Supporting information

Neutral BODIPY-Chitosan Films for Photodynamic Food Freshness Preservation

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1. Experimental section

Experimental Materials

Carboxymethyl chitosan (CMCS, deacetylation degree ≥ 90% Mw=50000)), Glycerol, N-Hydroxysuccinimide, N,N′-Dicyclohexylcarbodiimide, DMSO, Succinyl chloride, 2,4-Dimethylpyrrole, Boron trifluoride diethyl etherate, Iodine (99.8%), Iodic acid (99.5%), were purchased from the Aladdin Reagent Co. Ltd. (Shanghai, China). Dialysis Membrane (MW3500) was purchased from Hunan Yibo Biotechnology Co., Ltd.

All other chemicals were purchased from commercial suppliers and used without further purification unless otherwise noted.

Analytical techniques

^{1}H NMR, DOSY NMR and ^{13}C NMR spectra were recorded on a Bruker 510 NMR spectrometer, using $CDCl_3$ as the solvent. Fourier-transform infrared (FT-IR) spectra were acquired using a FT-IR spectrometer. All UV−vis spectrometric measurements were analyzed by a Shimadzu UV-2450 spectrophotometer. The fluorescence emission spectra were monitored with the PerkinElmer LS-55 spectrofluorometer.

1.1. Syntheses of BDP

Firstly, succinyl chloride (0.8 mL, 7.5mmol) was dissolved in anhydrous dichloromethane (25 mL) and 2,4-Dimethylpyrrole (1.35ml, 13mmol) was added under N_2 protection. The reaction was refluxed at 45 ^{o}C for 3 h. After the solution was cooled to room temperature（24℃）, Et_3N (8 mL) was added slowly and stirred for 1 h at room temperature. Then $BF_3 \cdot Et_2O$ (8.7 mL) was also added slowly and the mixture was refluxed at 45^{o}C for 2 h. When the reaction finished，60 mL of 0.2 M HCl was added to quench the reaction. The brown, viscous mixture was extracted with 40ml $NaHCO_3$ three times. The aqueous phase was acidified with HCl to pH 2. Finally, extracted the product with ethyl acetate The product was a brown solid. Yield: 10.5%

BDP（500 mg，1.56 mmol）was added to 100 mL of methanol, followed by the addition of I_2 (0.62 g, 2.44 mmol). Then, 330 mg of HIO_3 was dissolved in water, and the resulting iodic acid solution was added to the mixture. The reaction was stirred at 45°C for 3h. After the reaction was completed, methanol was removed by vacuum distillation. The crude product was purified via silica gel column chromatography to afford a red solid product. Yield: 73.5%. We performed proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy, diffusion-ordered nuclear magnetic resonance spectroscopy (DOSY), Fourier transform infrared (FT-IR) spectroscopy, and mass spectrometry (MS) analysis, as well as the determination of the maximum absorption and emission peaks for the synthesized COOH-BDP.

1.2. Syntheses of CMCS-BDP

The structure of COOH-BDP was verified by ^{1}H NMR, DOSY NMR and ^{13}C NMR and electrospray ionization mass spectrometry (ESI-MS) and FT-IR spectrum in Figure S1-S5. Photodynamic chitosan, designated CMCS-BDP, was synthesized via amide formation between carboxyl group in COOH-BDP and free amine group in CMCS(Carboxymethyl chitosan)as depicted in Scheme 1. In brief, COOH-BDP (35 μmol, 20 mg) was dissolved in dimethyl sulfoxide (DMSO) and activated by N,N′-dicyclohexylcarbodiimide and N-hydroxysuccinimide for 30 min. The activated COOH-BDP was added dropwise into CMCS (Mw=50000, 0.4 g, 1% w/w in pure water). After stirring for 24 h at room temperature, the reaction mixture was purified by dialysis against ethanol absolute for 3 days and the dialysate was supposed to be replaced every day. Finally，the CMCS-BDP was obtained from vacuum drying.

1.3. Water contact angle of the films

The water contact angle measurement used a Ramé-Hart 200-F1 standard goniometer by the sessile drop method at room temperature with deionized water (2 μL) as the medium.

1.4. Mechanical Testing.

The tensile strength of the nanofiber mats was measured using a universal testing machine (Instron 5944, ITW-Instron, USA). The load cell capacity was 100 N, with a crosshead speed of 10 mm/s. Knife-shaped clamps (2 mm thick) were used. For compressive testing, the crosshead speed was 10 mm/s with a 2 kN load cell. The tested samples were dumbbell-shaped, with a diameter of 15 mm and a height of 5 mm.

1.5. Water vapor permeability (WVP) of the films

The dried silica gel powder (5 g) was completely sealed in a 10 mL open weighing bottle with the films. The weighing bottle was then placed in a constant temperature and humidity chamber at 30 °C, 70% RH, and weighed every 12 h during 36 h test. WVP was calculated as follows:

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

In the formula, Δm was weight gain of the weighing bottle (kg), D was the film thickness (m), t was the time (s), S was the centrifugal tube opening area (m^2), and ΔP was the saturated vapor pressure at 30 °C.

