Research Article

Inhibitory Effect of Chlorogenic Acid on Lipid and Protein Oxidation in Rabbit Meat during Cold Storage

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Abstract
The application of natural phenolic compounds (NPCs) in the meat industry has attracted increasing attention. In this study, the effects of chlorogenic acid (CGA) at different ratios (0.006% (w/w), 0.01% (w/w), and 0.02% (w/w)) on protein and lipid oxidation in rabbit meat during cold storage at –4 °C were investigated. The results showed that the addition of 0.01% (w/w) CGA significantly reduced protein and lipid oxidation during cold storage. Compared with the control group, the thiobarbituric acid-reactive substances (TBARS) values, lipoxygenase (LOX) activity, and carbonyl content of rabbit meat supplemented with CGA during cold storage were lower, while the sulfhydryl level was higher than that of the control group. Furthermore, *a** and *a*/b** ratios—which could show the degree of protein oxidation and color stability in meat samples—were positively impacted by CGA. Additionally, it was noted that the addition of CGA lowered the pH and prevented microbes from growing. Therefore, the addition of CGA significantly improved the quality of refrigerated rabbit meat, and the appropriate addition amount of 0.01% CGA (100 mg/kg) means that the CGA cost of 1 kg of meat can be as low as 0.8 yuan, providing a theoretical basis for the application of chlorogenic acid in the meat industry.

Keywords: Chlorogenic acid, Inhibition, Lipid oxidation, Protein oxidation, Rabbit meat

1 Introduction

Europe, North Africa, and Asia have sizable consumer markets for rabbit meat [1]. It is regarded as a highly nutritious and healthy "functional food" with the characteristics of "three highs and three lows", which means that rabbit meat has the properties of high protein, high lysine content, high digestibility, low fat, low cholesterol and low calories [2]–[4]. However, it is easily oxidized or impacted by bacteria to decrease the meat quality, especially after 6 days of refrigeration. *Brochothrix* and *Psychrobacter* were the dominant spoilage bacteria, significantly affecting the sensory quality of fresh rabbit meat [5]. Conventional methods try to halt this degradation using artificial antioxidants or preservatives. This conflicts with the desires of customers for clean label foods [6], [7]. Natural phenolic compounds (NPCs) have gained notice for their potential use in the application of meat and meat products [8], [9]. For example, beef patties kept in a refrigerator can retain their good sensory qualities, pH, and oxidative stability with the addition of black cumin (*Nigella sativa*) extract for fifteen days [10]. 2% microencapsulated bamboo leaf extract (MBLE) can effectively prevent the spoilage of fat-rich Moo Yor (Vietnamese-style sausage) stored at 4 °C for 8 days [11]. During 16 days of cold storage, rabbit meat's lipid oxidation and color deterioration were

significantly inhibited by the aqueous extracts of several spices and their mixes [12]. When 6 log CFU/g was used as the microbial limit, the shelf life of rabbit patties at 4 °C was less than 6 days, while the shelf life of rabbit meat with nanocapsule clove essential oil composite film reached 12 days, the shelf life was extended by 2 times, which can significantly reduce industrial production costs and improve the economic benefits of products [13].

Chlorogenic acid (CGA) is a kind of NPCs formed by the shikimate pathway and is frequently present in potatoes, tea, honeysuckle, coffee beans and fruits (apples, mangoes, pears, strawberries and blueberries) [14]. Its five phenolic hydroxyl structures, which can both scavenge free radicals directly and impact the antioxidant signaling system indirectly, are primarily responsible for its antioxidant activity [15], [16]. In addition, it can suppress microbes by altering the structure of bacterial cell membranes, interfering with their cell cycle, and impairing their regular metabolism [17]. Previous research has looked at the impact of adding CGA to rabbit diets on the meat's growth performance [18] and the liver's protective effect [19]. Studies on the antibacterial and antioxidant properties of CGA on fresh beef [20], fish [21], and roasted lamb [22] have also been performed. However, the effects of NPCs on meat are affected by the matrix [23]. For instance, the antioxidant effects of caffeic acid in different matrices differ due to differences in alkyl chain lengths [24], [25]. Therefore, it is necessary to conduct antioxidant and antibacterial studies on CGA in rabbit meat to enrich the effect of CGA in different matrices.

In order to explore the role of CGA in the preservation of rabbit meat, this study analyzed the antioxidant activity of CGA at different concentrations(w/v), determined the effects of CGA at different ratios (w/w) based on the weight of fresh meat on the lipid and protein oxidation of rabbit meat during refrigeration for 9 days, as well as product characteristic indicators closely related to oxidation, including pH, color and aerobic plate count (APC), to provide a theoretical reference for the application of CGA in rabbit meat.

