Protein fibrillation and hybridization with polysaccharides enhance strength, toughness, and gas selectivity of bioplastic packaging

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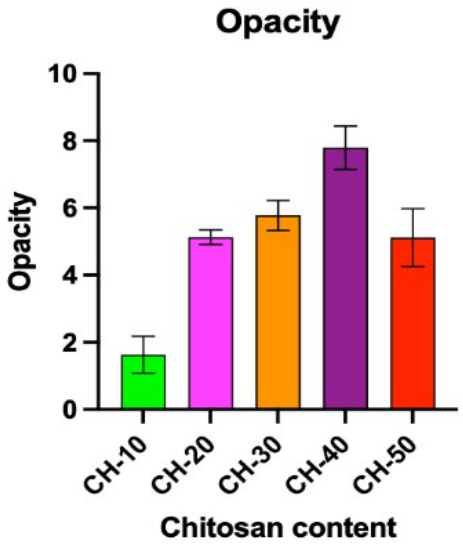

Fig. S1. The opacity measured at 550 nm of the bioplastic films composed with soybean protein isolate (SPI) and different contents of chitosan (CH) from 10% to 50%.

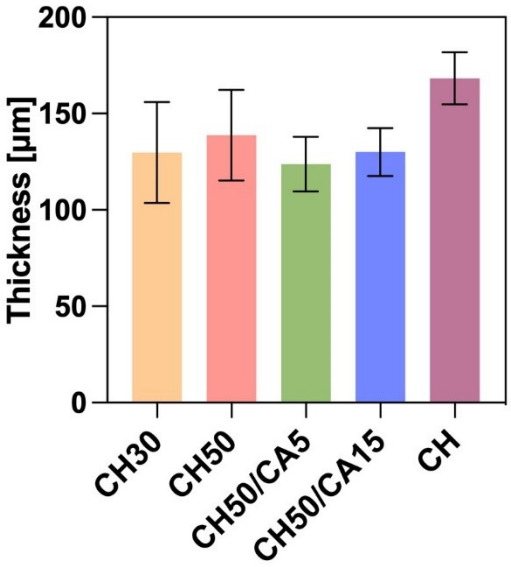

Fig. S2. Thickness of the SPI-chitosan hybrid bioplastic films and the chitosan film for measurement of gas permeability.

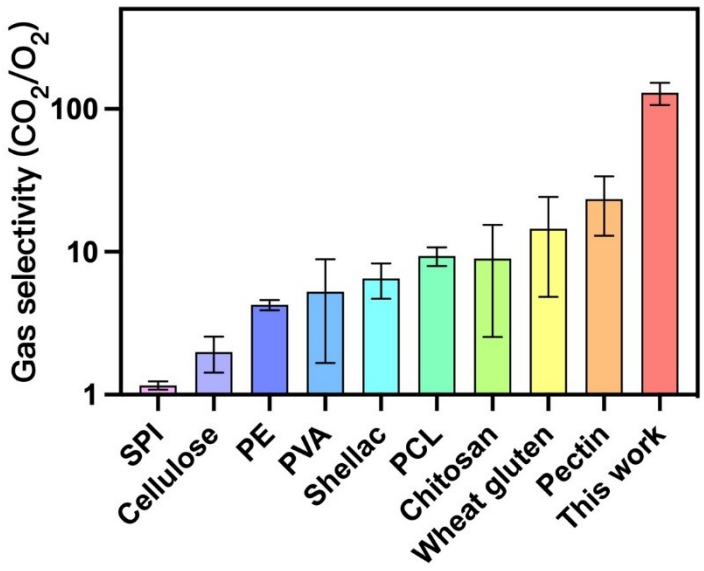

Fig. S3. The CO_2/O_2 selectivity of the bioplastic films prepared by soybean protein isolate (SPI)[1], cellulose[2], polyethylene (PE)[3], polyvinyl alcohol (PVA)[4], shellac[5], polycaprolactone(PCL)[3], chitosan[6,7,8,9], wheat gluten[7] and pectin[7,10] in previous other studies.