1.6. Oxygen permeability (OP)

The 3 g of deoxidizer (1.5 g NaCl, 1 g activated carbon, and 0.5 g reduced iron powder) was placed in a weighing bottle, and the mouth of the bottle was sealed with a film sample. The weighing bottle was then placed in a constant temperature and humidity chamber at 30 °C, RH = 70% and weighed every 12 h during 36 h test

$$OP = \frac{\Delta m \times D}{t \times S \times \Delta P}$$

In the formula, Δm was weight gain of the weighing bottle (kg), D was the film thickness (m), t was the time (s), S was the centrifugal tube opening area (m^2), and ΔP was the partial pressure of oxygen.

1.7. Assessment of ROS generation of films.

A total of 10 mg of photodynamic films was immersed in 10 mL of DPBF solution to serve as an indicator of overall ROS generation. The films were affixed to the bottom of a 24-well plate, and 600 μL of DPBF phosphate-buffered saline (PBS) solution was added to each well. The changes in UV absorbance at 420 nm were recorded after green-light irradiation for different time intervals. We also performed ROS test for the pure CMCS films.

1.8. Antibacterial evaluation of photosensitizer.

After ultraviolet sterilization, 1mg COOH-BDP were co-incubated with 10 ml bacterial suspensions (10^5 CFU mL^{-1}) for 2 hours, followed by 20 minutes of green light irradiation, the bacteria suspensions were smeared into LB ager plates. The number of colonies of different groups was characterized by the conventional plate counting method after 24 h culture.

1.9. Film-contact antibacterial test of the films

The films (10mm×10mm) were sterilized by UV irradiation, transferred to bacteria-coated agar plate (10^7 CFU mL^{-1}). After incubation at 37 °C for 30 min, the plate was irradiated with a green LED light for 15min. The diameter of the inhibition zone was determined after 24 h of incubation at 37 °C. In Figure S6, the UV-region spectra confirm that UV sterilization has no impact on the photosensitizer function of BODIPY itself.

1.10. Preparation and experimental conditions of the fruit preservation test

Specifically, the fresh-keeping films with a uniform size were all prepared by casting in petri dishes with a standard dimension of 90×90 mm. Fresh cherries with homogeneous sizes (5–6 g per fruit, 3 cherries per group) were placed on sterile petri dishes (9 cm in diameter), and then tightly wrapped with pre-cut film pieces (90×90 mm for each piece). Three film pieces were used for one group of cherries (one laid on the bottom of the petri dish and two wrapped around the cherries on the top), and the

edges were sealed with sterile parafilm to ensure airtightness. To ensure consistent contact area between the film and fruit, we marked the actual contact positions of the preservative film on cherries in each group during wrapping. Horizontal comparison of these positions and areas guaranteed nearly identical contact areas across all groups. The same film pieces were used for each cherry group throughout the 8-day storage period, and the films were inspected daily to confirm no breakage or loosening. All cherry preservation experiments were conducted under constant temperature and humidity conditions (25 ± 1 °C, 50 ± 5% relative humidity), which is a typical ambient storage condition for fresh fruits.

1.11. The VC content of cherries

Cherries (5 g) were mashed in each group, 4% metaphosphoric acid (5 mL) was added, ultrasound was performed for 5 min and swirled for 2 min. After centrifugation, the samples were prepared by diluting 4% metaphosphate 20 times, and the absorbance at 242 nm was measured. The standard curve was constructed using 4% metaphosphoric acid.

1.12. The firmness of cherries

Fruit firmness was measured using a GY-3 portable fruit ripeness test, which was equipped with a stand for stable operation. The instrument features a measuring range of 0–12 kg/cm² (equivalent to 0–117.68 N/cm²), a resolution of 0.1 kg/cm², and a standard 8 mm diameter probe (with optional 5 mm and 11 mm probes available). It is equipped with a digital LCD screen for real-time data display and is powered by 2×AA batteries, ensuring convenient portability for on-site or laboratory measurements.

1.13. Weight loss

The weighing method was used to determine the weight change of winter jujubes during storage, and the weight loss rate was calculated by the following formula：

$$\text{Weight loss rate } (\%) = \frac{m_0 - m}{m_0} \times 100 \qquad (9)$$

where m is the weight of winter jujubes after being stored for a storage time (day 0-day 8), and m_0 is the initial weight.