2 Materials and Methods

2.1 *Materials*

Rabbit longissimus dorsi muscle was provided by Shandong Kangda Food Co., Ltd., chlorogenic acid (≥95%) was purchased from Sigma-Aldrich (Cat. No. C3878-250MG, molecular weight, 354.31, extracted from honeysuckle in China), other analytical-grade chemical reagents were provided by Chengdu Kelong Chemicals Co., Ltd., and the kit was purchased from Beijing Solarbio Science & Technology Co., Ltd.

2.2 *Methods*

2.2.1 Preparation of chlorogenic acid solution

Various amounts of 3, 5, 10, and 30 mg of chlorogenic acid were dissolve in 10 mL of ultrapure water to obtain 0.3, 0.5, 1.0, 3.0, 5.0, and 7.0 mg/mL chlorogenic acid solutions. The solutions were kept in refrigerator as the test solution for free radical scavenging ability.

2.2.2 Determination of free radical scavenging ability (FRSA)

DPPH FRSA Assay Kit （DPPH: 2,2-Diphenyl-1-picrylhydrazyl）was used. The positive control was vitamin C, and anhydrous ethanol was used as the blank control. A 96-well plate was filled with varying quantities of extracts and working solutions, left in the dark for half an hour, and then measured at an absorbance of 515 nm. Three tests were performed on each group, and the average result was calculated according to equation (1).

$$\text{DPPH FRS rate (\%)} = \frac{[\text{A blank} - (\text{A determination} - \text{A control})]}{\text{A blank}} \times 100\% \quad (1)$$

Total Antioxidant Capacity Assay Kit with ABTS method (ABTS ： 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) was used, vitamin C as positive control, distilled water as blank control, different concentrations of extracts and ABTS+ working solution were added to 96-well plates, mixed thoroughly, kept still in the dark for 6 min, and the absorbance was measured at 405 nm according to formula (2).

$$\text{ABTS + FRS rate (\%)} = \frac{[\text{A blank} - (\text{A assay} - \text{A control})]}{\text{A blank}} \times 100\% \quad (2)$$

2.2.3 Samples preparation

The fascia of the longissimus dorsi muscle of the rabbit was removed and minced (5 mm pore meat

grinder), and the meat was divided into 4 groups on average. 2% salt and CGA were added into the sample. The contents of CGA were 0, 0.006% (w/w), 0.01% (w/w) and 0.02% (w/w) based on the weight of meat (expressed as Control, CGA6, CGA10 and CGA20). The salt and CGA were mixed and dissolved in 10 mL of ultrapure water. The solution was added to the rabbit meat, mixed and sealed with a polyethylene sealing bag and refrigerated for 9 days (4 °C). The relevant indicators were measured for fresh meat (0 day) and samples after adding CGA and refrigerated for 1, 3, 5, 7, and 9 days.

2.2.4 pH value

The procedure was followed as reported in Ji *et al*., [26]. 5 g of sample was added in 50 mL of ultrapure water. Following homogenization, a pH meter (FE28, Mettler Toledo) was used to determine the mixture's pH value.

2.2.5 Aerobic plate count (APC)

25 g minced meat was added into a sterile homogenizing bag containing 225 mL of sterile saline. The sample was homogenized for 2 min to prepare a 1:10 sample solution (XY-05 Sterile homogenizer, Ningbo Xinyi Ultrasonic Equipment Co., Ltd. China). 1 mL of the solution was added to a test tube containing 9 mL of sterile saline. The sample was mixed well to make a 1:100 diluted sample. According to the above operation, 10-fold serial dilution sample solutions were prepared. At the same time, 1 mL of sterile saline was added to a sterile culture dish containing plate count agar (PCA) as a blank control [27]. After the agar solidified, the plate was turned over and incubated at 37 °C for 48 h. The dilution multiple and the corresponding colonies were counted, and express them as Log (CFU/g) (colony forming units, CFU).

2.2.6 Thiobarbituric acid reactive substances (TBARS)

5 g minced meat and 50 mL trichloroacetic acid mixture were added to a 100 mL conical flask, mixed and sealed, placed on a constant temperature oscillator at 50 ℃ for 30 min, cooled to room temperature, and filtered with double-layer quantitative filter paper. 5 mL of the above filtrate and standard series solution were transferred to 25 mL stoppered colorimetric tubes, and 5 mL of trichloroacetic acid mixture was taken as sample blank, and 5 mL of thiobarbituric acid (TBA) aqueous solution was added to each, stoppered, mixed, placed in a 90 ℃ water bath for 30 min, and cooled to room temperature [28]. The absorbance at 532 nm (FlexA-200 ELISA, Shandong Solai Technology Co., Ltd., same below) was measured. A standard curve was prepared and expressed as: y = 0.4158x-0.0081 (R^2 = 0.9997), as shown in Equation (3).

$$\text{TBARS (mg/kg)} = \frac{C \times V \times 1000}{\text{m} \times 1000} \quad (3)$$

C (ug/mL): The sample solution's malondialdehyde concentration as determined by the standard curve; *V* (mL): The sample solution's constant volume; *m* (g): The final sample solution represents the mass of the sample; 1000: conversion factor.