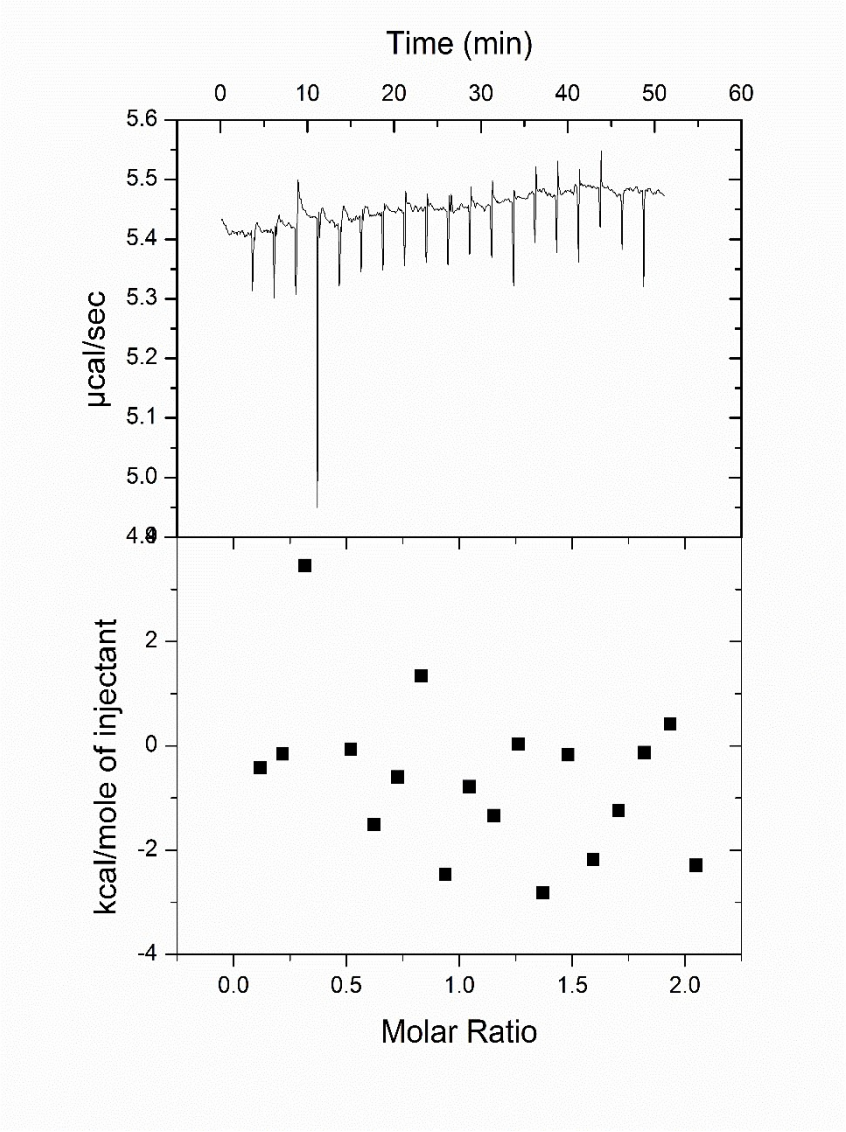

Fig. S4. Isothermal titration calorimetry (ITC) results for the interaction between the chitosan sample and the soybean protein isolate sample heated at acidic environment.

