Incorporating ε-Polylysine Hydrochloride, Tea Polyphenols, Nisin, and Ascorbic Acid into Edible Coating Solutions: Effect on Oxidation and Structure of Marinated Egg Proteins

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Research Article

Keywords: Edible coating, Chitosan, Protein oxidation, Protein structure, Egg products, Tea polyphenols

Posted Date: January 30th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-3186108/v2

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Additional Declarations: The authors declare no competing interests.
Abstract

Egg processing products lose their attraction due to protein oxidation and degradation, while the edible coating incorporating antioxidant and antimicrobial agents can address this deficit. In this work, chitosan (CS) in combination with ε-polylysine hydrochloride, tea polyphenols, nisin, and vitamin C (CS-NH-TC) was applied as a novel edible coating material for preserving marinated eggs for 20 days and its effects on egg quality, and protein oxidation and structure were determined. The CS-NH-TC coating exhibited the highest antioxidant and antibacterial activities among all coatings. Compared to uncoated marinated eggs (Sulfhydryl: 12.02 μmol/g, dityrosine: 6251.8, protein hydrophobicity: 3691, turbidity: 0.13 mg/mL, particle size: 342.13 nm), CS- and CS-NH-TC-coated eggs exhibited improved protein stability, as evidenced by a higher sulfhydryl (22.20 and 30.92 μmol/g) and lower dityrosine content (4722 and 4216), protein hydrophobicity (2891.72 and 865.75), turbidity (0.06 and 0.03 mg/mL), and particle size (292.06 and 256.57 nm). Additionally, edible coatings, especially CS-NH-TC coatings, were positively correlated with smaller intermolecular force changes in marinated eggs, corresponding to suppressed protein oxidation and conformational changes. Furthermore, CS-NH-TC-coated samples showed more stable protein secondary structures and less disorderly and aggregated protein microstructure, and markedly protected the color, texture, and sensory scores of marinated eggs. Our study could provide a theoretical basis for stabilizing the protein structure of egg processing products.

Introduction

Marinated egg is a processed egg product and has obtained enormous attention in research and daily life due to its appealing flavor and nutritional value. It is a casual egg processing product prepared by eggs using spices and seasonings through a series of processes (Liu et al., 2022). Consumers prefer marinated eggs with high lightness, smooth surface, delicate taste, and unique flavor (Zhao, Zhang, Liu, & Zhang, 2021). However, marinated eggs may be susceptible to protein oxidation and structural changes after exposure to air for a period of time and lose these desirable characteristics, demonstrating that they may have a short shelf-life and demand immediate sale and delivery to prevent spoilage (Rysman, Van Hecke, Van Poucke, De Smet, & Van Royen, 2016). Besides, quality loss and deterioration of marinated eggs might occur due to microorganism growth and metabolism that induce protein decomposition and off-flavor (Delbarre-Ladrat, Chéret, Taylor, & Verrez-Bagnis, 2006). Therefore, there is an urgent need for quality preservation methods in the egg processing industry, especially in casual egg product industry, without sacrificing the quality and protein conformation of the products.

Edible coatings are regarded as excellent delivery systems containing several active agents, including spices, antioxidants and antimicrobials (Zhang, Li, & Kang, 2019). It forms a film on the product surface and thus plays a role in slowing protein oxidation, suppressing microorganism growth and reproduction, and preventing changes in protein structure of various foods, such as fishes (Li et al., 2022), meat (Ruan et al., 2019), fruits (Ranjith et al., 2022), vegetables (Rather, Makroo, Showkat, Majid, & Dar, 2022) and egg products (Liu et al., 2022). Chitosan (CS) is derived from the partial removal of the acetyl groups of the natural polysaccharide chitin and has been reported to exhibit excellent antibacterial and antioxidant
capacities against hydroxyl radicals and pathogens in several studies (Ebadi, Khodanazary, Hosseini, & Zanguee, 2019; Luo et al., 2023). In last decade, several scholars have explored the combination of chitosan with other agents to be used as coatings to improve the quality of food products, such as carboxymethyl chitosan-pullulan-galangal essential oil coating and chitosan-carvacrol coating (Wang, Lei, Ma, Yuan, & Sun, 2018; Zhou et al., 2021). Among numerous active substances, ε-polylysine hydrochloride (ε-PLH) is an active peptide produced from Streptomycetaceae and Ergot fungi and shows broad-spectrum antibacterial capacities against microorganisms, yeasts, and molds (Gao et al., 2022). Nisin, a polypeptide isolated from Lactococcus lactis subsp. Lactis, is effective in suppressing the growth and metabolism of Gram-positive bacteria and Listeria monocytogenes (Gharsallaoui, Oulahal, Joly, & Degraeve, 2016). These two antimicrobial agents are considered safe and thus have been widely applied in the preservation of pork, fish, fruits, and vegetables, with the aim of improving qualities and extending shelf-life (Cao, Warner, & Fang, 2019; Eldib, Khojah, Elhakem, Benajiba, & Helal, 2020; Song et al., 2017; Xiao et al., 2023; Zheng, Tang, Yang, Ran, & Li, 2023). However, the study of incorporating these two antimicrobial agents simultaneously to CS solution for preparing edible coatings has not been investigated. Tea polyphenols (TP) are natural polyphenols extracted from tea leaves and are highly favored for their multiple beneficial functionalities, such as antioxidant, antibacterial, and anti-inflammatory activities (Zhang, Jiang, Rhim, Cao, & Jiang, 2022). Similarly, vitamin C (VC), which mainly exists in fruits and vegetables, is an antioxidant that can efficiently scavenge free radicals, thereby preventing damage to micronutrients such as lipids and proteins (Duzzioni, Franco, Lenton, & Sylos, 2018). It is hypothesized that mixtures of antioxidants and antimicrobials with various chemical characteristics might act in a synergistic manner (Bayram & Decker, 2023; Du et al., 2019), protecting foods from oxidation and decomposition. The study of Liu, Zhang, Bhandari, Xu, and Yang (2020) revealed that the antimicrobial effect of antimicrobial mixture is greater compared to single agent alone because of the synergistic effects between polylysine, nisin and anise essential oil. Ran, Chi, Huang, He, and Ren (2020) also found that combination of glutathione and caffeic acid showed synergistic effect in 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), oxygen radical absorbance capacity (ORAC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. Thus, the incorporation of ε-PLH, nisin, TP and VC to the CS edible coatings may create a very promising combination to fully utilize the synergistic effects of antioxidants and antimicrobials, thereby minimizing the quality loss of marinated eggs during storage.