2.2.7 Lipoxygenase activity (LOX)

0.1 g of rabbit meat samples from each group were taken and the absorbance at 234 nm was measured according to the instructions of the lipoxygenase (LOX) test kit, and the activity was calculated according to the sample mass. One unit of enzyme activity was defined as a change of 0.05 units in A234 absorbance per gram of tissue per minute.

2.2.8 Carbonyl content

0.1 g of rabbit meat sample from each group was taken and the absorbance at 370 nm was measured according to the instructions of the protein carbonyl content detection kit. Calculation was shown as in Equation (4).

$$\text{Carbonyl content (nmol/mg)} = \frac{(A_{370\ \text{determination}} - A_{370\text{control}})}{\varepsilon \times \rho} \times 10^6 \quad (4)$$

Among them, A370 determination: absorbance of the experimental group at 370 nm; A370 control: absorbance of the control group at 370 nm; ε: molar absorption coefficient, 22000/L/(mol·cm); ρ: protein mass concentration in the sample solution, mg/mL; 10^6, unit conversion.

2.2.9 Thiol content

0.1 g of rabbit meat from each group was taken, and the absorbance at 412 nm was measured according to the instructions of the total thiol content detection kit. Reduced glutathione (GSH) was used as the standard to draw a standard curve: y = 1.4714x + 0.0527 (R^2 =

0.9994), and the calculation was performed according to equation (5).

$$\text{Thiol content (nmol/mg)} = \frac{y}{\rho} \times 10^6 \quad (5)$$

Y (μmol/mL): sample concentration calculated by substituting sample absorbance into the standard curve; ρ (mg/mL): protein mass concentration in sample solution; 10^6: unit conversion.

2.2.10 Color

Using a CR-400 colorimeter (Konica Minolta (China) Investment Co., Ltd., Japan), the meat samples' lightness value *L**, redness value *a**, and yellowness value *b** were measured. The *a**/*b** ratio was then obtained.

2.2.11 Statistical analysis

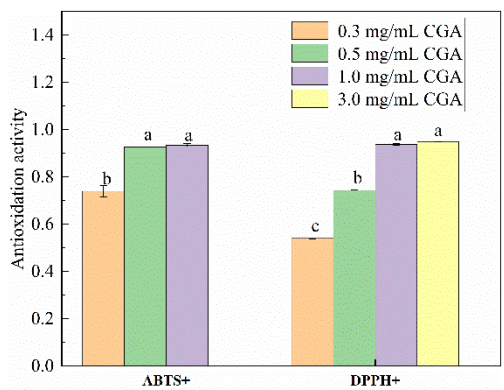
All experimental groups were repeated three times. Microsoft Excel 2019 was used for data preparation, data are expressed as mean ± standard deviation (S.E), Origin 2018 was used for graph preparation, and IBM SPSS Statistics 23.0 was used for data significance analysis, Duncan's post hoc multiple comparisons were performed in the one-way ANOVA test, and *p*-value < 0.05 was considered to be a significant difference.

3 Results and Discussion

3.1 *Evaluation of the antioxidant activity of chlorogenic acid in vitro*

The antioxidant activity of CGA solutions of different concentrations is shown in Figure 1. The higher the FRSA of NPCs, the higher the antioxidant activity, and the better the effect of inhibiting lipid and protein oxidation on meat [12], [29]. The DPPH and ABTS FRSA increased with the increase in CGA concentration. The DPPH FRSA increased from 0.54 (0.3 mg/mL CGA) to 0.938 (1.0 mg/mL CGA), and then there was no significant difference with the increase of CGA concentration. The ABTS FRSA increased from 0.74 (0.3 mg/mL CGA) to 0.93 (0.5 mg/mL CGA), and then there was no significant difference with the increase in CGA concentration. This shows that 0.5～1.0 mg/mL CGA has a strong antioxidant ability.

No similar studies on the antioxidant activity of CGA were found, but kinetic simulations showed that 0.05 mg/mL of CGA and caffeic acid had equivalent antioxidant activity, and when the concentration increased to 2 mg/mL, the activity of caffeic acid was stronger [30]. The economically attractive cost of CGA is estimated to be between 0.06–0.38 USD/mg [31], and the lowest market cost in China is 0.008–0.08 yuan/mg. Under the premise of obtaining the same effect as synthetic antioxidants, the amount of CGA added should be as low as possible. Therefore, based on the preliminary experiment, in order to ensure the problem of insufficient uniformity of mixed meat samples caused by low addition of CGA, 0.006% (w/w), 0.01% (w/w) and 0.02% (w/w) of CGA and salt based on the weight of fresh meat were dissolved in the same mass of water (10mL) and then added to the matrix for mixing.



