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Influence of Chitosan/Lycopene on Myoglobin and Meat Quality of Beef During Storage

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Abstract: Myoglobin (Mb) is easily oxidized, which causes the discoloration of meat. In addition, various microorganisms are responsible for meat spoilage. Chitosan and lycopene can be used to protect the color and extend the shelf life of meat. In this study, a series of coatings with different ratios (1:0, 3:1, 1:1, 1:3, 0:1) of chitosan to lycopene were prepared. Beef was treated with different coatings. The changes in color, relative content of different Mb forms, thiobarbituric acid-reactive substances (TBARS), sulfhydryl content, carbonyl content, microbial count, cooking loss, and sensory evaluation during storage were investigated. The results showed that after 8 days, compared to the control, the relative content of oxymyoglobin (OxyMb), the lightness (L^*) value, the redness (a^*) value, and the composite index (CI) value of beef treated with chitosan/lycopene of 1:3 (w:w, the concentration of lycopene was 0.75% (w:v)) increased by 6.34%, 34.73%, 67.25%, and 116.27%, respectively. Meanwhile, the relative content of metmyoglobin (MetMb) and the yellowness (b^*) value decreased by 11.67% and 23.21%, respectively. Additionally, beef treated with chitosan/lycopene of 1:3 also performed well in protein oxidation, fat oxidation, microbial count, and cooking loss. Generally, the beef treated with chitosan/lycopene of 1:3 showed the best comprehensive quality. The coating was suitable for application in beef. These results are promising for food preservation.

Keywords: beef; lycopene; chitosan; myoglobin; color; meat quality

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1. Introduction

Due to its complex composition and rich nutrition, meat is prone to spoilage and is not resistant to storage. With the improvement of people's living standards, consumers have put forward higher requirements for the quality of meat and meat products. In addition to nutrition and safety, a good meat color, suitable tenderness, juicy taste, and distinctive flavor are essential requirements for an excellent product. Among them, meat color is the consumers' first impression of the product, which often affects their purchasing intention [1]. Especially for red meat, such as beef, lamb, pork, etc., the natural red color is an indicator of freshness and quality. However, the color of fresh meat is not very stable, and the natural red color can only be maintained for a short time without any protection treatment. The same problem also exists in the processing and storage of meat products. Discoloration causes a decrease in the sensory quality of meat products, which causes more than USD 1 billion in revenue loss to the US meat industry every year [2].

The color of red meat is mainly determined by the content and the state of myoglobin (Mb) in the meat [3]. Mb is a natural pigment protein in sarcoplasmic proteins (SP), composed of globin and heme cofactors, with a relative molecular weight of approximately 16.7 kDa [4]. In general, meat with higher Mb content will have a redder color, but the redox state of Mb also has a significant impact on meat color [5]. Deoxymyoglobin (DeoxyMb) shows a purple-red color, which is not a desirable color for consumers. Metmyoglobin (MetMb) shows a brown-red color, which is also not a desirable color for consumers. Oxymyoglobin (OxyMb), carboxymyoglobin (COMb), and nitrosylmyoglobin (NOMb)

can provide a bright-red color that is accepted by consumers [6]. For a piece of meat, the content of Mb is constant, but its redox state is influenced by multiple factors. Therefore, keeping Mb in an appropriate state is the key to maintaining good meat color.

Various physical and biochemical methods have been widely applied to protect meat color. The conventional physical methods for protecting meat color are packaging, such as modified atmosphere packaging, vacuum packaging, and active packaging. Modified atmospheric packaging with high O_2 and low CO can promote the formation of OxyMb and COMb, resulting in an acceptable red color. However, high O_2 can lead to lipid oxidation and affect the flavor of meat [7]. Vacuum packaging can inhibit the growth of aerobic microorganisms, as well as the occurrence of chemical reactions involving oxygen. However, it can promote the formation of DeoxyMb, thus, meat shows an undesirable purple-red color [8]. Active packaging usually embeds antibacterial, antioxidant, deodorizing, or other active substances into packaging materials, allowing them to be slowly released during storage and then in contact with the contents to extend their shelf life. Many active packaging materials have not yet been used in production due to their high costs or uncertain security issues [9]. Additionally, other physical processing techniques, such as high-pressure treatment, illumination, and mild cold atmospheric plasma, have complex impacts on meat color, closely related to their specific parameters [10–12].

Compared with physical methods, biochemical methods are feasible to implement and more effective in protecting meat color. At present, the method of adding nitrite is widely used in meat products to obtain desirable meat color [13]. However, excessive consumption of nitrite carries a risk of cancer [14]. Therefore, many researchers have been committed to finding alternatives to nitrite. Various small metabolites, such as ascorbate, vitamin E, succinate, and lactate, have been proven to improve the stability of meat color by oxygen consumption and controlling electron transfer [15–17]. In addition, many plant extracts, especially plant polyphenols, can also protect meat color due to their antioxidant activity. Various plant polyphenols, such as catechin, gallic acid, ferulic acid, caffeic acid, and grape seed extract, have been proven to improve the stability of meat color by inhibiting the oxidation of lipids and proteins [18]. Therefore, plant extracts with antioxidant activity have great potential in meat color protection.

Lycopene is a natural red pigment that is oil-soluble and belongs to the carotene family found in plant-based foods like tomatoes, papaya, watermelon, guava, and other red fruits and vegetables [19]. Research has shown that lycopene is highly effective in combating free radicals and singlet oxygen in laboratory settings [20]. Its antioxidant potency is ten times greater than alpha-tocopherol and twice that of beta-carotene [21]. Studies have demonstrated that incorporating unrefined tomato products or by-products containing lycopene can enhance food quality [22,23]. However, the low lycopene content in unrefined tomato products or by-products limits their antioxidant effectiveness.

Nateghi, Zarei and Pahlevan Afshari [24] studied the possibility of lycopene as a sodium nitrite replacement. Different dosages of lycopene pigment were added into sausage with 40% red meat. After 30 days of storage, no significant ($p > 0.05$) difference was found in physicochemical properties, microbial tests, lightness index (L^*), and sensory properties between samples and the control (40% meat sausage contain 120 ppm sodium nitrite). However, the application of refined and extracted lycopene oils and crystals in high-moisture foods such as fresh aquatic products and meat is limited due to the oil solubility of lycopene. Ehsani et al. [25] found that soaking fresh food in a solution containing lycopene for a period of time could effectively extend its shelf life. Furthermore, lycopene can serve as an emerging natural antioxidant for preparing composite coatings. Ehsani et al. [26] used a composite coating of sodium alginate and lycopene to preserve rainbow trout and found that samples coated with alginate containing lycopene showed better quality during storage. Canché-López et al. [27] prepared chitosan/tomato extract/moringa extract films. The preservation effect of those films on pork loin was studied. The results showed that the coatings formulated with both extracts and higher concentrations of glycerol presented good material properties, making them suitable for application in pork loin.

Chitosan is a linear polysaccharide formed of chitin by alkali treatment, which is very soluble under acidic conditions. Chitosan has excellent film-forming properties and can be used to produce coating materials [28]. In addition, chitosan is widely used as an antibacterial that protects food from spoilage. In this work, beef was selected as a representative meat sample. Firstly, several composite coatings with different ratios of chitosan to lycopene were prepared. Then, beef slices were immersed in composite coating solutions for some time, followed by draining and packaging. Finally, beef samples were stored at 4 °C, and their changes in quality were tested. These results can reveal the effects of chitosan/lycopene on Mb and meat quality, which provide a basic theory for applying chitosan/lycopene in meat preservation.