1.14. The cherry sensory evaluation

The cherry sensory evaluation was conducted by 10 trained experts (5 men and 5 women) from the population. A 0–10 scoring scale, where scores increase sequentially from 0 to 10, with higher values indicating better sample quality was used to assess the four key indicators used to evaluate the preservation effect of cherries.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 (Solvent), 3.41(t, 2H), 2.66(t, 2H), 2.62(s, 6H), 2.51(s, 6H), 2.05, 1.25, 0.88(impurities). The impurity peak (0-2.5 ppm region) is caused by residual solvent from rotary evaporation during the synthesis of the photosensitizer. The peaks corresponding to the hydrogen atoms of the other four methyl groups and two methylene groups are visible in the spectrum.

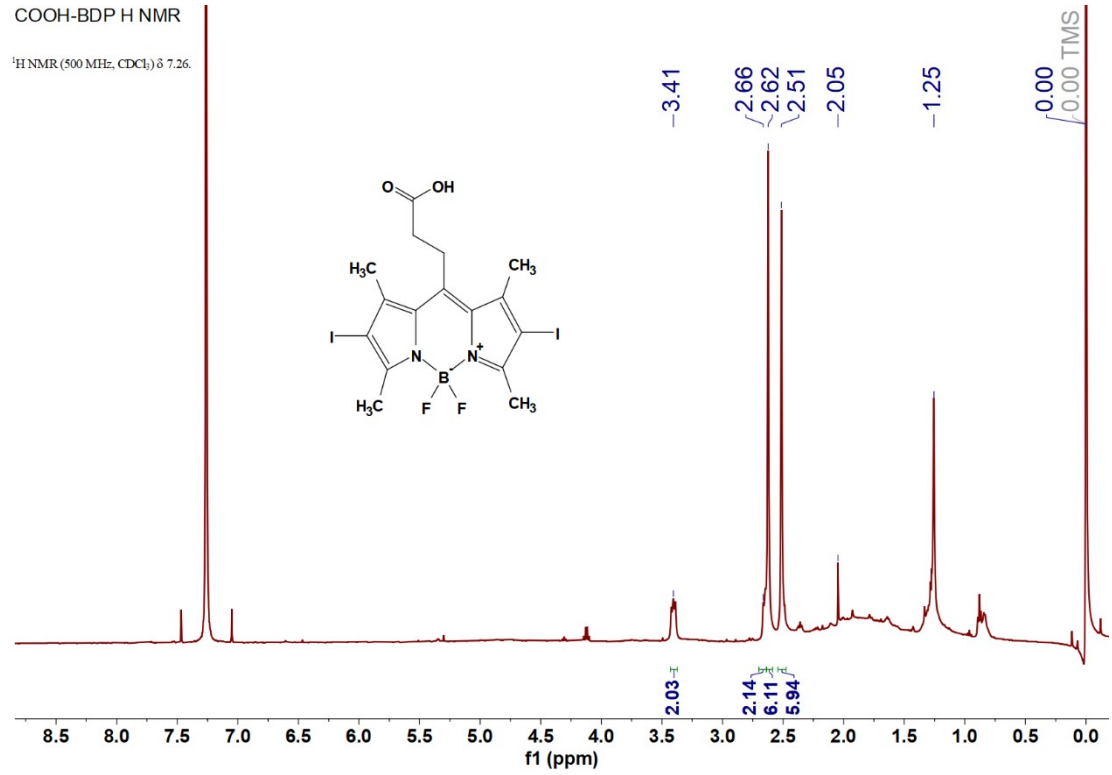

Figure S1. The ^{1}H NMR spectrum of COOH-BDP (Solvent: $CDCl_3$).

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.77 (C=O), 156.82 (C), 146.78 (C), 138.51 (C), 129.80 (C), 97.10 (C), 77.12 (Solvent), 57.83, (CH_2-CO), 47.18 (CH_2), 18.21, 10.26 (CH_3). The signal at δ 174.77 ppm corresponds to the carboxylic acid carbon (-COOH). The aromatic/sp^2 carbon region shows signals at δ 156.82 (C – I carbons), 146.78 (meso-sp^2 carbon of the BODIPY core), 138.51 (methyl-substituted pyrrole carbons), 129.80 (unsubstituted pyrrole sp^2 carbons), and 97.10 ppm (aza carbons adjacent to BF_2), all characteristic of the BODIPY core structure. The signal at δ 77.12 ppm is assigned to the $CDCl_3$ solvent. The aliphatic region shows signals at δ 57.83 and 46.80 ppm, corresponding to the two methylene carbons of the propanoic acid side chain (-CH_2CH_2COOH). The signals at δ 18.21 and 10.26 ppm are assigned to the four pyrrole ring methyl groups, with slight differences in chemical shifts arising from the asymmetric substitution pattern of the BODIPY core. All signals are accounted for and consistent with the expected molecular structure.