Note: Different lowercase letters indicate the significant difference in antioxidant activity under different CGA addition amounts (*p*-value < 0.05).

Figure 1: The DPPH and ABTS free radical scavenging abilities at different contents.

3.2 *Effect of CGA on pH value and aerobic plate count of rabbit meat*

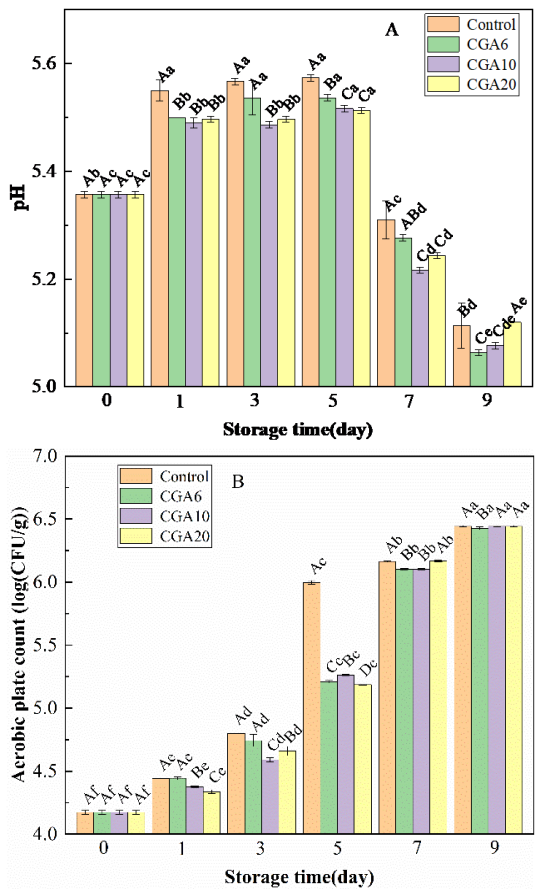
The effect of CGA on the pH value and APC of rabbit meat is shown in Figure 2. As the storage time progressed, the pH value of the four groups initially increased and subsequently fell, increasing from 5.357 (day 0) to 5.49～5.55 on the 1st day, and stabilized at 5.517～5.573 on the 5th day of refrigeration, then decreased to 5.063–5.12 on the 9th day. The pH value of groups with CGA was significantly lower than that of the control group (*p*-value < 0.05). On the 5th and 7th day of refrigeration, there was no discernible pH variation between the group of CGA10 and CGA20.

On the 9th day of refrigeration, however the pH value in the group of CGA20 was much higher than the lowest value of 5.077 in the group of CGA10. The pH value of fresh rabbit meat is neutral, generally greater than 6, and then it drops to between 5.54 and 5.9 after 24 h of storage [32]–[34]. This is quite different from the pH values in this experiment, which may be caused by the initial microbial load of the raw meat and its metabolic effects on the pH reduction, as well as pH differences of up to 0.7 units caused by the different types of rabbit muscle fibers [32], [35]. When the pH value is between 5.3 and 6.0, it indicates that the meat sample has sufficient glycogen reserves [36].

Microorganisms and changes in the pH of meat products are strongly connected, the alkaline substances produced by microbial metabolism and the degradation of proteins and amino acids lead to increased ammonia nitrogen levels, which in turn leads to increased pH values [20], [37], lactic acid bacteria's metabolic activity in the meat can lower pH levels [38]. As a result, within 5 days of refrigeration, the pH value climbed and stabilized due to the addition of CGA, which also enhanced the antibacterial behavior. Nevertheless, as carbon dioxide from microbial metabolism accumulated, the pH value fell. The main aerobic spoilage bacteria in rabbit meat during cold storage are *Psychrobacter* and *Brochothrix* [5], and their optimal growth ranges are pH 6.5～8.0 [39]–[41]. Therefore, controlling the pH below 6 can significantly inhibit the growth of psychrotrophic spoilage bacteria, which is consistent with the experimental results of APC.

The samples' APC dramatically increased with the increase of refrigeration time (p-value < 0.05), from 4.173 log (CFU/g) in fresh meat (day 0) to 6.427–6.443 log (CFU/g) on the 9th day, which is less than 7 log (CFU/g) that is thought to be the highest allowable limit for meat products [42]. The same initial bacterial count was observed in the study by Chen *et al*., [13]. It is also observed that rabbit meat burgers with garlic (*Allium sativum* L.) and ramson (*Allium ursinum* L.) had a total bacterial count below the maximum threshold of 7 within 7 days of refrigeration [43]. Within 5 days of refrigeration, the addition of CGA dramatically reduced the growth of APC. This is happening as CGA can disrupt cell membranes and alter their permeability, which effectively stops both Gram-positive and Gram-negative bacteria from proliferating [44]. Compared with the control group, the APC in the CGA group had the largest decrease percentage on the 5th day, ranging from 12.2% to 13.5%.

