Protective effect of based on chitosan coating with grape peel extract on cattle rumen induced by freeze-thaw cycles

Yinjuan Cao
Gansu Agricultural University
Zhaoyang Song
Gansu Agricultural University
Ling Han
Gansu Agricultural University
Qunli Yu (yuqunli9394@163.com)
Gansu Agricultural University

Research Article

Keywords: Cattle rumen, freeze-thaw, chitosan, grape peel extract, quality

Posted Date: April 22nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1573655/v1

Abstract

This study aimed to investigate the protective effect of chitosan (CH) containing grape peel extract (GPE) coating on cattle rumen induced by freeze-thaw cycles. It can be concluded that pH, water holding capacity, *L**, *a**, and cutting force of the sample without CH were significantly decreased, Total aerobic count (TCA), total volatile base nitrogen (TVB-N), *b** and oxidation reactions were increased with the increasing in F-T cycles ($p < 0.05$). The pH, thawing loss, pressing loss, cooking loss, *b**, cutting force, TCA and TVB-N of CH-GPE coating samples were decreased by 5.2%, 10.9%, 13.0%, 11.4%, 8.2%, and 18.9%, and *L**, *a** and sensory evaluation were increased by 151.2%, 3.9% and 17.0% than the sample without CH coating after five F-T cycles. In addition, CH coating could effectively restrain the destruction of the microstructure of cattle rumen during F-T cycles. More importantly, the inhibitory effect of CH-GPE coating on oxidation was verified from the decrease in carbonyl contents (16.5%), sulfhydryl contents (8.7%), TBARS (17.5%) and POV value (27.8%) of cattle rumen compared with CH coating after five F-T cycles. Therefore, these results illustrated the potential benefits of CH-GPE coating in meat products.

1. Introduction

With the development of China's livestock industry, the production of cattle by-products has been increasing. The rumen, the main by-product of cattle, is the largest gastric chamber in the intestine, accounting for 80% of the entire stomach (Li et al., 2019). At present, most of the cattle by-products are discarded, causing environmental pollution and economic losses. If the meat value of the smooth muscle can be exploited, this will increase the output value of the beef cattle industry and promote the healthy development of the beef cattle and food industries.

After the cattle are slaughtered, the rumen cannot directly meet the needs of consumers and needs to be frozen for storage, transport and retail before it can be consumed by the consumer (Wang, Luo, Shi, & Shen, 2015). However, Repeated F-T cycles often occur due to imperfections in cold chain technology and temperature fluctuations, which has certain effects on meat quality, such as discoloration, mechanical damage to tissues, protein denaturation, increased water loss after thawing and lipid oxidation (Kim et al., 2013; Pan, Dong, Du, Kong, & Xia, 2020). This reduced the acceptability to the consumer. Thus, developing a cost-effective green solution to extend the shelf life of products during the freeze-thaw process would be valuable in meeting time-bound challenges and reducing waste.

Currently, edible coatings are considered to be a promising and eco-friendly means of food preservation and commonly used coating substrates include mainly polysaccharides, proteins and lipids (Ju et al., 2018). Among these, Chitosan is a non-toxic, non-polluting material with good barrier properties and a wide range of antibacterial and antioxidant activities for use as a food additive in meat products (Yuan, Chen, & Li, 2016). Chitosan has become an important coating matrix due to its superior properties, and there are also many successful cases of meat preservation (Mehdizadeh, & Langroodi, 2019; Langroodi et al., 2018). However, the main issue limiting the application of single matrix coatings of chitosan is the relatively limited bioactivity when faced with food systems. In other words, the application performance of chitosan coatings alone is not sufficient to achieve acceptable targets, and combination with exogenous preservatives is often considered a promising strategy to overcome coating preservation limitations (Yu et al., 2021). The processing of grapes produces by-products such as peel, which are discarded as waste, which to some extent affects the burden on the environment and economic development. However, studies have shown that grape peels extract (GPE) is rich in polyphenols such as anthocyanins and flavonoids (Baca-Bocanegra, Hernández-Hierro, Nogales-Bueno, & Heredia, 2019). These polyphenols not only have a strong antioxidant capacity, but also have an antibacterial effect on foodborne spoilage bacteria (Kim et al., 2019). In addition, it has a variety of beneficial health effects such as cardioprotective, anti-cancer, anti-aging and immune-boosting effects, and can effectively prevent and treat diseases associated with free radicals (Rodrigo, Miranda, & Vergara, 2011). So it has certain nutritional and pharmacological effects and is safe to use. Therefore, the addition of PPE to chitosan solutions can bring out the multifunctional properties of the coating and improve meat quality. However, the improvement of rumen quality in cattle with the combination of chitosan and PPE is not known.

The objective of our study was to investigate the inhibitory effect of chitosan coating combined with grape peel extract (CH + GPE-coated solution, CH-coated solution and deionized water)on the deterioration of rumen quality (WHC, cutting force, TCA, TVB-N, color and texture properties) and oxidative reactions (protein and lipid oxidation) in the bovine rumen in multiple F-T cycles. Changes in microstructure were observed using scanning electron microscopy (SEM).

2. Material And Methods

2.1. Materials

Chitosan (CH) powders were purchased from Fuyang Biological Technology Co., Ltd. (Jinan, Shandong, China), with molecular weight and degree of deacetylation close to 200 kDa and 85% respectively. Grape peel extract (food grade, GPE) was purchased from Xi'an Huilin Biotechnology Co., Ltd. (Xian, Shanxi, China). Other analytical grade chemical reagents were purchased from Sinopharm Group Chemical Reagent Co., Ltd (Lanzhou, Gansu, China).

2.2. Coating solution preparation and sample treatment

The coating solution was prepared by mixing CH and GPE powders and dissolving them in deionized water using a magnetic stirrer, then mixing glycerol as a plasticizer (25% CMCS, m/m) and centrifuging (4°C, 3000 g, 15 min) using a Sigma H1850 centrifuge (Yancheng Kite Experimental Instruments, Nanjing, China) to remove insoluble impurities, resulting in CH and GPE concentrations of 2% and 0.4% (m/v), respectively. The selection of PPE concentrations was based on preliminary trials in which the organoleptic quality of the fillets, including colour, odour and texture, was compared after coating with a range of PPE concentrations (0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5% and 0.6% (m/v)). When the PPE concentration was ≥ 0.5%, the rumen samples were significantly darker and less acceptable to the senses after coating, so the maximum working concentration was determined to be 0.4%. The healthy local yellow cattle (average body weight: 450 kg; average age: 3 year) from Kang Mei Meat Co. Ltd. (Kangle, China) were slaughtered according to the standard procedure (GB/T 19477 – 2018). The bovine rumen was taken immediately after slaughter and washed with distilled water to remove internal dirt, and the rumen was immediately transferred to the laboratory in an icebox. Next, removing the villi and mucous layer on the surface and removing the visible fat, and then the muscles were cut into 10 ×

10 cm pieces for following coating treatment. All samples (in total 48 samples)were randomly divided into three groups of groups and immersed in CH-coated solution, CH + GPE-coated solution and deionized water (labelled as CK) under the same conditions, with a rumen slice/solution ratio ~ 1:3 (m/v). Repeat 3 times for each sample group. For each coated sample group, the pieces were dipped in 500 mL of coating solution for 30 s, removed and left to dry for 2 min, then repeated 1 more time. After 30 s of impregnation in the coating solution, the fillets were then removed and placed in a 4–8◦ C incubator with airflow for 30 min (LRH-250, Shanghai Yiheng Instruments Co., Ltd., Shanghai, China) to form an edible coating. After that, the drained rumen pieces are packed individually in sterile bags. All rumen pieces were stored at -30°C until the core temperature of the rumen piece fell below − 18°C. Pre-frozen cattle rumen pieces were stored at -18°C for 7 days and then thawed at 4°C. Randomly selected samples (4 of 16) from each group were used for measurements and the first F-T cycle was completed. The remaining rumen slices were again frozen separately and stored as described above until the third and fifth consecutive F-T cycles were completed.

2.2 Cutting force

The cutting force of the cattle rumen was measured using a TA. XT Plus texture analyzer (Isenso, USA). The measurement conditions were: a probe of P/50, a probe downward speed of 2 mm/s, a test speed of 1 mm/s, a return speed of 1 mm/s, a compression ratio of 60% of the sample thickness, a height calibration of 40 mm, a time interval between two compressions of 6 s, a trigger force of 5 g, and with samples with an area of 1 cm^2.

2.3 pH and colour

The pH of cattle rumen was measured by using the method of Xu et al. (2020). 3 g of sample were homogenized with 30 mL of KCl (0.1 M pH 7.0) for 60 s and then the pH was measured with a PB-10 pH meter (PHS-3+, Century Fangzhou Science & Technology Co., Ltd., Chengdu, China).

The colour was assessed using the procedure described by Aminzare et al. (2018). A colourimeter (SP60; X-Rite, Shanghai, China) was used to determine lightness (L^*), redness (a^*) and yellowness (b^*) values. The colourimeter was calibrated using a white standard plate prior to measurement. Each sample was analyzed at three different locations and at least three samples were measured to obtain an average value.

