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Citric acid vapor-assisted crosslinking of zein/PEG composite nanofiber membrane embedded with nisin by electrospinning for the cooled goose meat preservation

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A R T I C L E I N F O

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A B S T R A C T

This study demonstrated the fabrication of zein/polyethylene glycol/nisin (zein/PEG/nisin) nanofiber membrane and cross-linked by citric acid vapor (zein/PEG/nisin/C). The distribution within the whole nanofiber membranes was monitored by scanning electron microscopy (SEM). Studies using thermogravimetric analysis (TGA) and Fourier transform infrared spectroscopy (FTIR) validated the effectiveness nisin encapsulation and molecular interactions. The water vapor permeability (WVP) and oxygen permeability (OP) of zein/PEG/nisin/C are 150.47 $\pm$ 7.14 (g m^{-2} 24h) and 59.74 $\pm$ 3.10 (cm3 m^{-2} 24h), respectively. Antibacterial experiments have shown that the antibacterial effect of zein/PEG/nisin/C on *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) and the diameters of the bacteriostatic circle were 11.52 $\pm$ 0.44 mm and 10.67 $\pm$ 0.46 mm, respectively. During 10 days of the storage of the cooled fresh goose meat, compared with the control group, the pH of zein/PEG/nisin/C nanofiber membrane was 5.7, the concentration of the total volatile basic nitrogen (TVB-N) and the value of total viable count (TVC) and thiobarbituric acids (TBARS) are 11.28 mg/100g, 5.01 $\pm$ 0.69 log (CFU g^{-1}), and 0.83 mg kg^{-1}, respectively. These results point to the possibility of using functionalized nanofiber membranes for the cold fresh goose meat preservation facilitated by vaporized citric acid cross-linking.

1. Introduction

Generally, plastic has been widely applied to food industry for packaging, transport, and distribution so that prevent the foods from pollution and can guarantee quality. However, plastic is now regarded as a planetary emerging due to their non-renewable sources and their substantial environmental pollution (Liu et al., 2022). Hence, bio-polymers have attracted the gaze of people in the whole word to take place of synthetic plastic for food packaging. There are mainly two kinds of bio-polymers one part are naturally occurring substances including proteins, carbohydrate, fats and another part are chemically synthesized from natural derived monomers (Garavand et al., 2018). However, the composite bio-polymers membranes are limited in application due to poor mass transfer properties, which weaken their strength and stability under a variety of handling conditions (J.X. Li et al., 2024).

Hence, other natural or synthetic biopolymers are biocompatible with zein, and the functionality increases (Hajji et al., 2021). Due to the zein the characteristics of good bio-compatibility, biodegradability, and sustainability, what's more, it has an amphiphilic nature, which leads to unique polymerization characteristics, bioconjugation, and complex development. However, the use of corn zein membranes in cost-effective food packaging applications appears impossible with the current status due to poor mechanical properties (Lan et al., 2023). Electrospinning is considered to be a handy, current, and spacious method to get continuous nanofibers, owing to the integrity of nanofiber structural and the high surface area to volume, the electrospinning nanofibers has enormous potential for application in many fields (Fabra et al., 2016). The reason why zein can be electrospinning is that it is one kind of

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alcohol-soluble protein and can be dissolved in different solvents involving ethanol, acetic acid, and aqueous methanol (Cruz et al., 2024). Although pure zein nanofibers were successfully synthesized, their mechanical and solvent resistance properties were poor when compared to literature (Zhang et al., 2015). Hence, to solve this problem, other polymer compatibilizers like amphiphilic, the compounds of low-molecular-mass (Deng et al., 2017), ionomers (Q. Li et al., 2024), or partially miscible polymers in alloying solutions (Azizi et al., 2023) were added and mixed with zein-named hybrid electrospinning, which was regarded as a suitable method to improve the properties of the compound membranes. Polyethylene glycol (PEG) consists of ethylene glycol units $[CH_2CH_2O]_n$, which is one kind of biocompatible polymer with flexible, water-soluble, and non-immunogenic, so results in the properties of versatility, tunable and well-established safety profile and widely-applied to numerous fields (Cheng et al., 2022).

The polymer membranes of cross-linking have potential in package applications. Therefore, there is an important role in the field of cross-linking in selecting appropriate cross-linker. Even though the aldehyde cross-linker has a positive impact on composite membrane properties, its usage is limited due to its toxicity, on the other hand, because of the high reaction activity and they cannot be added in the electrospinning solution, which is a difficulty in direct electrospinning (Zhang et al., 2016). The citric acid cross-linker have been widely applied own to their safety, reliability, and cost-effectiveness (Ma et al., 2024) The addition of citric acid can dramatically improve the water resistance and mechanical properties of membranes (Wang et al., 2024) Nisin is one kind of natural food-preserving agent without toxicity and with highly effective, side-effect-free so that own the characteristic of a broad range of antimicrobial and bio-safety, hence it is widely applied to act as potential material to prepare antimicrobial membranes for food packaging. Nevertheless, as far as we know, there are few studies showing that the zein/PEG-based membrane-embedded nisin is an active packaging material applied to preserve cold fresh goose meat preservation. The main purpose of this study was to prepare an active membrane, determine its physical, chemical, structural, and antibacterial properties, as well as investigate the possibility of increasing goose breast meat's shelf life by using an active membrane. Afterward, the composite membranes were further characterized using Fourier transform infrared spectroscopy (FTIR) spectroscopy, scanning electron microscopy (SEM), and crystallinity. The water vapor permeability (WVP) and oxygen permeability (OP) of the composite membranes were also determined.

2. Material and methods

2.1. Materials

All chemicals and reagents were of analytical grade. Zein (97% mass purity), and polyethylene glycol (PEG, average Mw: 20000) were obtained from MACKLIN Reagent Co. Ltd (Shanghai, China). Nisin was purchased by Zhejiang Silver-Elephant Bio-engineering Co. Ltd (Zhejiang, China). Citric acid and sodium chloride were obtained from Tianjin Yong-da Chemical Co. Ltd (Tianjin, China). Bromocresol green, methyl red, hydrochloric acid (HCL), boric acid (H_3BO_3), 2-thiobarbituric acid ($C_4H_4N_2O_2S$), trichloroacetic acid (Cl_3CCOOH, TCA), EDTA disodium, trichloromethane ($CHCl_3$), magnesium oxide (MgO) and anhydrous ethanol (CH_3CH_2OH) were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Plate Count Agar (PCA) was obtained from Biowell Technology Co. Ltd (Shanghai, China). Nutrient Broth (NB) was purchased from Hopebiol Co. Ltd (Qingdao, China). Cooled Goose Breast (Lander Goose) was obtained from Longxiangmsw Co. Ltd (Luan, China).