Nevertheless, following a week of refrigeration, all samples' APC exceeded 6.0 log (CFU/g), with the groups of CGA10 and CGA6 having significantly lower numbers than the other two groups. On the 9th day of refrigeration, the APC of group CGA6 was substantially lower than the other three groups, and there was no difference between them. This could be the result of high dosages of CGA altering protein structures over extended periods of storage, which changes the characteristics of cell membranes and lessens their antibacterial action [8], [45].



Note: Significant differences between different additions of CGA are shown by different capital letters (p-value < 0.05); significant differences within a group of different storage times are indicated by different lowercase letters (p-value < 0.05).

Figure 2: Effect of CGA on pH and APC of rabbit meat during refrigeration for 9 days.

3.3 *Effect of CGA on lipid oxidaiton of rabbit meat*

Figure 3 illustrates the impact of diverse amounts of CGA on LOX and TBARS during the refrigerated

storage of rabbit meat. With an increase in storage time, the TBARS values of the four groups rose dramatically, from 0.117 mg/kg on the 5th day to 0.346–0.463 mg/kg on the 9th day, the LOX activity declined as the storage time increased, from 573.33 (U/g fresh weight) to 133.33~326.67 (U/g fresh weight). Malondialdehyde (MDA) was utilized as a marker to quantify the amount of fat oxidation, and TBA was measured to reflect its level [46]. During the 9th days of refrigeration, the TBARS of the three groups treated with CGA were considerably lower than those of the control group (p-value < 0.05). Particularly, the value of the CGA6 group did not significantly alter from the control group's results on the first day (p-value > 0.05) and shows that even after one day of refrigeration, the addition of 0.006% CGA is insufficient to significantly affect the lipid oxidation of rabbit meat. Within 9 days of refrigeration, the TBARS of groups of CGA10 and CGA20 were significantly lower than the other two groups (p-value < 0.05) and there was no significant difference between the group of CGA10 and CGA20 after 5 days of refrigeration (p-value > 0.05), suggesting that the addition of 0.01% CGA can achieve the same lipid antioxidant effect as the addition of 0.02% CGA. Compared with the control group, TBARS in the group of CAG10 decreased by 13%–24% during 9 days of refrigeration.

The degree of lipid oxidation is positively correlated with LOX activity [47], compared with fresh meat (0 days), LOX continued to increase after 1 day of refrigeration and then decreased to the lowest value after 9 days of storage. The same trend was also reported in related studies on aquatic products [48]. The three groups with CGA had LOX activities that were considerably lower than the control group's (p-value < 0.05), and the reduction in LOX activity declined as the amount of CGA increased. However, on the 3rd and the 9th days, no significant change was seen in the LOX activities between the group of CGA10 and CGA20 (p-value > 0.05). This suggests that the effect of CGA on LOX activity is the same as that of TBARS, and 0.01% and 0.02% addition are equal. To summarize, the highest possible inhibition of lipid oxidation in rabbit meat can be obtained by adding 0.01% CGA.

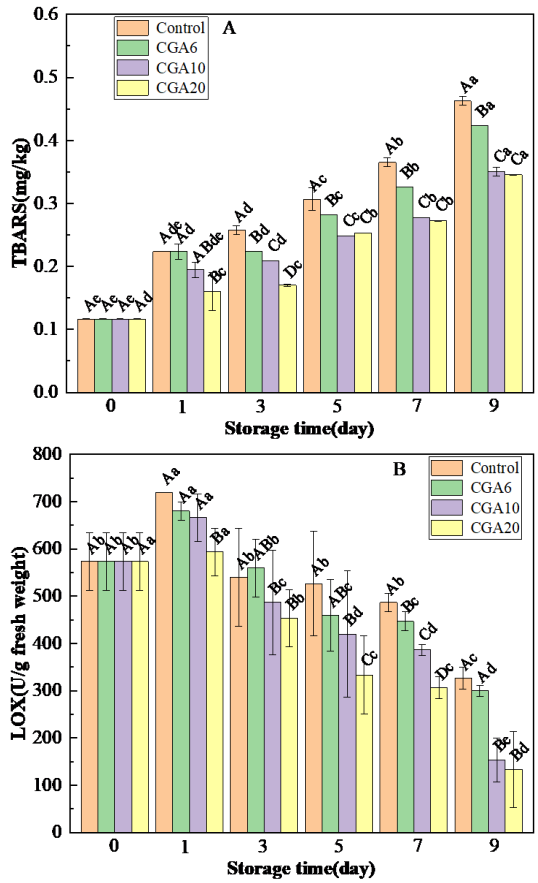


Figure 3: Effect of CGA on TBARS (A) and LOX(B) of rabbit meat during refrigeration for 9 days.

