Incorporating ε-polylysine hydrochloride, tea polyphenols, nisin, and ascorbic acid into edible coating solutions: Effect on quality and shelf life of marinated eggs

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Abstract

This study aimed to develop and characterize the chitosan-edible coating solution (CS-NH-TC) incorporated with ε-polylysine hydrochloride, tea polyphenols, Nisin, and ascorbic acid, then investigate their effect on physicochemical, antibacterial, and sensory properties of marinated eggs stored at 4 °C for 24 days, marinated eggs without coating were set as control. The CS-NH-TC edible coating solution exhibited the lowest particle size (582.6 ± 16.6 nm) and polydispersity index (0.49) and highest zeta potential (30.96) among the coatings. The FT-IR spectra showed that TP, VC, Nisin, and ε-PLH were successfully encapsulated in the CS edible coating solution. The moisture content and water holding capacity of the marinated eggs coated with CS-NH-TC remained stable up to 20 days. Marinated eggs coated with CS-NH-TC edible coating solution exhibited lower total viable counts of microorganisms, and more stable color and pH values compared to the control during the entire storage time. Furthermore, the texture and sensory evaluation results demonstrated that marinated eggs coated with CS-NH-TC edible coating solution had low degradation rate and improved overall acceptability scores. This study confirmed that CS-NH-TC edible coating solution is a promising coating for improving the quality and shelf life of marinated egg products and may have a broad application prospect in the preservation of other food products.

1 Introduction

Marinated eggs are regarded as traditional Chinese egg products with a long processing history. It is a casual egg product made from fresh eggs via boiling, shelling, seasoning, marinating, pickling and other processes. Marinated eggs have rich nutrition, unique flavor, and balanced amino acid ratio and are easy to be digested and absorbed by the human body, which indicates these casual egg products may have certain food therapy (Zhao et al. 2020). However, marinated eggs are equally susceptible to the effect of microbial growth and reproduction, resulting in very short shelf life. Enzymes produced by harmful microorganisms can cause the breakdown of products into amines, aldehydes, organic acids, and other substances, thereby affecting the color, flavor, and texture of marinated eggs (Scalone et al. 2019; Zwolan et al. 2020). Nevertheless, there is little research about preserving marinated eggs using peptides, tea polyphenols and ascorbic acid complexes. Hence, the casual eggs products industry urgently needs quality preservation techniques that do not compromise the quality and sensory properties of the product.

Edible coating refers to the edible liquid material that can form a film on the appearance of various foods by spraying, dipping, or brushing (Zhang et al. 2015). It is also a suitable carrier of several food additives such as antimicrobial agents and antioxidants, which may improve food quality by slowing lipid oxidation, preventing protein degradation, and protecting the color and flavor of food (Dehghani et al. 2018). Chitosan (CS), a linear cationic polysaccharide resulting from chitin deacetylation, is considered a suitable coating material due to its antimicrobial capacity and uniform film-forming ability (Lai et al. 2022). Chitosan exhibits antioxidant activity and broad-spectrum antibacterial activity against Salmonella, Lactobacillus, Listeria monocytogenes, and yeast (Chein et al. 2019; Lin et al. 2020; Molamohammadi et al. 2019). In addition, several studies have reported that the combination of chitosan
with other substances may show enhanced antibacterial and antioxidant effects compared to either single agent alone. Therefore, a chitosan edible coating solution (prepared by mixing chitosan with several small-molecule substances) was developed in this study with a view to improving its protective effect against pathogenic bacteria.

ε-polylysine hydrochloride (ε-PLH) is an FDA-listed antimicrobial peptide and has been widely used in the preservation of noodles, cakes, sliced fish, boiled rice and so forth, due possibly to its antioxidant properties and broad antimicrobial activity (Chheda and Vernekar 2015). Nisin is a polypeptide produced by Lactococcus lactis subsp lactis and shows substantial antimicrobial activity against Gram-positive bacteria. It is generally considered safe and thus has been FDA-approved for food use (Chandrasekar et al. 2017). Tea polyphenols (TP) are also extensively studied for their antioxidant and antibacterial capacity and for their contribution in preserving foods (Yi et al. 2011; Wang et al. 2022). Ascorbic acid (also known as vitamin C (VC)), is a plant-derived antioxidant mainly found in fruits and vegetables, especially citrus fruits. VC can act as scavengers of reactive oxygen species, thus contributing to prolonging the shelf life of the food product (Zou et al. 2016). Furthermore, VC and TP are both well-acknowledged natural antioxidants that play an essential role in preventing fat oxidation and enzymatic reaction. Therefore, they are also of interest to the food industry and are widely investigated because of their potential in improving appearance and sensory properties of numerous foods (Song et al. 2011).