2.2. Preparation of the zein/PEG/nisin nanofiber

Initially, zein (2.5 g), PEG (1.5 g), and nisin (0.2 g) were weighed and dissolved in 10 mL 90% ethanol solution; subsequently, stirred at 35 °C until transparent, then cooled at room temperature, and zein/PEG/nisin electrospinning solution was obtained. After being loaded into the glass syringe, which was fixed with a steel needle the tip diameter was 0.33 mm. Finally, the electrospinning conditions were followed: the high voltage was 22 kv, the distance between the tip of the needle and collector was 15 cm, and the flow rate of mixed electrospinning solution was 2.0 mL h^{-1}. The nanofibers were collected on the collector and dried at 60 °C in drying box.

2.3. Citric acid vapor-assisted crosslinking of the zein/PEG/nisin nanofiber membrane

The zein/PEG/nisin nanofiber membrane was crosslinked by using citric acid vapor. During the drying process, the citric acid solution in a Petri dish was placed in a dryer and covered with a metallic mesh. Meanwhile, the zein/PEG/nisin nanofiber membrane was placed on the metallic mesh. Finally, the entire dryer including citric acid and nanofiber was heated and maintained at 60 °C. During the crosslinking procedure, the zein/PEG/nisin nanofiber membrane was exposed to vaporized citric acid under 60 °C for 30 min. After vapor-assisted crosslinking, the zein/PEG/nisin/C nanofiber membrane was stored at room temperature.

2.4. Characterization of the whole composite membranes

2.4.1. Scanning electron microscopy (SEM)

After the prepared composite nanofiber membranes dried, they were cut into 1.0 cm × 1.0 cm square pieces and stuck on a glass slide with conductive adhesive (gold spray treatment). Scanning electron microscopy (SEM, Supra55, Zeiss, German) was used to observe the morphology of zein, zein/PEG, zein/PEG/nisin, and zein/PEG/nisin/C nanofibers membrane at 20kv and ranged from 20 to 200000.

2.4.2. Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR, NicoletiS10, ThermoFisher, USA) was used to confirm the functional groups of the nanofiber membranes and the spectrum was analyzed at a resolution of 6 cm^{-1} in the wave number range of 500–4000 cm^{-1}.

2.4.3. X-ray diffraction (XRD)

XRD patterns of membrane samples were recorded using an X-ray diffraction technique (XRD, XD-3X, Persee General, China). The measurement conditions are followed: CuKα target, scanning method was stacked scanning, and the scanning range was from 5° to 90° with a step of 0.02°. In conclusion, we measured a current of 24 mA and a voltage of 36 KV during the measurement process.

2.4.4. Thermogravimetric analysis (TGA)

The thermal behavior of different kinds of nanofibers was observed by the advantage of the thermogravimetric analyzer, TGA (TG 209 F3, NETZSCH, Germany). The flow of continuous nitrogen for thermogravimetric analyses was 20 mL min^{-1}, and the temperature ranged from 25 °C to 800 °C, and the heating rate was 10 °C min^{-1}.

2.5. Physical properties

2.5.1. The method of measuring water vapor permeability (WVP)

The method of measuring the membrane WVP was according to the reference Chaparro and Suchdev (2019). In the first step, 20 mL of distilled water was added to the 50 mL centrifuge tube that opened and the membrane samples were sealed tightly. Secondly, the centrifuge tubes with the membrane samples were laid in a desiccator, which contained dried silica gel. Finally, the weight of the centrifuge tubes with the membrane samples was determined every 1.0 h for 24 h. The WVP of each membrane sample was evaluated followed by the equation:

$$\mathrm{WVP} = \frac{\mathrm{M} \times \mathrm{d}}{\mathrm{S} \times \mathrm{t} \times \triangle \mathrm{p}} \tag{1}$$

M: the change in the weight of the centrifuge tube (g), d: the average thickness of the membrane (mm), S: the area of membrane surface (m^2), t: time (s), Δp: the water vapor pressure difference between the distilled water and atmosphere.

2.5.2. The method of measuring oxygen permeability (OP)

To measure the oxygen permeability (OP) of the membrane, an automated oxygen permeability testing machine (Labthink Co., Ltd., Jinan, China) was used. Oxygen was on one side of the membrane sample, meanwhile the nitrogen was on the other side. The rate of oxygen transmission (ROT) was determined and OP was evaluated followed by the equation:

$$\mathrm{OP} = \frac{\mathrm{OTR} \times \mathrm{d}}{\Delta \mathrm{P}^*} \tag{2}$$

d: the of thickness membrane (mm), ΔP*: the oxygen partial pressure.

2.6. Antibacterial experiment

As a first step, the prepared nanofiber membranes zein, zein/PEG, zein/PEG/nisin and the cross-linked nanofiber zein/PEG/nisin/C were cut into circular sheets with a diameter of 5 mm, and irradiated under ultraviolet light for 30 min. Secondly, *Escherichia coli* and *Staphylococcus aureus* were inoculated in liquid culture medium under the sterile environment and incubated at 38 °C and shook for 24 h, 48 h, respectively, then 50 μL bacterial fluid was coated on solid culture medium plates. Three parallel samples were made for each sample after the sterilized thin membranes were placed on the coated solid culture medium incubated at 37 °C for 24 h. Finally, the sizes of the antibacterial zones were recorded and the antibacterial performance of the composite membranes was analyzed.

2.7. Application

As shown in Fig. 1, the goose breast meat was taken out and cut into pieces of an appropriate size on the sterile operating table. After the fascia have been removed, the goose breast samples were rinsed with sterile physiological saline and dried with sterile filter paper, cut into 7.0 ± 0.5 g square pieces. The composite nanofiber membranes were cut into circular pieces of around 8.0 cm in diameter, and then were irradiated under UV light (254 nm, 70 μW/cm^2) for 30 min. Following their sterilization, the nanofiber membranes were placed at the bottom of the culture plate covering the goose breast samples. Next, the culture medium plate was stored at 4 °C for 0, 2, 4, 6, 8, and 10 days. Meanwhile, the unpacked goose breast was used as blank control.