Protein molecules undergo oxidation during storage due to the attack of free radicals and several secondary by-products formed in the process of oxidation (Wang, He, Emara, Gan, & Li, 2019), thus causing changes in the degree of oxidation and structure of proteins, ultimately affecting the quality and properties of proteins and protein-rich foods (Xiong & Guo, 2021). Previous studies that investigated the CS coatings incorporated with active substances mainly focused on the quality of food products during storage, such as improving texture, color, sensory properties and inhibiting microbial growth (Hatab et al., 2018). However, few studies have determined the oxidation and structure changes of protein associated with the protective effect of edible coatings. Moreover, most studies have revolved around using edible coatings for the preservation of fresh foods, such as vegetables (Pholsin et al., 2024), beef (B. Zhang et
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with the protective effect of edible coatings. Moreover, most studies have revolved around using edible coatings for the preservation of fresh foods, such as vegetables (Pholsin et al., 2024), beef (B. Zhang et al., 2021), fishes (Chaijan et al., 2022) and fruits (Ranjith et al., 2022), while less attention has been given to the storage of processed egg products. Marinated eggs have a unique protein gel structure that is far different from that of protein-rich fresh foods (Xue et al., 2021), so it is innovative to study the effect of edible coating solutions with multiple active agents on oxidation and structural changes of marinated egg proteins.

Herein, a CS-loaded edible coating (CS-NH-TC) was designed via blending CS with antibacterial agents of ε-PLH and nisin, and antioxidant agents of TP and vitamin C and used on marinated egg surface to investigate its effects on marinated egg quality, protein oxidation and structure changes. The protein oxidation and protein structure-related parameters, including total sulfhydryl groups, dimeric tyrosine, solubility, hydrophobicity, particle size, and turbidity were measured to evaluate the protection of coatings against protein oxidation and structural stability. Besides, the changes in interaction forces between protein molecules were assessed using intermolecular forces and fourier transform infrared spectroscopy (FT-IR) experiments to determine the protection mechanism of coatings. Furthermore, the surface of marinated eggs was also recorded using a scanning electron microscope (SEM) to directly evaluate the combined effect of storage and edible coatings on the protein gel structure. Lastly, the color, texture and sensory attributes of marinated eggs were examined to assess the protective effects of edible coating against quality loss due to protein instability.

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1. Materials

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Fresh eggs laid within 24 h and seasonings were purchased from a local distributor (Ouya, Changchun, Jilin, China). Halogen materials and spices, including cooking wine, soy sauce, spring onion, ginger, garlic and salt, were supplied by Huachang online store. TP (> 99%), acetic acid (≥ 99.8%), and glycerol (> 99%) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Nisin (99%) and ε-PLH (99%) were bought from Yi Nuo Biological Technology Co., Ltd. (Zhejiang, China). Microbial media was purchased from Beijing Land Bridge Technology Co., Ltd. (Hebei, China) and phosphate buffer saline (PBS, pH=7.4) was obtained from Beijing Solarbio Science and Technology (Beijing, China). VC (≥ 99%), DPPH (≥ 99.7 %), ABTS (≥ 99.7 %), 8-anilino-1-naphthalene sulfonic acid (ANS, 96%), potassium bromide (KBr, ≥ 99%), and chitosan (> 99%) were purchased from Aladdin Chemical Co. (Shanghai, China).

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2.2 Edible coating preparation
Two kinds of edible coating solutions were firstly prepared according to the method of Liu et al. (2022). An aliquot of chitosan (1.5 g) was fully mixed with 99 mL acetic acid (1% (v/v)) and 1 mL glycerol, and then covered with plastic wrap to prevent evaporation and stirred overnight at 60 °C using a magnetic stirring. Next, the solution was sonicated for 5 min using an ultrasonic cleaner (Jinghong mod XMTD-8222, Shanghai, China) to eliminate visible air bubbles. Following the treatment, the sample solution (100 mL), which was referred to as CS, was further added with 0.2 g TP and 1 g VC to prepare CS-TP-VC, with 0.8 g Nisin and 1.5 g ε-PLH to prepare CS-N-PLH, and with 0.8 g Nisin, 1.5 g ε-PLH, 0.2 g TP and 1 g VC to prepare CS-NH-TC.

2.3 Antioxidant activity analysis

2.3.1 DPPH radical scavenging activity

The DPPH free radical scavenging activity of edible coatings was determined using previously published methods with some modifications (Yeganegi et al., 2018). An aliquot of 0.5 mL of edible coating was well mixed with 2.5 mL of DPPH solution (0.2 mmol/L, dissolved in absolute ethanol) and then incubated in darkness for 0.5 h. The absorbance was recorded using a UV-Vis spectrophotometer (UV-2500, Shimadzu Corp., Kyoto, Japan) at 517 nm and the DPPH radical scavenging capacity was calculated from the following formula:

\[
\text{DPPH radical scavenging capacity (\%) = } \frac{A_2 - A_0}{A_1} \times 100
\]

Where \( A_0 \) was the absorbance of absolute ethanol without DPPH solution, and \( A_1 \) and \( A_2 \) were the absorbance of distilled water and samples with DPPH solution, respectively.