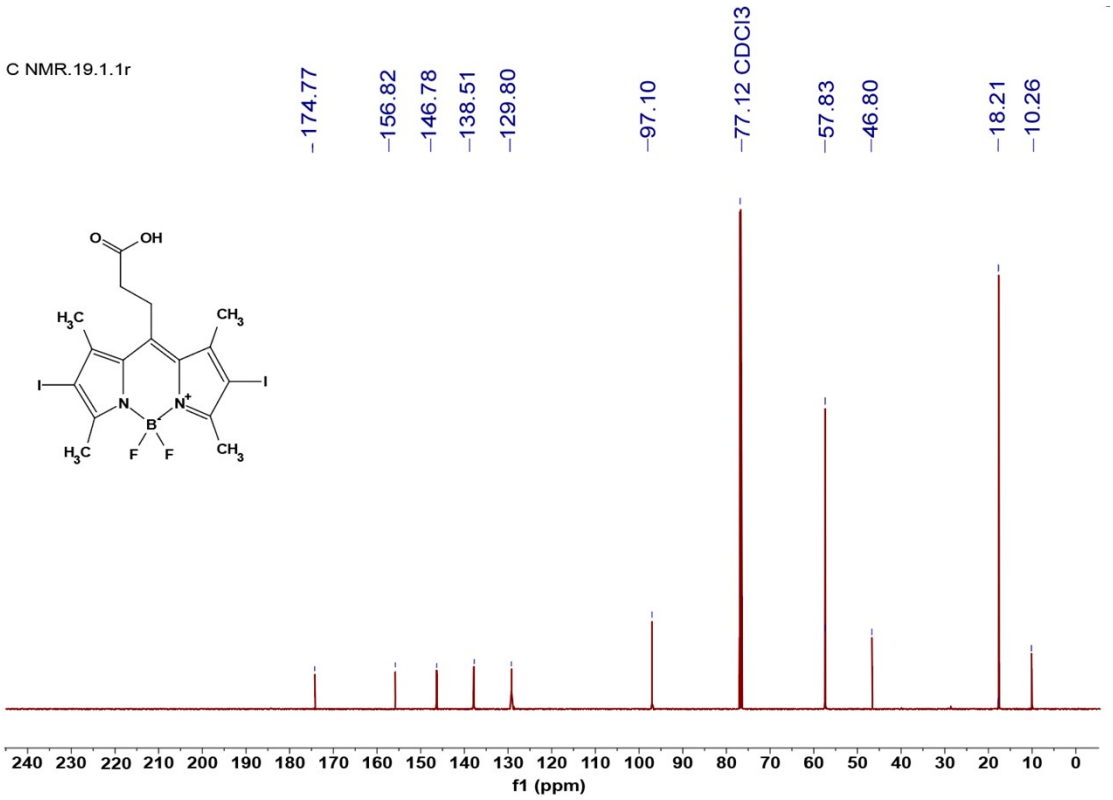

Figure S2. The ^{13}C NMR spectrum of COOH-BDP (Solvent: $CDCl_3$).

