



## Research Article

**Shelf life extension of chicken fillet using gelatin-nanochitosan film enriched with cumin essential oil during refrigerated storage**

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**Abstract**

One of the greatest challenges in food industry is the loss of quality of food products during storage, especially perishable foods such as meat and poultry products. Edible films are a potential alternative for maintaining food quality and enhancing shelf life by delaying microbial spoilage. In this study, the combined effect of different concentrations of *Cuminum cyminum* essential oil (CEO; 0, 0.3, 0.6, and 0.9%) and gelatin-nanochitosan film on chicken meat packaging was investigated during 12 days of refrigerated storage. The results showed that the addition of CEO to the gelatin-nanochitosan significantly influenced the thickness, tensile strength, and elongation at break ( $p < 0.05$ ). The results indicate that the use of gelatin-nanochitosan films containing 0.6% and 0.9% CEO had a significant inhibitory effect on total bacterial count, coliforms, psychrophilic, and lactic acid bacteria. Based on the results of the application of gelatin-nanochitosan film with 0.6% and 0.9% CEO to chicken fillets, the quality of chicken fillets could be maintained during storage without any adverse sensory effects.

**Keywords:** Active packaging, Cumin essential oil, Gelatin nanochitosan film, Poultry products, Shelf life.

**Introduction**

Chicken meat is a good source of protein, essential amino acids, and unsaturated fatty acids. Chicken meat has very low microbial and oxidative stability and is easily exposed to microbial and chemical spoilage during processing and storage. The most common pathogens that contaminate chicken meat are *Salmonella* spp., *Campylobacter jejuni*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7, which can cause severe foodborne diseases (Safari et al., 2023). Therefore, improving the storage life of fresh meat is an important challenge in the meat industry today. In this regard, the use of biopolymer-based films is proposed as an important solution owing to

their barrier, mechanical, optical, and biodegradability characteristics.

Edible films and coatings are novel and efficient approaches for improving the shelf life of meat and meat products. Several proteins and polysaccharides have been used to make films, including gelatin, whey protein, alginate, and chitosan (Yaghoubi et al., 2021). Gelatin is a water-soluble protein derived from collagen via controlled hydrolysis at high temperatures in the presence of water. Gelatin is one of the most practical coating materials because of its low permeability to oil, aroma, and gas, and its appropriate gelling and film-forming properties (Yu et al., 2019; Mohammadian et al., 2020).

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Furthermore, the active properties of polysaccharides enable the film to release antioxidants and antimicrobial agents into packaged food. Chitosan is a polycationic polysaccharide obtained from chitin via deacetylation. Chitosan is recognized as Generally Recognized as Safe (GRAS) by the FDA. Chitosan has a unique film-forming ability, low gas permeability, good mechanical properties, and nontoxicity. In particular, because it possesses strong antioxidant and antimicrobial activities against a wide range of microorganisms, it is applied in meat and meat products (Zhang et al., 2020). Different reports have revealed indirect antimicrobial activity and shelf life enhancement of gelatin and chitosan in various food products because of their role in reducing water activity (Ranjbar & Azizi, 2017). The effectiveness of such combinations can be remarkably increased by incorporating antimicrobial and antioxidant compounds into packaging.

Hence, researchers have widely focused on developing natural additives and preservatives, such as bacteriocins, essential oils, and plant extracts, using direct addition or active packaging methods (Qiu et al., 2022). The use of plant, herb, and spice extracts and Essential oils has gained popularity in recent years for creating edible films with natural antioxidant and antimicrobial properties (Zhang et al., 2023) such as ginger (Noori et al., 2018), clove (Mulla et al., 2017), rosemary (Sirocchi et al., 2017), basil, and thyme (Sharma et al., 2017).

Recently, several essential oils have been employed as promising natural preservatives in the packaging of chicken meat. Cumin (*Cuminum cyminum*) is a well-known herb due to its nutritional value and functional and pharmaceutical properties, such as antimicrobial, anti-inflammatory, anticancer, antihypertensive, and antidiabetic properties (Ravi et al., 2013). Cumin is used as a digestive stimulant by increasing bile acid production and excretion and enhancing digestive enzymes (amylase, trypsin, chymotrypsin, and lipase) in the pancreas. Cumin essential oil (CEO) contains bioactive compounds such as cuminaldehyde, terpinenes, carvone, D-limonene, polyphenols, and flavonoids. Many researchers have indicated that CEO significantly reduces the growth of some foodborne pathogens (Gotmare & Tambe, 2018).

