 cm^{-1} was the peak produced by overlapping the C-O of PPP and the C-N stretching vibration of CS. After the addition of PPP, no new stretching vibration peaks or obvious wavelength drifts were observed. In contrast, PPP and CS were physical interactions, and no new bonds were formed. However, this phenomenon may be related to the electrostatic interactions between the NH_4 group of CS and the negatively charged COOH side chain in gelatine to form polyelectrolyte complexes under acidic conditions.

The XRD patterns of the PPP-CS composite membrane solutions with different concentrations are shown in Figure 3B. The diffraction peak positions and crystal structures of the composite membrane solutions with different concentrations were determined using XRD. When 2θ of the control was 20.07°, the diffraction peak at this position was the characteristic amorphous polymerisation peak of CS. In contrast, the control had two smaller diffraction peaks at 2θ of 9.56 and 12.51°, respectively. The diffraction peak could be observed at 2θ of 22°, and the diffraction absorption peak was broad after adding PPP. No obvious diffraction absorption peaks appeared at other positions, and only a few small peaks were observed, illustrating that the crystalline structure after the addition of PPP was amorphous.

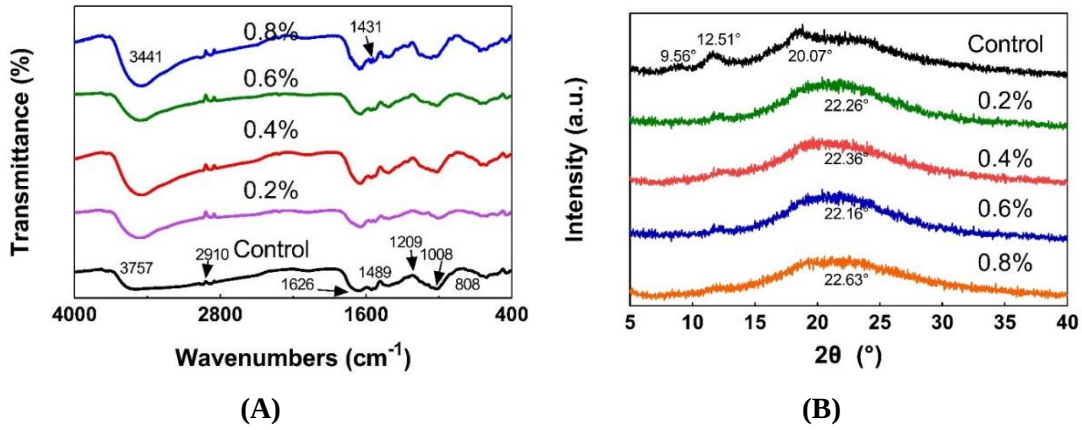

Figure 3. FTIR and XRD assay of PPP-CS composite membrane solution at different concentrations. **(A)** FTIR, and **(B)** XRD.

pH, juice loss rate, TBRAS, and TVB-N analysis of beef

pH is an important indicator of beef quality. Beef with a pH greater than 6.7 is spoiled. With prolonged storage time, all groups showed consistent changes in pH with an increasing trend (Figure 4A). The initial pH value of beef was 5.89. On day 6 of storage, the pH of the beef in the control group was 6.75, indicating that the beef in the control group had deteriorated. In contrast, the pH of beef in the different concentrations of the PPP-CS composite membrane solution was significantly lower than that of the control group ($p < 0.05$). On day 8 of storage, the pH values of beef in the 0.2, 0.4, and 0.8% treatment groups were all greater than 6.7, indicating that beef in 0.2, 0.4, and 0.8% treatment groups had deteriorated. In comparison, beef in the 0.6% treatment group maintained good quality. The pH value of beef treated with different concentrations of PPP-CS composite membrane solution was more stable; the 0.6% treatment group delayed the increase in beef pH; and the beef shelf life was extended by two to three days compared with that in the control group.

The juice loss rate of beef treated with different concentrations of PPP-CS composite membrane solution increased with increasing storage time (Figure 4B). However, the juice loss rate of the PPP-CS composite membrane solution-treated group was significantly lower than that of the control group from day 6 of storage ($p < 0.05$). Therefore, different concentrations of the PPP-CS composite membrane solution could delay the increase in beef juice loss rate.