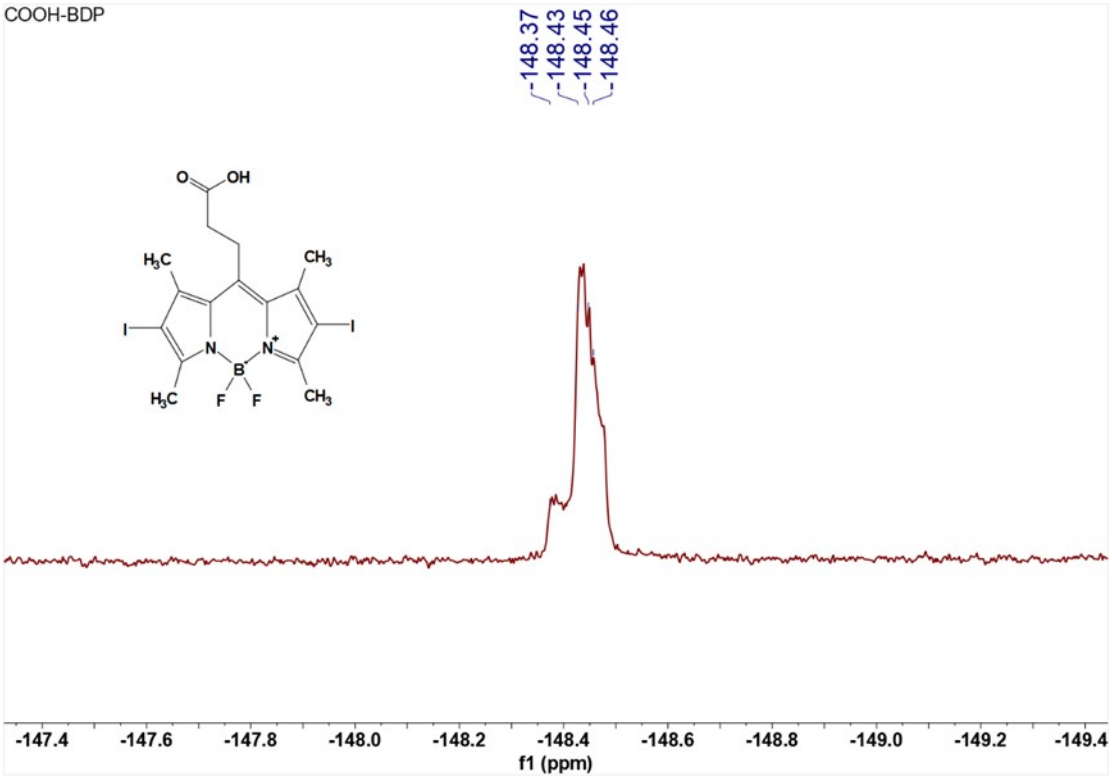

Figure S3. The ^{19}F NMR spectrum of COOH-BDP (Solvent: $CDCl_3$).

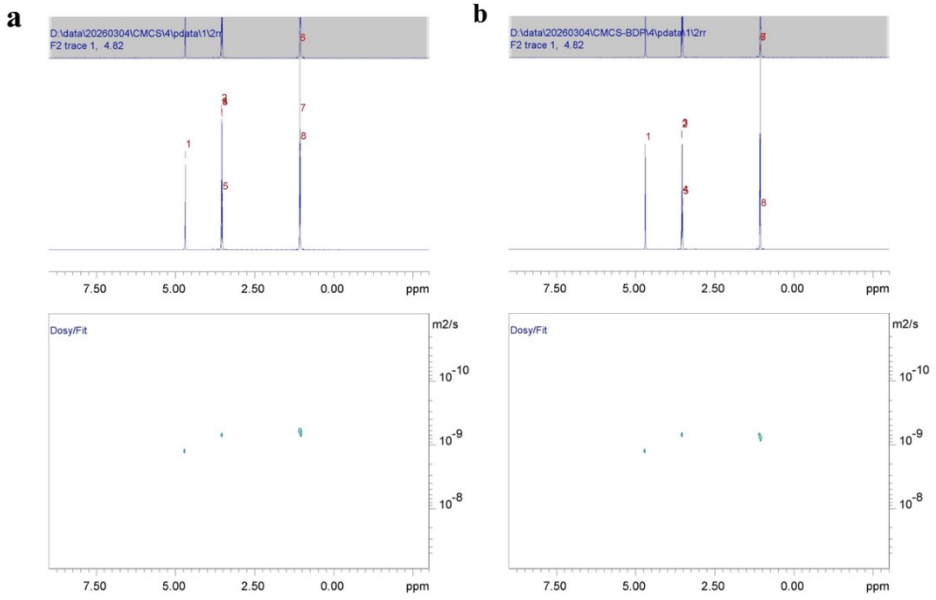

Figure S4. The DOSY NMR spectra of CMCS and CMCS-BDP (Solvent: D_2O).

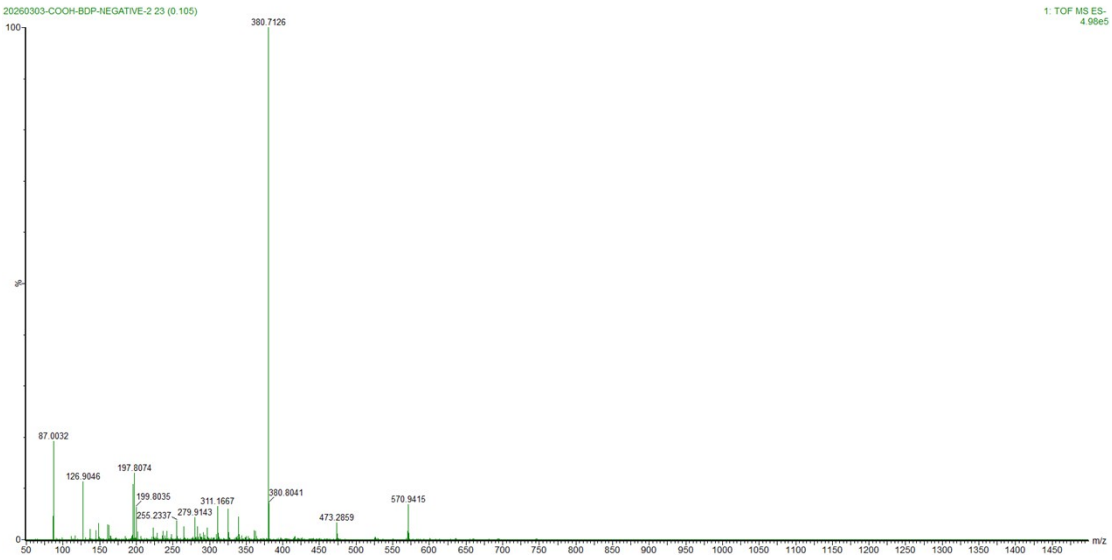

Figure S5. The ESI-MS spectrum of COOH-BDP.