2. Materials and Methods

2.1. Materials and Chemicals

Fresh Longissimus dorsi muscle of beef was obtained from Henan He Sheng He Food Co., Ltd. (Xinxiang, China). All ingredients used in the processed beef were of food grade. All other chemicals used in this study were of analytical reagent grade, which were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Sample Preparation, Treatment, and Storage

The fresh beef cooled down to 4 °C quickly after being sent to the laboratory. The visible connective tissues and fat on the surface were removed. Then, the beef was cut into small pieces with 1.0 cm thick and a mass of 30.00 ± 5.00 g. Chitosan and lycopene were mixed in ratios of 1:0, 3:1, 1:1, 1:3, and 0:1, respectively (the total mass of chitosan and lycopene was 1 g). Then, they dissolved into a 1% acetic acid solution (100 mL) and a series of lycopene suspensions with different concentrations were prepared. Finally, the suspensions were homogenized (10000 rpm, 2 min). The beef samples were divided into six groups randomly: (i) control (no treatment), which were recorded as CK; (ii) treatment I (the beef pieces were immersed in the suspension with chitosan/lycopene of 1:0 for 10 min at room temperature, followed by draining), which were recorded as CS-LP-1; (iii) treatment II (the beef pieces were immersed in the suspension with chitosan/lycopene of 3:1 for 10 min at room temperature, followed by draining), which were recorded as CS-LP-2; (iv) treatment III (the beef pieces were immersed in the suspension with chitosan/lycopene of 1:1 for 10 min at room temperature, followed by draining), which were recorded as CS-LP-3; (v) treatment IV (the beef pieces were immersed in the suspension with chitosan/lycopene of 1:3 for 10 min at room temperature, followed by draining), which were recorded as CS-LP-4; (vi) treatment V (the beef pieces were immersed in the suspension with chitosan/lycopene of 0:1 for 10 min at room temperature, followed by draining), which were recorded as CS-LP-5. The samples were packaged with polyethylene bags and stored for 8 days at 4 °C. Two pieces of beef from each group were randomly sampled for further analyses on days 0, 2, 4, 6, and 8.

2.3. Color Measurement

A colorimeter (CR-400, Konica Minolta, Tokyo, Japan) was used to measure five randomly selected areas (both sides) on the surface of the beef samples. The color values included lightness (L^*), redness (a^*), and yellowness (b^*).

2.4. Relative Content of Different Mb Forms

The relative content of different Mb forms was measured according to a method previously described [29]. Each beef sample (2 g) was homogenized with 10 mL of phosphate buffer (pH 8.0, 0.01 M), and then placed in an ice bath for 1 h. After that, the homogenate was centrifuged for 25 min at 8000 rpm, and then the supernatant was filtered. Finally, a UV-visible spectrophotometer was used to measure the absorbance at 504 nm, 557 nm, 582 nm, and 525 nm. The relative content of different Mb forms were calculated via the following Equations (1)–(3):

$$\text{DeoxyMb (\%)} = (-0.543\ R1 + 1.594\ R2 + 0.552\ R3 - 1.329) \times 100 \quad (1)$$

$$\text{OxyMb (\%)} = (0.722\ R1 - 1.432\ R2 - 1.659\ R3 + 2.599) \times 100 \quad (2)$$

$$\text{MetMb (\%)} = 100 - \text{DeoxyMb (\%)} - \text{OxyMb(\%)} \quad (3)$$

where $R_1 = A_{582}/A_{525}$, $R_2 = A_{557}/A_{525}$, $R_3 = A_{504}/A_{525}$.

2.5. Determination of Thiobarbituric Acid-Reactive Substances (TBARS)

The TBARS assay was conducted following a previously described method [30]. Each beef sample (3.00 ± 0.01 g) was added to trichloroacetic acid (TCA) solution (15 mL, 7.5% (*w*/*v*)) containing 0.1% EDTA-2Na, which was homogenized, and then centrifuged (10,000 rpm, 10 min) at 4 °C. After that, the supernatant was added to the same volume of thiobarbituric acid (TBA) (0.02 mol/L). The mixture was cooled to room temperature after it reacted at 90 °C for 30 min, then the absorbance was measured at 532 nm. The standard curve was established by 1,1,3,3-tetrathoxypropane and the TBARS of samples was calculated using it. TBARS of samples were expressed as mg malondialdehyde (MDA)/kg sample.

2.6. Protein Oxidation Assays

2.6.1. Preparation of Myofibrillar Protein

Myofibrillar protein was extracted according to a method previously described [31]. Beef (3 ± 0.2 g) was minced and rinsed in a low-salt buffer (10% (*w*/*v*), 20 mM Tris-Maleic acid, 0.05 M KCl, pH 7.0). The homogenate was centrifuged (1000 rpm, 10 min). After that, the precipitates were extracted in a high-salt buffer (20 mM Tris-Maleic acid, 0.6 M KCl, pH 7.0) at 4 °C for 60 min. Then, the homogenate was centrifuged (1000 rpm, 30 min) and the supernate was collected. The concentration of myofibrillar protein was measured by the biuret method.

2.6.2. Determination of Carbonyl Content

The carbonyl content of the myofibrillar protein solution was determined according to a method previously described [32]. To 1 mL sample solution, 2,4-dinitrophenylhydrazine (DNPH) solution (2 mL, 0.01 M, 2 M HCl) was added, which then reacted in the dark for 1 h at room temperature. Trichloroacetic acid (2.5 mL, 20%) was added to precipitate protein before centrifuging (10,000 rpm, 3 min). Then, the precipitates were washed in ethyl acetate/ethanol (2 mL, 1:1 (*v*/*v*)) three times. After that, guanidine hydrochloride (6 mL, 6 M) was added to the precipitates, which were incubated for 10 min at room temperature. Finally, the mixture was centrifuged (10,000 rpm, 3 min) and the absorbance of the supernatant was measured at 370 nm. The carbonyl content was calculated by using the following Equation (4):

$$\text{Carbonyl content (nmol/mg protein)} = A_{370} \times 3 \times 10^6/(0.5 \times \varepsilon \times C) \quad (4)$$

where ε represents a molar extinction coefficient of 22,000/M/cm and C represents the concentration of myoglobin (mg/mL).

2.6.3. Sulfhydryl Content

The sulfhydryl content of the myofibrillar protein solution was determined according to a method previously described [33], using 5,5-dithiio-bis(2-nitrobenzoic acid) (DTNB). Firstly, myofibrillar solution (0.5 mL, 4 mg/mL) was added to Tris-HCl buffer (4.5 mL, 0.2 M, pH 6.8, containing 2% SDS, 8 M urea, and 10 mM EDTA). Then, 4 mL of the mixture and 0.4 mL of 0.1% DTNB were added into 0.2 M Tris-HCl (pH 8.0). After that, the mixture was incubated for 25 min at 40 °C. Finally, the absorbance of the mixture was measured

at 412 nm. A blank was conducted by replacing the sample with 0.6 M KCl. The total sulfhydryl content was calculated by using the following Equation (5):

$$\text{Sulfhydryl content (nmol/mg)} = A_{412} \times D/(\varepsilon \times C) \quad (5)$$

where C represents the concentration of myofibrillar protein, ε represents the molar extinction coefficient of 13,600/M/cm, and D represents the dilution factor.

2.7. Microbial Count

The total viable count of samples was determined according to a method previously described [34], using the standard spread plate count agar method. Briefly, each sample (10 g) was blended with saline (90 mL, 0.85 g/100 mL). Then the mixture was inoculated on plates after it was diluted to an appropriate dilution. Finally, it was incubated for 24 h at 37 °C for total viable count determination.