2.7.1. The determination of color

Colorimetric measurements of cooled goose breasts for each group were carried out using handheld colorimeter (NR110, 3nh Global, Shenzhen, China). After the black and white buttons of the instrument were calibrated, 5 points (the different corners and the center of the cooled goose breast) of each sample were selected, and then the L*, a*, and b* values of goose breast were calculated.

2.7.2. The rate of weight loss

The rate of weight loss of pork was determined by reference to the method (Hu et al., 2022) and the rate of weight loss of goose meat was determined as followed:

$$\text{Weight loss } (\%) = \frac{W_0 - W_t}{W_0} \times 100 \tag{3}$$

W_0: the initial weight of the goose meat sample, W_t: the weight of pork sampled at a specific time. Triplicates were performed for each goose meat sample.

2.7.3. Thiobarbituric acids (TBARS)

For the TBARS analysis, 1.0 g goose breast was added in 10 mL of 7.5% trichloroacetic acid (TCA) solution and homogenized at 10000 rpm for 1.0 min, then vibrated for 30 min. Next, the mixture was filtered with double-layer filter paper. Supernatant was collected and around 5.0 mL of supernatant was mixed with 0.02 mol L^{-1} of thiobarbituric acid solution (TBA) and incubated at 90 °C for 40 min. After the reaction was completed, the system stewed 1.0 h with 5.0 mL trichloromethane, then centrifuged at 3000 rpm for 5.0 min and the supernatant was collected. The absorbance of the supernatant was measured at 530 nm.

$$\mathrm{TBARS}(\mathrm{mg}/\mathrm{kg}) = \frac{A_{530nm}}{m} \times 9.48 \tag{4}$$

2.7.4. pH

The goose breast was mixed with distilled water with a ratio of 1:10 (w/v), then homogenized at 5000 rpm for 0.5 min. The system was stewed for 10 min and then the pH measurements were done using the pH meter.

2.7.5. Total volatile basic nitrogen (TVB-N)

The first step involved adding 2.0 g of goose breasts to 30 mL of deionized water and homogenizing them at a speed of 10000 rpm for 1.0 min and vibrating evenly for 30 min. After the vibration, the mixture was filtered by the two layers of gauze, and the filtrate was collected. Secondly, the 10 mL of filtrate was mixed with 10 mL (1.0%) magnesium

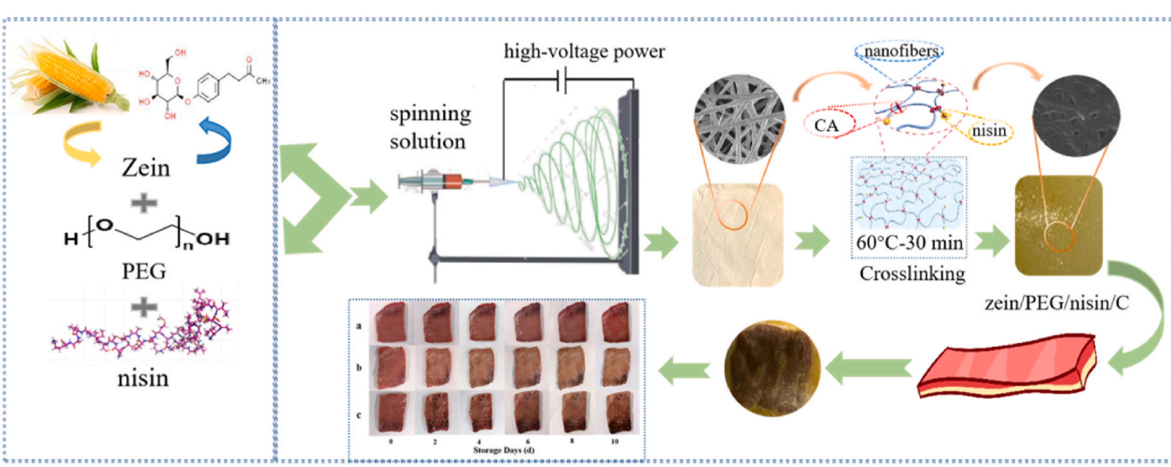

Fig. 1. Synthesis of Zein/PEG/nisin/C composite nanofiber membrane and illustration of goose breast preservation.

oxide solution and 10 mL magnesium oxide solution, and then the system was added to the reaction tube, which was connected to the distillation device. A 50 mL conical flask was filled with 20 mL of 2% boric acid solution and 2.0 drops of indicator, and the end of the condenser tube of the Kjeldahl nitrogen analyzer was immersed in this solution then the whole system was distilled for 2.0 min and the distillate was collected. Finally, the above distillate was titrated by the 0.01 mol L^{-1} hydrochloric acid standard solution until the color of the system changed from blue-green to grayish-red, which was regarded as the endpoint.

2.7.6. Total viable count (TVC)

A sample of 1.0 g goose breast meat was crushed and mixed with 10 mL physiological saline and homogenized at a speed of 10000 rpm for 1.0 min. After diluting the above mixture with the gradient ten times it was inoculated at 37 °C for 24 h and the number of colonies (in log CFU/g) was counted; meanwhile, three parallels were made.

2.8. Statistical analysis

In this study, all of the data were analyzed by Software Origin-Lab, version 8.5. The experimental errors were solved by recording triplicate values and he significance of the statistical difference between means was determined through variance analysis (one-way ANOVA). Differences were supposed to be statistically significant as $p < 0.05$.

3. Results & discussion

3.1. Structural characterization

3.1.1. Scanning electron microscope (SEM)

SEM was used to explore the morphology of the nanofiber membranes ad the images are displayed in Fig. 2. It can be seen that the pure zein nanofiber is slender and the degree of thickness is uneven. As zein/PEG nanofibers are arranged in an increasingly chaotic manner after being modified with PEG, the network structure is tighter due to the strengthening of intermolecular forces. Furthermore, the uniformity and smoothness of the zein/PEG composite membrane surface can be attributed to the suitable dissolution of zein and PEG in ethanol. The structure of zein/PEG/nisin nanofibers are arranged neatly and distributed uniformly without any small ball joints or spindle-shaped phenomenon, indicating that the addition of nisin has almost no effect on the structure of the nanofibers. The surface of the zein/PEG/nisin/C composite membranes after crosslinking treatment is flat, uniform, and smooth.