Bacteria are prokaryotic microorganisms that can pose a serious threat to human health, industrial operation and clinical medicine (Wei et al. 2019). Antibiotics are widely used to treat infectious bacteria to devastate destroy bacterial cell membranes or inhibit their growth. However, some bacteria strains have developed resistance against the penetration of antibiotics and are currently a global health crisis. Therefore, plenty of effective therapeutic methods, especially natural ingredients with synergistic antibacterial effects, have been proposed to promote the antimicrobial activity of antimicrobial agents. Previous studies have proven that the antibacterial capacity of carboxymethyl pachyman could be improved due to the presence of low concentration of oligomeric procyanidins, indicating a significant synergistic effect between them (Wang et al. 2019). Moreover, researchers also revealed a synergistic antibacterial activity exists between nisin and polylysine, suggesting an enhanced protective effect against harmful microorganisms when the two substances were applied together in different food systems (Alirezalu et al. 2019; Liu et al. 2020). Thus, there is a need to investigate the synergistic antibacterial activity between natural ingredients for copious food and biomedical applications.

In this research, an edible coating solution was developed by blending chitosan, ε-PLH, Nisin, TP, and VC and was applied to marinated eggs to extend their shelf-life. The physicochemical properties, including polydispersity index (PDI), average droplet size, zeta potential (\(\zeta\)), pH, FT-IR characterization, and rheological properties of the edible coating solutions, and their protective effect against moisture loss, pH change, color variation, microbial growth and reproduction, and textural and sensory changes in marinated eggs during cold storage was measured to evaluate their important role in extending shelf life.

2 Materials And Methods
2.1 Materials

Fresh eggs (50-60 g) and seasonings were brought from a local supermarket (Changchun, China). Halogen materials and other spices were purchased from Hua Chang flagship store. Ascorbic acid, potassium bromide (KBr), and chitosan were purchased from Aladdin Chemical Co. (Shanghai, China). Nisin and ε-polylysine hydrochloride were supplied by Yi Nuo Biological Technology Co., Ltd (Zhejiang, China). Tea polyphenols, acetic acid, and glycerol were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Microbial media (plate count agar) was obtained from Beijing land bridge Technology Co., Ltd (Hebei, China). Micropore filter was supplied by Ai Ke instrument Co., Ltd (Hebei, China).

2.2 Preparation of edible coating solutions and marinated eggs

A series of edible coating solutions were prepared as described by Xiong et al. (2020) with modifications. Briefly, 1.5 g chitosan was dispersed in a mixture of 99 mL of 1 % sterile glacial acetic acid and 1mL of glycerol, followed by constantly stirring at 60 °C overnight. After that, the mixture was filtered through micropore filters and was used as the CS edible coating solution. Five batches of experimental edible coating solutions were prepared as follows:

- **CS**: CS edible coating solution;
- **CS-TP-VC**: CS edible coating solution + 1 g VC + 0.2 g TP;
- **CS-Nisin-ε-PLH**: CS edible coating solution + 0.8 g Nisin + 1.5 g ε-PLH;
- **CS-NH-TC**: CS edible coating solution + 1 g VC + 0.2 g TP + 0.8 g Nisin + 1.5 g ε-PLH;

The marinated soup formulation was as follows: 2000 g water, 30 g sugar, 1.5 g monosodium glutamate, 35 g cooking wine, 20 g soy sauce, one marinade packet of about 20 g, 2.2 g spring onion, 1.65 g ginger, 1.65 g garlic, 2.5 % salt. Fresh eggs were washed with tap water and then heated in a boiling water bath for 8 min. Then the boiled eggs were dehulled and mixed with marinated soup at a ratio of 1:4 (w/w), before cooking with an electric cooker powdered at 600 w for 2 h.

2.3 Edible coating of the marinated eggs

Marinated eggs were soaked in sterile distilled water for 1 min to remove the surface impurities and then left to dry at room temperature. Thereafter, the marinated eggs were randomly divided into five groups for different coating treatments: the control group (CK), CS group, CS-TP-VC group, CS-Nisin-ε-PLH group, and CS-NH-TC group. Marinated eggs were submerged in different edible coating solutions for 3 min and were then allowed to dry at room temperature. Finally, different marinated eggs were kept in the fresh-
keeping boxes (Length 180 mm, width 100 mm) at 4 ± 1 °C and were taken out for analysis on days 0, 4, 8, 12, 16, 20, and 24.