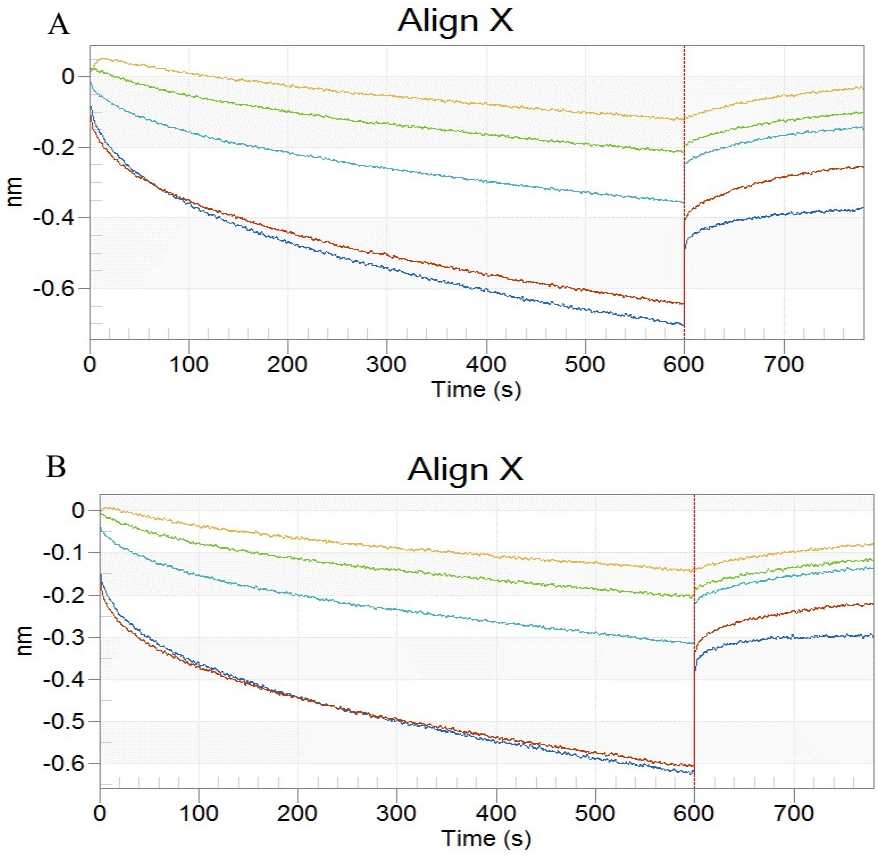

Fig. S5. Measurement of affinity of chitosan to the sensor chip coated with (A) or without (B) the soybean protein isolate samples which were heated in the acidic environment.

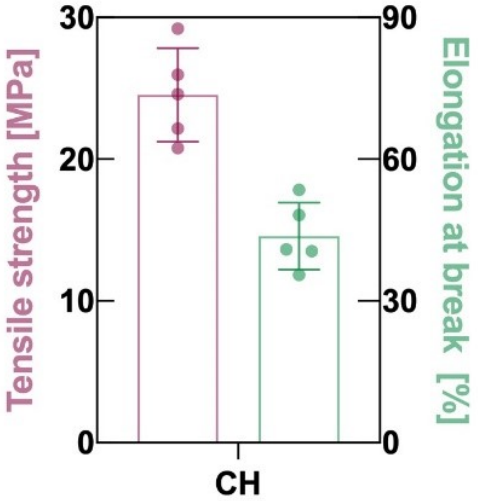

Fig. S6. Mechanical properties of the tensile strength and elongation at break of the bioplastic films prepared with chitosan.