3.1.2. Fourier transform infrared spectroscopy (FTIR)

Composite nanofibers containing zein, zein/PEG, zein/PEG/nisin and zein/PEG/nisin/C were analyzed using FTIR and the results are presented in Fig. 3. The absorption band at 3275 cm^{-1} was attributed to -OH and -NH stretching vibration, while C-H stretching vibration were responsible for the absorption peaks around 3075 cm^{-1} and 2959 cm^{-1}.

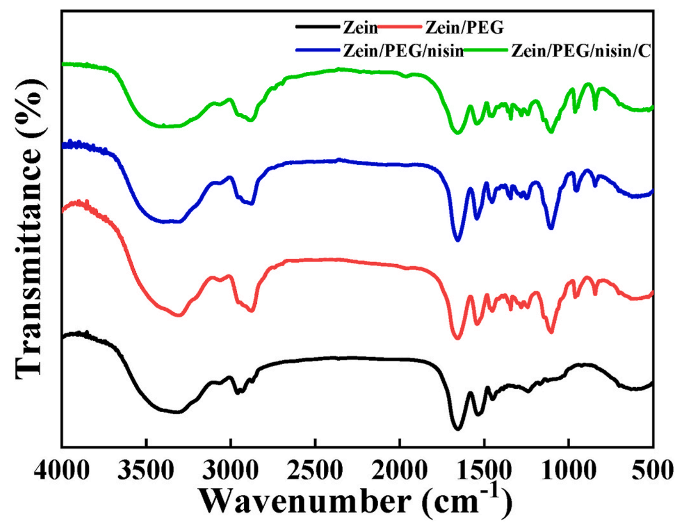

Fig. 3. FTIR images of different kinds of composite nanofiber membranes.

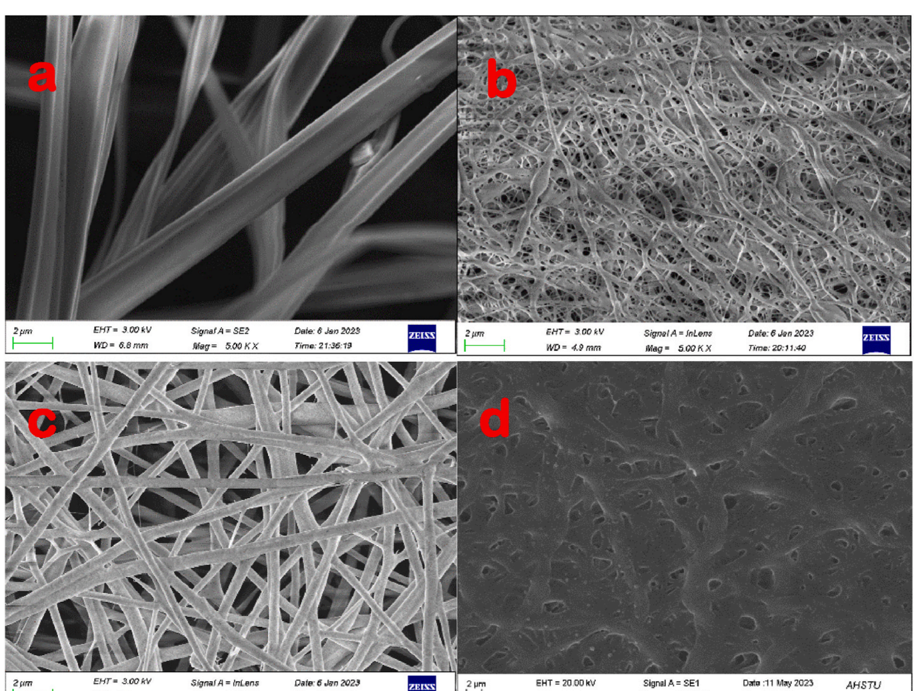

Fig. 2. SEM images of nanofiber membranes with different treatment. The pure zein nanofiber (a), zein/PEG nanofiber (b), zein/PEG/nisin nanofiber (c), zein/PEG/nisin/C nanofiber (d).

The stretching vibrations absorption peak of amide I, C-O, and C-N were at 1631 cm^{-1}. Meanwhile the stretching vibrations absorption peak of amide II, N-H bending, and C-H were at 1532 cm^{-1}, and the amide III was at 1240 cm^{-1}. According to literature, Zein exhibits the stretching vibration absorption peak of O-H bonds at 3275 cm^{-1} (Wang et al., 2019). When PEG, nisin, and crosslinking were added, the O-H bond absorption peak shifted slightly under the above treatment and was seen at 3280 cm^{-1}, 3285 cm^{-1}, and 3291 cm^{-1}, respectively, indicating that the hydrogen bonding between the composite membranes gradually enhanced.

3.1.3. X-ray diffraction (XRD)

XRD pattern is used to categorized the atomic and molecular structure of different materials (Garavand et al., 2017). The crystal structure of different nanofiber membranes is shown in Fig. 4. Typical diffraction peaks of pure zein nanofiber appeared around at $2\theta = 9°$ and $2\theta = 20°$. Compared with pure zein nanofiber, the addition of PEG causes to increase the crystallinity of zein/PEG composite membrane and the diffraction peaks were at $2\theta = 18°$ and $2\theta = 22°$, respectively. This is due to the intermolecular interaction caused by the hydrogen bonding between polyethylene glycol and zein. Compared with the zein/PEG composite nanofiber membrane, the original crystal structure of the zein/PEG composite nanofiber membrane was not changed; however, the new characteristic diffraction peaks appeared at $2\theta = 30°$, which was mainly caused by the addition of nisin (Zheng et al., 2019). The original diffraction peaks of zein/PEG/nisin/C nanofiber membrane did not show a significant change with the crosslinking treatment. Still, the diffraction peaks of zein/PEG/nisin/C nanofiber membrane appeared at $2\theta = 44°$, indicating that the crosslinking treatment strengthened the molecular interactions. To sum up, the results indicate that the composite of zein, PEG, and nisin generated strong molecular interactions with good compatibility.