2.4 Water holding capacity

2.4.1 Thawing loss

The thawing loss of cattle rumen was measured by using the method of Xu *et al.* (2021), and expressed as:

$$\text{Thawing loss}(\%)=\frac{m_1 - m_0}{m_1}\times 100\%$$

where m_1 is the sample weight before thawing and m_0 is the sample weight after thawing.

2.4.2 Cooking loss

The cattle rumen is placed in a plastic cooking bag and cooked in a water bath at 80°C until the internal temperature reaches 75°C for 20 min. A thermometer probe was inserted into the geometric center of the cattle rumen to measure its temperature of the cattle rumen. Cooking loss is measured by the rate of weight loss before and after cattle rumen cooking, and is expressed as:

$$\text{Cooking loss}(\%)=\frac{m_3 - m_2}{m_3}\times 100\%$$

where m_3 is the weight of uncooked cattle rumen and m_2 is the weight of cooked cattle rumen.

2.4.3 pressing loss

Pressing loss was measured by the method of Li *et al.* (2012). Briefly, a 2 g sample was placed between 18 layers of filter paper and pressed with a pressure loss rate meter (YYW-2, Shanghai Soil Instrument, Shanghai, China) at a pressure of 343 N for 5 min. Pressing loss is calculated based on the percentage of weight loss before and after compression of the sample, and is expressed as:

$$\text{Pressing loss }(\%)=\frac{m_5 - m_4}{m_5}\times 100\%$$

where m_5 is the sample weight before pressing and m_4 is the sample weight after pressing.

2.6 Texture properties

Texture properties of cattle rumen samples, i.e. elasticity, chewiness and hardness, were run in TPA mode using the TA-XT plus Texture Analyzer (Yingsheng Hengtai Technology Co., Ltd., Beijing, China): 50 mm diameter aluminium cylindrical probe, test speed 1 mm/s, pre/post-test speed 2 mm/s, trigger force 5 g, compression ratio 50%. Sample measurements are the average of five separate measurements.

2.7. Total volatile basic nitrogen content

Total volatile basic nitrogen (TVB-N) content was determined according to the method of Cao et al. (2022). Briefly, 5 g of sample was dispersed in 50 mL of distilled water and stirred for 25 min, and then the mixture was filtered. The TVB-N content is based on the consumption of hydrochloric acid and is calculated as

$$\text{TVB-N (mg/100 g)} = C \times 2800 \times (Y_1\text{-}Y_2),$$

where Y_1 and Y_2 are the titration volumes of hydrochloric acid for the sample and blank, respectively, and C is the concentration of hydrochloric acid (= 0.01 M).

2.8. Total aerobic count

Total aerobic count (TAC) was performed as described by Fan et al. (2019). Briefly, approximately 5 g of cattle rumen sample was homogenized in 90 mL of sterile saline (0.85% NaCl) for 2 min. The homogenate was serially diluted 10 times by adding 1 mL of homogenate to 9 mL of 0.85% sterile saline. The colonies were counted after incubation at 37 ± 1°C for 48 h. Results are expressed as the logarithm of colony forming units (CFU) per gram of cattle rumen (log CFU/g).

2.9. Sensory assessment

The sensory evaluation was carried out by eight postgraduate students from our team in a well-lit, well-ventilated room; they assessed three samples in a single session at each freeze-thaw cycle point and each sample was tested a total of seven times. All team members were trained in sensory assessment prior to being included in this study. For this sensory test, samples were cut into 1 cm thick slices and heated in a water bath at a controlled temperature until the core temperature of the meat reached 75°C. After this, the cooked meat was offered to the panelists for sensory evaluation on a white plate. Briefly, samples were presented to each panelist in random order and water was provided between samples for gargling. Panelists simultaneously assessed the colour, odour and tissue status of the samples on a hedonic scale of 10 - from 1 (did not like it at all) to 10 (liked it a lot) (Cao et al., 2022).

2.10 Protein oxidation

2.10 .1 Carbonyl content

The carbonyl content was detected spectrophotometrically after the samples were mixed with 2,4- dinitrophenylhydrazine (DNPH) to form protein hydrazine according to the method of Huang, Liu, Xia, Kong, and Xiong (2015). The absorbance values of the DNPH-treated samples were measured at 365 nm using hydrochloric acid as a control. The total carbonyl content was expressed as nanomoles per milligram of protein with an absorption coefficient of 22,000 $M^{-1}cm^{-1}$ for hydrazine.

2.10 .2 Sulfhydryl content

The sulfhydryl content was assessed using the method of Ali et al. (2015). Briefly, 1 g of sample was homogenized in 20 mL of 50 mM phosphate buffer (pH 7.2) at 6000 rpm for 15 seconds. 1 mL of homogenate was then mixed with 9 mL of 50 mM phosphate buffer (pH 7.2) containing 0.6 M NaCl, 6 mM EDTA and 8 M urea. After centrifugation at 5000 rpm and 4°C for 20 min, 2 mL of supernatant is mixed with 30 µL of 50 mM sodium acetate containing 10 mM 5,5'-dinitrobi(2-nitrobenzoic acid) and incubated at 45°C for 10 min. The absorbance of the resulting mixture was measured at 412 nm. An absorption coefficient of 13 600 M^{-1} cm^{-1} was used to estimate the total mercaptan content.

2.11 Lipid oxidation

2.11 .1 TBARS

Thiobarbituric acid reactive substances (TBARS) were measured according to the method of Xin et al. (2022). A 1 g sample of rumen was homogenized in 10 mL of 0.15 M potassium chloride. Then 2 mL of thiobarbituric acid (TBA)/trichloroacetic acid (TCA) stock solution [0.375% (w/v) TBA and 15% (w/v) TCA dissolved in 0.25 M HCl] and 3 mL of 2% (w/v) butylated hydroxytoluene (BHT) in ethanol were added to the 1 mL homogenate and immediately mixed with a vortex mixer. The mixture was then incubated in a boiling water bath for 20 min to develop colour and then cooled to 25°C. The absorbance of the solution was measured at 532 nm using a spectrophotometer (Model 721; Beijing Jolyhang Technology Co., Ltd., Tongzhou, China). TBARS levels were expressed as nanomoles per gram of meat with a molar extinction coefficient of 1.56 × 105 $M^{-1}cm^{-1}$.

2.11 .2 POV

Peroxide value (POV) was assessed using the method of Wang, He, Gan and Li (2018), briefly, 5 g of minced meat samples were homogenized in 20 mL of a 1:1 cold chloroform-methanol mixture for 2 min. After the addition of 6 mL of 0.5% NaCl solution, the resulting solution was mixed on a vortex mixer (MIX1000; Hangzhou Ruicheng Instruments Co., Ltd., Zhejiang, China) for 30 s and then centrifuged at 3000 g for 5 min at 4°C. Next, 5 mL of the bottom chloroform layer was extracted using a glass syringe and transferred to a glass tube. 3 mL of a 1:1 chloroform-methanol mixture, 100 µL of 4 M ammonium thiocyanate and 100 µL of 20 mM $FeCl_2$ were added in turn and vortexed for 10 s. The resulting solution was incubated at 25°C for 15 min and the absorbance was measured at 500 nm using a UV-5500PC spectrophotometer (Model 721; Beijing Jolyhang Technology Co., Ltd., Tongzhou, China).

2.12 Microstructure analysis

The samples (2 mm × 2 mm × 5 mm) were fixed in 2.5% glutaraldehyde for at least 3 d at 4°C. All samples were washed three times with 0.1 M phosphate buffer. The specimens were then post-fixed with 1% osmic acid solution for 2 h at 4°C. After dehydration in graded ethanol (from 30%, 50%, 70%, 90–100%, v/v), the samples were plated with gold and observed by scanning electron microscopy (SU2080, Hitachi, Japan) with an accelerating voltage of 20 kV at a magnification of × 300.

2.13 Statistical analysis

Three batches (replicates) of cattle rumen were produced independently to study the effects of CH + GPE on the quality of quick-frozen cattle rumen subjected to F-T cycles. For each batch, the indices of samples were measured three times as technical repetition. A completely randomized design included treatment groups (CK, CH, CH + GPE) and F-T cycles (0, 1, 3, 5 F-T cycles) as fixed effects and three replications as random effects. Results were expressed as the mean

values ± standard error. Statistical analysis was obtained using the IBM SPSS version 19.0 Software (SPSS, Inc., Chicago, IL, USA). Origin 9.0 Software was utilized to analyze the dynamics and plot the graphs. The significance of the main effects was determined by Analysis of variance (ANOVA). Significant differences ($p < 0.05$) among means were determined using the Tukey procedure.

3. Results And Discussion

3.1 Effects of CH + GPE on the pH, WHC, color, and cutting force of cattle rumen

Meat qualities are the main indicators that affect meat processing and consumption, including indicators such as pH, WHC, color and cutting force (Lee, Owens, & Meullenet, 2009). The pH can influence the color, cooking loss and the cutting force of cattle rumen (Guo et al., 2021). As shown in Table 1, the pH of the three treatments all increased and then significantly decreased ($p < 0.05$) during cattle rumen F-T cycles. This is primarily attributed to the decomposition of proteins will form ammonia and amines during the meat storage process, resulting in a pH increase (Guo, Han, Yu, & Lin, 2020). With the F-T cycles increased, the inorganic phosphate from ATP degradation and generation of lactic acid from anaerobic glycolysis dropped the pH (Bahmani et al., 2011). The pH of CH + GPE treatment was significantly lower than that of CK and CH treatments during F-T cycle ($P < 0.05$). This is due to the antioxidant activity of chitosan being enhanced by the phenolic substances in grape peel extract. These results are in agreement with those found by Lorenzo et al. (2014) and Garrido et al. (2011), who reported that the incorporation of antioxidants from grape pomace extract reduced the pH values of pork patties in refrigerated storage throughout storage time.