2.8. Cooking Loss Determination

The cooking loss of samples was determined according to a method previously described [35]. Each sample (30.00 ± 5.00 g) was weighed accurately, and the mass was recorded as m_1. Then, the sample was put in a cooking bag and heated in a water bath (72 °C) to the core temperature of 70 °C. After that, the sample was cooled for 30 min with running water. Finally, the sample was put in a refrigerator for 12 h at 4 °C and weighed accurately again, and the mass was recorded as m_2. The cooking loss of samples was calculated using the following Equation (6):

$$\text{Cooking loss}(\%) = (m_1 - m_2)/m_1 \times 100\% \quad (6)$$

2.9. Sensory Evaluation

Sensory evaluation was performed using a nine-point hedonic scale, according to a method previously described [36]. The evaluation team consisted of 20 non-trained panelists from Henan University of Animal Husbandry and Economy, including 10 males and 10 females, whose ages ranged from 20 to 25 years old. A single piece of beef sample (10 g) from each treatment was served on a piece of white paper. Panelists were asked to give a liking score (1–9) for color, odor, springiness, and adhesiveness. The sensory analysis was performed in a test room at 25 °C. Panelists were not requested to eat the samples. From each index, the highest rating and the lowest rating were removed, and the final score originated from the average value of other ratings and was reported as means ± standard deviations (SD). In order to obtain a single score that described the whole impression of consumers, the four sensory indexes were combined into a composite index called CI. The color and odor of meat are the consumers' first impressions of the product, which often affect their purchasing intention. Compared to springiness and adhesiveness, color and odor are more important. Therefore, the relative weight of color and odor was set to 0.3 and the relative weight of springiness and adhesiveness was set to 0.2. CI was calculated using the following Equation (7):

$$\text{CI} = 0.3\ \text{color} + 0.3\ \text{odor} + 0.2\ \text{springiness} + 0.2\ \text{adhesiveness} \quad (7)$$

2.10. Statistical Analysis

All the analyses were performed in triplicate, and the data were reported as means ± standard deviations (SD) for triplicate treatments. One-way ANOVA by SPSS 18.0 was used for the statistical significance ($p < 0.05$) of variables. Figures were created using Origin 2018 software (Origin Lab Inc., Northampton, MA, USA).

3. Results

3.1. Color of Beef Samples During Storage

The color of meat is a crucial indicator of meat products for freshness. As the storage days increased, the color of all samples with different treatments gradually darkened (Figure 1). During the storage period, the color of CS-LP-4 sample and CS-LP-5 sample was better than that of other groups. Since day 2, the appearances of the CS-LP-1 sample and the CS-LP-2 sample were significantly worse than those of the other groups. The reason might be that soaking in solution harmed beef color, thus CK sample without soaking treatment had better color. The colors of the CS-LP-1 sample treated with chitosan/lycopene of 1:0 and the CS-LP-2 sample treated with chitosan/lycopene of 3:1 were greatly affected by the soaking treatment due to the low concentration of lycopene. However, the colors of the CS-LP-3 sample treated with chitosan/lycopene of 1:1, the CS-LP-4 sample treated with chitosan/lycopene of 3:1, and CS-LP-5 sample treated with chitosan/lycopene of 0:1 were less affected by the soaking treatment due to the high concentration of lycopene.

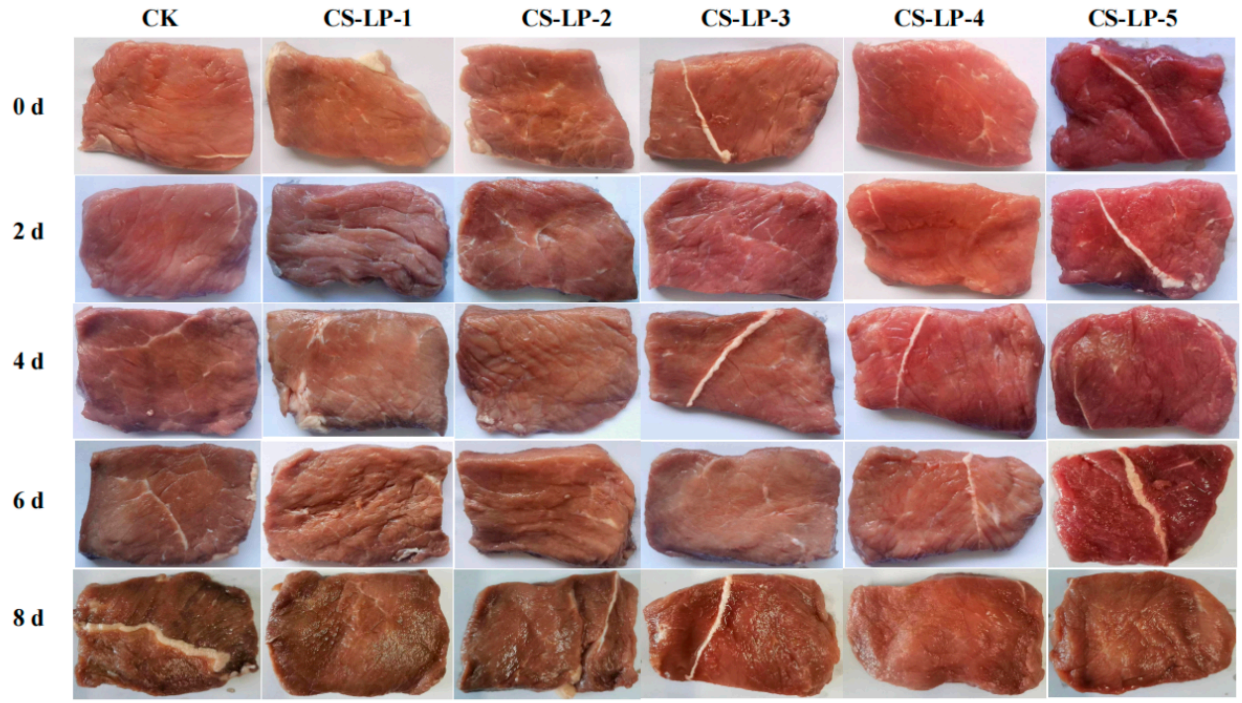

Figure 1. Photo of beef samples during storage.

Similar phenomena could also be observed from the color parameters of samples (Figure 2). The most important color traits of fresh meat at the time of sale are the lightness (paleness) measured by CIE-L^*; the redness measured by CIE-a^*, and the yellowness measured by CIE-b^*. It was observed that the L^* value of the CK sample was first increased from 42.70 to 48.54 and then decreased to 31.50. Other treatment groups exhibited a similar change pattern with the increasing number of storage days, except for the CS-LP-1 sample. The L^* value of the CS-LP-1 sample continuously decreased with the extension of storage time. As the proportions of lycopene in the soaking solution gradually increased, the amplitude of the change in the L^* values of the samples gradually decreased. The L^* value of the CS-LP-5 sample did not change significantly throughout the entire storage period.