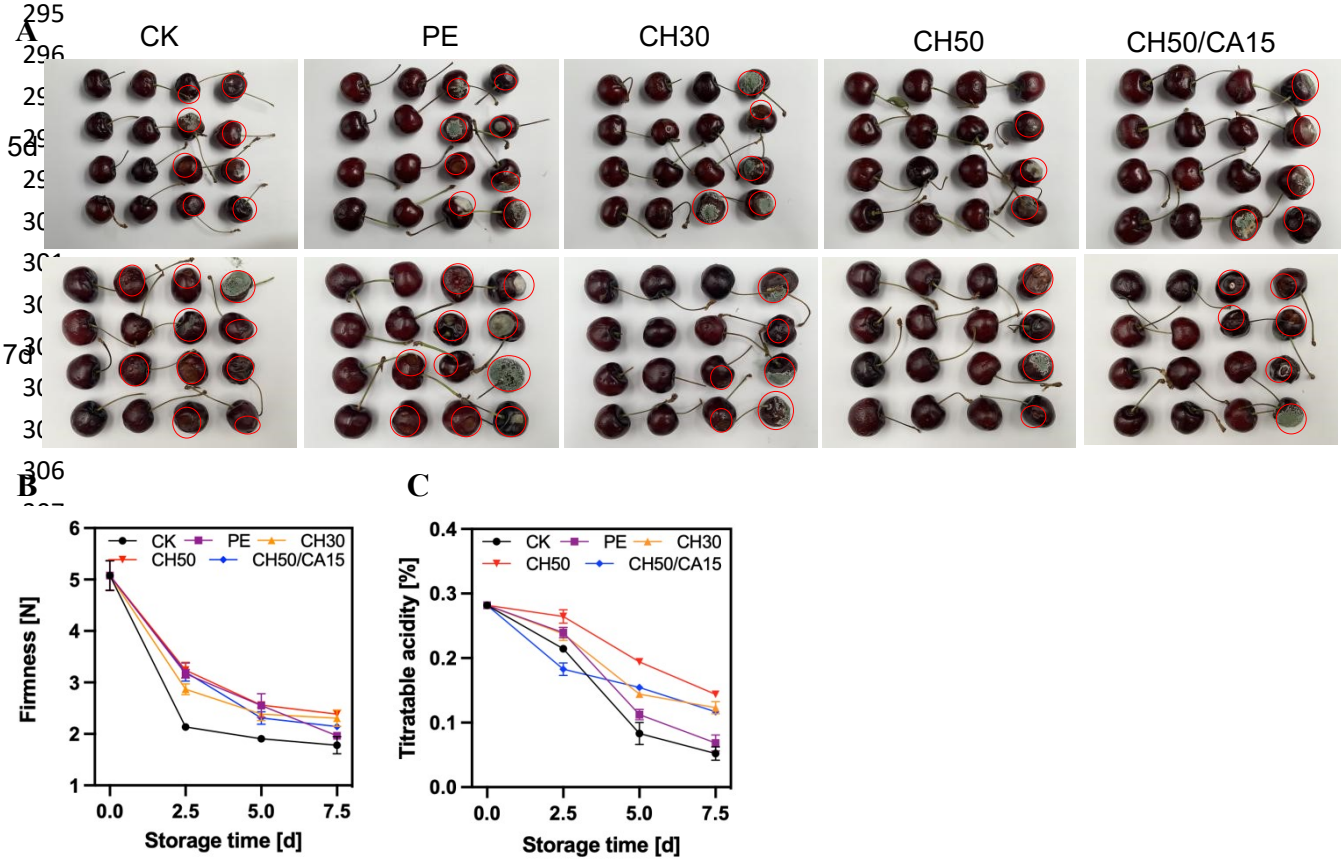

Fig. S7. The hybrid bioplastic films as a passively modified atmosphere packaging of cherry. (A) Photographs of ambient (CK) and packaged cherries by polyethylene films (PE), the bioplastic films composed with soybean protein isolate (SPI) and chitosan (CH) with different contents of 30 % (CH30), 50 % (CH50) and the one crosslinked by 15 % citric acid (CH50/CA15) after 5 and 7 days of storage at room temperature. The firmness (B), titratable acidity (C) of cherry in different treatment groups during storage.