Thawing loss, pressing loss and cooking loss of meat is essential indicators of WHC, which is defined as the ability to retain water in samples (Xu et al., 2020). The changes in WHC of cattle rumen with different treatments during F-T cycles are shown in Table 1. The thawing loss, pressing loss and cooking loss of cattle rumen all significantly increased with the increase in the number of F-T cycles ($p < 0.05$). This result indicated that the increase in water loss due to F-T cycles lead to a reduction in the WHC of the cattle rumen. Similar results are obtained by Jiang et al. (2019), who report that the regeneration and melting of ice crystals during F-T cycles cause mechanical damage to muscle tissue, resulting in moisture migrating to the outside of the cell. In addition, the WHC of CH + GPE treatment is significantly higher than CH and CK treatments ($p < 0.05$). This is due to the polyphenols in the GPE that increase the antioxidant effect of CH and slow down the water loss of cattle rumen during F-T cycles. Kong et al. (2017) showed that plant extracts treatment decreased the WHC of cherries and had potential for frozen food preservation, this result is consistent with ours. Therefore, CH + GPE treatment could effectively reduce the loss of moisture content.

Color stability is an important component of meat quality that also can improve overall consumer's acceptability (Pateiro et al., 2018). The changes in the color of cattle rumen induced by F-T cycles are illustrated in Table 1. All treatments presented a significantly decreasing trend for a^* values of cattle rumen, and redness of CH + GPE was significantly higher than CK and CH groups ($p < 0.05$). CH + GPE treatment could prevent the growth of ice crystals in F-T cycles to maintain cellular integrity. Therefore, the degree of myoglobin denaturation is weakened, resulting in the formation of metmyoglobin being decreased (Medić et al., 2018). These results indicated that CH + GPE inhibited the decrease in a^* values and improved the color stability of cattle rumen during the F-T cycles. However, L^* values of three groups cattle rumen all significantly decreased, the lightness of CH + GPE treatment is significantly higher than CK and CH treatments ($p < 0.05$). This is because the water loss declines, which leads to less moisture accumulation on the surface of the cattle rumen and reduces the lightness of cattle rumen (Wang et al., 2021). In addition, b^* values increased for three treatments studied as the F-T cycles (Table 1), and the yellowness of CH + GPE treatment is significantly lower than CK and CH treatments ($p < 0.05$). Alirezalu et al. (2019) observed ε-PL, chitosan and natural antioxidants on color of frankfurter sausages, reporting that treated samples had lower b^* values than control, these are in agreement with our result.

Cutting force is an importantly used index to evaluate meat tenderness (Guo et al., 2021). As shown in Table 1, the cattle rumen cutting force of three treatments decreased when the number of F-T cycles increased, the cutting force of CH + GPE and CH treatments are significantly lower than CK ($p < 0.05$). This is due to F-T cycles might change muscle structure and reduce the cutting force (Zhang et al., 2017). The muscle tissue damage due to the increase in ice crystal formation during freezing is the major reason for the decrease in cattle rumen cutting force during the F-T cycles. In addition, phenolic components in GPE are known as functional ingredients with antimicrobial and antioxidant properties. Rusak, Komes, Liki´c, Hor˘zi´c, & Kova˘c (2008) found that ε-PL coated samples with the highest displayed significantly ($p < 0.05$) highest amount of Cutting force, which could be related to the high phenolic compound in plant extracts. These results are consistent with we obtained.

3.2 Changes in TAC of cattle rumen during F-T cycles

TAC changes in cattle rumen during the F-T cycles are listed in Fig. 1. TAC of three treatments of cattle rumen increased with the increase in the number of F-T ($p < 0.05$). Coating with GPE and CH had a significant effect on microbial counts during the F-T cycles of cattle rumen, and the TAC of CH + GPE treatment is significantly lower than CH and CK treatments ($p < 0.05$). These results indicated that CH and GPE treatments accelerated microbial growth in cattle rumen during F-T cycles. This may be due to the increased temperature during the thawing of cattle rumen, which favors microbial growth. Jia et al. (2019) found an increase in the fats and water-soluble proteins of nutrients, resulting in the increased TAC. However, the results of this study showed that CH + GPE treatment showed the highest inhibitory effect on TAC of cattle rumen during F-T cycles. Alirezalu et al. (2019) found that the combination of mixed plant extract with chitosan displayed effective inhibitory effects against microbial growth, these results are in agreement with our study. The initial TAC of three treatments for cattle rumen during F-T cycles was no different ($p > 0.05$), which after 5 times of F-T cycles reached 6.77, 6.30 and 5.54 log CFU/g, respectively. Fernández et al. (2009) reported that a microbial threshold close to 6 log CFU/g is acceptable. Therefore, CH + GPE treatment could increase the F-T times of cattle rumen.

3.2 Changes in TVB-N of cattle rumen during F-T cycles

The TVB-N is one of the most fundamental indicators for the evaluation of meat freshness and stability (Alirezalu et al., 2021). The cattle rumen TVB-N of the different treatments during F-T cycles is shown in Table 1. During F-T cycles the TVB-N values of cattle rumen increased significantly, being this increase lower

in samples coated with CH and CH + GPE compared to the CK group ($p < 0.05$) (Fig. 2). The TVB-N mainly is the protein decomposition and production of alkaline nitrogen-containing compounds, which are from microbial growth and enzymatic degradation (Li, Tang, Shen, & Dong, 2019). The initial values of TVB-N in the three treatments were no different ($p > 0.05$), and after 5 times F-T cycles TVB-N was increased to 11.87, 10.67 and 9.54 mg/100 g, respectively. This could be due to the antioxidant and antimicrobial properties of CH and GPE reduce the increase of cattle rumen TVB-N during F-T cycles. Alirezalu et al. (2019) showed that the combined use of ε-PL and chitosan with natural plants extracts in frankfurter sausages could significantly delay the increase of TVB-N, the result are in agreement with our study.

3.3 Effect of CH + GPE on the texture of cattle rumen during F-T cycles

The effect of CH + GPE on the hardness, springiness, cohesiveness, chewiness and resilience parameters of the cattle rumen during F-T cycles are demonstrated in Table 2. The hardness of three treatments all significantly increased during the F-T cycles ($p < 0.05$), and the hardness of CH + GPE was significantly higher than CK and CH ($p < 0.05$). With increasing F-T times, the springiness, cohesiveness, chewiness and resilience of cattle rumen were all significantly decreased during the F-T cycles ($p < 0.05$). This is due to the growth of ice crystals breaking the myofibrils apart and leading to water migration outside the cell (Sun et al., 2019), which causes muscle tissue and structure damage. Furthermore, the protein denaturation that is induced by F-T cycles alters the tight structure of cattle rumen, which decreases the hardness of the meat. This phenomenon agrees with the results of Huang et al. (2015). However, CH + GPE and CH had a positive effect on inhibiting the degradation of cattle rumen quality. CH + GPE treatment restricts the growth of ice crystals by binding to the cattle rumen surface during F-T cycles, this is consistent with Regand & Goff (2006). Therefore, CH + GPE can modify the normal ice crystal growth patterns and inhibit ice recrystallization, which helps to preserve cattle rumen texture.

3.4 Protein oxidation of cattle rumen during F-T cycles

Carbonyl content and sulfhydryl content may determine the protein oxidation of cattle rumen during F-T cycles, and they are the most important signs of protein oxidation (Jin et al., 2020; Xia et al., 2009). Figure 3A expresses the changes in carbonyl contents of cattle rumen from all treatments subjected to different F-T times. There was a significant increase in the carbonyl content throughout the F-T cycles of cattle rumen for three treatments, but sulfhydryl contents decreased ($p < 0.05$). The formation of large ice crystals during F-T cycles might induce protein denaturation by the moisture migration (Leygonie et al., 2012), this was consistent with our results. The carbonyl content and sulfhydryl content of the CH + GPE group are significantly lower than CK and CH groups ($p < 0.05$). These results illustrated that GPEs bind to the surface of ice crystals and inhibit the growth of ice crystals, which effectively inhibited the protein oxidation of cattle rumen (Hassas-Roudsari & Goff, 2012). Therefore, the CH + GPE treatment can inhibit protein oxidation by suppressing the growth of ice crystals.