2.4 Characterization of edible coating solutions

2.4.1 Droplet size, zeta potential, polydispersity index and pH

The polydispersity index (PDI), average droplet size, and zeta potential (ζ) of the marinated eggs were determined by Nano ZS90 particle size analyzer following the method of Wen et al. (2022) with minor modifications. The particle refractive index was set at 1.453, particle absorption rate at 0.001, and dispersant refractive index at 1.330, respectively. Each sample was diluted with distilled water at a ratio of 1:100 before subjecting to the analyzer. For each assay, three independent samples were prepared. The pH of edible coating solutions was measured using a pH meter (ST310, OHAUS, Shanghai, China).

2.4.2 Fourier-transform infrared (FTIR) spectroscopy

FT-IR was determined as per the method of Jiang et al. (2022) with some modifications. Each edible coating solution, including CK, CS, CS-TP-VC, CS-Nisin-ε-PLH, and CS-NH-TC, was freeze-dried with a freeze-drier (NAI-T1-50, Shanghai Na ai Precision Instrument Co., Ltd., Shanghai, China), followed by being pressed into tablets together with 100 mg KBr. Afterward, the FT-IR spectra of the edible coating solutions were recorded using an IRPrestige-21 Spectrometer (Shimadzu, Japan) in the range of 4000 to 400 cm⁻¹ (64 scans at a resolution of 4 cm⁻¹).

2.4.3 Rheological analysis

The rheological properties of edible coating solutions were characterized with a rheometer (AR-1000, TA, 133 USA) as per the method of Sun et al. (2021). For determining the frequency and apparent viscosity, the samples were well mixed before measurement and the shear rate was increased linearly from 1.5 to 100 s⁻¹.

2.5 Effects of edible coating solutions on the quality of marinated eggs during storage

2.5.1 Determination of moisture content

As indicated in the literature of Dong et al. (2020), the moisture content of marinated eggs was determined by drying 2 ± 0.1 g of samples in an oven at 105 °C to a constant weight. Then, the moisture content of marinated eggs was calculated on wet basis (%):
Where \( M_1 \) indicates the weight of a dry blank weighing bottle, \( M_2 \) and \( M_3 \) express the overall mass of the samples and weighting bottle before and after drying separately.

2.5.2 Determination of water holding capacity (WHC)

The WHC of marinated eggs was determined as per the method of Zhang et al. (2020) with some modifications. An aliquot of 90 mL deionized water was added to 10 g of the sample and homogenized using a homogenizer, and then allowed to sit for 30 min. After the suspension was centrifuged at 10,000 rpm for 10 min, the supernatant was filtered through Qualitative filter paper (150 mm, Shanghai, China) and the resulting solution was collected and measured using a pH meter (ST310, OHAUS, Shanghai, China). The following formula was used to calculate the WHC of marinated eggs:

\[
WHC (\%) = \frac{M_1 - M_0}{M_0} \times 100 \%
\]

In formulas, \( M_0 \) indicates the mass of the samples before centrifugation (g), and \( M_1 \) represents the mass of the samples after centrifugation (g).

2.5.3 Determination of total viable counts (TVC)

TVC of marinated eggs during cold storage was determined using the plate counting method following the method of Ruan et al. (2019) with some modifications, and the results were expressed in \( \log_{10} \) CFU/g. Briefly, 25 g of marinated eggs were transferred to a sterile plastic bag containing 225 mL of sterile saline and homogenized for 1-2 min. After that, each slurry (0.1 mL) was aseptically added to 10 mL of standard plate count agar and incubated at 37 °C for 48 h.

2.5.4 Determination of color

The color intensity of marinated eggs was analyzed using a Minolta Chroma Meter (CR-400, Minolta, Japan) following the method of Zhang et al. (2021) with some modifications. The surface color of marinated eggs was taken at 4 external and 4 internal locations of the eggs on days 0, 4, 8, 12, 16, 20, and 24 of storage. Before measurement, a white reference tile (\( L^* = 95.25, a^* = 0.49, b^* = 2.53 \)) was used for calibration of data. The color of marinated eggs was expressed as \( L^* \) (black to white), \( a^* \) (green to red), and \( b^* \) (yellow to blue).