TBARS of beef showed an increasing trend in each group (Figure 4C). The initial TBARS value of

beef TBARS was 0.23 mg/kg. During the first two days of storage, there was no significant difference in the TBARS content among all groups ($p > 0.05$), and the rate of beef fat oxidation was slow. From day 4 of storage, the TBARS value of the PPP-CS composite membrane solution-treated groups was significantly lower than that of the control group, and the TBARS value of the 0.6% treatment group was significantly lower than that of the 0.2, 0.4, and 0.8% treatment groups ($p < 0.05$). The results showed that different concentrations of the PPP-CS composite membrane solution could delay the increase in the TBARS value. The increase in the TBARS value could be delayed by two to three days in the 0.6% treatment group.

The TVB-N content of the fresh beef did not exceed 20 mg/100 g. The TVB-N content in beef increased with prolonged storage (Figure 4D). The initial value of TVB-N content in beef was 8.11 mg/100 g. On day 6 of storage, the TVB-N content in the control group was 21.16 mg/100 g, indicating that the beef in the control group had deteriorated. In contrast, the beef in the different concentrations of PPP-CS composite membrane solution had not. On day 8 of storage, beef in the 0.2, 0.4, and 0.8% treatment groups was spoiled; however, the beef in the 0.6% treatment group maintained good quality. The results showed that different concentrations of the PPP-CS composite membrane solution delayed the increase in the TVB-N content of beef. The 0.6% treatment group had a lower TVB-N value, and beef shelf life was extended by two to three days compared with that in the control group.

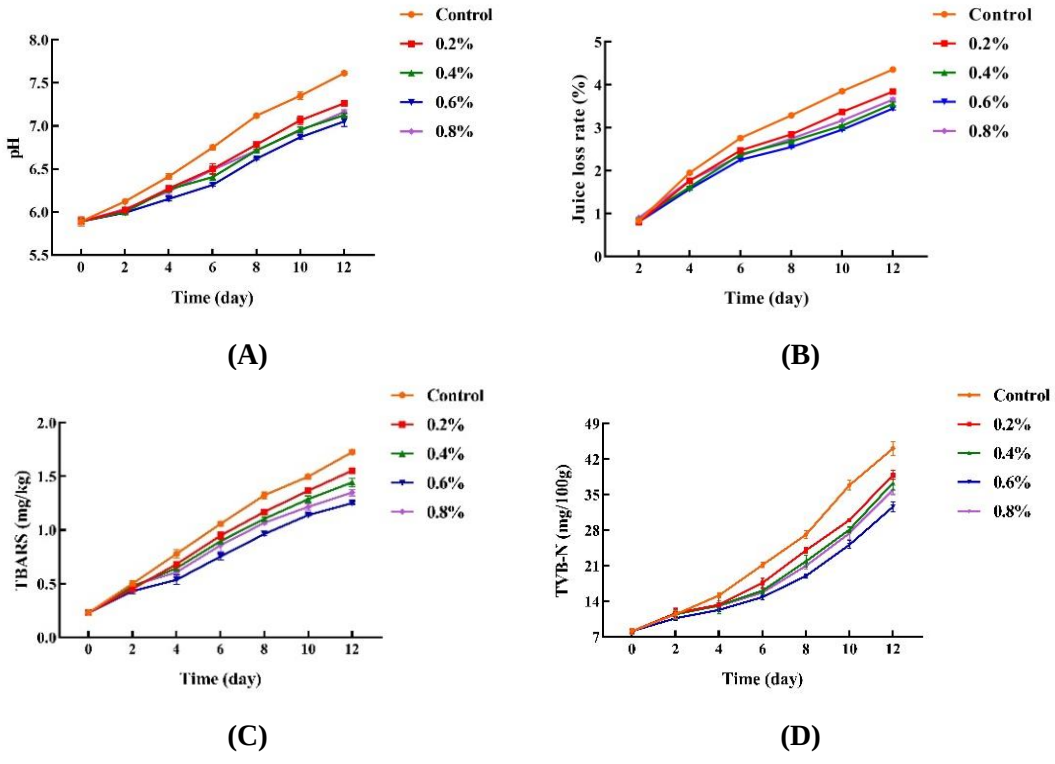

Figure 4. Effect of PPP-CS composite membrane solution at different concentrations on pH, juice loss rate, TBRAS, and TVB-N of beef. **(A)** pH, **(B)** juice loss rate, **(C)** TBARS, and **(D)** TVB-N.