The combination of gelatin, chitosan, and essential oils create an active packaging film that offers several advantages. First, the film is biodegradable and environmentally friendly, addressing the growing concerns regarding plastic waste. It can be easily disposed of without harming the environment, unlike traditional plastic films, which take hundreds of years to decompose. Second, the film exhibits excellent mechanical and barrier properties, which help preserve the freshness and quality of packaged food. The film also prevents the degradation of sensitive food components, maintains its nutritional value and sensory attributes, and prolongs the shelf life of food products. (Ghaderi et al., 2024). The aim of this study was to investigate the effect of a gelatin-nanochitosan film containing CEO on the microbial, chemical, and organoleptic properties of chicken meat under refrigeration as a form of active packaging.

## Materials and Methods

### Essential oil extraction and analysis

*C. cyminum* L. (Linnean society of London Herbarium No. LIMN-HS511.1) was collected from Kerman province of Iran at flowering stage of plant in June 2024 and identified by Institute of Medicinal Plants of Tehran University, Tehran, Iran. First, the dried cumin plant was crushed and transferred for steam distillation to a Clevenger-type apparatus (Corning, Mexico) for 4h. The obtained essential oil was stored in a dark glass tube under refrigeration ( $4 \pm 1$  °C).

The components of the essential oil were analyzed by gas chromatography-mass spectrometry (GC-MS) (Agilent 6890N, USA), equipped with a silica capillary column (30 mm × 0.25 mm inner diameter; film thickness of 0.25 µm) and coated with DB-5. The MS was scanned in electron impact mode using an ionization energy of 70e-V. The Retention Index (RI) for each of the separated materials was calculated based on the extraction time, and the identification of the major components of the essential oil was confirmed by comparing their relative Kovats RI and mass spectra with the standard of the National Institute of Standards and Technology (NIST 05) mass spectral library and those reported in the literature (Kasaiyan et al., 2022).

### Preparation of gelatin/nano chitosan/CEO films

To create the nanocomposite film solution, an aqueous solution of acetic acid (1% v/v) was used to dissolve (2% w/v) nano-chitosan and (4 % w/v) gelatin. Next, glycerol (1% v/v) was added to the solution as a plasticizer and magnetically stirred on a magnetic stirrer at 50 °C for 2 h. Different concentrations of CEO (0, 0.3, 0.6, and 0.9 % v/v) were incorporated into the final solution. The mixtures were homogenized at 12000 rpm for 2 min. Following the, films were formed and dried in 12 cm diameter glass Petri dishes at room temperature (25 °C) and allowed to evaporate and create a film for 48 h at room temperature. (Kasaiyan et al., 2022). The treatments were named as follows.

C0: G/NanoCh; T<sub>1</sub>: G/NanoCh/ 0.3% EO; T<sub>2</sub>: G/NanoCh/ 0.6% EO; T<sub>3</sub>: G/NanoCh/ 0.9% EO

### Preparation of chicken fillets with different film treatments

Fresh chicken fillets (50 g) were placed between two layers of each film in a sterile Petri dish and stored in a refrigerator at 4 °C for 12 days. Each specimen was tested in triplicate (Takma & Korel 2019).

### Characterization of the films' properties

#### Thickness

The thickness of the films was measured using a digital micrometer (Comecta Electronic Digital Micrometer, Cod. 5900602, Spain) with a precision of 0.001 mm was used. The measurements were performed in triplicate (Takma & Korel, 2019).

#### Tensile strength

Tensile strength and elongation at break analyses were performed using a universal testing machine (UTM). The test films were cut into 10 mm wide and 80 mm long strips, and the initial gauge length and speed were fixed at 10 mm/min, and the load cell was 5 kg. The tensile strength ( $\sigma$ ; TS) and elongation at break (EAB) were determined using the following equation (Azizah et al., 2023):

$$\sigma = F/A$$

$$E = F L_0 / A \Delta L$$

where F is the force exerted on an object under tension,  $L_0$ : Original length, A is the cross-sectional area, and  $\Delta L$  is the change in length of the object.

$$EAB = \frac{L_f - L_i}{L_i} \times 100$$

where,  $L_f$  = final length at break;  $L_i$  = initial length of the film.