2.3.2 ABTS radical scavenging activity

The ABTS radical scavenging activity of edible coatings was determined following the method of Zhang et al. (2017) and Liu et al. (2018). Briefly, ABTS•+ stock solution was generated via the interaction between equal volume of ABTS solution (7 mmol/L) and potassium persulfate (2.45 mmol/L). The obtained mixture was incubated in the dark at ambient temperature for 16 h and was further adjusted to an absorbance of 0.7 ± 0.02 (734 nm). Then, an aliquot of 180 µL ABTS•+ working solution was mixed with 20 µL edible coating solution and then incubated under dark conditions for 10 min before measuring.
for absorbance with a microplate reader (Synergy HT, BioTek, USA) at 734 nm. The ABTS+ radical scavenging capacity was calculated using the following equation:

\[
ABTS+ \text{ radical scavenging capacity (\%)} = \frac{(A_C - A_S)}{(A_C - A_B)} \times 100
\]

Where \(A_S\) = absorbance of edible coating solution with ABTS+ working solution, \(A_C\) = absorbance of PBS with ABTS+ working solution, and \(A_B\) = absorbance of PBS without ABTS+ working solution.

2.4 Antibacterial activity analysis

The antibacterial activity of edible coating against *Escherichia coli* and *Staphylococcus aureus* was determined using colony counting assay following the experimental procedure of Sureshjani, Yazdi, Mortazavi, Behbahani, and Shahidi (2014) and Behbahani, Shahidi, Yazdi, Mohebbi, and Production (2013) with some modifications. The two bacteria were incubated in LB broth medium at 37 °C for overnight and sub-cultured twice continuously to ensure vitality. Next, the bacterial solution was diluted to an OD value of 0.50 (595 nm) and 100 μL of diluted bacterial suspension was evenly spread on the agar plate surface. Moreover, the sterilized roundish filter paper with a diameter of 1 cm was soaked in the CS, CS-TP-VC, CS-N-PLH, and CS-NH-TC edible coating solutions for 2 min, respectively, and the sterile saline was used as a control. Subsequently, the filter paper was placed onto the agar with a sterile tweezer and then incubated at 37 °C for 24 h. The inhibition zone diameters of each edible coating were recorded and compared. Based on the results of antioxidant and antibacterial activity, the CS-NH-TC edible coating solution was used for further marinated egg preservation studies.

2.5 Preparation of marinated eggs

Marinated soup formulation was prepared as per described by Liu et al. (2022). Each egg was washed twice with distilled water and put in the boiling water for 10 min. Afterward, the eggs were collected, peeled, and blended with soup formulation at a ratio of 1:4 (w/w), followed by heating in a 600 w electric cooker for 2 h.

The marinated egg samples were gently washed with mobile water and dried in air. Thereafter, the marinated egg samples were randomly sub-grouped as control (CK, without treatment), CS, and CS-NH-TC, and were submerged in corresponding solutions for 3 min before drying in air. Lastly, these egg samples were stored in a crispant at 4 °C and measured at days 0, 4, 8, 12, 16, and 20.
Results And Discussion

3.1 Antioxidant activity of edible coatings

The antioxidant activity of various edible coating is given in Figure 1A and 1B. Both DPPH and ABTS results followed the order of CS-NH-TC (DPPH: 97.42%, ABTS: 95.93%) > CS-TP-VC (DPPH: 94.80%, ABTS: 98.30%) > CS-N-PLH (DPPH: 21.81%, ABTS: 30.09%) > CS (DPPH: 7.64%, ABTS: 7.53%). Notably, the DPPH and ABTS radical scavenging activities of the CS-NH-TC coating were around 14 times higher than those of the CS coating, whereas the DPPH and ABTS radical scavenging activities of the CS-N-PLH coating solution was 2.8-fold and 4-fold higher, respectively, than those of the CS coating solution. The above results demonstrated that the addition of TP, VC, Nisin and ε-PLH to the CS solution yielded an edible coating with the highest antioxidant activity.

3.2 Antibacterial activity of edible coatings

Figure 1C and 1D summarize the antibacterial activity of CS, CS-N-PLH, CS-TP-VC and CS-NH-TC edible coating solutions. CS solution exhibited an inhibition zone diameter of 7.99 mm for *E. coli* and 6.18 mm for *S. aureus*, which was possibly attributed to the ability of natural polysaccharides to damage the structure of bacteria and inhibit bioenergetic metabolism (Wang et al., 2021). Similar views were given by Palanisamy, Vinosha, Marudhupandi, Rajasekar, and Prabhu (2017), who reported that sulfated polysaccharides isolated from *Spatoglossum asperum* inhibited the growth of *Aeromonos hydrophila*, and by Khemakhem, Abdelhedi, Trigui, Ayadi, and Bouaziz (2018), who found that olive leaf polysaccharides exhibited excellent antibacterial activity against *Salmonella enterica* and *Micrococcus luteus*. The optimized edible coatings exhibited higher antimicrobial activity compared to the CS coatings, with CS-TP-VC showing an inhibition zone diameter of 9.04 mm for *E. coli* and 7.76 mm for *S. aureus*, CS-N-PLH having an inhibition zone diameter of 10.10 mm against *E. coli* and 9.10 mm against *S. aureus*, and CS-NH-TC exhibiting an inhibition zone diameter of 9.43 mm and 10.29 mm for *E. coli* and *S. aureus*, respectively. According to Keykhosravy, Khanzadi, Hashemi, and Azizzadeh (2020), ε-PLH and nisin might disrupt the cell membrane integrity of microorganisms, thus leading to microbial death. This explains the comparatively high antimicrobial activity of CS-N-PLH and CS-NH-TC. Our results were consistent with those of Huang et al. (2023), who found that the epigallocatechin gallate-pectin (EGP) edible coating exhibited excellent DPPH free radical scavenging and linoleic acid autoxidation inhibition capacity as well as effectively inhibited the growth of *E. coli* and *S. aureus*, thereby preventing the decline of grape quality. Based on these results, CS-NH-TC was chosen to protect marinated eggs in the following studies.