Beef hardness and colour difference analysis

The hardness of all groups showed a downward trend with prolonged storage time (Figure 5A), and there was no significant difference among the hardness values of all groups during the first two days of storage ($p > 0.05$). From day 6 of storage, compared to that in the control group, the hardness of the PPP-CS composite membrane solution treatment group was relatively high, indicating that different concentrations of the PPP-CS composite membrane solution could delay the decrease in the hardness of beef during storage.

The colour difference of beef treated with different concentrations of the PPP-CS composite membrane solution is shown in Figure 5. With the extension of storage time, the L* value first increased and then decreased (Figure 5B). From day 4 of storage, the L* value of the PPP-CS composite membrane solution-treated group was significantly higher than that of the control group ($p < 0.05$),

indicating that PPP-CS composite membrane solutions of different concentrations could delay the decrease in beef L* value, and that the brightness of beef could be improved. The a* values of beef in each group showed a decreasing trend (Figure 5C). On days 4 and 6 of storage, the a* value of beef in the control group was significantly lower than in the other groups ($p < 0.05$). On day 8 of storage, the a* values of the 0.4, 0.6, and 0.8% treatment groups were significantly higher than those of the control and 0.2% treatment groups ($p < 0.05$), indicating that higher concentrations of the PPP-CS composite membrane solution could delay the a* value. With prolonged storage, there was no obvious change in the b* value of beef (Figure 5D).