#### Antibacterial activity

The disk diffusion method was used to assess the sensitivity of microbes to the gelatin-nanochitosan film. First, some amount of fresh culture of standard bacteria was dissolved in the physiological serum until reaching the 0.5 McFarland turbidity, and the suspension was cultured using a sterile swab on the surface of Muller Hinton Agar completely. Discs from films (with and without CEO) with a diameter of 10 mm were placed on the medium to form the inhibition zone. After incubation at 37 °C for/18 h, the inhibition zone was measured and interpreted following the guidelines (CLSI, 2022).

### Physicochemical and microbial analysis of chicken fillets

#### Determination of pH

Chicken samples (10 g) were homogenized with 100 ml of distilled water for 1 min, and the pH of the sample was measured using a pH meter (Seven Compact, Mettler Toledo, USA), (Ghaderi et al., 2024).

#### Total protein and fat analysis

The total protein and fat content of the samples were evaluated using the Kjeldahl and Soxhlet methods, respectively, as described by the AOAC (AOAC,1995).

#### The 2-thiobarbituric acid assay

The 2-thiobarbituric acid (TBA) assay was used to assess lipid oxidation and was expressed as mg of malondialdehyde (MDA) per kg of chicken meat samples. Meat samples (10 g) were mixed with 50 mL of distilled water, homogenized, and left for 30 min. Then, 20 mL of 20% TBA was added and placed for 10 min at ambient temperature. The samples were filtered, and distilled water was added until the solution level reached 100 mL. The absorbance of

the obtained solution was measured at 532 nm using a spectrophotometer (Ultrospec 2000, Scintec, UK) (Alizadeh-Sani et al., 2020). TBA content was expressed as  $\mu\text{g}$  MDA per gram of chicken meat. The ability to lipid oxidation was calculated using the following equation (Shahvandari et al., 2021):

$$\text{MDA } (\mu\text{g/g}) = A532 \times 12.9$$

#### Determination of peroxide value (PV)

Meat samples (5 g) and 30 mL of acetic acid and chloroform (3:2) were added to 0.5 mL potassium iodide and kept for 1 min. Titration was performed with sodium thiosulfate (0.1 N) until a yellow color appeared, and 0.5 mL of starch solution was added to produce a purple color. The peroxide value was expressed as milliequivalents (meq) of peroxide oxygen per 1 kg of lipids. The peroxide value was calculated using the following equation (Shahvandari et al., 2021):

$$\text{PV} = \frac{(S-B) \times N \times 1000}{W}$$

S: The volume of titrant ( $\text{Na}_2\text{S}_2\text{O}_3$  standard solution) consumed by sample (mL)

B: The volume of titrant ( $\text{Na}_2\text{S}_2\text{O}_3$  standard solution) consumed by control sample (mL)

N: Normality titrant ( $\text{Na}_2\text{S}_2\text{O}_3$ )

W: weight sample (fat extracted, g)

#### Total volatile nitrogen

To measure total volatile nitrogen (TVN), the samples (10 g) were boiled for 25 min with magnesium oxide, and distilled water was added to 2% boric acid, including methyl red and Bromocresol Green as indicators, which was back titrated with 0.1 M sulfuric acid. The control sample did not contain chicken meat. TVN value was calculated using the following equation (Shahvandari et al., 2021):

$$\text{TVN } \left( \frac{\text{mg}}{100\text{g}} \text{ sample} \right) = V(\text{titrant}) \times 14$$

#### Microbial analysis

Chicken meat samples (10 g) were mixed with 90 mL of 0.1% peptone water. Serial dilutions (1:10) were prepared in 0.1% peptone water solution, and appropriate dilutions of homogenates were transferred onto plates. Total mesophilic and psychrophilic bacteria, *Enterobacteriaceae*, lactic acid bacteria (LAB), and *Pseudomonas* spp. were determined using plate count agar, violet red bile lactose agar, de Man Rogosa and Sharpe (MRS) agar, and *Pseudomonas* agar, respectively. Microbiological counts were expressed as logarithms of the number of colony-forming units per gram of sample ( $\text{Log}_{10}$  CFU/g). (Takma & Korel, 2019; Kasaiyan et al., 2022). Microbiological analysis was performed in duplicate.

#### Sensory analysis

For sensory evaluation of the samples, a trained 15-person panel was used. The results were expressed on a 9-point hedonic scale from 9 (highest score) to 1 (lowest score). The assessment was based on the total acceptability (color, taste, odor, and texture) (Shahvandari et al., 2021).

#### Statistical analysis

The data were analyzed using SPSS software (SPSS 22.0, SPSS Inc., Ill., U.S.A.) through analysis of variance (ANOVA).