3.5 Lipid oxidation of cattle rumen during F-T cycles

TBARS and POV are the secondary products of lipid oxidation (Song et al., 2022). The evolution of lipid oxidation of cattle rumen is shown in Fig. 4. TBARS and POV values significantly increased over the F-T time for three treatments, and the degree of lipid oxidation in group CH + GPE was significantly lower than that in group CH and CK ($p < 0.05$). This result can be explained by the active compounds present in the GPE such as polyphenols. A previous study demonstrated that natural extracts are able to more effectively delay oxidative reactions in lipids (Fernandes et al., 2017). With increasing numbers of F-T cycles, muscle cell integrity of cattle rumen is disrupted to accelerate the formation of malondialdehyde (Leygonie et al., 2012). As seen in Fig. 4A, the TBARS of three treatments are increased to 0.57, 0.54 and 0.47 mg MDA/kg after five F-T cycles, respectively. Lan et al. (2016) observed that the threshold levels of TBARS for rancidity that can be detected by trained sensory panels are 0.50 mg MDA/kg. Therefore, CH + GPE treatment delayed the quality deterioration of cattle rumen during freeze-thaw cycles.

3.5 Sensory evaluation of cattle rumen during F-T cycles

Sensory evaluation is one of the most traditional food detection methods, and directly affects the purchase orientation of consumers (Cao et al., 2022). The influence of the CH + GPE treatment on sensory attributes of cattle rumen is shown in Fig. 6. Significant differences ($p < 0.05$) were for color, odor and tissue state among the different treatments of cattle rumen during F-T cycles. The sensory scores for all three treatments decreased with F-T cycles, with the CH + GPE treatment showing a significantly lower rate of decrease than did the other two treatments ($p < 0.05$). This is because the grape peel extracts contained polyphenolic substances, which exhibit strong antioxidant activity and antibacterial properties. These results are consistent with those obtained by Liu et al. (2019) who evaluated that *P. oleracea* extract has high antioxidant activity on fresh-cut potato slices by significantly inhibiting lipid oxidation and thereby restraining color deterioration. A previous study showed that the ε-PL is a natural plant extract, and combined ε-PL and SNE could be recommended as useful antimicrobial and antioxidant ingredients without a negative effect on meat sensory properties (Alirezalu et al., 2019). In addition, samples containing ε-PL had better sensory properties compared to ice-stored samples (Jia et al., 2019). Therefore, the CH + GPE synergistically delayed the deterioration of cattle rumen sensory properties, thereby extending cattle rumen F-T cycles.

3.6 Changes in SEM of cattle rumen during freeze-thaw cycles

The cross-sectional microstructure of all samples was obtained by SEM in Fig. 5. Prior to the F-T cycle, the samples had a denser myofibrillar structure with small gaps between myofibrils. After five F-T cycles, the microstructure of all samples changed: the gaps between the muscle fibres increased and the muscle fibres were disrupted. This deterioration of muscle structure may be due to the formation of large and uneven ice crystals during freezing and thawing, which destroy cellular and fibrous tissue (Wang, He, Zhang, Chen, & Li, 2020).

The CH-coating bovine rumen muscle microstructure was less disrupted than the samples without CH-coating at the same F-T cycle. The CH-GPE coating samples had smoother muscle fibres and smaller gaps than the other two groups after five F-T cycles. These observations suggest that the CH-coating inhibits the disruption of bovine rumen microstructure caused by the F-T cycle by limiting the growth of ice crystals. This phenomenon may be due to the fact that the chitosan coating inhibits ice formation and controls the expansion of ice volume (Kong et al., 2017), thereby reducing damage to muscle tissue

structure. Similar observations were seen in the work of Wang et al. (2021), where ferulic acid-grafted chitosan coatings maximally inhibited microstructural damage to pork during refrigeration.

4. Conclusion

The inhibitory effects of CH on the quality deterioration of cattle rumen were verified by the increase in WHC, *b**, texture property, TAC, TVB-N, sulfhydryl contents and the decrease in pH, *L**, *a**, cutting force, carbonyl contents, TBARS and POV value during the same F-T cycles. CH + GPE was most effective in inhibiting the deterioration of rumen quality in cattle. It was demonstrated that CH + GPE prevents water loss and inhibits ice formation, thereby protecting muscle microstructure (SEM observation) and inhibiting protein and lipid oxidation. It is possible and acceptable that CH + GPE can be used in frozen meat products to improve quality and extend shelf life.

Declarations

Funding

This work was supported by the Fostering Foundation for the Excellent Ph.D. Dissertation of Gansu Agricultural University, the Gansu Province Science and Technology Plan Funded Project (20YF3NA016).

Data Availability

Not applicable.

Author Contribution

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the fnal version.

Declarations Conflict of Interest

The authors declare no competing interests.

References

1. Ali, S., Zhang, W., Rajput, N., Khan, M. A., Li, C. B. & Zhou, G. H. (2015). Effect of multiple freeze-thaw cycles on the quality of chicken breast meat. Food Chemistry, *173*, 808–814.
2. Alirezalu, K., Hesari, J., Nemati, Z., Munekata, P. E., Barba, F. J., & Lorenzo, J. M. (2019). Combined effect of natural antioxidants and antimicrobial compounds during refrigerated storage of nitrite-free frankfurter-type sausage. Food Research International, *120*, 839–850.
3. Alirezalu, K., Movlan, H. S., Yaghoubi, M., Pateiro, M., & Lorenzo, J. M. (2021). -polylysine coating with stinging nettle extract for fresh beef preservation. Meat Science, *176*, 108474.
4. Aminzare, M., Tajik, H., Aliakbarlu, J., Hashemi, M., & Raeisi, M. (2018). Effect of cinnamon essential oil and grape seed extract as functional-natural additives in the production of cooked sausage-impact on microbiological, physicochemical, lipid oxidation and sensory aspects, and fate of inoculated Clostridium perfringens. Journal of food safety, *38*(4), e12459.
5. Baca-Bocanegra, B., Hernández-Hierro, J. M., Nogales-Bueno, J., & Heredia, F. J. (2019). Feasibility study on the use of a portable micro near infrared spectroscopy device for the “in vineyard” screening of extractable polyphenols in red grape skins. Talanta, *192*, 353–359.
6. Bahmani, Z. A., Rezai, M., Hosseini, S. V., Regenstein, J. M., Böhme, K., Alishahi, A., & Yadollahi, F. (2011). Chilled storage of golden gray mullet (Liza aurata). LWT-Food Science and Technology, *44*(9), 1894–1900.
7. Cao, Y., Hao, R., Guo, Z., Han, L., Yu, Q., & Zhang, W. (2022). Combined effects of superchilling and natural extracts on beef preservation quality. LWT, *153*, 112520.
8. Fan, X., Liu, S., Li, H., He, J., Feng, J., Zhang, X., et al. (2019). Effects of *Portulaca oleracea* L. extract on lipid oxidation and color of pork meat during refrigerated storage. Meat Science, *147*, 82–90.
9. Fernandes, R. P. P., Trindade, M. A., Tonin, F. G., Pugine, S. M. P., Lima, C. G., Lorenzo, J. M., & De Melo, M. P. (2017). Evaluation of oxidative stability of lamb burger with Origanum vulgare extract. Food chemistry, *233*, 101–109.
10. Fernández, K., Aspé, E., & Roeckel, M. (2009). Shelf-life extension on fillets of Atlantic Salmon (Salmo salar) using natural additives, superchilling and modified atmosphere packaging. Food control, *20*(11), 1036–1042.
11. Garrido, M. D., Auqui, M., Martí, N., & Linares, M. B. (2011). Effect of two different red grape pomace extracts obtained under different extraction systems on meat quality of pork burgers. LWT-Food Science and Technology, *44*(10), 2238–2243.
12. Guo, Z., Ge, X., Yang, L., Ma, G., Ma, J., Yu, Q. L., & Han, L. (2021). Ultrasound-assisted thawing of frozen white yak meat: Effects on thawing rate, meat quality, nutrients, and microstructure. Ultrasonics Sonochemistry, *70*, 105345.
13. Guo, Z., Han, L., Yu, Q. L., & Lin, L. (2020). Effect of a sea buckthorn pomace extract-esterified potato starch film on the quality and spoilage bacteria of beef jerky sold in supermarket. Food chemistry, *326*, 127001.
14. Hassas-Roudsari, M., & Goff, H. D. (2012). Ice structuring proteins from plants: mechanism of action and food application. Food Research International, *46*(1), 425–436.