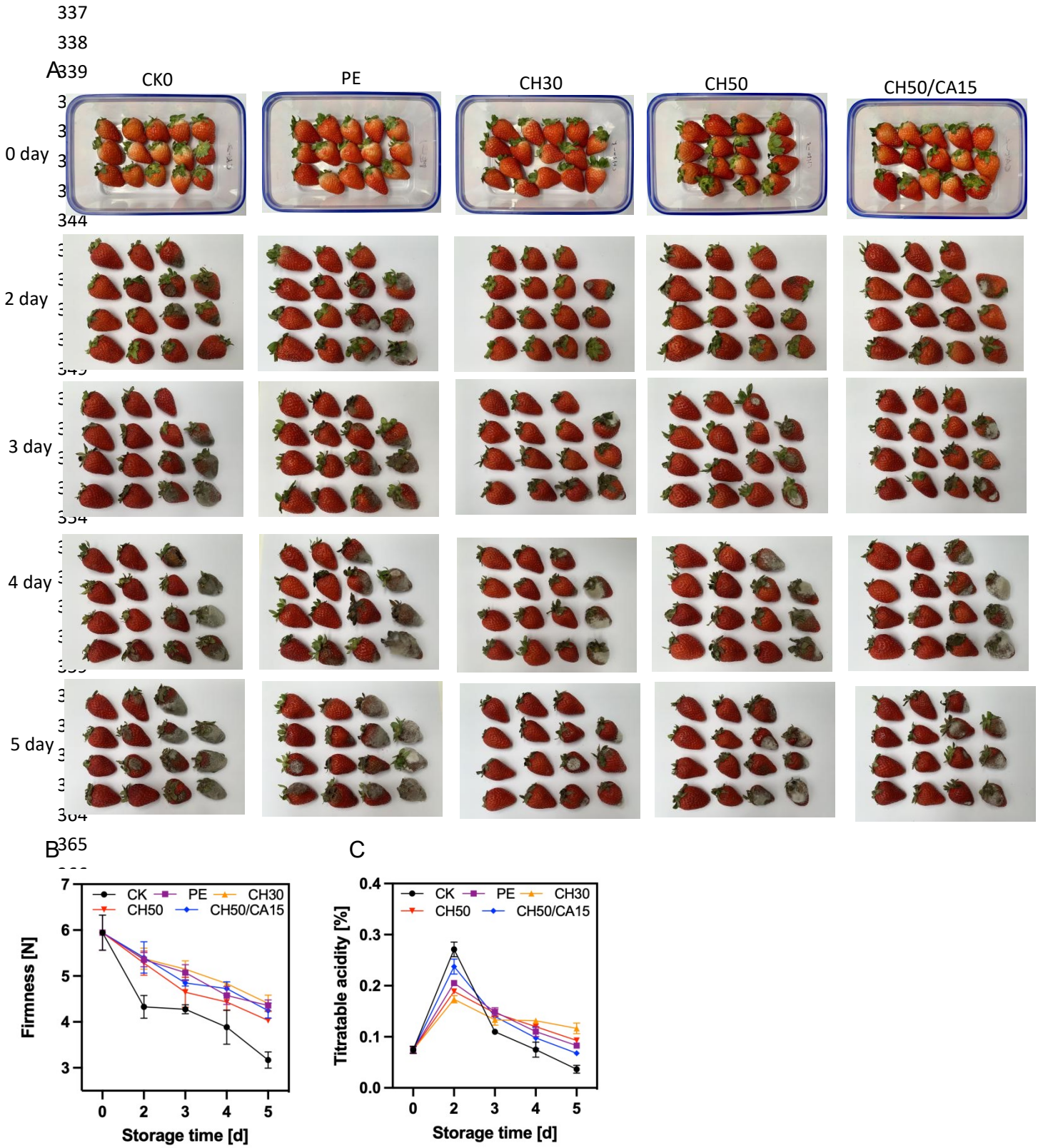

Fig. S8. The hybrid bioplastic films as a passively modified atmosphere packaging of strawberry. (A) Photographs of ambient (CK) and packaged strawberry by polyethylene films (PE), the bioplastic films composed with soybean protein isolate (SPI) and

chitosan (CH) with different contents of 30 % (CH30), 50 % (CH50) and the one crosslinked by 15 % citric acid (CH50/CA15) on 0, 2, 3, 4 and 5 days of storage at room temperature. The firmness (B), titratable acidity (C) of strawberry in different treatment groups during storage.

Methods for characterization of the interactions between the SPI sample and the chitosan sample during the thermal acidic treatment.

Isothermal titration calorimetry (ITC). ITC was performed on a MicroCal iTC200 (MicroCal Inc., England). The diluted SPI sample (20 μM) after the thermal acidic treatment was maintained in the sample cell. Chitosan (200 μM) was kept in a PCR tube and introduced into the sample cell through injection by syringes. Both of the SPI sample and the chitosan sample were prepared in 0.8% acetic acid solution. The temperature was set at 25 ℃ in "Instrument Controls".