## Results and Discussion

#### CEO components

The results showed that the major components of CEO were *cuminaldehyde* (28.96%), *beta-terpinylbutanoate* (22.17%),  *$\beta$ -pinene* (14.93%), *p-cymene* (9.40%), and *1,3-cyclohexadiene* (8.51), (**Table 1**). Cuminaldehyde has been reported as the most important component of CEO in several studies (Wanner et al., 2010). In a study, Cuminaldehyde (49.4%), *p-cymene* (17.4%),  *$\beta$ -pinene* (6.3%),  *$\alpha$ -terpinen-7-al* (6.8%),  *$\gamma$ -terpinene* (6.1%), *p-cymen-7-ol* (4.6%) and *thymol* (2.8%) were identified as main components in CEO (Rana, 2014). Gotmare & Tambe (2018) reported that the main components of CEO were cuminaldehyde (74.62%),  *$\gamma$ -Terpinen-7-al* (7.95%), *p-cymene* (6.67%),  *$\alpha$ -Terpinen-7-al*

(3.98%), 4-Hydroxy cryptone (1.68),  $\beta$ -pinene (1.58) and p-Menth-3-en-7-al (1.11%) (Gotmare & Tambe, 2018). In numerous research papers, CEO has been mentioned as having antioxidant, antibacterial, anticancer, and anti-inflammatory properties (Mohammadpour et al., 2012). The difference in the percentage of components of each essential oil, including cumin, in this investigation and other researches can be influenced by the harvest season, drying method, plant age, geographical area of plant growth, etc. However, in numerous articles, including our study, the most important component of cumin essential oil has been reported to be cumin aldehyde.

**Table 1.** The main chemical components of cumin essential oil

Composition	Value (%)
$\alpha$ -Thujene	0.42
$\alpha$ -Pinene	0.94
Sabinene	0.96
$\beta$ -pinene	14.93
Myrcene	0.57
Alpha-phelandrene	0.56
p-cymene	9.40
$\beta$ -phelandrene	0.61
$\beta$ -terpinylbutanoate	22.17
$\gamma$ -terpinene	3.6
Cumin aldehyde	28.96
1,3-cyclohexadiene	8.51
1-phenyl-1,2 ethanediol	6.30
<b>Total compounds</b>	<b>97.93</b>

### Mechanical properties of films

Tensile strength and elongation at break are important mechanical properties for determining the strength and flexibility of a film. The higher the tensile strength, the better the edible film can withstand mechanical damage (Azizah et al. 2023). The mechanical properties, that is, TS and EAB, of

the edible gelatin nanochitosan films with different concentrations of cumin essential oil are shown in **Table 2**. The results indicated that the TS and EAB of the gelatin-chitosan films incorporated with CEO (0.3, 0.6, and 0.9% v/v) increased, probably due to the formation of a denser and more compact film matrix owing to the increase in CEO concentration. This can be attributed to the formation of a firm network in which CEO molecules were homogeneously entangled within the gelatin-nanochitosan molecules (Khah et al., 2021). As shown in **Table 2**, the addition of essential oil increased the TS of the edible films ( $p < 0.05$ ). This indicates that EOs can act as crosslinking agents. Cross-linking occurs because molecules with low molecular weights can more easily enter the gelatin gel network. The intermolecular interactions between gelatin-nanochitosan molecules and CEO resulted in crosslinks between the chains, improving the film properties, which is in accordance with a previous study that reported that essential oils, when interacting with protein films, increased the tensile strength of the film (Nurilmala et al., 2017).

Elongation at break is the maximum length change experienced by the film until it breaks. The EAB of the edible films ranges from 74.13–124.45%, as shown in **Table 2**. The EAB of edible films increased with the addition of essential oil and with increasing essential oil concentration ( $p < 0.05$ ). This is because EO may act as a plasticizer and lead to increased elasticity of the films. EO containing monoterpenes of hydrocarbons interact with protein-polysaccharide chains and reduce peptide bonds, which are useful for stabilizing gelatin-nanochitosan based edible films, thus increasing the stretchability of the films. A previous study also indicated that gelatin films incorporated with essential oils showed an increased EAB (Tongnuanchan et al., 2016).