HR-MS Peak assignment:

m/z calcd for $C_{16}H_{16}BF_2I_2N_2O_2^-$ $[M–H]^-$ 570.9254, found 570.9415;

m/z calcd for $C_{15}H_{16}BFIN_2^-$ $[M–H–CO_2–IF]^-$ 381.0327, found 380.7126;

m/z found 126.9046 (I^-)**.**

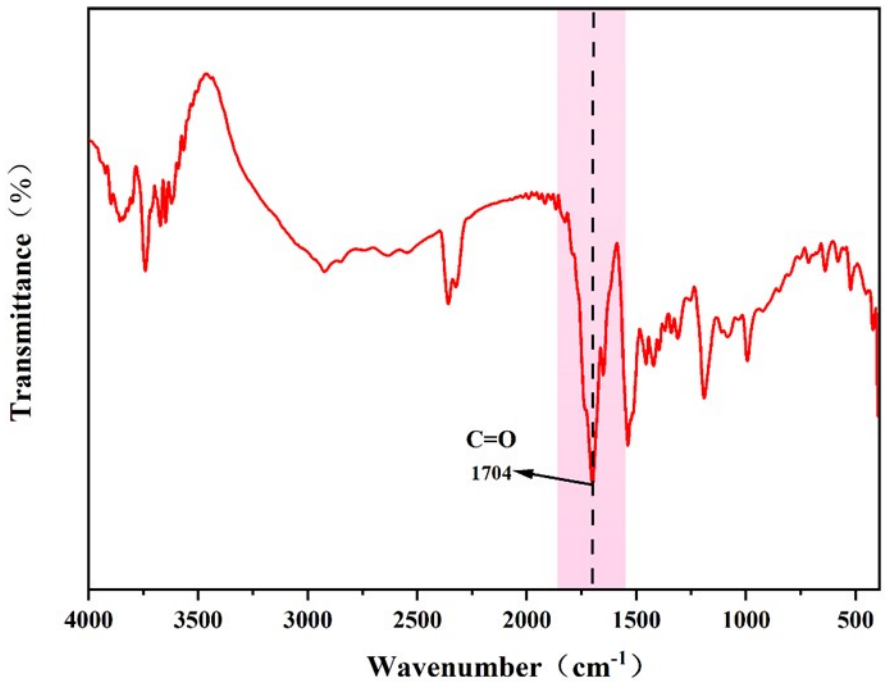

Figure S6. FT-IR spectrum of free COOH-BDP

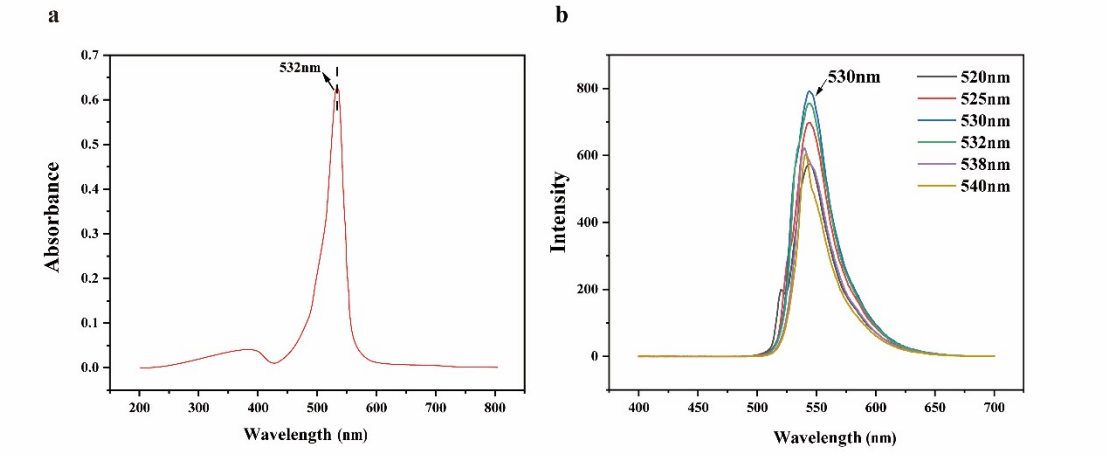

Figure S7. UV–vis absorption (a) and fluorescence spectra (b) of COOH-BDP in methanol.