15. Huang, L., Liu, Q., Xia, X. F., Kong, B. H., & Xiong, Y. L. (2015). Oxidative changes and weakened gelling ability of salt-extracted protein are responsible for textural losses in dumpling meat fillings during frozen storage. Food Chemistry, *185*, 459–469.
16. Jia, S., Liu, Y., Zhuang, S., Sun, X., Li, Y., Hong, H., & Luo, Y. (2019). Effect of ε-polylysine and ice storage on microbiota composition and quality of Pacific white shrimp (Litopenaeus vannamei) stored at 0° C. Food microbiology, *83*, 27–35.
17. Jiang, Q., Nakazawa, N., Hu, Y., Osako, K., & Okazaki, E. (2019). Changes in quality properties and tissue histology of lightly salted tuna meat subjected to multiple freeze-thaw cycles. Food Chemistry, *293*, 178–186.
18. Jin, S., Pang, Q., Liu, R., Yang, H., Liu, F., Wang, M., & Shan, A. (2020). Dietary curcumin decreased lipid oxidation and enhanced the myofibrillar protein structure of the duck (Anas platyrhynchos) breast muscle when subjected to storage. LWT, *133*, 109986.
19. Ju, J., Xie, Y., Guo, Y., Cheng, Y., Qian, H., & Yao, W. (2018). Application of edible coating with essential oil in food preservation. Critical Reviews in Food Science and Nutrition, *59*(15), 2467–2480.
20. Kim, G. D., Jung, E. Y., Lim, H. J., Yang, H. S., Joo, S. T., & Jeong, J. Y. (2013). Influence of meat exudates on the quality characteristics of fresh and freeze-thawed pork. Meat Science, *95*(2), 323–329.
21. Kim, J., Oh, J., Averilla, J. N., Kim, H. J., Kim, J. S., & Kim, J. S. (2019). Grape peel extract and resveratrol inhibit wrinkle formation in mice model through activation of Nrf2/HO-1 signaling pathway. Journal of food science, *84*(6), 1600–1608.
22. Kong, C. H., Hamid, N., Ma, Q., Lu, J., Wang, B. G., & Sarojini, V. (2017). Antifreeze peptide pretreatment minimizes freeze-thaw damage to cherries: An in-depth in vestigation. LWT, *84*, 441–448.
23. Lan, Y., Shang, Y., Song, Y., & Dong, Q. (2016). Changes in the quality of superchilled rabbit meat stored at different temperatures. Meat Science, *117*, 173–181.
24. Langroodi, A. M., Tajik, H., Mehdizadeh, T., Moradi, M., Kia, E. M., & Mahmoudian, A. (2018). Effects of sumac extract dipping and chitosan coating enriched with Zataria multiflora Boiss oil on the shelf-life of meat in modified atmosphere packaging. LWT, *98*, 372–380.
25. Lee, Y. S., Owens, C. M., & Meullenet, J. F. (2009). Changes in tenderness, color, and water holding capacity of broiler breast meat during postdeboning aging. Journal of food science, *74*(8), E449-E454.
26. Leygonie, C., Britz, T. J., & Hoffman, L. C. (2012). Impact of freezing and thawing on the quality of meat. Meat science, *91*(2), 93–98.
27. Li, S., Yu, Q., Han, L., Zhang, Y., Tian, X., & Zhao, S. (2019). Effects of proteome changes on the tenderness of yak rumen smooth muscle during postmortem storage based on the label-free mass spectrometry. Food research international, *116*, 1336–1343.
28. Li, Y., Tang, X., Shen, Z., & Dong, J. (2019). Prediction of total volatile basic nitrogen (TVB-N) content of chilled beef for freshness evaluation by using viscoelasticity based on airflow and laser technique. Food chemistry, *287*, 126–132.
29. Liu, X., Yang, Q., Lu, Y., Li, Y., Li, T., Zhou, B., & Qiao, L. (2019). Effect of purslane (Portulaca oleracea L.) extract on anti-browning of fresh-cut potato slices during storage. Food Chemistry, *283*, 445–453.
30. Lorenzo, J. M., Sineiro, J., Amado, I. R., & Franco, D. (2014). Influence of natural extracts on the shelf life of modified atmosphere-packaged pork patties. Meat Science, *96*(1), 526–534.
31. Medić, H., Kušec, I. D., Pleadin, J., Kozačinski, L., Njari, B., Hengl, B., & Kušec, G. (2018). The impact of frozen storage duration on physical, chemical and microbiological properties of pork. Meat Science, *140*, 119–127.
32. Mehdizadeh, T., & Langroodi, A. M. (2019). Chitosan coatings incorporated with propolis extract and Zataria multiflora Boiss oil for active packaging of chicken breast meat. International journal of biological macromolecules, *141*, 401–409.
33. Pan, N., Dong, C., Du, X., Kong, B., & Xia, X. (2020). Effect of freeze-thaw cycles on the quality of quick-frozen pork patty with different fat content by consumer assessment and instrument-based detection. Meat Science, *172*(5), 108313.
34. Pateiro, M., Vargas, F. C., Chincha, A. A., Sant'Ana, A. S., Strozzi, I., Rocchetti, G., & Lorenzo, J. M. (2018). Guarana seed extracts as a useful strategy to extend the shelf life of pork patties: UHPLC-ESI/QTOF phenolic profile and impact on microbial inactivation, lipid and protein oxidation and antioxidant capacity. Food Research International, *114*, 55–63.
35. Regand, A., & Goff, H. D. (2006). Ice recrystallization inhibition in ice cream as affected by ice structuring proteins from winter wheat grass. Journal of dairy science, *89*(1), 49–57.
36. Rodrigo, R., Miranda, A., & Vergara, L. (2011). Modulation of endogenous antioxidant system by wine polyphenols in human disease. Clinica Chimica Acta, *412*(5–6), 410–424.
37. Rusak, G., Komes, D., Likić, S., Horžić, D., & Kovač, M. (2008). Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used. Food Chemistry, *110*(4), 852–858.
38. Song, Z., Cao, Y., Zhang, Y., Zhang, Z., Shi, X., Zhang, W., & Wen, P. (2022). Effects of storage methods on the microbial community and quality of Sichuan smoked bacon. *LWT*, 113115.
39. Sun, Q., Sun, F., Xia, X., Xu, H., & Kong, B. (2019). The comparison of ultrasound-assisted immersion freezing, air freezing and immersion freezing on the muscle quality and physicochemical properties of common carp (Cyprinus carpio) during freezing storage. Ultrasonics Sonochemistry, *51*, 281–291.
40. Wang, B., Li, F., Pan, N., Kong, B., & Xia, X. (2021). Effect of ice structuring protein on the quality of quick-frozen patties subjected to multiple freeze-thaw cycles. Meat Science, *172*, 108335.
41. Wang, G., Liu, Y., Yong, H., Zong, S., Jin, C., & Liu, J. (2021). Effect of Ferulic Acid-Grafted-Chitosan Coating on the Quality of Pork during Refrigerated Storage. Foods, *10*(6), 1374.
42. Wang, H., Luo, Y., Shi, C., & Shen, H. (2015). Effect of different thawing methods and multiple freeze-thaw cycles on the quality of common carp (cyprinus carpio). Journal of Aquatic Food Product Technology, *24*(2), 153–162.

43. Wang, Z., He, Z., Zhang, D., Chen, X., & Li, H. (2020). Effect of multiple freeze-thaw cycles on protein and lipid oxidation in rabbit meat. International Journal of Food Science & Technology, *56*(6), 3004–3015.
44. Xia, X., Kong, B., Liu, Q., & Liu, J. (2009). Physicochemical change and protein oxidation in porcine longissimus dorsi as influenced by different freeze–thaw cycles. Meat science, *83*(2), 239–245.
45. Xin, K. Q., Ji, X. Y., Guo, Z., Han, L., Yu, Q. L., & Hu, B. (2022). Pitaya peel extract and lemon seed essential oil as effective sodium nitrite replacement in cured mutton. LWT, *160*, 113283.
46. Xu, D., Wang, Y., Jiao, N., Qiu, K., Zhang, X., Wang, L., & Yin, J. (2020). The coordination of dietary valine and isoleucine on water holding capacity, pH value and protein solubility of fresh meat in finishing pigs. Meat Science, *163*, 108074.
47. Yu, D., Zhao, W., Yang, F., Jiang, Q., Xu, Y., & Xia, W. (2021). A strategy of ultrasound-assisted processing to improve the performance of bio-based coating preservation for refrigerated carp fillets (Ctenopharyngodon idellus). Food Chemistry, *345*, 128862.
48. Yuan, G., Chen, X., & Li, D. (2016). Chitosan films and coatings containing essential oils: The antioxidant and antimicrobial activity, and application in food systems. Food Research International, *89*, 117–128.

Tables

Table 1 Changes in pH, water holding capacity, color and cutting force of cattle rumen in chitosan coating containing grape peel extract during freeze-thaw cycles