3.1.4. Thermogravimetric analysis (TGA)

TGA was used to access the thermal stability of zein, zein/PEG, zein/PEG/nisin, and zein/PEG/nisin/C nanofiber materials. The results are shown in Fig. 5, and the process of weight loss for pure zein nanofiber was mainly divided into two stages. The first stage temperatures ranged from 50 °C to 120 °C, primarily due to the evaporation of water contained in nanofibers. In the second stage, the temperature was ranged between 210 °C and 380 °C, which caused the decomposition of high polymers in the nanofiber membranes. Meanwhile, compared with pure zein nanofiber as the bio-compality PEG added, the weight loss was decreased at the range of 210 °C and 380 °C. Moreover, the nisin contained nanofiber membrane did not exhibit significant difference in its thermal stability. However, the thermal stability of the zein/PEG/nisin/C nanofiber membrane increased with the treatment of cross-linking was evident it is mainly because that the structure of zein/PEG/nisin/C nanofiber membrane was more compact than others so that both of the initial degradation temperature and the ending degradation temperature were shifted to higher side were about 100 °C and 400 °C, respectively. The analysis of microstructural (Fig. 2 (a and d)) showed the fact that the cross-linking owned the ability to create more confined and compact network structures (Garavand et al., 2017).

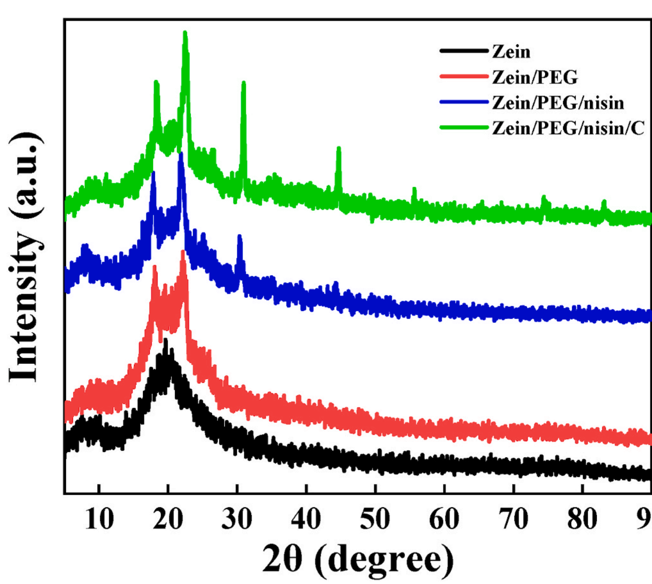

Fig. 4. XRD images of different kinds of nanofiber membranes.

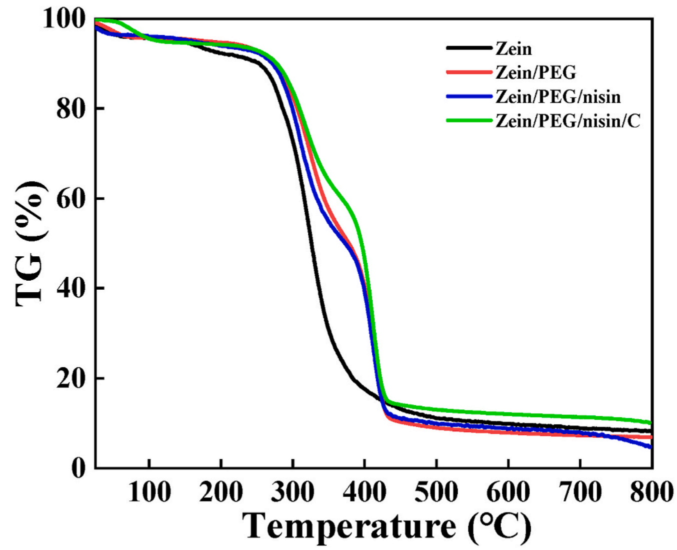

Fig. 5. Thermogravimetric analysis of different kinds of nanofiber membranes.

3.2. Physical properties of the composite membranes

According to Table 1, the addition of PEG and crosslinking treatment reduced the WVP and OP of the composite nanofiber membranes. The WVP and OP of zein/PEG/nisin and zein/PEG/nisin/C composite nanofiber membranes were 259.46 (g/m^2 24h), 178.37 (cm^3/m^2 24h) and 150.47 (g/m^2 24h), 59.74 (cm^3/m^2 24h), respectively. This may be due to the interaction between PEG and the zein matrix, making the network structure more obvious. Additionally, by adding citric acid as a crosslinking agent, the network structure was strengthened, resulting in stronger intermolecular forces and stronger barrier properties of the composite nanofiber membranes (Garavand et al., 2017). The composite membrane's thickness is generally affected by the way the membranes are prepared and the drying environment. As shown in Table 1, the thickness of pure zein membrane, zein/PEG composite membrane, and zein/PEG/nisin composite membrane are 0.24 ± 0.04 mm, 0.22 ± 0.06 mm, 0.24 ± 0.02 mm and not changed as the PEG and nisin added. However, the thickness of the zein/PEG/nisin/C composite membrane is 0.18 ± 0.04 mm; compared with the above membranes, the treatment of crosslinking produced a thinner membrane.

Table 1
The water resistance of different nanofiber membranes.

Membranes	WVP g/m^2·24h	OP cm^3/m^2·24h	Thickness (mm)
zein	299.11 ± 5.25	235.16 ± 7.13	0.24 ± 0.04
zein/PEG	271.89 ± 9.13	186.76 ± 5.14	0.22 ± 0.06
zein/PEG/nisin	259.46 ± 6.10	178.37 ± 6.11	0.24 ± 0.02
zein/PEG/nisin/C	150.47 ± 7.14	59.74 ± 3.10	0.18 ± 0.04

3.3. Antibacterial activity

By employing antibacterial zone measurements, the antibacterial activity of zein, zein/PEG, zein/PEG/nisin, and zein/PEG/nisin/C composite nanofiber membranes against *Staphylococcus aureus* and *Escherichia coli* were evaluated. As shown in Fig. 6, the antibacterial zone diameters of zein/PEG and zein/PEG/C nanofiber membranes against *Staphylococcus aureus* and *Escherichia coli* were almost 0 mm. However, from Table 2, the antibacterial zone diameters of zein/PEG/nisin and zein/PEG/nisin/C nanofiber membranes against *Escherichia coli* were 7.91 ± 0.35 mm and 84 ± 0.19 mm. In addition, the antibacterial zone diameters of zein/PEG/nisin and zein/PEG/nisin/C nanofiber membranes against *Staphylococcus aureus* were 11.52 ± 1.20 mm and 10.67 ± 0.26 mm, respectively. Due to electrostatic and hydrophobic interactions between the nisin and bacteria membrane, the cell contents were revealed and caused cell death (Martínez et al., 2020). The zein/PEG/nisin/C composite nanofiber owned the best antibacterial ability against *Staphylococcus aureus* and *Escherichia coli* compared with other groups after being cross-linked, the thickness being thinner which led to the nisin being more easily released.