3.3 Viability of microorganisms in marinated eggs
The growth and propagation of microorganisms induce degradation of proteins in foods, thus causing disruption of the protein conformation (Ruan et al., 2019). Figure 1 exhibits the total viable count (TVC) changes of uncoated and coated marinated eggs. Obviously, the viability of microorganisms increased gradually in all marinated eggs, especially in the CK group. The initial viable counts of all marinated eggs were 0 log CFU/g, similar with the previous report (Liu et al., 2022). However, the TVC reached 5.03, 4.71 and 3.93 log CFU/g, respectively, for the CK, CS and CS-NH-TC groups after 4 °C storage for 12 days, indicating that the uncoated marinated egg was the first to exceed the threshold (> 5 log CFU/g) (Liu et al., 2022). Then, the TVC values of the CS-coated marinated eggs also exceeded this threshold on day 16, whereas CS-NH-TC-coated marinated eggs always exhibited total bacterial numbers below the threshold during the whole period. These findings demonstrated that the supplement of TP, VC, nisin, and ε-PLH into chitosan coatings inhibited the growth and propagation of microorganisms, possibly resulting in less disturbed protein conformation. Better antimicrobial activity and longer shelf-life of CS-NH-TC-coated marinated eggs may be attributed to the more abundant antibacterial ingredients in the solutions as well as the synergistic antibacterial effects among these components (Yang, Cheng, Tong, & Chen, 2017; Zhao et al., 2019). As reported by Zhou et al. (2022), chitosan-bacterial cellulose edible coatings containing TP exhibited excellent antibacterial activity and can significantly extend the shelf-life of grass carp. This result supports our findings.

3.4 Total sulfhydryl of marinated eggs

Total sulfhydryl groups are mainly composed of two sulfhydry forms, hidden sulfhydryl groups and free sulfhydryl groups (He et al., 2018). During cold storage, SH groups, especially cysteine residues, could form disulfide bonds under the action of hydroxyl radicals, which in turn induces a continuous reduction in the total SH of marinated eggs. Therefore, its content represents the degree of protein oxidation to a certain extent (Nyaisaba et al., 2019). Figure 5A showed that total sulfhydryl decline was significant ($p < 0.05$) in all treatments due to the attack of hydroxyl radicals (Nyaisaba et al., 2019), but the degree of significance of the total sulfhydryl decline during storage varied. In detail, the total sulfhydryl group content of CK decreased from 38.10 μmol/g to 12.02 μmol/g, CS from 38.11 μmol/g to 22.20 μmol/g, and CS-NH-TC slightly dropped from 38.10 μmol/g to 30.92 μmol/g, demonstrating that the application of edible coating solution can effectively prevent the oxidation of marinated eggs. The slower rate of decrease in total SH content during storing can be explained by the following two reasons. Firstly, edible coating solution may form a dense film on the marinated egg surface, thus preventing the contact of oxygen with the eggs and reducing the oxidation of sulfhydryl groups induced by hydroxyl radicals (Nyaisaba et al., 2019). Secondly, antioxidants of TP and VC can compete with sulfhydryl groups to react with hydroxyl radicals, thereby inhibiting the oxidation of sulfhydryl groups. Similar views were expressed by Jia, Kong, Liu, Diao, and Xia (2012), who reported that extracts of black tea with a high antioxidant capacity could compete with SH for trapping hydroxyl radicals to prevent oxidation of sulfhydryl groups.

3.5 Dimeric tyrosine of marinated eggs
Tyrosine, an amino acid-sensitive to reactive oxygen species (ROS), is susceptible to being attacked by free radicals in protein oxidation, and the resulting tyrosine radicals and tyrosine residues can interact through covalent and non-covalent bonds to form dimeric tyrosine (Davies, Lin, & Pacici, 1987). Thus, the content of dimeric tyrosine reflects the degree of protein oxidation and the changes in protein conformation to some extent. As indicated in Figure 5B, the content of dityrosine in the marinated egg proteins increased with the increase of cold storage time, implying a higher degree of protein oxidation and greater protein structural changes. When stored for the same length of time, the CK group obtained the highest level of dimeric tyrosine, revealing that marinated eggs in CK group underwent severe oxidation during the refrigerated storage period. This was because these marinated eggs were directly exposed to the air and the proteins were severely oxidized, resulting in the polymerization of tyrosine residues with each other to form numerous dimeric tyrosines, which eventually induces alterations in protein structures. However, the upward trend of dimeric tyrosine was curtailed after the use of CS and CS-NH-TC coatings. These findings were also likely due to the above coatings could prevent the attack of free radicals on protein molecules while maintaining the stability of protein conformation.