2.5.5 Textural profile analysis (TPA)
The hardness, springiness, cohesiveness, and chewiness of marinated eggs were conducted as described by Zhao et al. (2021) with a Texture Analyser (BROOKFIELD CT-3, Hongkong) with some modifications. Marinated egg cubes (0.8 × 1 × 1 cm$^3$) were taken from the marinated eggs and placed on the stage of the texture analyzer. Texture analysis of samples was carried out under the following instrument parameters: trigger force value—5 g, pre-test speed—5.0 mm/s, test speed—2.0 mm/s, post-test speed—2.0 mm/s, and the compression ratio—50%.

2.5.6 Sensory evaluation

A plastic container containing different marinated eggs was presented to the assessor for sensory evaluation on days 0, 4, 8, 12, 16, 20, and 24 of storage. Ten assessors were trained in marinated egg evaluation under the direction of a panel leader and were asked to assess the color, odor, and overall acceptability of the marinated eggs using a visual analog scale (1-5). In detail, the color value indicates color variation in marinated eggs: 5 = none; 4 = slight; 3 = small; 2 = moderate; 1 = extreme. The smell value indicates deterioration degree of the marinated eggs: 5 = none; 4 = slight; 3 = small; 2 = moderate; 1 = extreme. The overall acceptability indicates consumer acceptance towards marinated eggs: 5 = like extremely and 1 = dislike extremely. The sensory score of samples lower than 3 was considered to be unacceptable.

2.5.7 Appearance changes of marinated eggs

The changes in marinated eggs were observed visually and recorded using a camera at 0, 4, 8, 12, 16, 20, and 24 days of storage.

2.6 Statistical analysis

All the experiments were carried out in triplicates. The obtained values were expressed as mean ± standard deviation (n = 3) and were submitted to one-way analysis of variance (ANOVA) and (significant differences, Tukey, LSD)’s test using the BONC DSS Statistics 25 at the significant level of P < 0.05.

3 Results And Discussion

3.1 Characterization of edible coating solutions

3.1.1 Droplet size, zeta potential, PDI and pH

The zeta size, zeta potential, PDI, and pH of edible coating solutions were presented in Fig 1. As indicated in Fig. 1a, the average zeta size of CS-TP-VC, CS-Nisin-ε-PLH, CS-NH-TC was in the nanometer range, whereas CS edible coating solution had a zeta size of 1.1 μm. The addition of TP, VC, Nisin, and ε-PLH...
into the CS edible coating solution resulted in a decline in the mean zeta size to 582.6 nm, which may be attributed to lower pH values of the edible CS edible coating solution caused by the addition of equally charged VC and ε-PLH, thereby reducing the aggregation of these antibacterial active substances (Kong and Park 2011). Moreover, our results also showed that CS-NH-TC edible coating solution exhibited excellent uniformity.

As indicated in Fig. 1b, all the test edible coating solutions were positive for zeta potential value as a result of the cation nature of the polymers (Wang et al. 2018). The addition of TP, VC, Nisin and ε-PLH to the CS edible coating solution resulted in an increased ζ-potential of edible coating solutions from 18.70 to 30.96 mV. ζ-potential with absolute value greater than 30 mV are considered stable. While particles become unstable and start to flocculate when the repulsive energy is not sufficient to counteract the hydrophobic attractive and van der Waals attractive energy due to the low ζ-potential (Huang et al. 2020). The incorporation of TP, VC, Nisin, and ε-PLH into CS edible coating solutions significantly improves their zeta potential (p < 0.05) due to the dispersal of cationic groups of the VC and ε-PLH in the aqueous phase, which contributes to the stability of the solutions (Haghju et al. 2016).

The polydispersity index (PDI) reflects the distribution of particle size in a liquid system. Values of the index close to 1 represent heterogeneous size distribution, whereas values close to 0 represent homogenous size distribution. As indicated in Fig. 1c, the PDI value of CS edible coating solution was at low level (PDI = 0.63) compared to CS-TP-VC (PDI = 0.76) and CS-Nisin-ε-PLH (PDI = 0.85) edible coating solutions. However, the lowest PDI value (PDI = 0.49) was obtained in CS-NH-TC edible coating solution. This can be explained by the fact that the addition of TP, VC, Nisin and ε-PLH increased the intermolecular repulsion of the edible coating solution, further reducing the aggregation of the particles (Kong and Park 2011).