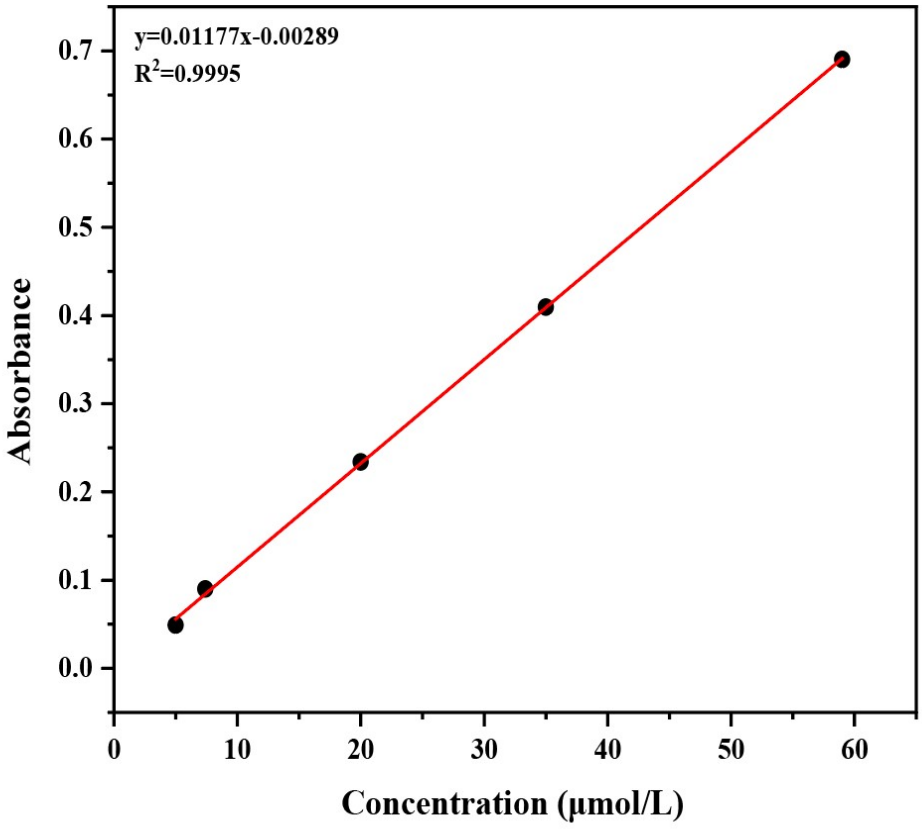

Figure S8. The standard curve of the concentration of COOH-BDP.

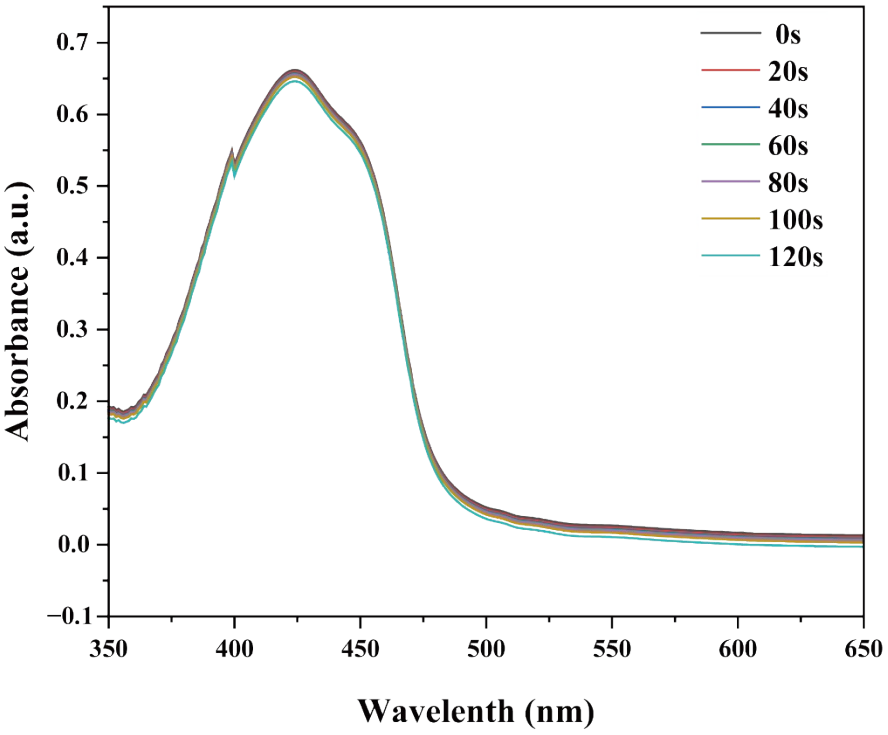

Figure S9. The absorption spectra of DPBF in the presence of pure CMCS films.