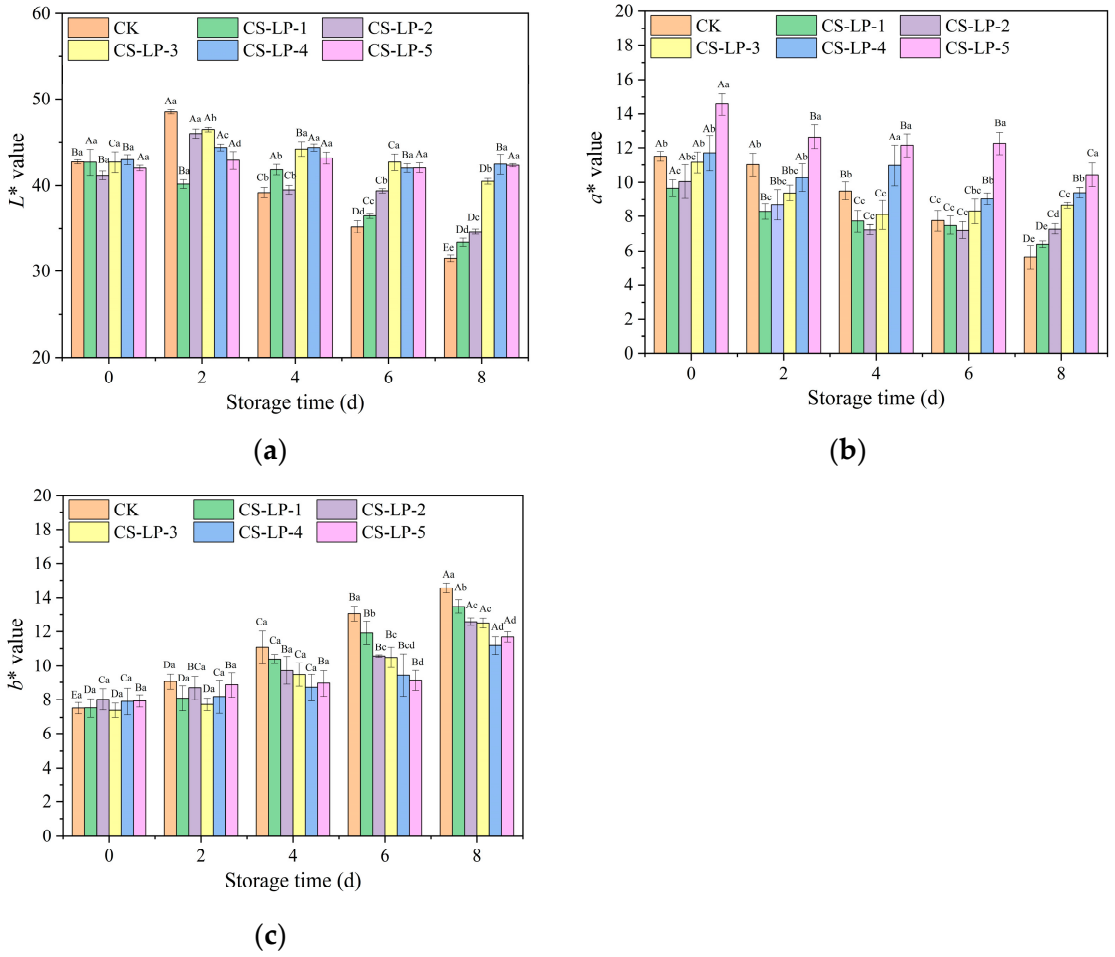

Figure 2. Color parameters of beef samples during storage. (**a**) *L** value. (**b**) *a** value. (**c**) *b** value. Different letters (a, b, c, d, e) indicate significant differences between the samples with different treatments at the same time ($p < 0.05$). Different letters (A, B, C, D, E) indicate significant differences between different storage days for the same sample ($p < 0.05$).

The value of *a** was an important indicator of the color stability of meat products, directly reflecting the color changes of beef samples during storage. It was observed that the *a** values of almost all samples decreased as the storage time prolonged. However, samples treated with higher concentrations of lycopene had smaller *a** value changes during storage. The *a** value of the CK sample decreased from 11.50 to 5.62 after 8 days while the *a** value of the CS-LP-5 sample decreased from 14.55 to 10.43. In contrast, the *b** values of almost all samples increased as the storage time was prolonged. Samples treated with higher concentrations of lycopene had smaller *b** value changes during storage. The *b** value of the CK sample increased from 7.52 to 14.56 after 8 days while the *b** value of the CS-LP-5 sample increased from 7.93 to 11.69. Overall, the CS-LP-4 sample and the CS-LP-5 sample showed good color stability. On day 8, compared to the CK sample, the *L** value of the CS-LP-4 sample increased by 34.73%, the *a** value increased by 67.25%, and the *b** value decreased by 23.21%. Meanwhile, the *L** value of the CS-LP-5 sample increased by 34.31%, the *a** value increased by 159.07%, and the *b** value decreased by 19.71%.

3.2. Changes in Relative Content of Different Mb Forms During Storage

The color of meat mainly depends on the chemical state of Mb and its relative content. There are three main types of Mb states: DeoxyMb, OxyMb, and MetMb. It was observed that the relative content of MetMb of all samples increased as the storage time prolonged while the relative content of OxyMb decreased and the relative content of DeoxyMb did

not change too much as the storage time prolonged (Figure 3). On day 0, there was no significant difference in the relative content of three forms of Mb among the samples of each group. However, as the storage time prolonged, the difference became increasingly significant. The relative content of OxyMb in the CK sample decreased from 34.75% to 29.98% while the relative content of OxyMb in the CS-LP-5 sample decreased from 34.36% to 32.21%. Meanwhile, the relative content of MetMb in the CK sample increased from 20.13% to 25.88% while the relative content of MetMb in the CS-LP-5 sample increased from 20.18% to 22.25%. Compared to the CK sample, other groups had a higher relative content of OxyMb and a lower relative content of MetMb after a period of storage time. In addition, samples treated with a higher lycopene concentration had a higher relative content of OxyMb and a lower relative content of MetMb. On day 8, the CS-LP-5 sample treated with chitosan/lycopenea of 0:1 had the highest relative content of OxyMb and the lowest relative content of MetMb, followed by the CS-LP-4 sample treated with chitosan/lycopene of 1:3. After 8 days, compared to the CK sample, the relative content of OxyMb of the CS-LP-5 sample increased by 7.44% and the relative content of MetMb decreased by 14.04%. The relative content of OxyMb of the CS-LP-4 sample increased by 6.34% and the relative content of MetMb decreased by 11.67%. It was suggested that the antioxidant activity of lycopene could prevent the oxidation of DeoxyMb and OxyMb to MetMb.

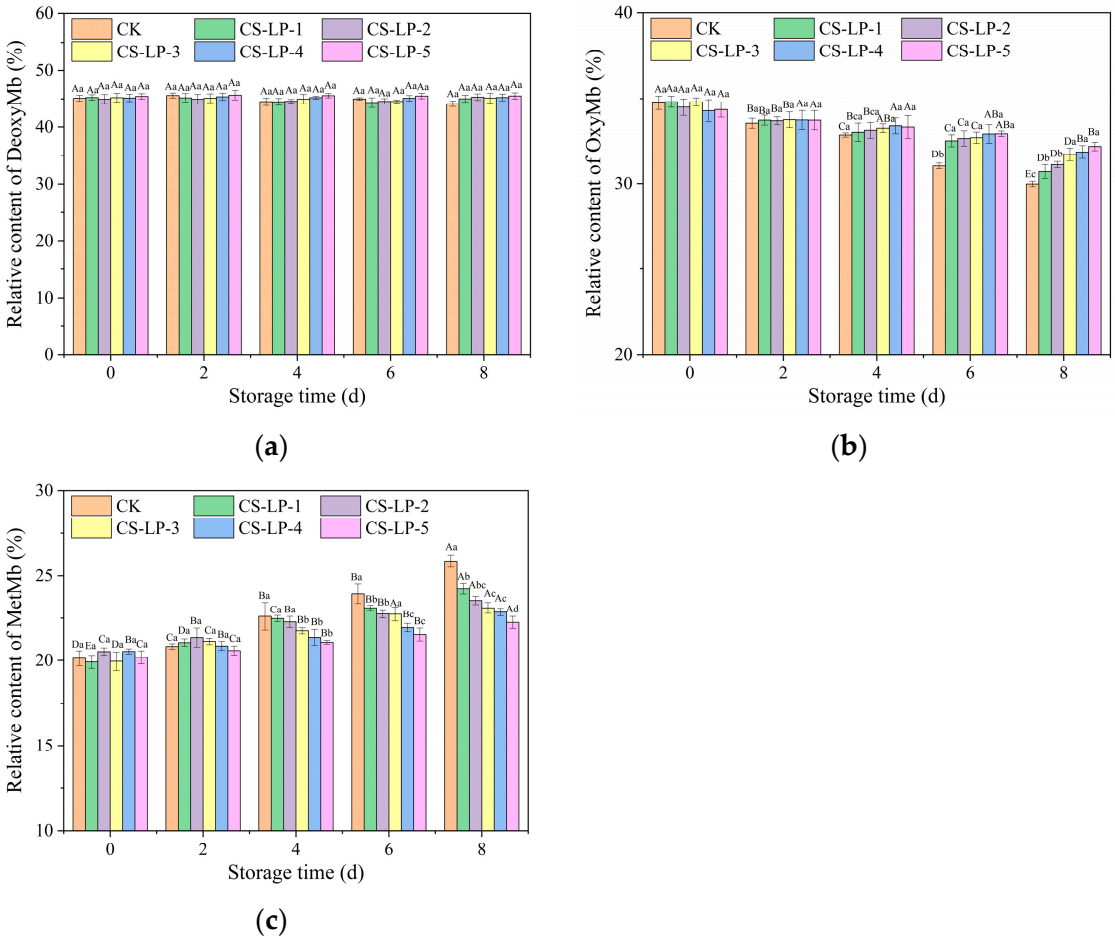

Figure 3. The relative content of different Mb forms during storage. (**a**) DeoxyMb. (**b**) OxyMb. (**c**) MetMb. Different letters (a, b, c, d) indicate significant differences between the samples with different treatments at the same time ($p < 0.05$). Different letters (A, B, C, D, E) indicate significant differences between different storage days for the same sample ($p < 0.05$).