As indicated in Fig. 1d, CS-NH-TC edible coating solution showed the lowest pH (p < 0.05). This can be explained by the fact that both the release of H⁺ from VC and the dispersal of the CS and ε-PL cationic groups decreased the pH values of the CS-NH-TC complex.

### 3.1.2 Rheological properties

The rheological properties of edible coating solutions can, to some extent, reflect the internal physicochemical properties of the system. Most fluids used in the food industry are characterized by shear variability, such as shear-thinning and shear-thickening fluids. Moreover, the viscosity change curve of the edible coating solutions is positively related to their kinetic stability (Sun et al. 2021). Fig. 2 showed the rheological properties of the edible coating solutions under the shear rate of 1.5 - 100 s⁻¹ and results demonstrated that all solutions exhibited distinctive shear thinning behavior. Upon the increment of shear rate, the viscosity of various edible coating solutions reduced, with CS edible coating solution reducing from 0.38 Pa s to 0.11 Pa s; CS-TP-VC from 0.35 Pa s to 0.063 Pa s; CS-Nisin-ε-PLH from 0.29 Pa s to 0.066 Pa s; CS-NH-TC from 0.16 Pa s to 0.050 Pa s. In addition, a significant decrease of viscosity in CS edible coating solution was accompanied by the addition of TP, VC,
Nisin, and ε-PLH. This phenomenon may be because that the addition of equally charged VC and ε-PLH in edible coating solution improved the repulsive forces existed between molecules, thus resulting in low viscosity (Huang et al. 2018). However, no significant differences were observed in the viscosity of all edible coating solutions as the shear rate continuously increased. One possible reason to account for this was that the formation rate and collapse rate of the edible coating solution remain in equilibrium (Sun et al. 2021). Finally, as reported by Du et al. (2016), high viscosity of the coating will lead to uneven dispersion of the solution, thereby affecting its diffusion ability. Hence, it is suggested that the apparent viscosity should be less than 0.7 Pa s to achieve a uniform coating. In our study, the viscosity of all the edible coating solutions was lower than 0.7 Pa s.

### 3.1.3 FT-IR characterization

Fourier-transform infrared could provide researchers with useful data regarding different functional groups that are involved in the edible coating solutions by measuring the vibrational frequencies of the chemical bonds (Mudunkotuwa et al. 2014). To explore the interaction between CS, TP, VC, Nisin, and ε-PLH in the edible coating solution, FT-IR measurement was performed, and the results were shown in Fig. 3. Broad-band between 3360 and 3406 cm\(^{-1}\) was observed in the FT-IR spectra of all the CS edible coating solutions, which may be due to the stretching of -OH and -NH, and the flexural vibration absorption peaks at 1420 cm\(^{-1}\) belongs to -CH\(_3\) and the bending-vibration absorption peak of -CH, respectively (Homez-Jara et al. 2018). In the IR spectrum of ε-PLH, the peaks observed between 3437-3261 cm\(^{-1}\) were attributed to characteristic peaks of the N-H stretching vibration and the absorption peak at 1676 cm\(^{-1}\) corresponded to the C = O group (Liu et al. 2018). In terms of VC, the absorption peak located at 1675 cm\(^{-1}\) was the C = C stretching vibration mode. While the absorption peak observed at 1658 cm\(^{-1}\) was attributed to the C = C group in the infrared spectrogram of Nisin.

The some characteristic spectra of TP were also observed in the IR spectra of the CS-TP-VC edible coating solution, suggesting that the TP molecule was successfully encapsulated in the CS network (Biao et al. 2019). The addition of Nisin and ε-PLH to the CS edible coating solution altered the amide I peak, speculating that these chemical groups were intensely involved in the polypeptide-biopolymer interactions (Yu et al. 2019). By contrasting the infrared spectrogram of CS, TP, VC, Nisin, and ε-PLH, the IR spectra of composite CS-NH-TC edible coating solution presented that the absorption peak of 1608 cm\(^{-1}\) was enhanced. A new characteristic absorption peak emerged at 1722 cm\(^{-1}\), which suggested that CS, TP, VC, Nisin and ε-PLH were successfully loaded into the CS edible coating solution (Sun et al. 2021).