Treatments	Freeze-thaw times	pH	WHC			Color			cutting force
			Thawing loss	Pressing loss	Cooking loss	*a**	*b**	*L**	
CK	0	7.1 ± 0.02^{Ab}	—	18.69 ± 0.66^{Ac}	25.8 ± 0.98^{Ad}	4.06 ± 0.1^{Aa}	9.06 ± 0.1^{Aa}	61.29 ± 0.5^{Aa}	8.1 ± 0.12^{Ab}
	1	7.34 ± 0.03^{Aa}	8.33 ± 0.25^{Ac}	24.37 ± 0.23^{Ab}	30.66 ± 0.65^{Ac}	3.29 ± 0.06^{Cb}	11.29 ± 0.06^{Ab}	56.76 ± 0.52^{Cb}	8.7 ± 0.25^{Aa}
	3	7.07 ± 0.06^{Ab}	10.56 ± 0.76^{Ab}	31.3 ± 0.41^{Aa}	34.48 ± 0.5^{Ab}	2.49 ± 0.06^{Cc}	13.72 ± 0.41^{Ac}	53.22 ± 0.03^{Cc}	6.38 ± 0.19^{Ac}
	5	6.88 ± 0.06^{Ac}	17.42 ± 0.58^{Aa}	33.33 ± 0.92^{Aa}	38 ± 0.6^{Aa}	0.41 ± 0.02^{Cd}	15.08 ± 0.57^{Ad}	50.74 ± 0.99^{Cd}	5.29 ± 0.11^{Ad}
CH	0	7.05 ± 0.07^{Ab}	—	18.35 ± 0.24^{Ad}	25.46 ± 0.65^{Ad}	4.11 ± 0.04^{Aa}	8.96 ± 0.1^{Aa}	61.56 ± 0.3^{Aa}	7.97 ± 0.08^{Ab}
	1	7.23 ± 0.02^{Aa}	7.93 ± 0.25^{Ac}	22.94 ± 0.54^{Bc}	29.25 ± 0.46^{Bc}	3.4 ± 0.02^{Bb}	11.09 ± 0.06^{Bb}	58.36 ± 0.45^{Bb}	8.44 ± 0.07^{ABa}
	3	6.91 ± 0.03^{Bc}	9.82 ± 0.09^{ABb}	29.3 ± 0.41^{Bb}	31.81 ± 0.26^{Bb}	2.53 ± 0.04^{Bc}	13.22 ± 0.03^{Bc}	54.02 ± 0.19^{Bc}	6.08 ± 0.03^{Bc}
	5	6.75 ± 0.05^{Bd}	16.09 ± 0.18^{Ba}	30.66 ± 0.59^{Ba}	36.67 ± 0.02^{Ba}	0.59 ± 0.03^{Bd}	14.74 ± 0.12^{Ad}	51.88 ± 0.13^{Bd}	4.92 ± 0.09^{Bd}
CH+GPE	0	7.08 ± 0.02^{Ab}	—	17.99 ± 0.26^{Ad}	24.8 ± 0.16^{Ad}	4.14 ± 0.05^{Aa}	8.89 ± 0.06^{Aa}	61.72 ± 0.33^{Aa}	7.24 ± 0.12^{Bb}
	1	7.14 ± 0.04^{Ba}	7.29 ± 0.16^{Bc}	21.37 ± 0.23^{Cc}	27.66 ± 0.38^{Cc}	3.78 ± 0.05^{Ab}	10.29 ± 0.06^{Cb}	59.43 ± 0.57^{Ab}	8.3 ± 0.15^{Ba}
	3	6.81 ± 0.02^{Ca}	9.46 ± 0.14^{Bb}	26.3 ± 0.41^{Cb}	30.14 ± 0.74^{Cb}	2.92 ± 0.03^{Ac}	12.49 ± 0.06^{Cc}	54.89 ± 0.57^{Ac}	5.77 ± 0.1^{Cc}
	5	6.52 ± 0.02^{Cc}	15.52 ± 0.21^{Ba}	29 ± 0.61^{Ca}	33.67 ± 0.02^{Ca}	1.03 ± 0.09^{Ad}	13.85 ± 0.4^{Cd}	52.74 ± 0.12^{Ad}	4.29 ± 0.11^{Cd}

Notes: The different capital letters (A–C) indicate significant differences between treatments at the same time point ($p < 0.05$), whereas the different lowercase letters (a–d) indicate significant differences between time points after the same treatment ($p < 0.05$). WHC: water holding capacity; CK: cattle rumen treated with deionized water; CH: cattle rumen treated with chitosan coating solution; CH+GPE: cattle rumen treated with chitosan coating containing grape peel extract solution.

Table 2 Changes in texture properties of cattle rumen in chitosan coating containing grape peel extract during freeze-thaw cycles

Treatments	Freeze-thaw times	Hardness	Springiness	Cohesiveness	Chewiness	Resilience
CK	0	3653.4 ± 92.39^{Ac}	1 ± 0.01^{Aa}	0.83 ± 0^{Aa}	4013.79 ± 104.16^{Aa}	0.5 ± 0.01^{Aa}
	1	3755.66 ± 33.67^{Cc}	0.94 ± 0.01^{Bb}	0.82 ± 0.01^{Bb}	3795.76 ± 64.28^{Cb}	0.45 ± 0^{Bb}
	3	4083.24 ± 89.44^{Cb}	0.91 ± 0.01^{Cbc}	0.77 ± 0.01^{Cc}	3083.17 ± 80.13^{Cc}	0.45 ± 0.01^{Ab}
	5	4150.16 ± 25.88^{Ca}	0.84 ± 0.01^{Cc}	0.75 ± 0.01^{Bd}	2214.73 ± 79.9^{Cd}	0.42 ± 0.01^{Cc}
CH	0	3673.4 ± 72.42^{Ad}	1.02 ± 0.03^{Aa}	0.84 ± 0^{Aa}	4047.13 ± 118.03^{Aa}	0.5 ± 0.01^{Aa}
	1	3862.33 ± 24.08^{Bc}	0.95 ± 0.01^{Bb}	0.83 ± 0^{Aa}	3852.56 ± 11.06^{Bb}	0.46 ± 0^{Bb}
	3	4216.58 ± 40.44^{Bb}	0.92 ± 0.01^{Bc}	0.79 ± 0^{Bb}	3249.84 ± 26.9^{Bc}	0.46 ± 0^{Ab}
	5	4350.16 ± 25.88^{Ba}	0.86 ± 0.01^{Bd}	0.76 ± 0^{Bc}	2448.07 ± 22.75^{Bd}	0.43 ± 0^{Bc}
CH+GPE	0	3700.07 ± 88.6^{Ad}	1.07 ± 0.05^{Aa}	0.84 ± 0^{Aa}	4060.46 ± 84.99^{Aa}	0.5 ± 0.01^{Aa}
	1	3955.66 ± 33.67^{Ac}	0.98 ± 0.01^{Ab}	0.84 ± 0^{Aa}	3919.43 ± 6.24^{Ab}	0.48 ± 0^{Ab}
	3	4313.24 ± 42.72^{Ab}	0.95 ± 0.01^{Ab}	0.8 ± 0.01^{Ab}	3516.5 ± 41.15^{Ac}	0.46 ± 0^{Ac}
	5	4453.5 ± 30.72^{Aa}	0.89 ± 0.01^{Ac}	0.78 ± 0.01^{Ac}	2848.07 ± 22.75^{Ad}	0.44 ± 0^{Ad}

Notes: The different capital letters (A–C) indicate significant differences between treatments at the same time point ($p < 0.05$), whereas the different lowercase letters (a–d) indicate significant differences between time points after the same treatment ($p < 0.05$). CK: cattle rumen treated with deionized water; CH: cattle rumen treated with chitosan coating solution; CH+GPE: cattle rumen treated with chitosan coating containing grape peel extract solution.

Figures

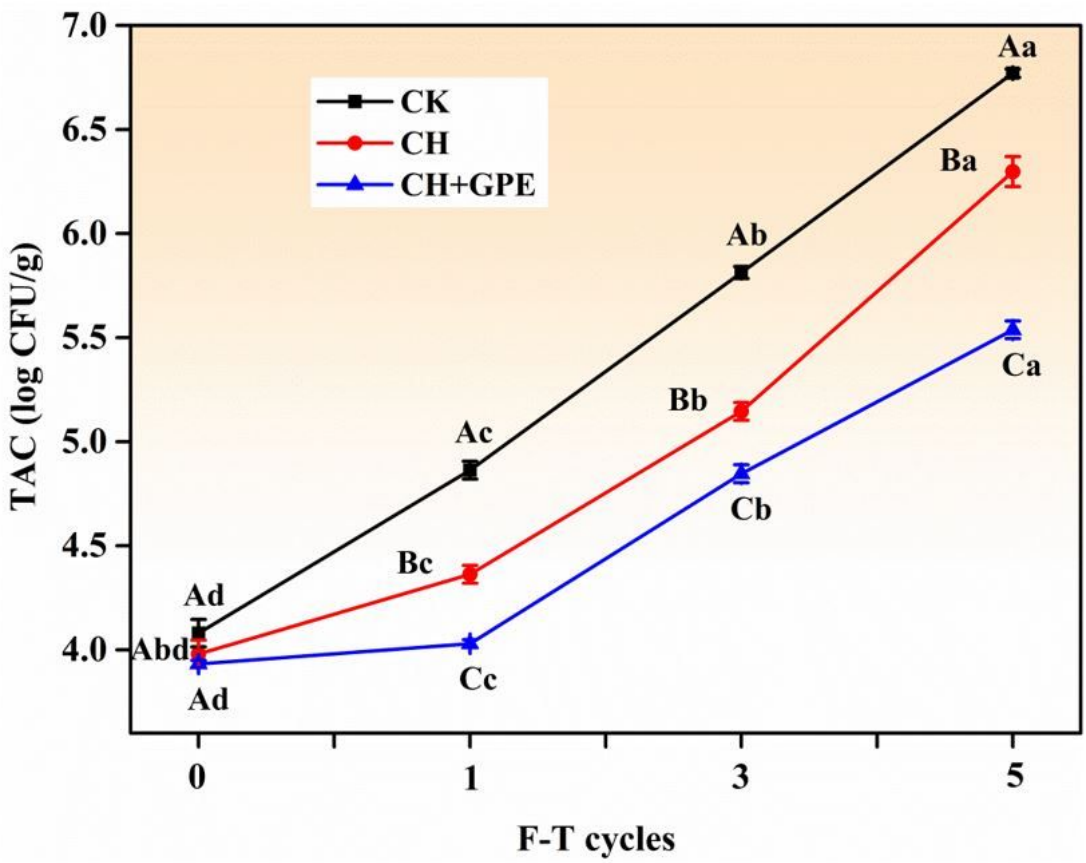

Figure 1

Changes in total aerobic count of cattle rumen in chitosan coating containing grape peel extract during freeze-thaw cycles. The different capital letters (A–C) indicate significant differences between treatments at the same time point ($p < 0.05$), whereas the different lowercase letters (a–d) indicate significant differences between time points after the same treatment ($p < 0.05$). CK: cattle rumen treated with deionized water; CH: cattle rumen treated with chitosan coating solution; CH+GPE: cattle rumen treated with chitosan coating containing grape peel extract solution.