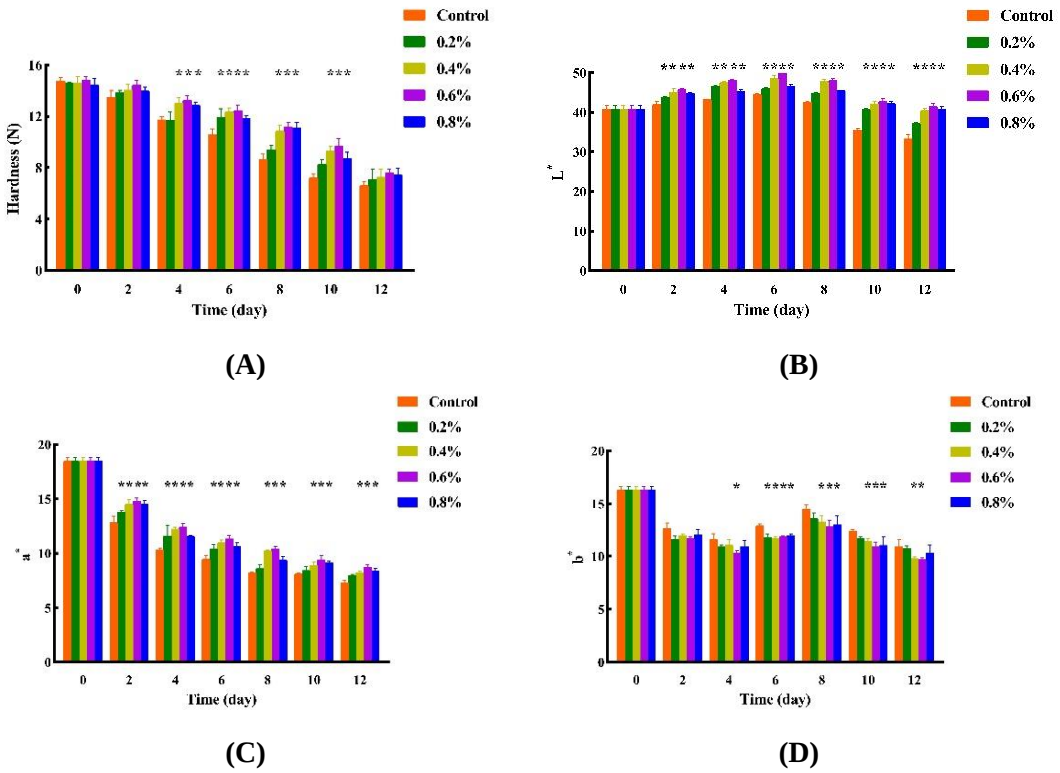

Figure 5. Effect of PPP-CS composite membrane solution at different concentrations on beef hardness and colour difference. **(A)** Hardness, **(B)** L*, **(C)** a*, and **(D)** b*. (*) represents a significant difference between the control group and the PPP-CS composite membrane solution treatment group ($p < 0.05$).

Discussion

In the present work, we investigated the physicochemical properties and preservation effects of a PPP-CS membrane solution on fresh beef. The PPP-CS composite membrane solution delayed the deterioration of beef, and the shelf life of beef was prolonged.

The pH values of the composite membrane solutions with different concentrations of PPP-CS were different, which might have been due to the amount of ionised H^+ in the solution; therefore, the pH of the composite membrane solution without PPP was significantly higher than that of the groups with PPP (Peng *et al.*, 2013). The viscosity response is a physicochemical property of the internal friction of a solution during flow, and the magnitude of the viscosity is related to the temperature, pH, and added substances (Loumprinis *et al.*, 2021). The friction and differential pressure resistance of the fluids in the PPP-CS composite membrane solution affected the viscosity of the PPP-CS composite membrane solution. In contrast, the internal friction and differential pressure resistance of PPP and CS in the high-concentration PPP-CS composite membrane solution increased; thus, the viscosity of the solution also increased.

Plant polyphenols, a class of chemicals containing polyphenolic hydroxyl groups, have powerful antioxidant properties and potent free radical scavenging capacity, and are effective nutrients for the prevention of oxidative stress-related

diseases (Gorzynik-Debicka *et al*., 2018). DPPH, $ABTS^+$, hydroxyl, and SA free radicals were used to evaluate the antioxidant activities of the PPP-CS composite membrane solutions. The results showed that the antioxidant activity of the composite membrane with PPP was significantly higher than that of the control group ($p < 0.05$), which might have been due to the active protons in PPP combined with the free radicals generated during the oxidation process to generate stable phenolic compounds (Yan *et al*., 2020). Moreover, Chen *et al*. (2022) reported that the antioxidant activity of CS composite membrane solution increased significantly after adding tea polyphenols, which was similar to the results observed in the present work.

FTIR and XRD were used to determine the intermolecular forces and crystal structure of the PPP-CS composite membrane solution, respectively. FTIR showed no extra stretching vibration peaks, and no obvious wavelength shift phenomenon after the addition of PPP, which might be because no chemical action occurred between the PPP and CS molecules, and it was likely that certain physical action occurred. In addition, this phenomenon may be related to the electrostatic interactions between the NH_4 group of CS and the negatively charged COOH side-chain group in gelatine to form polyelectrolyte complexes under acidic conditions (Asiamah *et al*., 2022). In summary, a comprehensive analysis of the FTIR spectra showed that most of the characteristic bonds of the control and PPP-CS groups were similar, and the main functional groups were not transformed. XRD analysis showed that the diffraction peak of the PPP-CS composite membrane solution decreased after the addition of PPP, which might have been due to the physical interaction between PPP and CS affecting the crystal structure of the PPP-CS composite membrane solution. Similar results were consistent with the changing trend of CS combined with macroalgal polyphenols (Meng *et al*., 2023).

Beef freshness can be determined based on its pH value. The pH value of beef tends to decrease which is due to the destruction of proteins, lipids, and other substances in beef by microorganisms as storage time increases, causing the proteins in beef to decompose into alkaline substances, such as amine compounds (Zhang *et al*., 2021a). Furthermore, the enzymatic reactions influence the increased pH in beef. However, PPP has strong antioxidant and antibacterial activities, and the activity of microorganisms in beef could be inhibited, and the increase in pH was delayed. Similar results were observed in the study of Amjadi *et al*. (2020).