3.4 *Effect of CGA on protein oxidation of rabbit meat*

The effect of different amounts of CGA on carbonyl content in rabbit meat during cold storage is shown in Figure 4(A). The change in carbonyl content is frequently employed as a key marker of protein oxidation because it can reflect the overall quantity of carbonyl compounds, such as aldehydes and ketones, generated by the oxidation of alkaline amino acids, such as lysine [49]. The carbonyl content of the four groups was the highest in fresh meat (0 day), and the lowest value was 0.019–0.048 μmol/g after one day, suggesting that the combination of salt and CGA, as well as the refrigeration storage, can greatly limit the synthesis of carbonyl groups. Among these, NaCl strengthens the protein structure's stability and raises the negative charge on the protein surface [50]. After that, it initially grew and then declined, the control group increased to a maximum value of 0.108 μmol/g on the 7th day and then dropped to 0.055 μmol/g on

the 9th day. The carbonyl content of the groups with CGA was significantly lower than that of the control group during the storage period, increasing from the lowest value on the 1st day to 0.059–0.064 μmol/g on the 5th day (p-value >0.05), and then decreased to 0.036–0.047 μmol/g on the 9th day (p-value >0.05). There is a lack of difference between the effects of 0.01% and 0.02% CGA on carbonyl groups (p-value >0.05), suggesting that the addition of CGA can significantly suppress the production of carbonyl groups in rabbit meat during refrigeration. After 5–7 days of refrigeration, the formation of protein carbonyl groups decreased, the same trend was observed in the refrigeration of chicken with coriander extract [51]. This may be due to the interaction between carbonyl compounds such as aldehydes and ketones with amino acids [52]. MDA formed by lipid oxidation is considered to be the main source of secondary protein carbonyl groups [49], the carbonyl value (COV) created by lipid oxidation likewise tends to grow initially and subsequently decline, which is caused by the polymerization, degradation or other reactions of carbonyl compounds [48]. Consequently, the addition of CGA greatly decreased both the MDA and total carbonyl contents. Compared with the control group, the carbonyl content in the group of CAG10 decreased by 17.8%–54.2% during 9 days of refrigeration.

Figure 4 (B) displays the impact of CGA on the thiol content of rabbit meat during cold storage. As the period of cold storage increased, the thiol content dramatically dropped, which is in line with the findings of several studies by Wang *et al*. [53], Cao *et al*. [48], Ca *et al*. [54], etc. Three groups with CGA decreased from 1.953 umol/g in fresh meat to 1.075–1.206 umol/g on the 9th day (p-value < 0.05), while the control group decreased to 0.798 μmol/g (p-value < 0.05). The thiol group of the treatment with CGA was higher than the control group's, but only on the 7th and 9th day, it became a meaningful difference (p-value < 0.05). Lipid peroxidation can be inhibited by the thiol group, its reduction is considered to be a key indicator of protein oxidation, while the disulfide bonds formed after oxidation can stabilize the protein structure [55]. Samples with CGA had a lower rate of thiol reduction than the control group. Free radicals can oxidize free thiols to create S-S groups, while phenolic compounds can prevent thiols from oxidizing by either scavenging free radicals or strengthening their capacity to compete with free radicals [24]. Thus, the loss of sulfhydryl groups in rabbit meat can be minimized by using CGA with five phenolic hydroxyl groups.

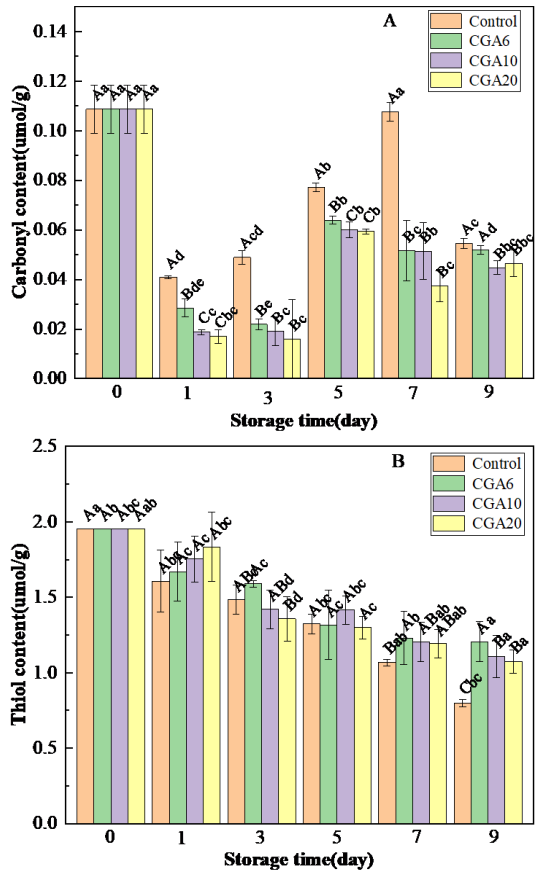


Figure 4: Effect of CGA on carbonyl content (A) and thiol content (B) of rabbit meat during refrigeration for 9 days.