Fortebio Octet system measurement. Binding kinetics between the chitosan sample and the SPI sample after the thermal acidic treatment were measured using the ForteBio Octet RED96 system (ForteBio) according to the operating manual. Biotinylation and desalination of the SPI samples were conducted as described in our previous study[11]. The pH 6 working solution was prepared by mixing 1% acetic acid solution and Bis-tris buffer. The desalted protein solution was diluted to 0.025 wt% using the above working solution. SA sensors coated with the SPI sample were transferred to different concentrations of chitosan solutions (dissolved in 1% acetic acid) such as 0.1 mg/mL, 0.05 mg/mL, 0.025 mg/mL, 0.0125 mg/mL and 0.00625 mg/mL, and then the sensors were transferred to the working solution to exclude non-specific binding. Chitosan solutions with different concentration gradients as described

406 above were directly bound to the SA sensor and dissociated to observe the signal ratio.

407

408 **The methods utilized in the wrapping preservation of beef by the bioplastic films**

409 The TVC measurement was performed for microbiological analysis based on the
410 method reported in previous study[12]. Briefly, a minced beef sample (25 g) was mixed
411 with 225 mL sterile normal saline in a sterile conical flask and homogenized for 1-2
412 min. Serial dilutions (1:10 v/v each time) were made, which was then used for spread
413 plate technique on standard plate count agar (PCA). TVC was determined after 48 h
414 incubation at 37 ℃. TVC values were expressed as CFU/g meat. All the tests were
415 carried out in triplicate per sample.

416 The total TVB-N was detected referring to the method reported in previous study
417 with some modifications[13]. Briefly, a minced beef sample (10 g) was added to 100 mL
418 distilled water in a conical flask. The conical flask was mixed by gently shaking for 30
419 min to obtain a homogeneous solution. The solution was filtered and then 5 mL filtrate
420 was alkalinized by adding 5 mL of MgO solution (10 g/L). Afterwards, the mixture
421 solution was determined to obtain the value of TVB-N by an Automatic Kjeldahl
422 Analyzer (Hanon, China). TVB-N values were expressed as mg/100 g meat and all tests
423 were carried out as triplicates per sample.

424 The pH of beef samples was determined by using 35634-40 PHSPEAR pH meter
425 (OAKTON Co., Ltd, USA), which was firstly calibrated using standard buffer solutions
426 (pH 4.0 and pH 7.0). Then the pH probe was rinsed with pure water and inserted into
427 the beef sample to make the electrode fully contacted with the sample muscle tissue.
428 Each sample was measured at three different points in the beef and the results were

averaged.

Lipid oxidation was measured by thiobarbituric acid reactive substances (TBARS). In brief, 5 g of minced beef was mixed with 50 mL trichloroacetic acid solution (7.5% trichloroacetic acid and 0.1% ethylenediaminetetraacetic acid) in a conical flask and the flask was shaken at 50 ℃ and the speed of 160 r/min in the thermostatic oscillator for 30 min. After cooling to the room temperature, the mixture was filtered using Whatman No.1 filter paper. Then 5 mL of filtrate and 5 mL of 20 mM 2-thiobarbituric acid (TBA) were mixed and incubated in a 90 °C water bath for 90 min. After that, the mixture was cooled to room temperature with water. The absorbance of the solution was determined at 532 nm with microplate reader (Thermo Fisher Scientific, USA). A standard curve was prepared using 1, 1, 3, 3- tetramethoxypropane and the TBARS values were expressed as mg of MDA equivalent per kg of meat. All the experiments were repeated in triplicate.

Color was measured directly on beef sample surface by Minolta colorimeter CR 400 (Minolta, Japan) with a D65, 10° illumination and an 8 mm aperture. White board calibration and background were used for standardization before test. Sample readings were taken at five different locations on each muscle and averaged. The L* (Lightness, black-white), a* (Redness, + to － from red to green), b* (Yellowness, + to － from yellow to blue).

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