### 3.2 Effects of edible coating solutions on the quality of marinated eggs during storage
3.2.1 Moisture content and WHC

The variation of moisture content in marinated egg samples during 4 °C storage is shown in Fig. 4a. No significant differences in moisture content were observed among these marinated egg white gels at day 0. As storage time increased, the moisture content of all the marinated egg white gels remained steady, except for the control. This phenomenon was possibly due to the designed edible coating solutions posed a physical barrier that could prevent weight loss and extend the shelf life of the foods (Lin et al. 2020).

WHC could be defined as the capacity of the food samples to hold water. Variations in WHC may result in changes in the structure and aggregation of the marinated egg white gel, which ultimately affects the palatability of marinated eggs (Nicolai et al. 2011). The WHC of all coated and uncoated marinated eggs decreased significantly with increased storage time (Fig. 4b). It was evident that the coated marinated eggs, particularly the CS-NH-TC group, showed higher WHC than the CK group. In detail, the marinated eggs coated with the CS-NH-TC edible coating solution underwent a water loss of 4.03 % during 24 days of storage, while this value was 25.61 % in the CK group, which indicated that the CS-NH-TC edible coating solution efficiently prevented the weight loss and extended the shelf life of the marinated eggs. Moreover, the protective effect of CS-NH-TC edible coating solution may also play an essential role in inhibiting the growth of harmful microorganisms and the decomposition of the protein matrix, thus hindering the downward trend of the water holding capacity (Xue et al. 2021).

3.2.2 pH

Fig. 5 shows the pH changes of the marinated eggs during 24 days of storage at 4 °C. The pH of marinated eggs without coating was slightly higher compared to that of the marinated eggs coated with various edible coating solutions at day 0. This can be concluded from the fact that the pH value of fresh marinated eggs was approximately 8.07, while the application of edible coating solutions reduced the pH of marinated eggs to 7.31 - 7.63 (p < 0.05). The reduction in pH was probably due to the acidic coating solutions (pH 3.85 - 4.39) attached to the surface of eggs. Besides, during 24 days of cold storage, the pH of the uncoated marinated egg samples significantly decreased from 8.07 to 7.12 (p < 0.05). A significant decrease in pH values was also observed in every coated marinated egg as the storage time increased, reaching a value of 7.14 - 7.39 at the end of storage. Furthermore, the pH value of marinated eggs coated with CS, CS-TP-VC, CS-Nisin-ε-PLH, and CS-NH-TC edible coating solutions also differs at day 24 (p < 0.05). The reduced pH was attributed to the decomposition of proteins and the production of lactic acid caused by spoilage bacteria (Dong et al. 2020). In summary, our edible coating solutions were demonstrated to slow down the spoilage rate and extend the shelf life of the foods, this finding was confirmed by Zhang et al. (2018) who also found that 2% CTS could inhibit pH changes in ready cook pork chops, suggesting CTS can suppress food spoilage caused by harmful microorganisms.

3.2.3 Microbiology
Total viable count (TVC) measurements were performed to investigate the inhibitory effect of different edible coating solutions on the growth of microorganisms in marinated eggs. **Fig. 6** shows the TVC results of the marinated egg coated with edible coating solutions. The viable bacteria increased gradually during cold storage for all the marinated eggs and was particularly pronounced from day 20 to day 24. On day 0, the microorganism counts of these marinated eggs were 0 log CFU/g because of the high-temperature sterilization processes. Afterward, the TVC value of samples coated with CK dramatically increased over time, exceeding the threshold on 16th day and reaching 8.30 log CFU/g on 20th day. However, the rapid growth trend of microorganism counts was suppressed when marinated eggs were coated with CS, CS-TP-VC or CS-Nisin-ε-PLH, as reflected by the microorganism counts of CS, CS-TP-VC and CS-Nisin-ε-PLH groups exceeded the threshold on day 16, 16 and 20, respectively. Notably, the TVC values of the CS-NH-TC group showed the slowest growth rate among all the marinated eggs, which can be concluded from the fact that the microorganism counts of marinated eggs coated with CS-NH-TC were always lower than the threshold. These results suggested that incorporating TP, VC, Nisin, and ε-PLH in the edible coating solution significantly suppressed the microbial growth in marinated eggs, thereby prolonging their shelf life. Similar results were reported by Xiong et al. (2020), who found that the chitosan-gelatin, nisin and grape seed extract coating could protect the pork against harmful microorganisms.