3.6 Protein solubility changes of marinated eggs

Protein solubility is an important measure of degree of protein denaturation during refrigeration and is negatively correlated with the level of protein oxidation, cross-linking and aggregation (Krämer, Torreggiani, & Davies, 2017). As indicated in Figure 5C, the solubility of marinated egg proteins in the CK and CS groups reduced during 20 days of storage, with the CK group exhibiting a greater decrease of 20.07%. However, the protein solubility of CS-NH-TC group remained steady during storage as a result of the protective effect of the edible coating. The lower protein solubility after storage could be explained by the oxidization of SH groups to disulfide bonds due to exposure to air and the polymerization of tyrosine residues with each other to form dimeric tyrosines. In addition, the rapid reproduction of microorganisms caused the disruption of the conformation of the marinated eggs and the exposure of SH groups (Delbarre-Ladrat et al., 2006), further leading to the oxidation of the SH groups to disulfide bonds. These changes induced increased oxidation, cross-linking and aggregation of proteins, which eventually reduced the soluble protein content. In terms of CS-NH-TC group, nisin and ε-PLH (Chheda & Vernekar, 2015; Wang et al., 2015), two antimicrobial substances, inhibited the growth and reproduction of microorganisms and thus reduced their destruction to protein structure, and TP and VC, two agents with strong antioxidant activity, can effectively scavenge free radicals and suppress their attack on sulfhydryl groups and tyrosine (Xu et al., 2010; Yi et al., 2021), which in turn prevented the formation of disulfide bonds and dimeric tyrosine, ultimately alleviating the reduction in protein solubility. These findings were consistent with the results of antioxidant and antibacterial activity as well as total sulfhydryl and dimeric tyrosine content. Similar views were observed by Huang, Qian, Jiang, and Zheng (2019), who stated that shiitake mushroom coated with 1% CS and 15% guar gum showed a slower decline rate of soluble protein compared to the control, thus delaying tissue senescence.

3.7 Protein hydrophobicity changes of marinated eggs
Hydrophobicity is a crucial parameter for assessing changes in protein conformation (Wen, Zhang, et al., 2023). During the initial period of storage, all marinated eggs exhibited comparatively low protein hydrophobicity as a result of the ordered protein structure (Figure 5D). As storage time progressed, the protein structure was disrupted by the action of oxygen and microorganisms, causing the exposure of hydrophobic groups and changes in protein secondary and tertiary structures, which in turn led to an increase in protein hydrophobicity. After coating treatment, the hydrophobicity of marinated eggs was in the order of CS-NH-TC > CS > CK, indicating that the designed edible coating with high antioxidant and antimicrobial properties effectively prevented the damage of protein structure induced by oxygen and spoilage bacteria, thereby reducing the exposure of hydrophobic groups. Similar views were expressed by Kang et al. (2016), who stated that oxidation alters the protein surface hydrophobicity and this process can be prevented or slowed down by edible coatings. The results of total sulfhydryl content, dimeric tyrosine content, and protein solubility and hydrophobicity confirmed the protein conformational changes of marinated eggs.

### 3.8 Intermolecular forces

A series of denaturing solutions, S1, which broke ionic bonds, S2, which broke hydrogen bonds, S3, which broke hydrophobic interactions, and S4, which broke disulfide bonds, were used to disrupt the interactions between proteins (Wen et al., 2022). Figure 6 gives the changes pattern of intermolecular forces of CK, CS and CS-NH-TC samples during cold storage. It can be observed that the proportion of intermolecular forces differed markedly depending on the time of storage. At day 0, disulfide bonds played a dominant role in the intermolecular forces of all marinated eggs, followed by ionic bonds > hydrophobic interactions > hydrogen bonds. Yang et al. (2020) also reported that disulfide bonds were critical in the alkali-induced egg yolk gels, which enabled the formation of a more stable gel system. Besides, all types of intermolecular forces in marinated eggs increased significantly ($p < 0.05$) during 20 days of storage, except for disulfide bonds. As storage time extended, the cross-linking and aggregation of egg white protein gel may lead to increase in ionic bonds and hydrogen bonds Yang et al. (2020). In terms of hydrophobic interactions, its increase might be assigned to the exposure of the hydrophobic groups induced by the unfolding of the proteins, which were initially buried within the protein (Kang et al., 2016). However, different trend was observed in the change pattern of disulfide bonds. The disulfide bond was the major force that maintained the protein gel structure, while oxidation and microbial action led to the disruption of the protein gel structure as the storage time extended, which in turn exhibited a decrease in the disulfide bond content (Zhao et al., 2016).

CS-NH-TC-coated marinated eggs exhibited the least obvious intermolecular force changes during storage, but not for CK and CS groups. As indicated above, the strong free radical scavenging and antimicrobial capacities of CS-NH-TC coating could effectively inhibit the protein oxidation and degradation caused by oxygen and microorganisms, respectively, thus promoting the maintenance of stable protein structures and intermolecular forces. Specially, the application of CS-NH-TC coating solutions caused an increase in the level of hydrogen bonds during the entire storage, except for day 20. In our previous study, polyphenols were found to enter the hydrophobic pocket of protein, thereby creating...
a stable complex via hydrogen bonding (Wen et al., 2022; Wen, Zhang, et al., 2023). Therefore, tea polyphenols in the edible coating solution may bind to the marinated egg proteins to form hydrogen bonds, ultimately enhancing the level of hydrogen bonding.

3.9 Storage stability of marinated eggs

3.9.1 Particle size changes of marinated eggs

The particle size of protein can reflect the degree of protein oxidation and aggregation, which is also positively correlated with the conformation changes of protein (Zhao et al., 2016). Figure 7A exhibits the particle size of marinated eggs coated with various coatings during 20 days of refrigerated storage. The particle size of CK groups was significantly \((p < 0.05)\) higher compared to that of the CS and CS-NH-TC groups. This can be concluded from the results that the particle size of all marinated eggs at day 0 was approximately 227, while this value climbed to 342.13 ± 13.96 for CK groups, 292.06 ± 13.96 for CS groups, and 256.57 ± 22.38 for CS-NH-TC groups at day 20. According to T. Zhang et al. (2023), several soluble protein aggregates were formed during storage due to oxidation, thereby promoting the interactions between egg white protein and water molecules, which finally caused a molten particle surface and thus further facilitated the aggregation of particles. The rise in protein particle size again proved the oxidation, cross-linking and aggregation of proteins that occurred during storage. In contrast, CS-NH-TC coating was capable of efficiently halting the above processes.