3.5 *Effect of CGA on color of rabbit meat*

Table 1 displays the impact of varying concentrations of CGA on the meat color of rabbits. After adding CGA, L^* and a^* are noticeably lighter than the fresh meat. Previous studies have shown that NaCl may modify meat color [56]. CGA is a pale yellow substance by nature, the lowest values of L^*, a^*, and b^* were found on the 1st day of refrigeration after the addition of salt and CGA, throughout the following nine days of refrigeration, L^* and a^* displayed an increasing trend and b^* showed an opposite pattern. Changes in meat's water retention and adhesion after protein oxidation have an impact on L^*. $L^*$is not much affected by the addition of CGA, and it is only considerably lower than the control group on the 9th day of refrigeration (p-value < 0.05), comparable outcomes were noted in Cao *et al*. [48]. Between the 7th and 9th day of refrigeration, there was not a

noticeable variation of a^* (p-value > 0.05), while a^* grew significantly (p-value < 0.05) from the 1st to the 7th day. Within 1 to 5 days of refrigeration, the changing trend of b^* in the four groups was consistent (p-value <0.05). The b^* of the four groups on the 7th and 9th day of refrigeration was lower than that of 5 days prior, with the exception of the group of CGA10, which saw a substantial drop (p-value <0.05). Only on the 1th and 9th day of refrigeration (p-value < 0.05) were there notable variations in a^* and b^* between the four groups. In this experiment, the L^* value was between 51.5 and 60. It was also observed that the L^* of fresh rabbit meat was 55 and 51.4, respectively, and the a^* and b^* varied greatly in different studies, but the trend of b^* being higher than a^* was consistent with this study [12, 57]. Therefore, the a^*/b^* ratio can be used to judge the color stability of the product.

Within the five days of refrigeration, there was no significant difference in the a^*/b^* ratio of the four groups (p-value > 0.05), the samples with CGA added on the 7th and 9th day of refrigeration were significantly higher than those of the control group (p-value < 0.05), but the ratio was not significantly affected by the CGA content (p-value > 0.05). Myoglobin's ongoing oxidation causes a rise in a^* as storage time increases, protein oxidation is intimately associated with the status of iron ions, where nitrosylmyoglobin (Fe^{2+}) can be continually oxidized to metmyoglobin (Fe^{3+}) and the higher the a^*/b^* ratio, the more Fe^{2+} in the sample indicating that the lower degree of meat oxidation [58]. Consequently, the presence of CGA contributes to preventing meat from oxidizing and minimizes the effect on color. The a^*/b^* ratios of the four groups increased significantly (p-value < 0.05) with increasing refrigeration time, suggesting that low-temperature refrigeration may also be beneficial for stabilizing Fe^{2+} and preserving product color. It is interesting to note that we observed differences in color development during the storage period, which is happening due to the fact that meat color may be contributed by a variety of factors, including diminished enzyme activity, protein denaturation, protein autooxidation, and other physical factors that impact moisture content [59].

Table 1: Effect of CGA on color of rabbit meat during refrigeration for 9 days (mean ± S.E.).