Juice loss is a phenomenon in which the structure of beef is destroyed, and water is lost under certain cold storage conditions (Liu *et al*., 2022). In the early stage of storage, there was no significant difference in the rate of juice loss among the groups ($p > 0.05$), which might be because the beef did not decay during the early stage of storage, and had relatively less juice loss. The juice loss rate increased with prolonged storage time. The beef juice loss rate after treatment with different concentrations of the PPP-CS composite membrane solution was significantly lower than that of the control group ($p < 0.05$), which might have been related to the inhibition of microbial growth and protein oxidation by PPP (Mphahlele *et al*., 2016). Recently, similar results have been reported for preserving fish fillet using composite coatings containing PPP (Yu *et al*., 2022).

The degree of oxidative fission of fat in beef affects beef quality. The thiobarbituric acid method is usually used to determine the degree of oxidative rancidity of beef fat, and the TBARS values can reflect the degree of oxidation in beef (Wang *et al*., 2021). The TBARS value of the PPP-CS composite membrane solution group was lower than that of the control group, which might have been due to the fact that the beef subjected to PPP-CS treatment formed a protective film containing polyphenols, and reduced the effect of air on the beef. Free radical chain reactions are an important factor in fat oxidation. The PPP-CS membrane solution might have provided hydrogen atoms to the free radicals, hindering free radical chain reactions (Zhou *et al*., 2019). Therefore, the PPP-CS membrane solution stopped the oxidative deterioration of beef during storage, resulting in lower TBARS values. Moreover, Wu *et al*. (2022) reported that beef treated with CS films rich in benzyl isothiocyanate and α-cyclodextrin inclusion complexes could maintain lower TBARS and TVB-N values, which was in good agreement with the results observed in the present work.

TVB-N is a bovine protein broken down into volatile basic nitrogen-containing substances such as amines by microbial action and various enzymes during storage (You *et al*., 2022). TVB-N is an important index of beef deterioration; the higher the volatile salt content, the greater the beef deterioration. The PPP-CS-treated group maintained a lower TVB-

N content during beef storage, which could have been due to the superior antioxidant and bacteriostatic effects of PPP-CS. The degradation of nucleic acids, proteins, and non-proteins in beef was impeded, and the TVB-N content in beef was reduced. This result was consistent with the TBARS values, and demonstrated that using PPP-CS in beef could effectively prevent the TBRAS and TVB-N values from increasing during storage.

The colour difference and hardness of beef can affect consumer discrimination based on beef quality, which can intuitively reflect quality. With longer storage time, the hardness and colour of beef could reduce to different degrees, due to microbial activity causing the protein to break down during storage. The myoglobin s oxidised to tan high-iron myoglobin and bright red oxygenated myoglobin under the effect of oxygen (Noushin *et al*., 2018). In the present work, after PPP-CS membrane solution treatment, beef colour was more stable, and the decrease in hardness was delayed. This was related to the antioxidant activity of the PPP-CS composite membrane solution and its inhibitory effect on microbial growth and reproduction. Similar results have been reported by Li *et al*. (2022).

Conclusion

The present work demonstrated that the PPP-CS composite membrane solution had excellent physicochemical properties and extraordinary preservative effects on beef. After the addition of PPP, the pH and L* of the PPP-CS composite membrane solution decreased significantly, whereas the viscosity, a*, and b* increased significantly ($p < 0.05$). A physical interaction between PPP and CS was observed, and the crystal structure of PPP-CS was amorphous. Compared with that in the control group, the increased rate of pH, juice loss rate, TBARS, and TVB-N in the treatment group of the PPP-CS composite membrane solution with different concentrations was relatively slow, and the hardness was well maintained. In addition, the oxidation of proteins and lipids in beef was better inhibited by the 0.6% PPP-CS membrane solution treatment group, and the shelf life of beef was delayed by two to three days. These findings could provide a strong theoretical basis for natural composite membrane solutions for preserving fresh beef.

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