3.9.2 Turbidity changes of marinated eggs

Measurement of turbidity was performed to investigate the inhibitory effect of different coating solutions on the cross-linking and aggregation of protein in marinated eggs and the results were given in Figure 7B. It can be observed that the coated marinated eggs, especially the samples coated with CS-NH-TC solution, exhibited lower turbidity than the marinated eggs without coating. Marinated eggs in the CK, CS and CS-NH-TC groups only show a small increase in protein turbidity from day 0 to day 8, demonstrating a low level of protein cross-linking and aggregation. At day 12, the protein turbidity of the marinated eggs in CK group increased suddenly, surpassing that of the marinated eggs in CS and CK-NH-TC groups. Moreover, marinated eggs in CS-NH-TC groups showed a 103.92% increase in turbidity within 20 days of storage, while this value was 698.10% in the CK group and 277.65% in the CS group, revealing that the cross-linking and aggregation of protein during short-term cold storage can efficiently be inhibited by highly antioxidant and antibacterial CS-NH-TC coating. The outcomes corresponded to the above protein solubility and particle size results.

3.9.3 FT-IR results of marinated eggs

To further evaluate information regarding protein conformation changes as affected by CK, CS and CS-NH-TC coating, the FTIR spectra of marinated egg samples was measured. As shown in Figure 8A, both the edible coating solution and the storage time affected the FTIR spectra of the protein gels, which can be reflected by fluctuations in the characteristic peaks of marinated eggs in the range of 4000-500 cm\(^{-1}\).
Broad typical peaks near 3300 cm⁻¹ were observed in the FT-IR spectra of all marinated eggs, which can be attributed to the stretching of -OH and -NH (Kong & Yu, 2007). The bands (3288.70 cm⁻¹) of CK at day 0 shifted to 3288.43 cm⁻¹, 3286.71 cm⁻¹, and 3284.77 cm⁻¹ for CK, CS, and CS-NH-TC at d 12, and to 3284.77 cm⁻¹, 3286.70 cm⁻¹ and 3288.63 cm⁻¹ for CK, CS, and CS-NH-TC at d 20, respectively, demonstrating that hydrogen bonds involved in the interactions between egg white protein molecules altered after different edible coating treatments or after a period of storage.

Additionally, significantly reduced intensity of the characteristic peak around 2930 cm⁻¹ was observed for the C-H stretching vibrational proteome of CH₃ and CH₂ with increasing storage time, and these changes demonstrated enhanced hydrophobic interactions. The intensity of absorption band at this region was markedly increased with the application of CS-NH-TC coating, while CS coating was not very effective in preventing hydrophobic interaction changes in marinated eggs. The FT-IR results of marinated eggs were consistent with that of the protein hydrophobicity, both indicating that CS-NH-TC coatings can prevent damage to protein structure of marinated eggs by the external environment, which was closely related to the excellent antioxidant and physical barrier ability of the coatings.

The amide I bands are located in the wave number range of 1600-1700 cm⁻¹, which represent the secondary structure of the protein skeleton (Phuhongsung, Zhang, & Devahastin, 2020). The detail information (α-helices, β-sheet, β-turn and irregular curls percentage) regarding secondary structure changes of the marinated egg proteins can be reflected in this vulnerable zone and is also given in Figure 8B. On day 0 of storage, α-helices, β-sheet, β-turn and irregular curls percentages of marinated egg proteins were 17.15%, 35.83%, 18.45% and 28.57%, respectively. As storage time progressed, the proportion of β-sheet was reduced to 28.75% and that of α-helices and irregular curls increased significantly (p < 0.05) and were 29.41% and 36.39%, respectively. Notably, the application of CS and CS-NH-TC coatings hindered changes in the secondary structure of marinated egg protein caused by storage. That is, less reduction in β-sheet and less increase in irregular curl were observed in the CS and CS-NH-TC groups, with β-sheet decreasing from 35.81% to 28.75% in the CS group and from 35.87% to 32.95% in the CS-NT-TC group, while irregular curl rose from 28.59% to 33.53% in the CS group and only rose from 28.66% to 31.45% in the CS-NT-TC group. Smaller changes in protein secondary structure suggested that edible coating solutions can attenuate the negative effects of storage time on proteins, thus hopefully ameliorating the quality loss of marinated eggs. Similar results were reported by T. Zhang et al. (2023).

3.9.4 Scanning electron microscope results of marinated eggs

The microstructure changes of marinated egg proteins treated with different edible coating solutions were recorded at different storage times and their microstructural characteristics were presented in Figure 9. Conventionally, the microstructures of protein in fresh marinated eggs with or without coating treatment had a continuous and ordered network structure as a result of protein stretching. However, on day 12 of storage, the surface morphologies of these marinated egg proteins without coating exhibited a disordered structure with numerous aggregates and enhanced the roughness of the overlapped surface. Furthermore, the microstructures of egg proteins without coating underwent more steric modifications
after 20 days of storage, thus forming a layered surface with bunched aggregation. As indicated in the literature of Shen, Elmore, Zhao, and Sun (2020), the presence of surface structures with highly oxidized protein gel was assigned to the cross-links and aggregates within proteins caused by oxidative modification, which in turn resulted in an uneven gel network structure after a period of cold storage.