Table 2
Different membranes composite membrane antibacterial circle diameters.

Membranes	The antibacterial zone diameters against *Escherichia coli*/mm	The antibacterial zone diameters against *Staphylococcus aureus*/mm
zein	–	–
zein/PEG	–	–
zein/PEG/C	–	–
zein/PEG/nisin	7.91 ± 0.35	7.84 ± 0.19
zein/PEG/nisin/C	11.52 ± 1.20	10.67 ± 0.26

3.4. Application

3.4.1. The color of cooled goose breast

The surface color of cooled goose breast is an important indicator to measure freshness (Bassey et al., 2021). As shown in Fig. 7 and Table 3, the L* values of all three groups decreased with the extension of refrigeration time. The experiment groups saw an overall declining trend, but the control group's a* value increased after first decreasing, and all groups' b* values indicate an overall rising tendency. Compared with the control group and zein/PEG/C group, the declining rate of the L* value of the zein/PEG/nisin/C group reduced effectively. The color change of cooled goose breast during refrigeration is related to hemoglobin oxidation. The gradual oxidation of hemoglobin is to methemoglobin (Cao et al., 2019), due to bacterial contamination and fat oxidation of the chilled goose breast is the reason for the increase in the a* value. It indicates that the nanofiber membrane isolates oxygen and prevents hemoglobin from being oxidized. Meanwhile, the b* value change of the zein/PEG/nisin/C group is minimal, mainly due to the nisin-loaded, which protects the meat from being contaminated by bacteria.

3.4.2. The rate of weight loss

In general, meat weight loss was caused by the hydrolysis of proteins and the evaporation of water. Marcinkowska-Lesiak et al. (2021). As shown in Fig. 8., the loss rate of meat juice of cooled goose breast packed with zein/PEG/C and zein/PEG/nisin/C nanofiber membranes were 9.4% and 6.2%, respectively, while the loss rates of the control group without composite nanofiber membranes was 11.8%, which was significantly higher than other groups. Because the molecules in the composite membranes were firmly bound together by hydrogen bonds; the water vapor permeability of the membranes was decreased and the amount of water migration between membranes and samples was limited. This could be the case because the structure of the zein/PEG/C and zein/PEG/nisin/C composite nanofiber membranes was ordered and dense in comparison to the pure zein nanofiber. The composite nanofiber membrane loaded with nisin had a good antibacterial performance that reduced the life activity of microorganisms, so it can effectively slow down the loss rate of meat juice and protect the quality of the cooled goose breast. It is consistent that zein/PEG/nisin/C composite membranes own good properties of antibacterial that can stop bacteria y so that keep it has strong weight retention Zhao et al. (2019).

3.4.3. The value of pH and the content of TBARS, TVB-N, TVC

One of the most important markers of the quality and freshness of refrigerated goose breast meat is the pH value (Pan et al., 2019). As shown in Fig. 9a, there was a certain decrease in pH value at the first two days. This was mainly because of the activity of acid-producing microbes in the refrigerated goose breast meat during the initial procedure of storage. The pH value of the samples increased when the refrigerated time was extended due to the build-up of alkaline chemicals created by the breakdown of protein by bacteria or endogenous enzymes, and an upward tendency was displayed. The pH value of refrigerated goose breast meat packed with zein/PEG/nisin/C nanofiber membranes was 5.7, which was increased slower than that of zein/PEG/C nanofiber membrane group and the control group as the storage time ranged from 4 days to 10 days and it was consistent with previous studies on pH of fresh pork (Xiong et al., 2019).The result indicated that the

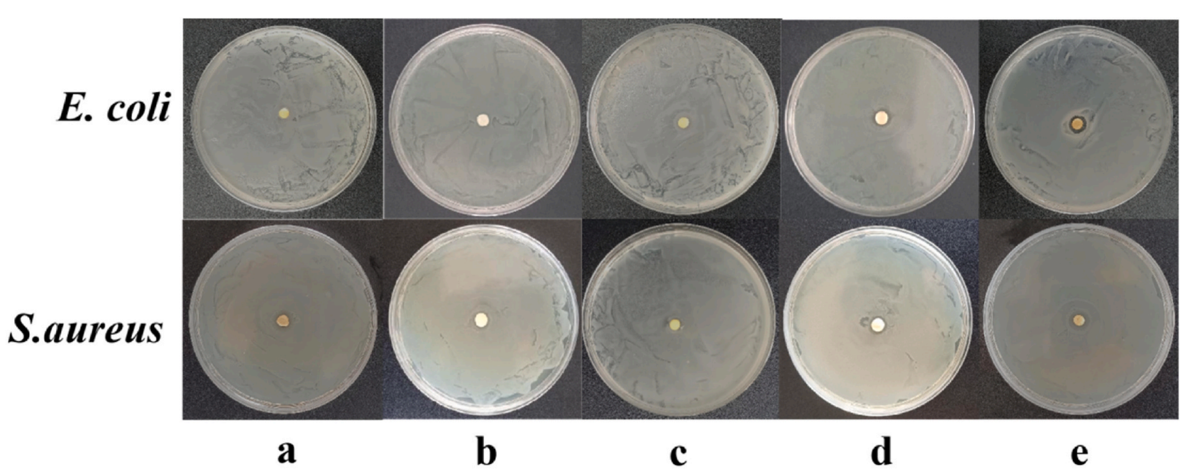

Fig. 6. The bacteriostatic zone of different antibacterial nanofiber membranes. Pure zein nanofiber (a), zein/PEG nanofiber (b), zein/PEG/C nanofiber (c), zein/PEG/nisin nanofiber (d) and zein/PEG/nisin/C nanofiber (e).