### 3.2.4 Color

The surface color of marinated eggs plays a vital role in consumers’ acceptance of the products (Pathare et al. 2013). During 24 days of storage, the influence of various edible coating solutions on lightness (L*), redness (a*), and yellowness (b*) values of marinated eggs is shown in **Fig. 7**. The color values of marinated eggs varied from 65.23 to 76.06, with marinated eggs coated with CS-Nisin-ε-PLH, CS-TP-VC, and CS-NH-TC typically having lower lightness than marinated eggs coated with CK and CS. Additionally, the L* values gradually increased in all samples along with storage time. Microbial propagation would lead to white spotted or mucus appearing on the surface of the marinated eggs, which further causes the degradation of the outer protein and the exposure of the inner protein, ultimately improving the lightness of marinated eggs. Our findings are consistent with those reported by Sezer et al. (2022).

The a* and b* value of all marinated eggs decreased significantly during storage (**Fig. 7b** and **Fig. 7c**). It was found that marinated eggs coated with CS-TP-VC and CS-NH-TC showed higher a* values, while the marinated eggs coated with CS-Nisin-ε-PLH and CS-NH-TC showed higher b* values than marinated eggs coated with CK. The improved redness and yellowness were because the tea polyphenols showed reddish-brown color and could improve the color a* and b* values of the marinated eggs.

### 3.2.5 TPA

Texture is recognized as one of the most crucial characteristics in determining the consumer acceptance of marinated eggs. Textural characteristics viz. hardness, springiness, cohesiveness, and chewiness of
the marinated eggs are given in Fig. 8. The hardness of marinated eggs increased at the early stage of storage, followed by a slight decrease along with the time extension. The increased hardness in the early stage was possibly due to the oxidation of proteins, while the decreased hardness may be attributed to microbial contamination and protein decomposition. Therefore, the fastest decline was observed in the hardness values of the CK group, followed by CS group, CS-TP-VC group, and CS-Nisin-ε-PLH group. Marinated eggs coated with CS-NH-TC exhibited the slowest declines due possibly to the CS-NH-TC coating could prevent the degradation of protein caused by microorganisms (Ghani et al. 2018).

Chewiness reflects the combined effects of hardness, springiness and cohesiveness of the test products and is defined as the required energy to chew the sample. Cohesiveness is defined as the deformation degree of the marinated eggs without breaking them in the mouth. The changing pattern of chewiness and cohesiveness was similar to that of the hardness, both exhibiting an upward trend first and then a downward trend. The breakdown of protein was positively related to the loose micro-structure of the egg network, resulting in a softer marinated egg with lower chewiness (Zhang et al. 2019).

There were no significant changes in the springiness of marinated eggs within the first 16 days. After that, the springiness of marinated eggs began to decline and the decline rate of springiness was in the order of CK > CS group > CS-TP-VC group > CS-Nisin-ε-PLH group > CS-NH-TC group. All the marinated eggs showed a similar cohesiveness changing pattern during the entire storage time. Our study demonstrated that the CS-NH-TC edible coating solution contributed to the improved texture properties of marinated eggs during cold storage.

3.2.6 Sensory evaluation

Sensory properties, especially color and odor are crucial indicators determining the conception of quality and consumer acceptance of a certain product (Jafarzadeh et al. 2021). The sensory scores of marinated eggs coated with various edible coating solutions were obtained with the assistance of assessors and were given in Fig. 9. It is obvious that the sensory scores (color, odor, and overall acceptability) of all marinated eggs decreased gradually during storage, especially the uncoated ones.

The senses of CK samples showed significant changes from the 12th day while not in other treatments. In terms of color, the CK, CS-TP-VC, and CS samples became unacceptable after 8 and 12 days of storage, respectively. In contrast, the marinated eggs coated with CS-Nisin-ε-PLH and CS-NH-TC were acceptable and showed great difference compared to the marinated eggs coated with CK, CS and CS-TP-VC between day 0 and day 16. Similar changing pattern was also observed in the odor scores of marinated eggs.

In terms of the overall acceptability, the shelf life of the CK, CS, and CS-TP-VC group was 8-12 days, while the marinated eggs coated with CS-Nisin-ε-PLH and CS-NH-TC were considered “acceptable for consumption” until day 20. Besides, differences were observed in the overall acceptability scores between
marinated eggs coated with various edible coating solutions and marinated eggs without coating after 8 days of storage. The suppression of microbial growth decreased rate of food spoilage, which contributed to better marinated egg quality. Similar results were reported by Basaglia et al. (2021) for processed pineapple coated with CS and cinnamon essential oil, Riaz et al. (2021) for strawberry coated with chitosan-based apple peel polyphenols, and Gedawatte et al. (2021) for beef coated with gelatin. Based on the assessment results, the edible coating solution containing TP, VC, Nisin, and ε-PLH had no unfavorable effects on the sensory properties of the marinated eggs.