**Table 2.** Tensile strength (TS) and elongation at break (EAB) of edible films loaded with Cumin essential oil

Sample	TS (MPa)	EAB (%)	Thickness (mm)
GNC	6.35 $\pm$ 0.33 <sup>c</sup>	74.13 $\pm$ 1.86 <sup>d</sup>	0.025 $\pm$ 0.003 <sup>d</sup>
GNC+ 0.3% EO	6.59 $\pm$ 0.74 <sup>c</sup>	88.35 $\pm$ 2.29 <sup>c</sup>	0.058 $\pm$ 0.001 <sup>c</sup>
GNC + 0.6% EO	7.46 $\pm$ 0.55 <sup>b</sup>	113.22 $\pm$ 3.42 <sup>b</sup>	0.077 $\pm$ 0.005 <sup>b</sup>
GNC + 0.9% EO	15.17 $\pm$ 0.83 <sup>a</sup>	124.45 $\pm$ 5.35 <sup>a</sup>	0.01 $\pm$ 0.008 <sup>a</sup>

\*GNC: Gelatin-anochitosan; EO: Cumin essential oil; Average values placed in columns marked with different letters are statistically different ( $P < 0.05$ ).

The thickness of the films gradually increased with increasing essential oil concentration. The thickness

of the gelatin-nanochitosan films increased slightly from 0.025 mm in the control to 0.01 mm at the



maximum concentration of CEO (**Table 2**). The increase in the thickness of the films incorporated with essential oils can be attributed to the increase in solid content (Aitboulahsen et al., 2020).

#### Antibacterial activities of films

The inhibition zones of the gelatin-nanochitosan film incorporated with higher concentrations of CEO for *E. coli*, *L. monocytogenes*, *S. aureus*, and *V. parahaemolyticus* were larger than those of the other microorganisms. The gelatin-nanochitosan film incorporated with CEO showed the strongest

inhibitory effect on *V. parahaemolyticus* (**Table 3**). The mechanism of the antimicrobial component of CEO is a breakdown of the cytoplasmic membrane, which increases its permeability and depolarization, causing cell leakage that can lead to bacteriocidal effects. In addition, CEO can damage cell membranes, encourage cell lysis, and accelerate the release of cell wall autolytic enzymes that induce lysis (Ghanbari et al., 2022). In a study by Kavoosi et al. (2013) using gelatin films with carvacrol, gelatin films alone had low antibacterial activity, while gelatin and carvacrol films had excellent antibacterial properties against both Gram-positive and Gram-negative bacteria.

**Table 3.** Antibacterial activities of different edible films

Sample	Inhibition zone (mm)			
	<i>E. coli</i> O157:H7 (ATCC 18776)	<i>L. monocytogenes</i> (ATCC 87946)	<i>S. aureus</i> (ATCC 36454)	<i>V. parahaemolyticus</i> (ATCC 43996)
GNC	-	9.21 ± 0.37 <sup>a</sup>	9.73 ± 0.97 <sup>a</sup>	12.46 ± 0.38 <sup>a</sup>
GNC+ 1% EO	-	11.33 ± 0.57 <sup>a</sup>	11.66 ± 1.15 <sup>a</sup>	14.33 ± 0.57 <sup>a</sup>
GNC + 2% EO	-	12.66 ± 1.15 <sup>ab</sup>	12.33 ± 0.57 <sup>a</sup>	14.66 ± 0.57 <sup>a</sup>
GNC + 3% EO	-	14.33 ± 0.57 <sup>bc</sup>	13.00 ± 1.00 <sup>a</sup>	16.00 ± 1.00 <sup>a</sup>
GNC + 4% EO	12.00 ± 1.00 <sup>a</sup>	16.00 ± 1.00 <sup>c</sup>	13.33 ± 1.15 <sup>a</sup>	18.67 ± 1.53 <sup>b</sup>

\*GNC: Gelatin-nanochitosan; EO: Cumin essential oil; Average values placed in rows marked with different letters are statistically different ( $P < 0.05$ ).