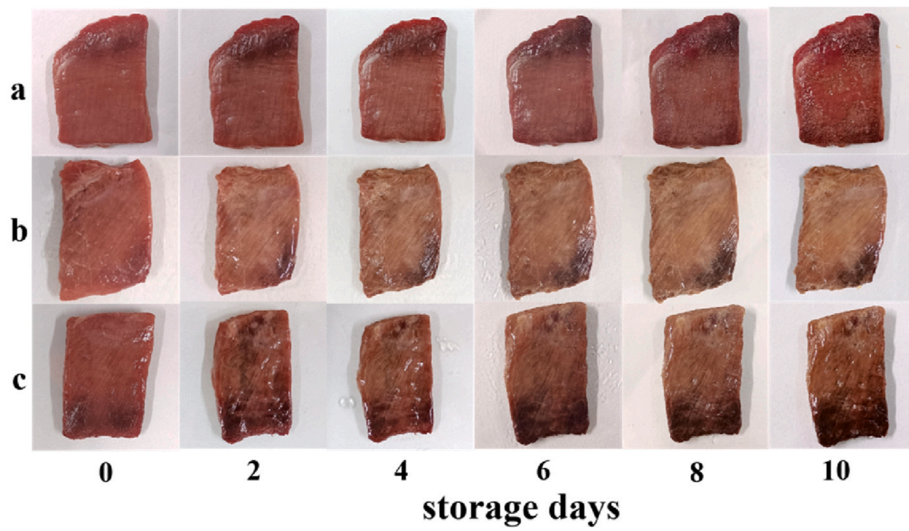

Fig. 7. Color change of cooled goose breast during storage. Without packaging (a, control) and packaged with zein/PEG/nisin (b) and zein/PEG/nisin/C (c) were stored at 4 °C for 0, 2, 4, 6, 8, and 10 d.

Table 3
The L*, a*, and b* values of cooled goose breast of the control group, the zein/PEG/C membrane, and the zein/PEG/nisin/C membrane at different storage times.

Groups		0 d	2 d	4 d	6 d	8 d	10 d
(L*)	control group	37.22 ± 0.86^{a}	36.87 ± 0.80^{a}	36.59 ± 0.60^{ab}	35.21 ± 0.54^{b}	33.36 ± 0.62^{c}	33.77 ± 1.71^{c}
	zein/PEG/C	39.55 ± 0.62^{a}	38.66 ± 0.84^{b}	37.06 ± 0.73^{c}	36.73 ± 1.04^{d}	35.52 ± 0.68^{d}	35.16 ± 0.87^{d}
	zein/PEG/nisin/C	37.62 ± 1.72^{a}	37.12 ± 0.65^{ab}	36.65 ± 1.21^{bc}	36.12 ± 0.76^{bc}	35.71 ± 0.69^{cd}	35.43 ± 0.82^{d}
(a*)	control group	12.63 ± 0.52^{a}	11.60 ± 0.46^{b}	10.88 ± 0.52^{b}	10.68 ± 0.48^{b}	11.15 ± 0.39^{b}	13.10 ± 0.47^{a}
	zein/PEG/C	12.86 ± 0.48^{a}	11.85 ± 0.51^{b}	11.15 ± 0.56^{bc}	10.01 ± 0.49^{c}	9.77 ± 0.52^{d}	9.28 ± 0.48^{d}
	zein/PEG/nisin/C	12.73 ± 0.37^{a}	11.34 ± 0.6^{b}	10.37 ± 0.54^{c}	10.34 ± 0.49^{c}	9.57 ± 0.53^{d}	9.47 ± 0.48^{d}
(b*)	control group	7.54 ± 0.32^{a}	7.65 ± 0.39^{b}	7.75 ± 0.29^{a}	7.89 ± 0.34^{c}	8.11 ± 0.4^{b}	8.39 ± 0.27^{ab}
	zein/PEG/C	7.55 ± 0.29^{b}	8.92 ± 0.27^{ab}	9.11 ± 0.31^{ab}	9.42 ± 0.27^{a}	9.44 ± 0.3^{b}	9.86 ± 0.21^{b}
	zein/PEG/nisin/C	7.69 ± 0.32^{c}	8.44 ± 0.29^{bc}	8.71 ± 0.26^{ab}	9.18 ± 0.31^{ab}	9.33 ± 0.37^{ab}	9.53 ± 0.28^{c}

Data is presented as mean SD (n = 3). Significantly different results ($p < 0.05$) are indicated by different letters in the same column.

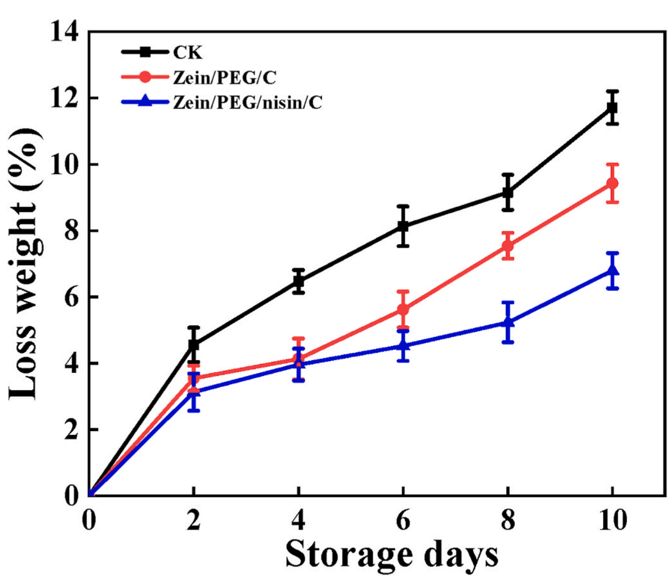

Fig. 8. Changes in juice loss rate of cooled goose breast stored for different days.

measurement of zein/PEG/nisin/C nanofiber membrane effectively inhibited the growth and reproduction of spoilage bacteria mainly because of the excellent antibacterial performance of nisin.

TBARS value indicates the degree of oxidation and rancidity of fat in meat (Wang et al., 2018). As shown in Fig. 9b the TBARS value of the zein/PEG/C group and zein/PEG/nisin/C group are both lower than the control group, because the degree of oxidation increased as more oxygen participated in oxidation reactions with the refrigerated goose breast meat in whole storage period (Hu et al., 2022). In contrast, the TBARS value of the control group increased significantly and exceeded 1.0 mg kg^{-1} at the end of storage. At the end of storage, the increasing trend of the TBARS value in the zein/PEG/nisin/C group remained stable with the lowest value, which is 0.83 mg kg^{-1}. The result indicates that the zein/PEG/nisin/C composite membrane has a certain barrier performance, limiting oxygen contact with meat samples and slowing down the degree of fat oxidation. At the same time, the nanofiber membrane loaded with nisin releases slowly, which effectively inhibits the growth of spoilage bacteria and prolongs their shelf life and it was consistent with the results of Hu et al. (2022).