3.2.7 Appearance

The appearance of coated marinated eggs during 24 days of storage is shown in Fig. 10. All the coatings were transparent and adhered well to the surface of marinated eggs. However, with the storage time increasing, the CK, CS, and CS-TP-VC coated samples became sticky and produced a rancid odor. Most of the samples were contaminated by microorganisms after 12 days of storage, while this shelf life was extended to 20 days, and marinated eggs maintained their good appearance and flavor after marinated eggs were coated by CS-Nisin-ε-PLH and CS-NH-TC edible coating solution. This experiment and the sensory evaluation experiment showed that CS-NH-TC edible coating solution was effective in inhibiting the growth and reproduction of microorganisms and maintaining the quality of marinated eggs.

4 Conclusion

The present study developed CS, CS-TP-VC, CS-Nisin-ε-PLH, and CS-NH-TC edible coating solutions for the preservation of marinated eggs. The results demonstrated that CS-NH-TC edible coating solution showed the best stability, highest zeta potential, and lowest particle size and PDI. The FT-IR spectra showed that TP, VC, Nisin, and ε-PLH were successfully encapsulated in the CS edible coating solution. Moreover, a more stable pH, color, texture, and appearance of marinated eggs were observed when coated with CS-NH-TC. The TVC values in the CS, CS-TP-VC, CS-Nisin-ε-PLH, and CS-NH-TC groups were suppressed and were 7.85, 8.01, 5.63 and 4.98 (log CFU/g), respectively. Finally, microbial analysis results indicated that CS-NH-TC edible coating extended the shelf life of marinated eggs from 12 to 20 days. This study confirmed that the CS-NH-TC edible coating solution is an ideal coating for marinated eggs to improve their quality and shelf life and provides a new strategy for developing an edible packaging with antibacterial activity.

Declarations

Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported on this paper.
Data availability statements

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

CRediT authorship contribution statement

**Jingbo Liu**: Conceptualization, Supervision and Writing - review & editing. **Dongkun Cheng**: Investigation, Methodology, Data curation and Writing - original draft. **Deju Zhang**: Writing - review & editing. **Lu Han**: Data curation, Formal analysis. **Yiming Gan**: Writing - review & editing. **Yiding Yu**: Methodology and Formal analysis. **Ting Zhang**: Conceptualization, Funding acquisition and Writing - review & editing.

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Figures

Figure 1

Zeta size (a), Zeta potential (b), PDI (c), and pH (d) of edible coating solutions incorporated TP, VC, Nisin, and ε-PLH. Bars with the different letters are significantly different (p < 0.05)

Figure 2

Rheological properties of CS, CS-TP-VC, CS-Nisin-ε-PLH, CS-NH-TC edible coating solutions

Figure 3

The FT-IR analysis of VC, TP, Nisin, ε-PLH and CS, CS-TP-VC, CS-Nisin-ε-PLH, CS-NH-TC edible coating solutions

Fig. 4

The Moisture contents (a) and WHC (b) analysis of the marinated eggs were treated with different edible coating solutions during 24 days of storage at 4 °C. Bars with different letters on the same day are significantly different (p < 0.05)
Figure 5

The pH variation of marinated eggs during 24 days of storage at 4 °C. Bars with different letters within each group are significantly different (p < 0.05)

Figure 6

The microbial analysis of the marinated eggs treated with during storage different edible coating solutions during 24 days of storage at 4 °C

Figure 7

The color L* value (a), a* value (b), and b* (c) of marinated eggs coated with different edible coating solutions during 24 days of storage at 4 °C. Bars with different letters within each group are significantly different (p < 0.05)

Figure 8

Effect of different edible coating solutions on texture properties of marinated eggs during 24 days of storage at 4 °C. Bars with different letters on the same day are significantly different (p < 0.05)

Figure 9

Sensory attributes of marinated eggs coated with different edible coating solutions on the 0th, 8th, 12th, 16th, 20th, and 24th day of storage at 4 °C

Figure 10

Appearance changes of uncoated (CK) and coated marinated eggs with different edible coating solutions during 24 days of storage at 4 °C