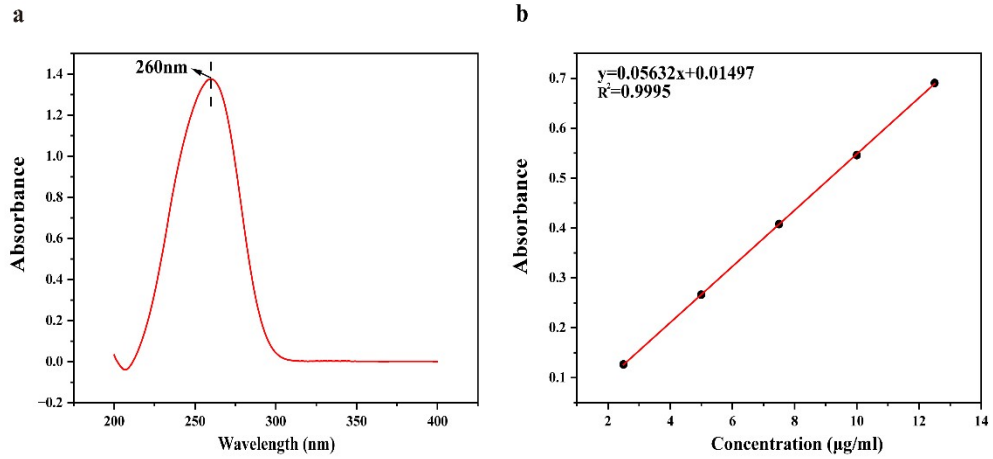

Figure S10. (a) Uv-vis absorption spectrum of VC and (b) standard curve of VC.

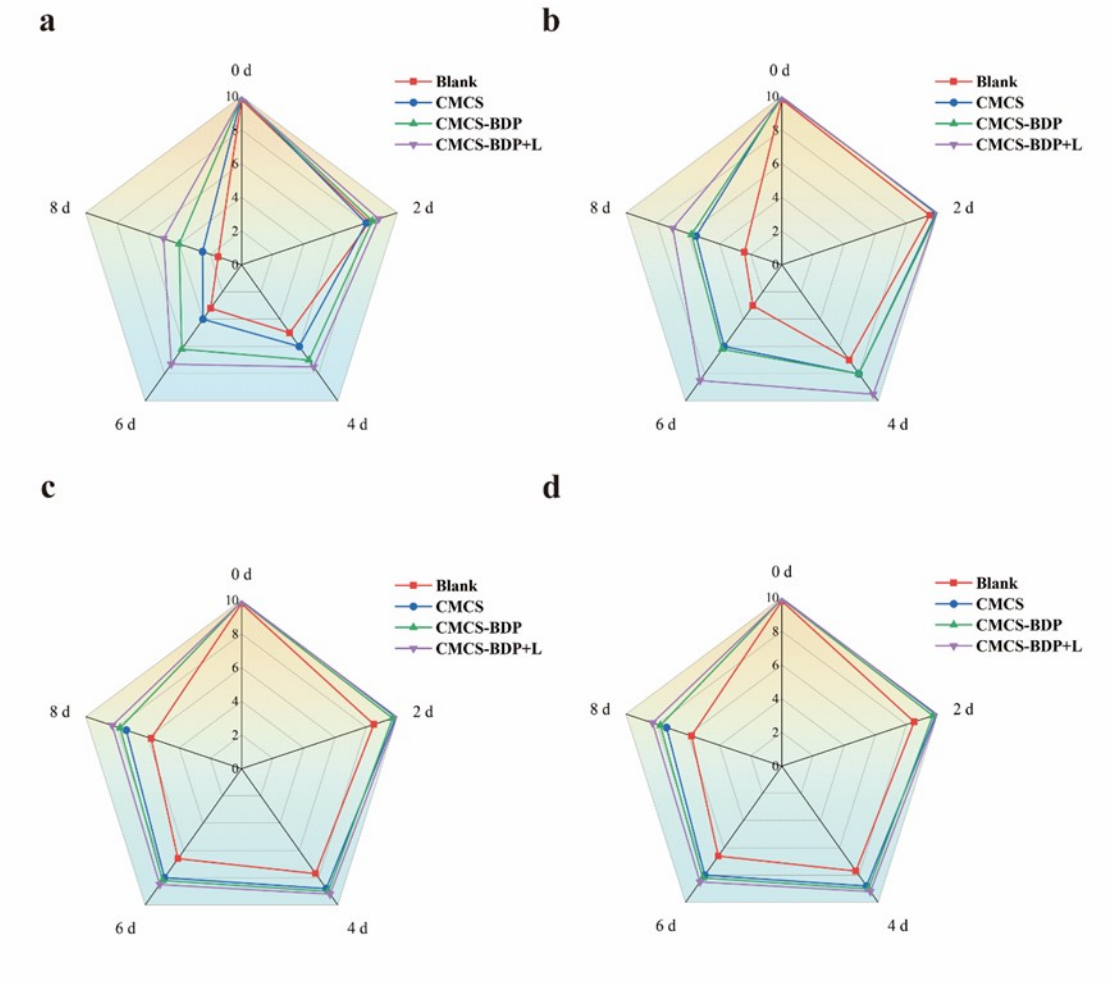

Figure S11. Sensory evaluation of the color (a), firmness (b), degree of shrinkage (c) and overall preservation integrity (d) of cherries in different groups during storage.