TVB-N, a type of volatile alkaline nitrogen-containing material derived from protein breakdown, functions as an important meat spoilage indicator and is used to evaluate the freshness of goose breast meat during storage (Wang et al., 2021). During the process of storage, the concentration of TVB-N for the whole experimental group increased due to the decomposition of proteins from spoilage bacteria (Ruan et al., 2019). As shown in Fig. 9c, the TVB-N of the control group increased significantly with the increasing storage time, which is 180.48 mg kg^{-1} and exceeded the acceptable upper limit of TVB-N in Chinese National Standard GB 2707–2016 is 150 mg kg^{-1} during the 8th day of storage (Zhao et al., 2019). On the other hand, compared to the control group and the zein/PEG/C group, the TVB-N of chilled goose breast meat packaged with nisin/C nanofiber membranes grew more slowly. After the 10th day of storage, the TVB-N content of zein/PEG/nisin/C

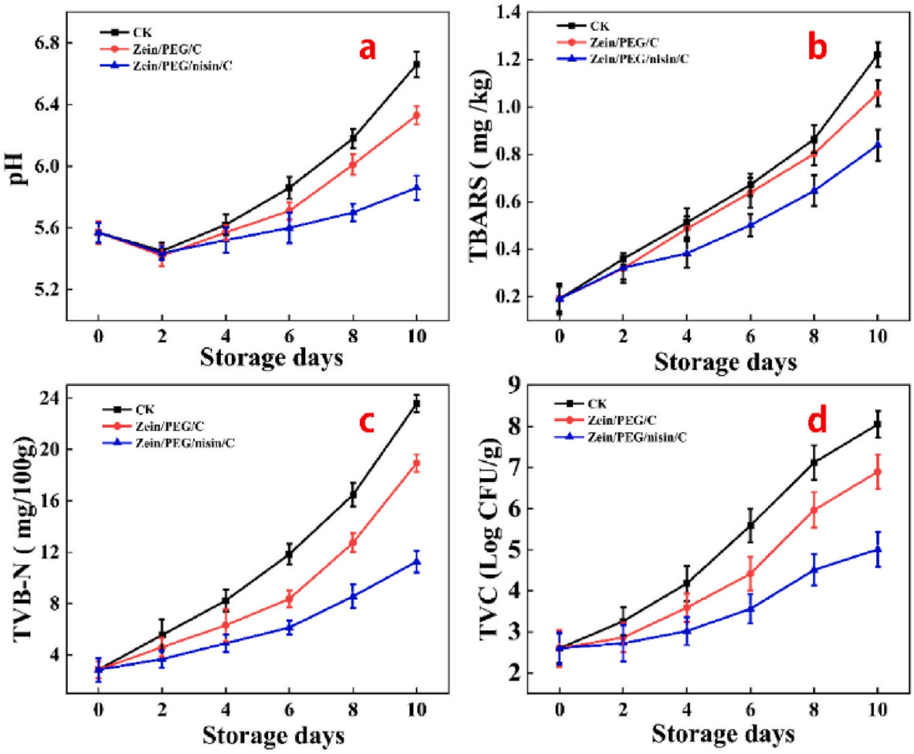

Fig. 9. (a) Changes in pH value of cooled goose breast stored for different days, **(b)** Changes in TBARS values of cooled goose breast stored for different days, **(c)** Changes in TVB-N values of goose breast meat stored for different days, **(d)** Changes in TVC of cooled goose breast stored for different days.

nanofiber membrane was 110.28 mg kg^{-1}. The rate of increase of TVB-N in the zein/PEG/C group and control group was lower than that in the zein/PEG/nisin/C group, which may be due to the composite nanofiber membranes were used to absorb water, and the slow releasing of biological activity inhibited microbial reproduction and delayed the spoilage of refrigerated goose meat.

As shown in Fig. 9d, the number of TVC of the control group significantly increased with the increase of storage and arrived at 7.31 $\pm$ 0.52 (log CFU g^{-1}) after the 8th day of storage, which corresponded to the TVB-N results. When compared to the control group and the zein/PEG/C group, the TVC value of refrigerated goose breast packaged with zein/PEG/nisin/C nanofiber membrane loaded with antibacterial agents was significantly lower. At the end of 10 days of storage, the TVC value of the control group, zein/PEG/C and zein/PEG/nisin/C nanofiber membranes were 8.52 (log CFU g^{-1}), 6.89 (log CFU g^{-1}), and 5.01 (log CFU g^{-1}), respectively. According to Cao et al. (2019), the TVC level should be lower than 7.0 (lg CFU g^{-1}). The results showed that the antibacterial action of nisin was well-executed by the zein/PEG/nisin/C nanofiber membrane loaded nisin, and that the preserved goose breast flesh in the refrigerator was noticeably superior to that of other groups.

4. Conclusion

Thus, in this research, nisin was encapsulated into the zein/PEG and zein/PEG/C nanofiber membrane with the citric acid vapor of electrospinning technique. According to the literature survey, this is the first work on the successful building-up of nisin to develop a zein/PEG/nisin/C active membrane. The findings of this study showed that, compared with other nanofiber membranes, the crosslinking-modified nanofiber membranes exhibited a denser microstructure. Meanwhile, with the addition of nisin the antimicrobial activity against both *E. coli* and *S. aureus* effectively suppressing their growth and proliferation. Results of application preservation exhibited that compared with the control group, the pH of zein/PEG/nisin/C nanofiber membrane was 5.7 and the concentration of TVB-N and the value of TVC and TBARS are 11.28 mg/100g, 5.01 $\pm$ 0.69 log (CFU g^{-1}), and 0.83 mg kg^{-1}, respectively, which were the lowest at the end of storage during 10 days of the storage of cooled fresh goose meat. Hence, serving as an eco-friendly, biodegradable packaging material the zein/PEG-based nanofilm has broad application prospects in food packaging applications.

CRediT authorship contribution statement

Lanlan Wei: performed the research, analyzed the data, wrote the manuscript, and designed the experiments. **Shuaijie Zhu:** performed the research, analyzed the data, wrote the manuscript, and designed the experiments. **Guoyuan Xiong:** analyzed and interpreted the data and participated in preparing samples, All authors have read and agreed to the published version of the manuscript. **Jingjun Li:** conceived the research. **Wangang Zhang:** conceived the research.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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