3.3. Lipid Oxidation and Protein Oxidation of Beef Samples During Storage

The TBARS value indicates the amount of lipid oxidation of secondary products, which is generally used to evaluate the degree of lipid oxidation of meat products [37]. Lipid oxidation is one of the important factors that cause discoloration and rancidity of meat products. The TBARS values of all samples were increased during storage (Figure 4). As the concentration of lycopene in the sample soaking solution increased, the rate of increase in the TBARS value of the sample decreased. The TBARS value of the CK sample increased from 0.024 to 0.048 mg/kg while the TBARS value of the CS-LP-5 sample increased from 0.024 to 0.038 mg/kg. On day 8, there were significant differences among different treatments of samples, except for CK and CS-LP-1 samples. The TBARS value of the CS-LP-5 sample was the lowest since day 2.

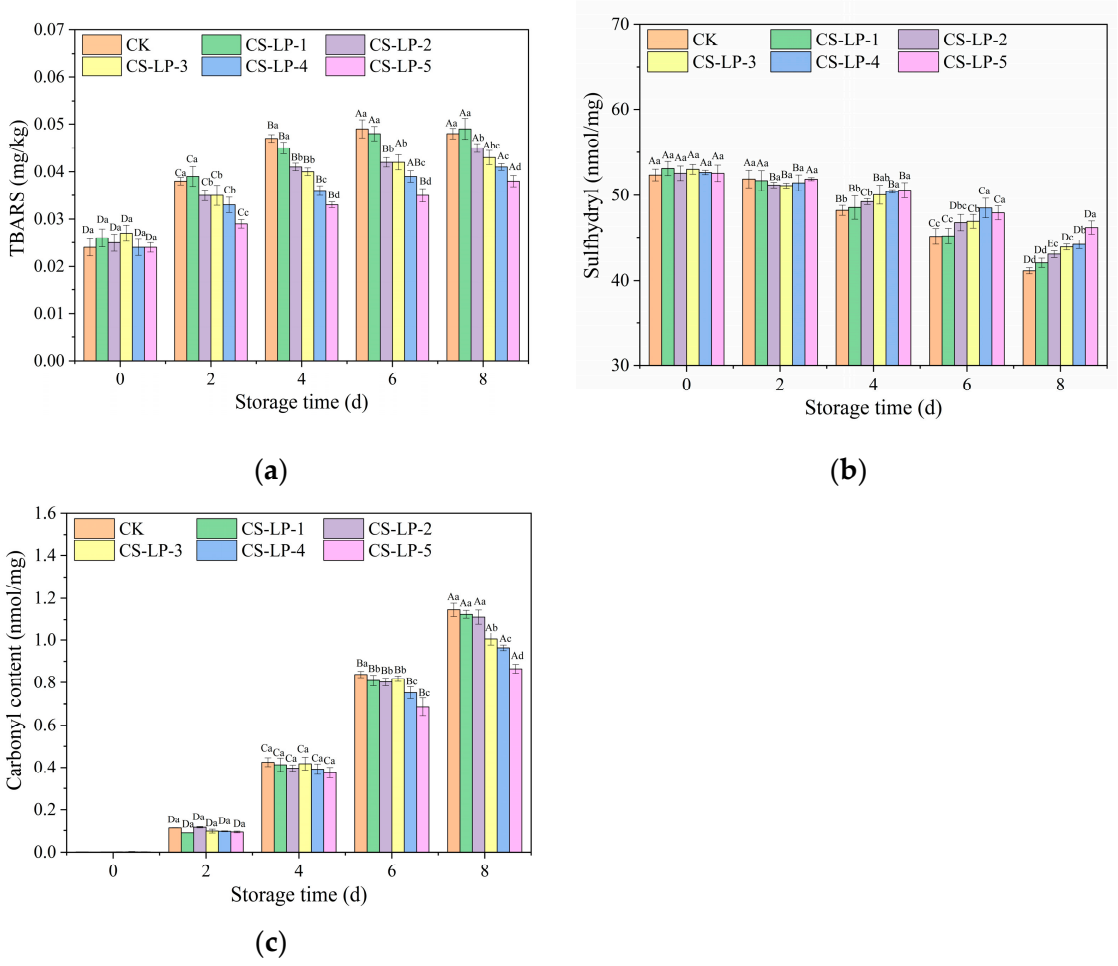

Figure 4. Lipid oxidation and protein oxidation of beef samples during storage. (**a**) TBARS. (**b**) Sulfhydryl content. (**c**) Carbonyl content. Different letters (a, b, c, d) indicate significant differences between the samples with different treatments at the same time ($p < 0.05$). Different letters (A, B, C, D, E) indicate significant differences between different storage days for the same sample ($p < 0.05$).

Meanwhile, it was observed that the sulfhydryl content of the samples decreased and the carbonyl content of the samples increased during storage. The sulfhydryl content of the CK sample decreased from 52.25 to 41.13 nmol/mg while the sulfhydryl content of the CS-LP-5 sample decreased from 52.55 to 46.18 nmol/mg. The carbonyl content of the CK sample increased from 0.002 to 1.146 nmol/mg while the carbonyl content of the CS-LP-5 sample increased from 0.002 to 0.865 nmol/mg. On day 8, there were significant differences in sulfhydryl content among different treatments of samples, except for CK and CS-LP-1 samples. Similarly, there were significant differences in carbonyl content among different treatments of samples, except for CK, CS-LP-1, and CS-LP-2 samples. The

sulfhydryl content of the CS-LP-5 sample was the highest since day 4 and the carbonyl content of the CS-LP-5 sample was the lowest since day 6.

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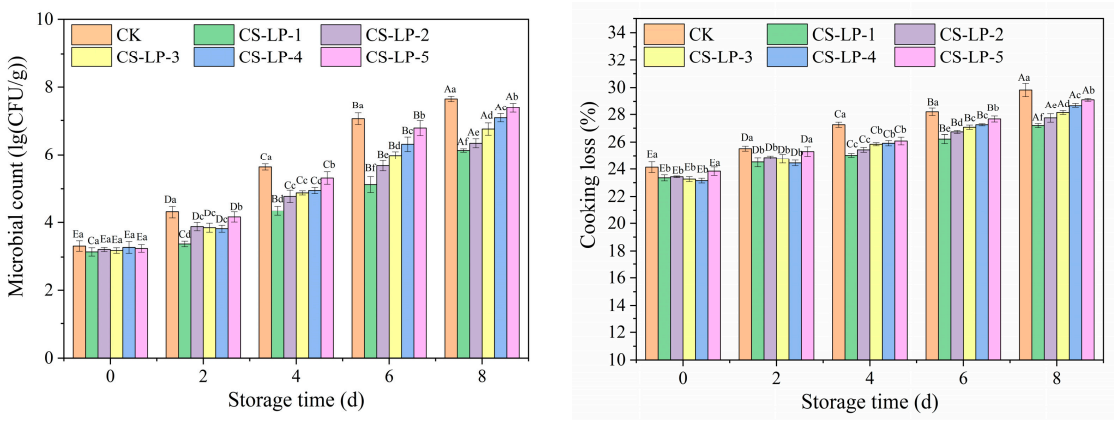
Meanwhile, it was observed that the sulfhydryl content of samples decreased and the carbonyl content of samples increased during storage. The sulfhydryl content of the CK sample decreased from 52.25 to 41.13 nmol/mg while the sulfhydryl content of the CS-LP-5 sample decreased from 52.55 to 46.18 nmol/mg. The carbonyl content of the CK sample increased from 0.002 to 1.146 nmol/mg while the carbonyl content of the CS-LP-5 sample increased from 0.002 to 0.865 nmol/mg. On day 8, there were significant differences in sulfhydryl content among different treatments of samples, except for CK and CS-LP-1 samples. Similarly, there were significant differences in carbonyl content among different treatments of samples, except for the CK, CS-LP-1 and CS-LP-2 samples. The sulfhydryl content of the CS-LP-5 sample was the highest since day 4 and the carbonyl content of the CS-LP-5 sample was the lowest since day 6.