Under the treatment of edible coating solutions, a less disorderly and aggregated protein structure occurred in CS and CS-NT-TC groups. Comparatively, a coarse cross-linked structure was observed in marinated egg proteins coated with CS group at day 12, while the gel structure became more relaxed and its original cross-linked strand was converted into an aggregated particle strand, showing a higher roughness. As storage time progressed, the roughness of marinated egg proteins treated with CS and CS-NT-TC coating further increased, but the microphotographs of the CS-NT-TC group always exhibited a lower degree of coalescence and a more ordered structure than those of the CK and CS groups, revealing that the CS-NT-TC coating solutions effectively suppressed the cross-linking and aggregation of marinated egg proteins during frozen storage. This was similar to the results reported by Shen et al. (2020), who found that higher oxidative attack caused an accelerated degree of aggregation, and also similar to the results of Nuerjiang et al. (2023), who stated that guava leaf polyphenol effectively decreased the cross-linking and aggregation of myofibrillar proteins. The SEM results confirmed the positive significance of the excellent antioxidant and antimicrobial activities of the CS-NT-TC coating in stabilizing the protein conformation, and also verified the particle size, turbidity and FT-IR results from a microscopic point of view.

3.10 Quality stability of marinated eggs

3.10.1 Color intensity changes of marinated eggs

The color of foods is a quality factor that determines consumers’ purchasing decisions and can be affected by oxidation and microbial propagation (Pathare, Opara, & Al-Said, 2013; Zhang et al., 2024). The color L*, a* and b* values of marinated egg samples with or without coating during refrigerated storage is shown in Figure 2. The lightness of CK group increased markedly (p < 0.05) with the prolongation of storage time. As a result, the highest L* value (75.28 ± 1.09) was obtained in this sample at the end of storage. Both CK and CS-NH-TC edible coatings slowed down the lightness increase of egg samples in comparison to the uncoated marinated eggs, with CS-NH-TC solutions exhibiting greater color stability effects. Moreover, the redness and yellowness of all marinated eggs decreased markedly (p < 0.05) during storage, except for a* values of CS-NH-TC group. At d 20, the redness and yellowness of uncoated marinated eggs decreased by 31.90% and 48.04%, respectively, and these two parameters decreased by 11.71% and 35.37% in CK-coated marinated eggs, and by 6.13% and 19.95% in CS-NH-TC-coated marinated eggs, indicating that CS-NH-TC solutions contributed to the color stability of the marinated eggs. The edible coatings maintain the color of the marinated eggs in two ways: firstly, they are excellent antibacterial agents to prevent microbial propagation. As indicated by Liu et al. (2022), microorganism reproduction induces the development of white spots and mucus on the surface of marinated eggs, which in turn leads to the degradation of external proteins and the exposure of internal...
proteins, ultimately heightening the whiteness of the egg products. Therefore, inhibition of microbial propagation was associated with a stable lightness of marinated eggs. Secondly, they acted as antioxidants to prevent the accumulation of oxidation products, which further maintained the color of the marinated eggs (Li et al., 2022). Similar views were given by Cardoso et al. (2019), who stated that the application of bilayer chitosan-gelatin coatings maintained color stability of meat during storage.

3.10.2 Texture parameter changes of marinated eggs

The changes in hardness, chewiness, cohesiveness and elasticity of marinated eggs from d 0 to d 20 were measured and their results were given in Figure 3. The hardness of all egg samples increased in the early stage, and then decreased during the later storage period. Protein oxidation causes protein cross-linking and fragmentation as well as modification of amino acid side chains during storage, thereby the promoting hardness of the marinated eggs (Li et al., 2020). The influence of microorganisms on the hardness of protein-rich foods originates from two points. On the one hand, microorganisms utilize water during growth and reproduction, resulting in foods with lower moisture and higher hardness. On the other hand, overpopulation of microorganisms near the end of storage might induce protein degradation and destruction, thereby reducing its hardness (Li et al., 2020). Therefore, in the early storage, the hardness of CS-NH-TC-coated samples exhibited the least noticeable changes among all marinated eggs due to the ability of CS-NH-TC coatings to resist free radical attack and microbial colonization, thus delaying protein oxidation and degradation. After 12 days of storage, the hardness of CS-NH-TC-coated marinated eggs was significantly higher than that of the CS-coated and control group, indicating that the CS-NH-TC edible coating system showed the greatest protective effect on the structure of marinated eggs. This was attributed to the better antimicrobial effect of the CS-NH-TC coating compared to the CS coating, which effectively hindered the marinated eggs to enter the stage where microorganisms were highly multiplied and the protein conformation was severely disrupted. According to Chaijan, Panpipat, Panya, Cheong, and Chaijan (2020), the fish coated with whey protein isolate-ginger extract coating solutions exhibited superior texture characteristics compared to the control, due to the inhibition effects of coating on microbial growth. Meanwhile, Jiang, Nakazawa, Hu, Osako, and Okazaki (2019) also reported that the combination of epigallocatechin-3-gallate and gelatin prevented the reduction of hardness in fish fillet.