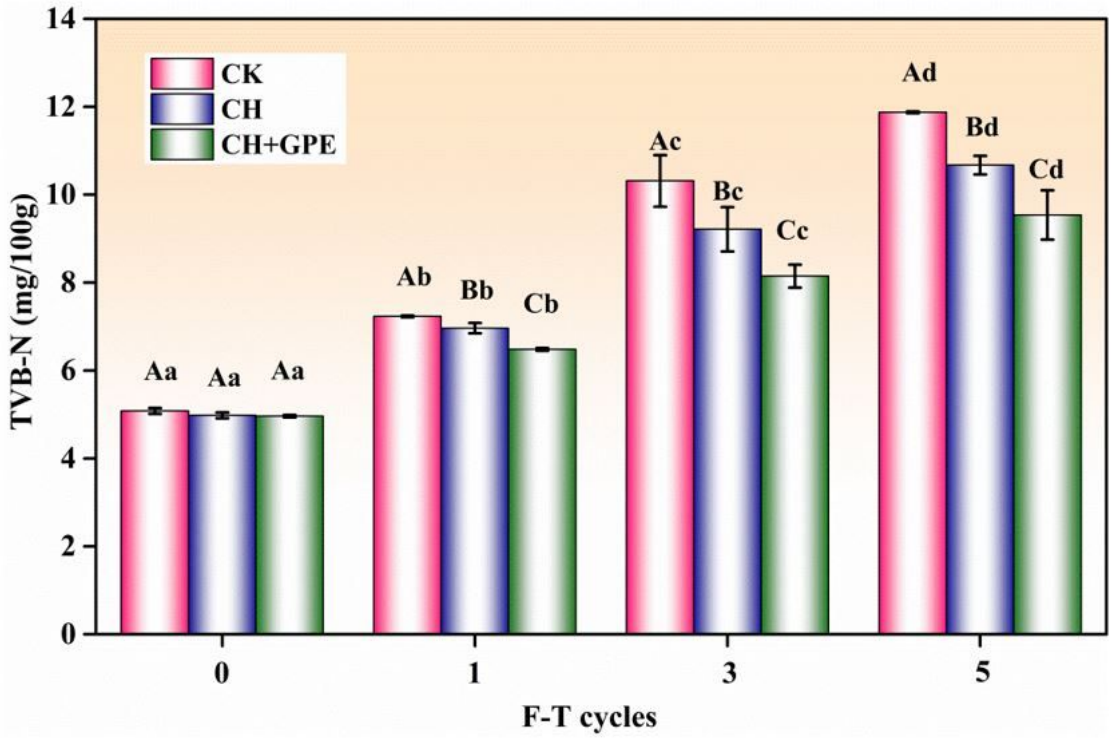

Figure 2

Changes in TVB-N of cattle rumen in chitosan coating containing grape peel extract during freeze-thaw cycles. The different capital letters (A–C) indicate significant differences between treatments at the same time point ($p < 0.05$), whereas the different lowercase letters (a–d) indicate significant differences between time points after the same treatment ($p < 0.05$). CK: cattle rumen treated with deionized water; CH: cattle rumen treated with chitosan coating solution; CH+GPE: cattle rumen treated with chitosan coating containing grape peel extract solution.

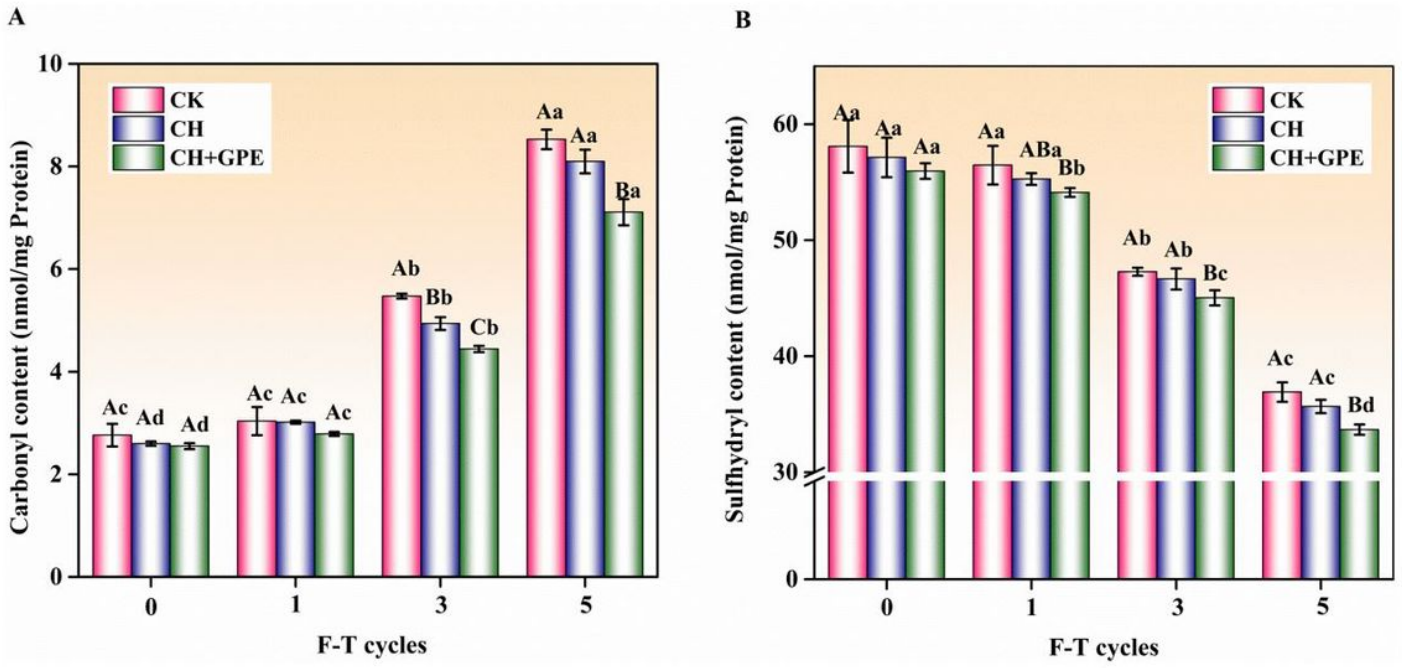

Figure 3

Changes in Carbonyl content (A) and sulfhydryl content (B) of cattle rumen in chitosan coating containing grape peel extract during freeze-thaw cycles. The different capital letters (A–C) indicate significant differences between treatments at the same time point ($p < 0.05$), whereas the different lowercase letters (a–d) indicate significant differences between time points after the same treatment ($p < 0.05$). CK: cattle rumen treated with deionized water; CH: cattle rumen treated with chitosan coating solution; CH+GPE: cattle rumen treated with chitosan coating containing grape peel extract solution.