3.4. Changes in Microbial Counts and Cooking Loss During Storage

The spoilage of fresh meat is primarily caused by the growth and reproduction of microorganisms, which results in protein degradation and fatty acid decay of the meat. The microbial counts of meat are closely related to the shelf life of meat, which can reflect whether the meat has spoiled. If the microbial counts exceed 7 lg (CFU/g), the surface of the meat is spoiled and the meat is not accepted [38]. On day 0, the microbial counts of samples were in the range from 3.19 to 3.32 lg (CFU/g) per sample and there was no significant difference among the samples (Figure 5). The microbial counts of the samples from different groups all increased as the storage time increased ($p < 0.05$). On day 2, the CK sample had the highest microbial counts, followed by the CS-LP-5 sample. The CS-LP-1 sample had the lowest microbial counts. However, there was no difference observed among the other samples ($p < 0.05$). On day 6, the CK sample had the highest microbial count, exceeding 7 lg (CFU/g). However, the microbial counts of the samples from other groups were below 7 lg (CFU/g), even for the CS-LP-5 sample, which was treated with chitosan/lycopene of 0:1. A similar trend was observed on day 4. On day 6, there were significant differences in microbial counts among different groups of samples ($p < 0.05$). The CK sample had the highest microbial counts, followed by the CS-LP-5, CS-LP-4, CS-LP-3, and CS-LP-2 samples, successively, and the CS-LP-1 sample had the lowest microbial counts. On day 8, the microbial counts of the CK, CS-LP-4, and CS-LP-5 samples exceeded 7 lg (CFU/g), while the microbial counts of the CS-LP-1, CS-LP-2, and CS-LP-3 samples were still below this limit value. The microbial count of the CS-LP-1 sample had remained at the lowest since day 2.

Water holding capacity is a key factor affecting the color, texture, and flavor of meat, and is important for evaluating meat quality. The cooking loss is regarded as an important indicator for evaluating water holding capacity. The cooking loss of beef samples increased gradually as the storage time increased ($p < 0.05$) (Figure 4). On day 0, the cooking loss of samples with treatment was significantly lower than that of the control group ($p < 0.05$), except for the CS-LP-5 sample. There was no difference observed among other treatment groups ($p < 0.05$), except for the CS-LP-5 sample. A similar trend was observed on day 2. On day 4, the CK sample had the highest cooking loss, followed by CS-LP-5, CS-LP-4, and

CS-LP-3 samples which did not observe a significant difference ($p < 0.05$). The CS-LP-1 sample had the lowest cooking loss, followed by the CS-LP-2 sample. On day 6, there were significant differences in cooking loss among different groups of samples, except for the CS-LP-3 and CS-LP-4 samples. The CK sample had the highest cooking loss and the CS-LP-1 sample still had the lowest cooking loss. A similar trend was observed at day 8 and there were significant differences among all groups ($p < 0.05$). The cooking loss of the samples with treatment was lower than that of the control group since day 0, except for the CS-LP-5 sample.



(**a**) (**b**)

Figure 5. Microbial count and cooking loss of the beef samples during storage. (**a**) Microbial count. (**b**) Cooking loss. Different letters (a, b, c, d, e, f) indicate significant differences between the samples with different treatments at the same time ($p < 0.05$). Different letters (A, B, C, D, E) indicate significant differences between different storage days for the same sample ($p < 0.05$).

3.5. Changes in Sensory Evaluation Scores During Storage

In order to comprehensively assess the quality of beef samples, sensory evaluation was organized. The sensory evaluation scores of all samples decreased as the storage time increased (Figure 6). On day 0, the CS-LP-4 sample had the highest color score. The reason was that soaking had a negative impact on the color of beef, but the staining effect of lycopene could make the beef appear redder. However, overly high lycopene concentration resulted in overly red color, which led to lower acceptance by consumers. It was observed in Figure 1 that the color of the CS-LP-5 sample treated with the highest concentration of lycopene was too red and appeared unnatural. The overall CI value of the CS-LP-4 sample was higher than that of other samples. On day 2, the CS-LP-4 sample had the highest color score, followed by the CS-LP-5. The CS-LP-1 sample had the lowest color score. The CK sample had the lowest adhesiveness score. The overall CI values of the CS-LP-4 sample and the CS-LP-5 sample were higher than that of other samples. On day 4, the CK sample had the lowest color, odor, springiness, and adhesiveness score. Therefore, the CK sample had the lowest CI value. The CI values of the CS-LP-4 sample and the CS-LP-5 sample were higher than those of other samples. A similar trend was observed on day 6. On day 8, the CS-LP-4 sample had the highest CI value, followed by CS-LP-5. In summary, the CS-LP-4 and CS-LP-5 samples had higher CI values than other groups of samples throughout the entire storage period. On day 8, compared to CK, the CI value of the CS-LP-4 sample increased by 116.27% and the CI value of the CS-LP-5 sample increased by 105.42%. It was suggested that when the ratio of chitosan to lycopene was 1:3, the beef had the best quality and storage stability.