Rabbit Meat		Storage Time (day)					
		0d	1d	3d	5d	7d	9d
L^*	Control	60.35±0.63 ^{Aa}	53.75±0.46 ^{Ac}	53.24±0.31 ^{Bc}	52.06±0.53 ^{Ad}	51.51±0.19 ^{Bd}	56.3±0.1 ^{Ab}
	CGA6	60.35±0.63 ^{Aa}	45.32±0.07 ^{Ab}	53.12±1.87 ^{Bb}	52.5±0.11 ^{Ab}	52.52±0.4 ^{Ab}	51.78±0.42 ^{Db}
	CGA10	60.35±0.63 ^{Aa}	51.31±0.75 ^{Bd}	55.26±0.88 ^{ABb}	53.15±1.25 ^{Ac}	52.33±0.83 ^{ABcd}	52.85±0.37 ^{Ccd}
	CGA20	60.35±0.63 ^{Aa}	55.93±0.9 ^{Bd}	55.93±0.9 ^{Ab}	52.9±1.07 ^{Ad}	52.76±0.35 ^{Ad}	54.3±0.63 ^{Bc}
a^*	Control	3.57±0.3 ^{Aa}	0.71±0.18 ^{Ae}	1.3±0.27 ^{Ad}	2.36±0.2 ^{Ac}	2.89±0.02 ^{Ab}	3.19±0.23 ^{Aab}
	CGA6	3.57±0.3 ^{Aa}	0.47±0.03 ^{Be}	1.49±0.61 ^{Ad}	2.12±0.06 ^{Ac}	2.84±0.21 ^{Ab}	2.58±0.08 ^{Bbc}
	CGA10	3.57±0.3 ^{Aa}	0.5±0.2 ^{ABe}	1.61±0.08 ^{Ad}	2.16±0.26 ^{Ac}	2.82±0.13 ^{Ab}	2.53±0.15 ^{Bb}
	CGA20	3.57±0.3 ^{Aa}	1.37±0.08 ^{ABe}	1.37±0.08 ^{Ad}	2.35±0.09 ^{Ac}	2.86±0.13 ^{Ab}	2.93±0.18 ^{Ab}
b^*	Control	6.72±0.11 ^{Aa}	3.53±0.63 ^{Ab}	3.39±0.28 ^{Abc}	2.86±0.24 ^{Acd}	2.31±0.22 ^{Ad}	2.69±0.42 ^{Ad}
	CGA6	6.72±0.11 ^{Aa}	2.95±0.1 ^{ABb}	2.92±0.7 ^{Ab}	2.63±0.5 ^{Ab}	1.91±0.34 ^{Ac}	1.73±0.22 ^{Bc}
	CGA10	6.72±0.11 ^{Aa}	2.98±0.17 ^{ABb}	3.06±0.09 ^{Ab}	2.74±0.42 ^{Ab}	2.1±0.09 ^{Ac}	1.55±0.15 ^{Bd}
	CGA20	6.72±0.11 ^{Aa}	3.33±0.19 ^{Bc}	3.33±0.19 ^{Ab}	3.2±0.18 ^{Ab}	2.25±0.17 ^{Ad}	2.05±0.36 ^{Bd}
a*/b*	Control	0.53±0.04 ^{Ac}	0.2±0.04 ^{Ad}	0.39±0.1 ^{Ac}	0.83±0.06 ^{Ab}	1.26±0.11 ^{Ba}	1.19±0.17 ^{Ba}
	CGA6	0.53±0.04 ^{Ac}	0.16±0.01 ^{Ad}	0.51±0.09 ^{Ac}	0.81±0.14 ^{Ab}	1.49±0.16 ^{Aa}	1.49±0.19 ^{ABa}
	CGA10	0.53±0.04 ^{Ac}	0.17±0.07 ^{Ad}	0.53±0.04 ^{Ac}	0.79±0.21 ^{Ac}	1.34±0.1 ^{ABb}	1.64±0.28 ^{Aa}
	CGA20	0.53±0.04 ^{Ad}	0.17±0.01 ^{Ae}	0.41±0.02 ^{Ad}	0.73±0.05 ^{Ac}	1.27±0.04 ^{Bb}	1.43±0.17 ^{ABa}

Note: Significant differences between different additions of CGA are shown by different capital letters (p-value < 0.05); significant differences within a group of different storage times are indicated by different lowercase letters (p-value < 0.05).

4 Conclusions

This paper evaluated the inhibitory effect of 0.006% (w/w), 0.01% (w/w) and 0.02% (w/w) CGA on lipid and protein oxidation during cold storage of rabbit meat. The results showed that the presence of CGA significantly inhibited the increase of TBARS value, LOX activity and carbonyl content, and also inhibited the loss of thiol groups. The addition of CGA also reduced the pH value of rabbit meat and inhibited the increase of APC with the best antibacterial effect on the 5th day of refrigeration (APC decreased by 12.29%-13.35%). The addition of CGA can inhibit the rate of change of a* and b* during cold storage, and the a*/b* ratio of the degree of protein oxidation is better than that of the control group. Compared with the control group, the carbonyl content and TBARS of rabbit meat supplemented with 0.01% CGA decreased by 17.8%–54.2% and 13%–24%, respectively, during 9 days of refrigeration, and its own color did not have

a negative impact on the color of the product. Therefore, 0.01% CGA (100 mg/kg) can significantly inhibit lipid and protein oxidation and delay further deterioration of rabbit meat quality, which means that the CGA cost of 1 kg of meat can be as low as 0.8 yuan. In general, this study determined the preservation effect and optimal addition amount of CGA in rabbit meat, and a lower addition amount can significantly reduce the production cost. At the same time, as a natural compound, it is used in the preservation and processing of rabbit meat to meet consumers' demand for clean label food. The subsequent research will focus on the effect of the interaction between CGA and protein on the functional properties of the matrix system, such as texture, flavor, antimicrobial and antioxidant activity.

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Author Contributions

T.B.: conceptualization, investigation, data analysis and curation, reviewing and editing; J. M. Z.: methodology, funding acquisition; supervision; Y. Z.: writing – review and editing; R. K.: writing—reviewing and editing; S. A.: Writing – review and editing; P. Y.: methodology; writing – review and editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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