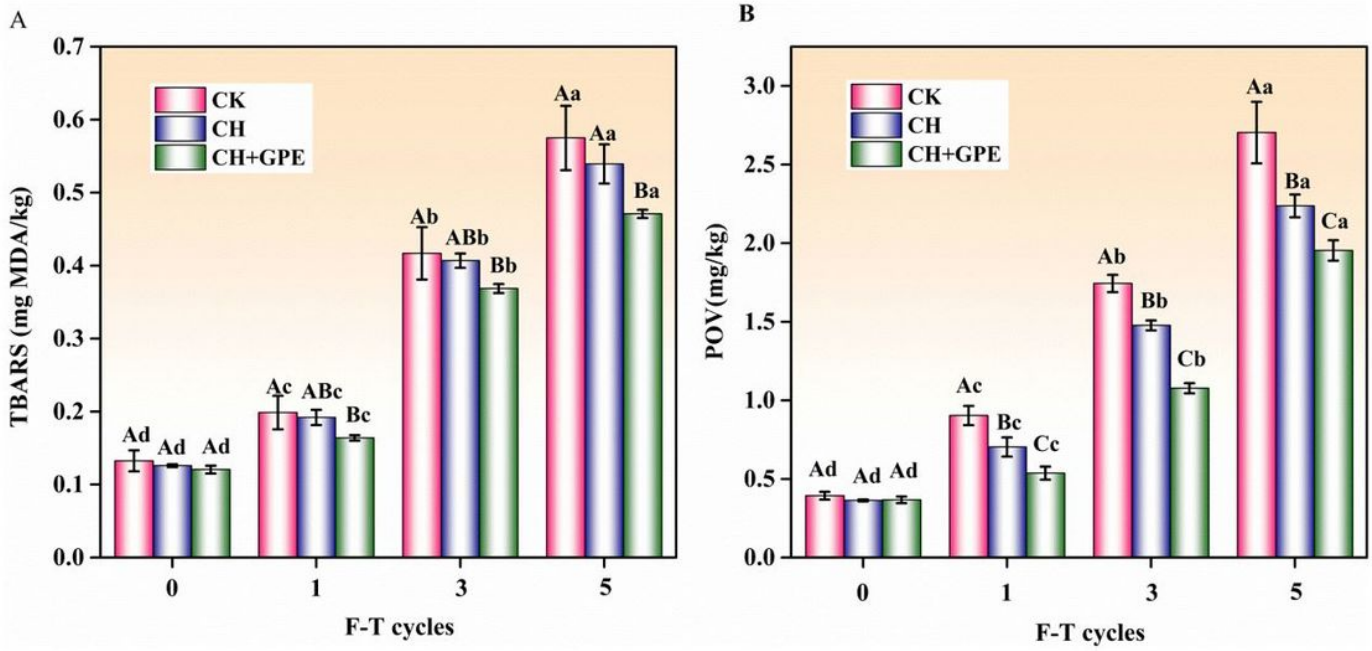

Figure 4

Changes in TBARS (A) and POV (B) of cattle rumen in chitosan coating containing grape peel extract during freeze-thaw cycles. The different capital letters (A–C) indicate significant differences between treatments at the same time point ($p < 0.05$), whereas the different lowercase letters (a–d) indicate significant differences between time points after the same treatment ($p < 0.05$). CK: cattle rumen treated with deionized water; CH: cattle rumen treated with chitosan coating solution; CH+GPE: cattle rumen treated with chitosan coating containing grape peel extract solution.

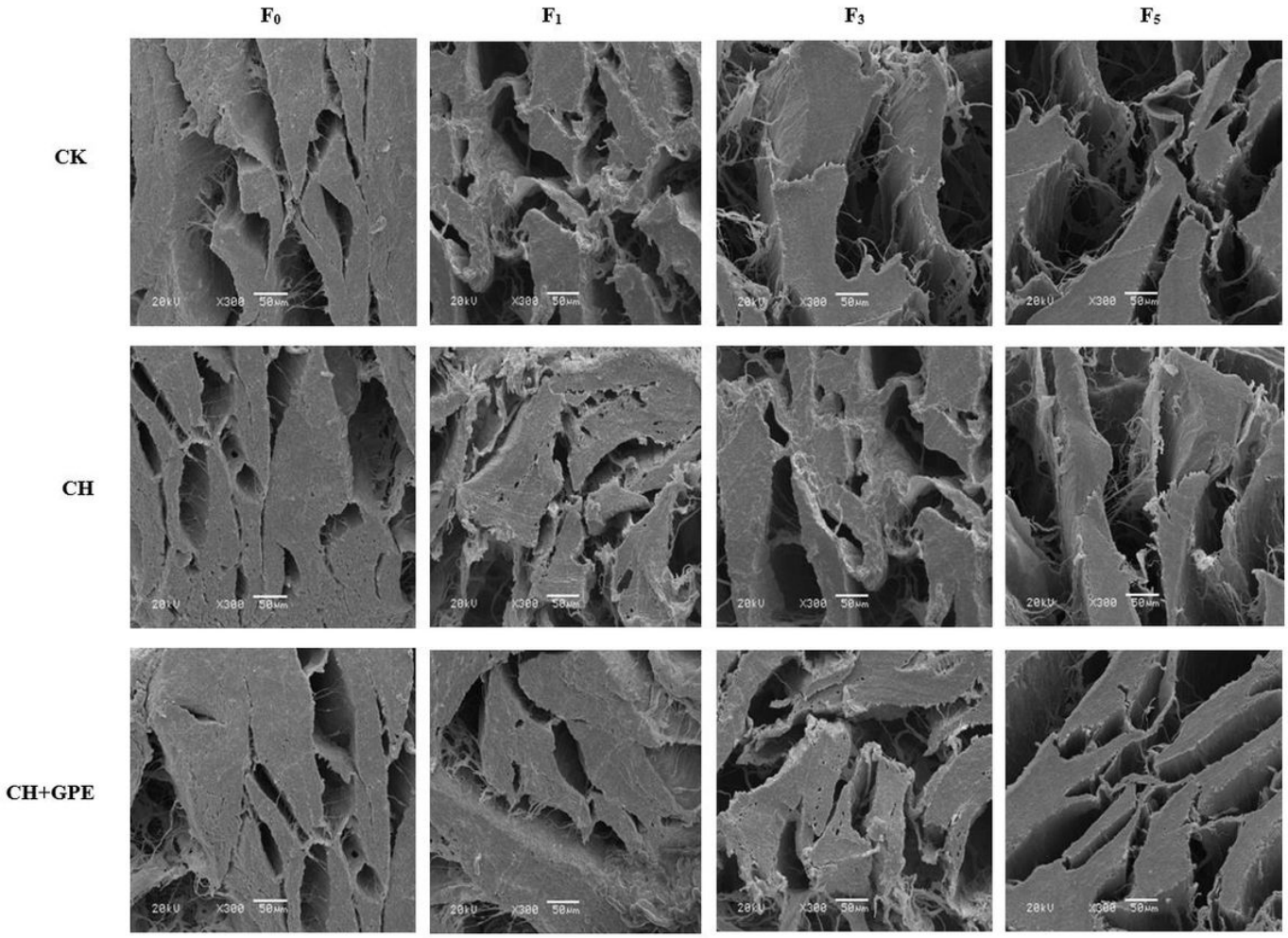

Figure 5

Changes in microstructure of cattle rumen in chitosan coating containing grape peel extract during freeze-thaw cycles. CK: cattle rumen treated with deionized water; CH: cattle rumen treated with chitosan coating solution; CH+GPE: cattle rumen treated with chitosan coating containing grape peel extract solution.

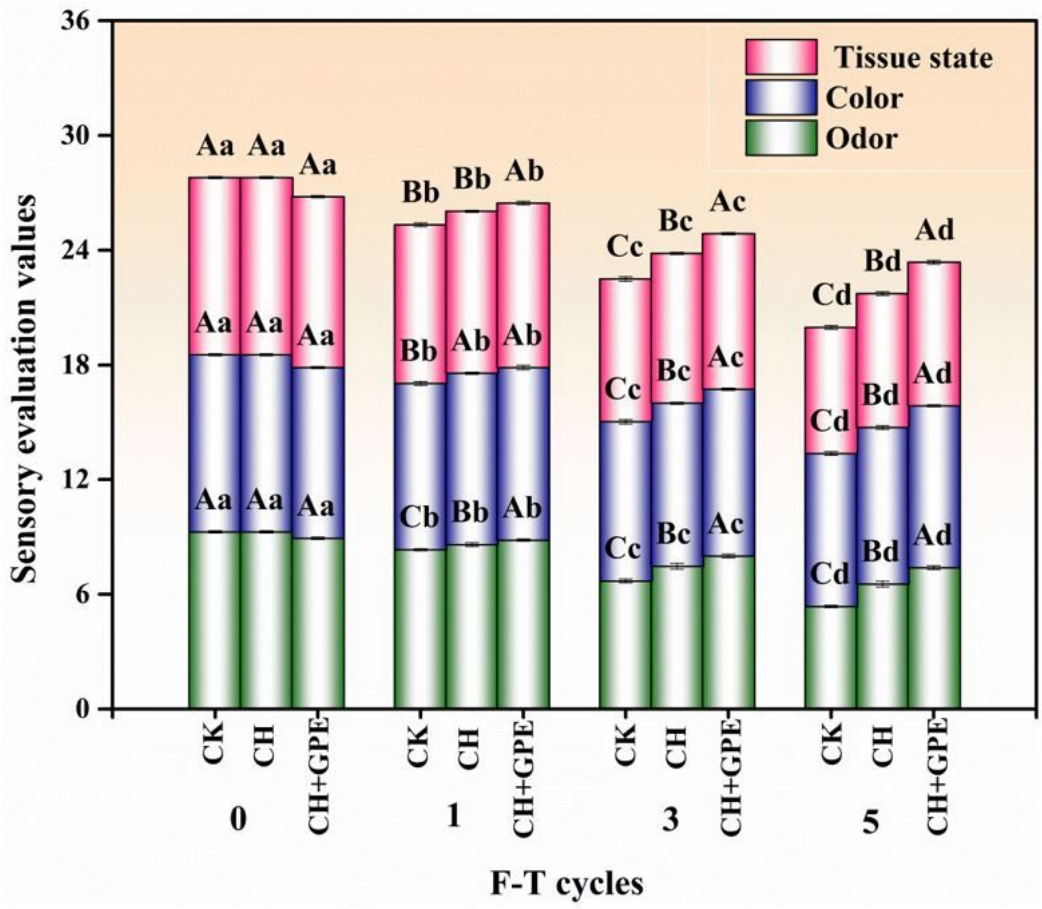

Figure 6

Changes in n sensory evaluation values of cattle rumen in chitosan coating containing grape peel extract during freeze-thaw cycles. CK: cattle rumen treated with deionized water; CH: cattle rumen treated with chitosan coating solution; CH+GPE: cattle rumen treated with chitosan coating containing grape peel extract solution.