Chewiness represents the energy needed to chew solid food to a state where it can be swallowed (Guénard-Lampron, Masson, & Blumenthal, 2021), while cohesiveness denotes the strength of the internal bonds making up food structures (Mahajan, Bera, & Panesar, 2022). Similar to the changing pattern of hardness, the chewiness and cohesiveness of marinated eggs increased first and then decreased during the whole period. Comparatively, the chewiness and cohesiveness of the CS-NH-TC groups were more stable than that of the CS and CK groups. Elasticity represents the recovery degree of foods under the action of a compression force (Yu et al., 2020). Significant decrease in elasticity occurred in CK group with the extension of storage, but the marinated eggs covered by CS and CS-NH-TC coatings retained their elasticity much better than the control samples. Wang et al. (2018) observed that elasticity of shrimp decreased gradually throughout the storage process, while the application of chitosan-carvacrol coating effectively retarded the elasticity change of shrimp. Wei et al. (2021) also revealed that
the carboxymethyl chitosan-coated ε-polylysine (ε-PL) nanoemulsion slowed down the reduction of elasticity and cohesiveness of donkey meat. The stabilizing effect of CS-NH-TC and CK edible coatings on the elasticity, chewiness and cohesiveness of marinated eggs can also be explained by the protective effects of edible coatings against protein oxidation and degradation.

3.10.3 Sensory property changes of marinated eggs

Sensory attributes of foods are of great significance for consumer acceptability (Khaledian, Basiri, & Shekarforoush, 2021). Alterations in sensory properties of marinated eggs treated by CS and CS-NH-TC coating solutions during keeping time are shown in Figure 4. Obviously, the sensory attributes (Odor, texture, color, taste and overall acceptability) of all egg samples decreased with the extension of storage. On the 0th day, it was found that the application of CS and CS-NH-TC edible coatings led to a slight decrease in the odor, color and taste of marinated eggs, while the texture and overall acceptance of the samples were not greatly affected. At day 4, there were almost no significant differences in the sensory scores between the CK, CS and CS-NH-TC group, with the exception of the color scores of CS-coated marinated egg being significantly ($p < 0.05$) lower than that of the control group and CS-NH-TC-coated group. However, an obvious inversion appeared on the 8th of storage, as CS-NH-TC group obtained the highest sensory ratings, followed by CS and CK groups. In terms of taste, the CK, CS, and CS-NH-TC samples became unacceptable after 12, 16, and 20 days of storage, respectively, while for texture rating, CK and CS were observed to show texture scores below 3 on day 16 and 20, respectively. Concerning overall acceptance, the shelf life of the CK and CS group was 12 and 16 days, respectively, while the samples coated with CS-NH-TC edible coating solutions were always regarded as “acceptable for consumption” during entire storage. It can be concluded that CS-NH-TC solutions were effective in inhibiting microorganisms and reducing the accumulation of oxidation products due to its high antioxidant and antimicrobial activity, thus stabilizing the protein conformation and improving the sensory attributes of marinated eggs. These results were in line with those for color and texture. Similarly, beef slices coated with ε-PL-glutathione-CS coating solutions exhibited lower microorganism growth rate, and higher sensory scores than those of the samples without coating (Cheng, Hu, & Wu, 2021), which further confirmed our results.

Conclusion

In our study, we designed CS-NH-TC edible coating and investigated its effects on the quality, oxidation and structure of marinated egg proteins. CS-NH-TC was the best among all coatings as it showed significantly higher antioxidant activity against DPPH and ABTS as well as antibacterial activity against E. coli and S. aureus. Comparatively, CS-NH-TC edible coating was more capable of suppressing the oxidative cross-linking and aggregation of marinated egg protein and it was positively correlated with stable intermolecular forces in marinated eggs, which represented an improvement in structural stability of the protein samples. Furthermore, FT-IR showed that storage time and edible coatings interacted to affect the protein secondary structures and CS-NH-TC edible coatings exhibited better protective effect against conformational changes than CS coatings. A less disorderly and aggregated microstructures was
observed in marinated eggs coated with CS-NH-TC solutions, corresponding to more pleasant color, texture and sensory scores of marinated eggs, demonstrating that CS-NH-TC can serve as a promising coating for obtaining high-quality egg processing products. This study not only reveals the mechanism by which edible coatings stabilize protein structure, but also offer feasible industrial application pathways for the preservation of egg processing products.

Declarations

Acknowledgments

This work was supported by Jilin Scientific and Technological Development Program (20210202056NC).

Conflict of interest

All authors have no conflict of interest.

Data availability statements

The datasets generated during the current study are available from the corresponding author on reasonable request.

Author contributions

Jingbo Liu: Investigation; Supervision; Project administration; Resources; Writing-review & editing. Lu Han: Investigation; Methodology; Validation; Writing-original draft. Dongkun Cheng: Validation; Formal analysis. Shengrao Li: Methodology; Formal analysis; Data curation; Validation. Xiumei Chen: Data curation; Formal analysis. Yiding Yu: Formal analysis. Deju Zhang: Data curation; Formal analysis; Writing-original draft. Ting Zhang: Conceptualization; Validation; Writing-review & editing.

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**Figures**
Figure 1

The sulfhydryl group content (A), dimeric tyrosine content (B), soluble protein content (C) and hydrophobicity (D) of marinated eggs at different storage time.

CK = marinated eggs without coating treatment, CS = marinated eggs coated with chitosan solutions, CS-NH-TC = marinated eggs coated with chitosan solution incorporating ε-polylysine hydrochloride, tea polyphenols, nisin, and vitamin C. A–E means for the same sample at different storage time without common letters are significantly different ($p<0.05$). a–c means for the different samples with same storage time without common letters are significantly different ($p<0.05$).
Figure 2

The ionic bonds (A), hydrogen bonds (B), hydrophobic interactions (C) and disulfide bonds (D) of marinated eggs at different storage time.

\[A-E\] means for the same sample at different storage time without common letters are significantly different \((p<0.05)\). \[a-c\] means for the different samples with same storage time without common letters are significantly different \((p<0.05)\).
Figure 3

The turbidity (A) and particle size (B) of marinated eggs at different storage time

Figure 4

The fourier transform infrared spectroscopy (A) and secondary structure proportion (B) of marinated eggs at different storage time
Figure 5

Microstructure of marinated eggs at different storage time