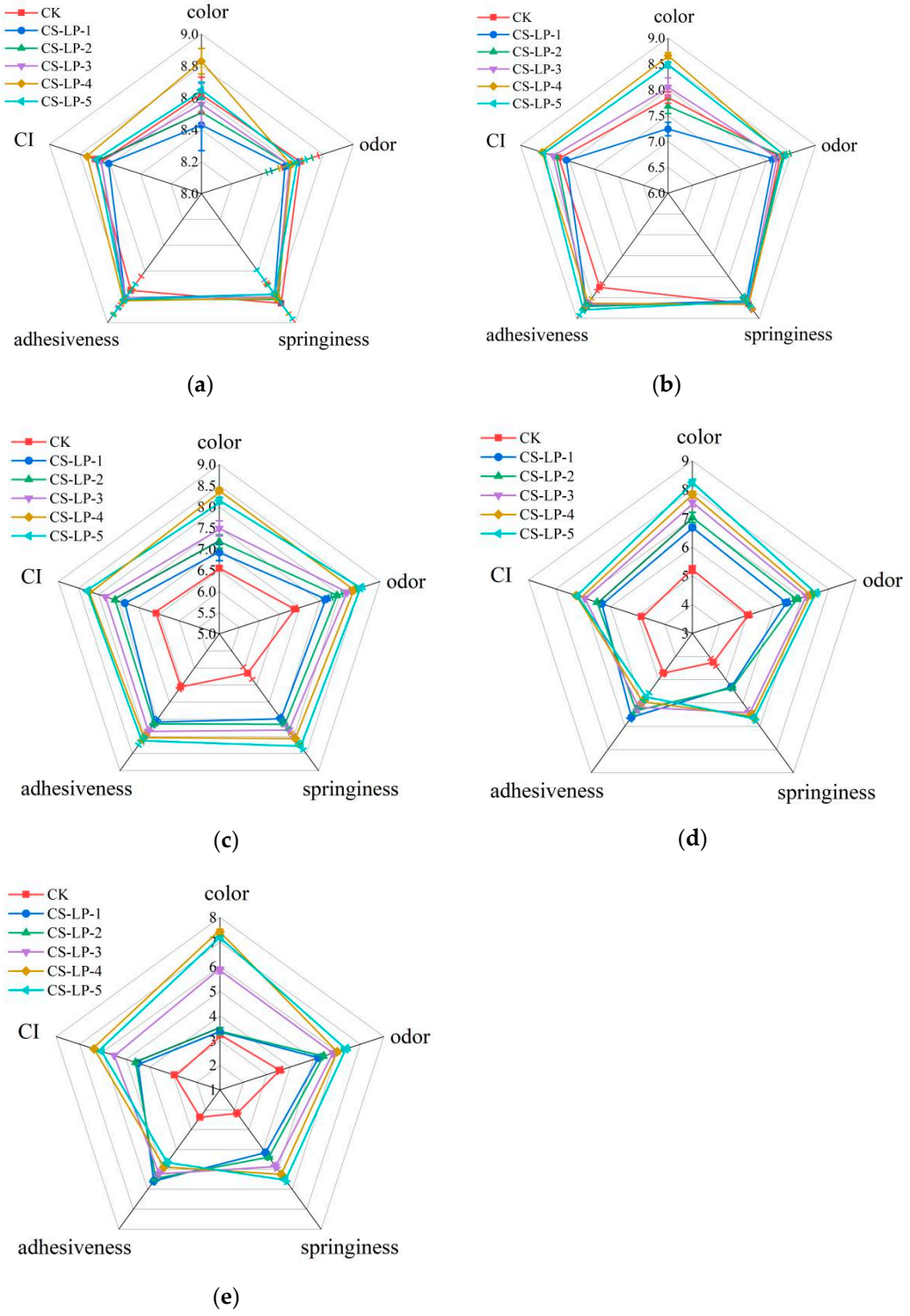
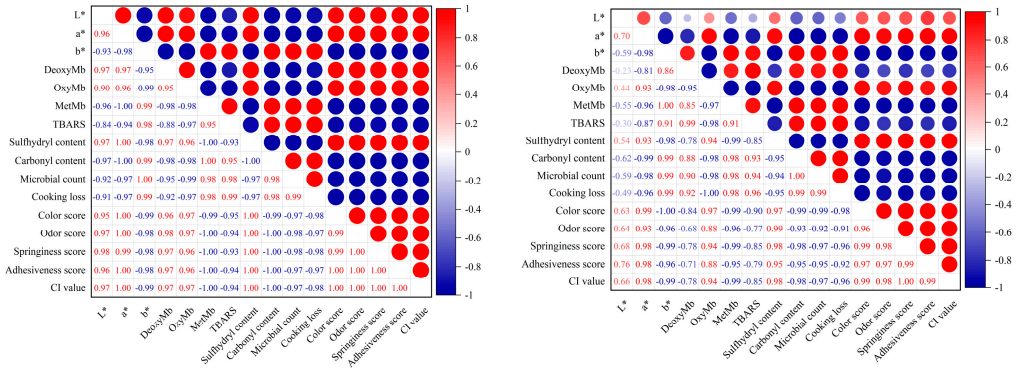


Figure 6. Sensory evaluation scores of beef samples during storage. (**a**) Day 0. (**b**) Day 2. (**c**) Day 4. (**d**) Day 6. (**e**) Day 8.

3.6. Correlation Analysis of Various Indicators

Correlation analysis of color parameters (L^*, a^*, b^*), the contents of three different forms of Mb (DeoxyMb, OxyMb, MetMb), lipid oxidation (TBARS), protein oxidation (sulfhydryl content, carbonyl content), microbial count, cooking loss and sensory evaluation score (color score, odor score, springiness score, adhesiveness score, CI value) was conducted. The correlation analysis results of different indicators in the storage of the CK sample

are shown in Figure 7. The results showed that the DeoxyMb content of the CK sample was significantly positively related to the L^* value, a^* value, odor score, springiness score, adhesiveness score, and CI value ($p < 0.01$) and was positively related to color score ($p < 0.05$). Still, it was negatively related to b^* value ($p < 0.05$). However, the DeoxyMb content of the CS-LP-4 sample was uncorrelated to the L^* value, a^* value, b^* value, and all sensory evaluation scores. The OxyMb content of the CK sample was positively related to the L^* value ($p < 0.05$). It was significantly positively related to the a^* value and all sensory evaluation scores, but it was significantly negatively related to the b^* value ($p < 0.01$). However, the OxyMb content of the CS-LP-4 sample was positively related to a^* value, odor score, springiness score, adhesiveness score, and CI value ($p < 0.05$), and it was significantly positively related to color score ($p < 0.01$), but it was significantly negatively related to b^* value ($p < 0.01$). It was uncorrelated to L^* value. The MetMb content of the CK sample was significantly positively related to the b^* value ($p < 0.01$) but was significantly negatively related to the L^* value, a^* value, and all sensory evaluation scores ($p < 0.01$). However, the MetMb content of the CS-LP-4 sample was significantly positively related to the b^* value ($p < 0.01$), while it was significantly negatively related to the a^* value, color score, odor score, springiness score, and CI value ($p < 0.01$). It was negatively related to adhesiveness score and uncorrelated to L^* value. The TBARS of the CK sample was significantly positively related to the b^* value ($p < 0.01$), but it was negatively related to the a^* value and all sensory evaluation scores ($p < 0.01$). It was uncorrelated to the L^* value. However, the TBARS content of the CS-LP-4 sample was positively related to the b^* value ($p < 0.01$), while it was negatively related to color score ($p < 0.01$). It was uncorrelated to the L^* value, a^* value, odor score, springiness score, adhesiveness score, and CI value. The correlation between sulfhydryl content of CK sample and color parameters, sulfhydryl content and sensory evaluation score was similar to that of CS-LP-4. The carbonyl content, microbial count, and cooking loss behaved similarly to sulfhydryl content.



(**a**) (**b**)

Figure 7. Correlation analysis of various indicators during storage. (**a**) The CK sample; (**b**) the CS-LP-4 sample.

4. Discussion

The purpose of this study was to investigate the influence of chitosan/lycopene on Mb and the overall quality of beef during storage. The color of red meat is mainly determined by the content and the state of Mb in the meat [3]. Red meat, such as beef, often becomes discolored due to the oxidation of Mb, resulting in a decline in the quality of the meat. In addition, the oxidation of lipids and myofibrillar proteins can also lead to a change in meat color. Moreover, the decline in meat quality was also reflected in the increase in microbial count and cooking loss. As a result, beef was treated with different ratios of chitosan to lycopene. The changes in color, relative content of different Mb forms, TBARS, sulfhydryl content, carbonyl content, microbial count, cooking loss, and sensory evaluation during storage were studied.

On the whole, the L^* value of samples first increased and then decreased during storage. The L^* value of the sample was affected by many factors, such as the type and content of pigment substances in the sample, the surface structure of the sample, and the distribution of moisture on the surface of the sample [39]. The initial increase in L^* value might be due to lipid oxidation [40]. The final decrease in L^* value might be due to changes in the state of Mb and hemoglobin [41]. Over an 8-day storage period, the L^* value of beef treated with chitosan/lycopene of 1:3 and beef treated with chitosan/lycopene of 0:1 only took place little change. With the extension of storage time, the a^* value decreased and the b^* value increased, which were manifestations of color deterioration. However, the a^* value and b^* value changed smaller when beef was treated with more lycopene. The reason might be that the changes of a^* value and b^* value were both related to oxidation. The decrease in a^* value could be due to the oxidation of Mb. The increase in the b^* value could be due to the increase in lipid oxidation [42]. Lycopene, a natural red pigment with high antioxidant activity, contributed to the good color stability of beef treated with a high concentration of lycopene. The results mentioned above could be further confirmed by the changes in the relative content of different Mb forms, lipid oxidation, and protein oxidation. With the extension of storage time, the relative content of OxyMb decreased along with the relative content of MetMb increased. It was also reported previously that DeoxyMb was dynamically and reversibly converted into OxyMb, and then OxyMb was irreversibly converted into MetMb, as the increasing time of storage [42]. Since consumers prefer the color of OxyMb over MetMb, this change is not ideal for the color of beef. However, less OxyMb changed to MetMb when beef was treated with a high concentration of lycopene. It was suggested that the antioxidant activity of lycopene could prevent the oxidation of OxyMb to MetMb, which was also supported by previous research [19].

With the extension of storage time, the TBARS value increased, indicating that the degree of lipid oxidation was deepened. The study found that beef treated with a high concentration of lycopene had lower TBARS values, indicating less lipid oxidation. This suggests that lycopene may delay the lipid oxidation of beef. Additionally, the beef treated with lycopene showed a smaller decrease in sulfhydryl content and a smaller increase in carbonyl content during storage. The decrease in sulfhydryl content was possibly due to the myofibrillar protein degradation and the disulfide bond formation or disulfide interchange [43]. The increase in carbonyl content was possibly due to the amino or imino groups in the side chains of amino acid residues in proteins being attacked by free radicals [44]. Those free radicals might originate from lipid oxidation. Lipid oxidation has been proven to accelerate the oxidation of Mb, leading to meat discoloration [45]. OxyMb (Fe^{2+}) can be oxidized to MetMb (Fe^{3+}) by free radicals produced by lipid oxidation. Meanwhile, the Fe^{3+} produced by Mb oxidation can act as a catalyst for lipid oxidation and aggravate Mb oxidation [46]. Our results of protein oxidation were consistent with the trend of lipid oxidation.

During the meat storage process, it was important to pay attention to the changes of color as well as other factors such as microbial count and cooking loss. During the growth and reproduction of microorganisms, it would produce a variety of enzymes, which would cause a lot of chemical reactions. Those reactions would not only cause the discoloration of meat but also the degradation of protein and the oxidation of fatty acids. It was observed that all treated groups had less microbial counts than the control after a period of storage, and beef treated with chitosan/lycopene of 1:0 had the least microbial counts. Generally, chitosan is known to possess excellent antimicrobial activity, mainly due to chitosan was a natural polymer with cations, which interacted with anions on the surface of microbial cell membranes through electrostatic interactions, disrupting the integrity of the cell membrane and causing leakage of intracellular substances, leading to metabolic disorders and cell death [47]. It is worth noting that the beef treated with chitosan/lycopene of 0:1 had less microbial counts than the control. It was reported that lycopene also had antibacterial activity [48]. However, it is evident that the antimicrobial activity of chitosan was much higher than that of lycopene.

The cooking loss of beef increased gradually as the storage time increased, which suggested that the color, texture, and flavor of samples deteriorated. It was observed that all treated groups had lower cooking loss than the control after a period of storage. The beef treated with chitosan/lycopene of 1:0 had the lowest cooking loss. Chitosan was instrumental in this process. Chitosan is a kind of natural polysaccharide, which contains many hydrophilic hydroxyl and amino groups in the structure. Thus, the chitosan treatment was beneficial for the water-holding capacity of samples, leading to lower cooking loss. However, the beef treated with chitosan/lycopene of 0:1 still had lower cooking loss than the control, which might be due to the antioxidant activity of lycopene. It was reported that lipid oxidation and protein oxidation could also lead to a decrease in water-holding capacity [49]. Thus, lycopene treatment could decrease the cooking loss of beef due to the antioxidants of lycopene. However, it was clear that lycopene was not as effective as chitosan in decreasing the cooking loss.

The conclusions above indicated that lycopene and chitosan played a positive role in different aspects of beef quality. Sensory evaluation was conducted to comprehensively evaluate the quality of beef. On the whole, the beef treated with chitosan/lycopene of 1:3 had a higher color score. The beef treated with chitosan/lycopene of 0:1 had a higher odor score and springiness score. The beef treated with chitosan/lycopene of 1:0 and the beef treated with chitosan/lycopene of 3:1 had higher adhesiveness scores. CI value showed that during the whole storage period, the overall quality of the beef treated with chitosan/lycopene of 1:3 was better than that of other groups.

Correlation analysis of various indicators further explained the influence of lycopene/chitosan on the color and overall quality of beef. The impacts of lycopene/chitosan treatment on beef color might be ascribed to the transformation of Mb forms. It was reported that the color of meat was affected mostly by the oxidation rate of OxyMb and the reduction rate of MetMb [50]. Hence, the oxidation stability of Mb was very important to the color and the shelf life of meat [51]. In this study, it was found that the relative content of OxyMb was positively related to a^* value, and the relative content of MetMb was negatively related to a^* value. It was also observed that the relative content of MetMb was positively related to TBARS, and TBARS was negatively related to a^* value, which suggested that lipid oxidation was another important factor that affected the color of the beef. Some previous studies also indicated that lipid oxidation could decrease the color stability of meat [52]. In addition, it was reported that the oxidation of Mb and the oxidation of lipids could promote each other [23]. In addition, the relative content of MetMb was significantly positively related to carbonyl content ($p < 0.01$) and was significantly negatively related to sulfhydryl content ($p < 0.01$), which suggested that the oxidation of Mb was closely related to the oxidation of proteins. Myofibrillar protein could interact with Mb through certain bonds. When Mb was adducted with myosin, myosin was prone to conformational changes [42]. Wang et al. [52] found that Mb could promote the oxidation of proteins. The oxidation of Mb had a great influence on the conformation of myofibrillar protein which induced more intense protein oxidation [46]. The growth of microbial counts and cooking loss also had adverse effects on the color and other sensory quality of meat.

The superior antioxidant activity of lycopene has attracted widespread attention for a long time. However, the application of lycopene in high-moisture foods is limited due to the oil solubility property. Chitosan is a natural biopolymer derived from chitin, which is non-toxic, biodegradable, and biocompatible. Chitosan solution has a certain viscosity that can help with the dispersion of lycopene in the aqueous phase. Furthermore, chitosan exhibits good antimicrobial property. Therefore, the preservation effect is more prominent when they are used together. The advantage of our study is that the preparation method of chitosan/lycopene coating and the coating of beef are simple. A large amount of meat can be processed in a short time. There are also shortcomings in our study. Firstly, the grain size distribution of lycopene in chitosan/lycopene coatings was not valuated. Secondly, the stability of chitosan/lycopene coatings was not evaluated. The stability of chitosan/lycopene coatings is mainly affected by the viscosity of chitosan solution. A low

concentration of chitosan results in a low viscosity, which will lead to low stability. Thus, meat needs to be processed immediately after the preparation of coatings. Otherwise, the preservation effect may be affected. Thirdly, the size of beef pieces in the experiment was small. The preservation effect may be affected if the size of samples is larger. These require further study.

5. Conclusions

In this study, a series of coatings with different ratios (1:0, 3:1, 1:1, 1:3, 0:1) of chitosan to lycopene were prepared. The influence of different coatings on Mb and meat quality of beef during storage was studied. The chitosan/lycopene coating with the ratio of 1:3 was particularly effective. The results showed that when applied on beef, the chitosan/lycopene coating with the ratio of 1:3 allowed the quality of beef to be retained for a longer time. Compared to beef treated with chitosan/lycopene of other ratios and beef without treatment, beef treated with chitosan/lycopene of 1:3 could maintain better meat color, lower cooking loss, and lower microbial counts during storage. Furthermore, beef treated with chitosan/lycopene of 1:3 also performed well in sensory characteristics (color, odor, springiness, adhesiveness). The results presented in this work represent a promising advancement in food preservation.

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