**Table 4.** Effects of gelatin-nanochitosan films on protein and fat values of refrigerated chicken meats stored at 4 °C for 12 days

Treatments	Chemical analysis	Days	
		0	12
GNC	Protein	20.30 ± 0.31 <sup>a</sup>	16.89 ± 0.14 <sup>a</sup>
	Fat	8.00 ± 0.18 <sup>a</sup>	6.10 ± 0.30 <sup>c</sup>
GNC + 0.3% EO	Protein	20.30 ± 0.31 <sup>a</sup>	17.10 ± 0.12 <sup>c</sup>
	Fat	8.00 ± 0.18 <sup>a</sup>	6.30 ± 0.27 <sup>cd</sup>
GNC + 0.6% EO	Protein	20.30 ± 0.31 <sup>a</sup>	17.60 ± 0.49 <sup>d</sup>
	Fat	8.00 ± 0.18 <sup>a</sup>	6.90 ± 0.33 <sup>a</sup>
GNC + 0.9% EO	Protein	20.30 ± 0.31 <sup>a</sup>	18.10 ± 0.22 <sup>b</sup>
	Fat	8.00 ± 0.18 <sup>a</sup>	7.20 ± 0.37 <sup>ab</sup>

\*GNC: Gelatin-nanochitosan; EO: Cumin essential oil; Average values placed in rows marked with different letters are statistically different ( $P < 0.05$ ).

#### Evaluation of qualitative characteristics of chicken meat

##### Chemical changes

The effect of active packaging on the fat and protein content of chicken meat is shown in **Table 4**. Overall, the fat and protein values of the treatments slightly decreased during 12 days of storage due to the oxidative deterioration of meat (Khan, 2014). Gelatin-nanochitosan films incorporated with higher concentrations of CEO show a great effectiveness to protect the chicken meat against oxidation, hence active-packaged samples with gelatin-nanochitosan

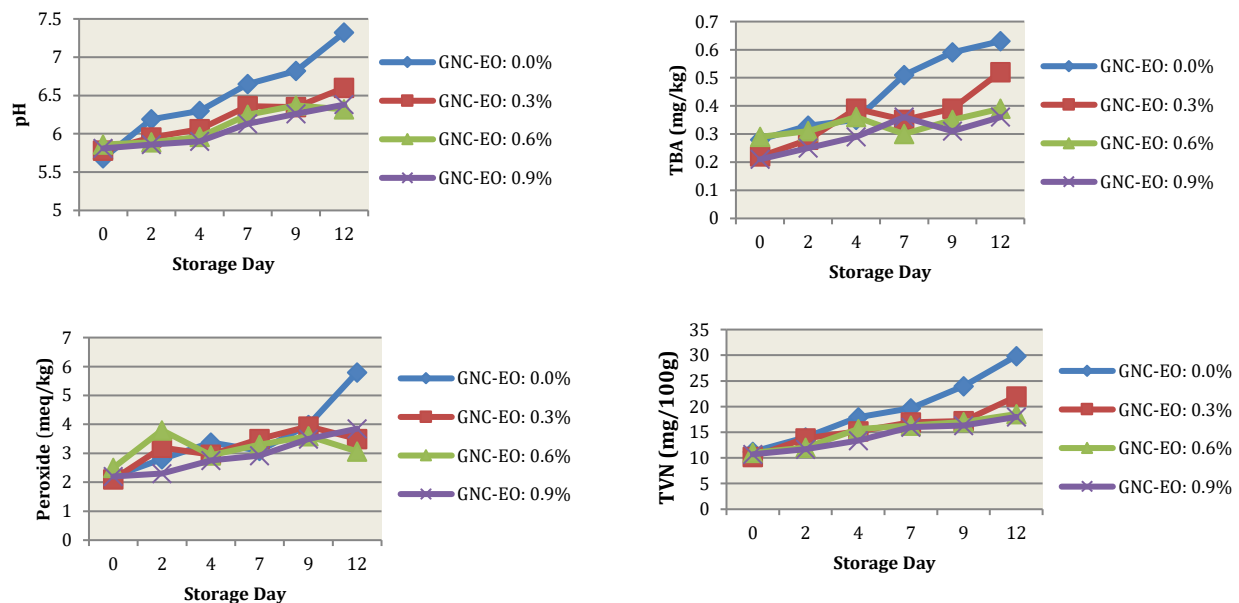
film containing 0.9% CEO had higher fat content than the other treatments. An increase in the protein content of the coated sample with the gelatin-nanochitosan film without EO after 12 d of storage compared to the first day can be attributed to protein degradation (Carvallho et al., 2020).

##### pH changes

Deterioration in the freshness of meat is accompanied by physicochemical changes, including oxidation, off-odors, discoloration, growth of food spoilage microorganisms, and formation of slime

(Shaik et al., 2022). The pH changes of the treatments during 12 days of storage at 4 °C are shown in **Figure 1**. The pH values increased ( $p < 0.05$ ) gradually during the 12 days of storage for all samples. An increase in pH was related to the spoilage bacteria that released free amino acids during protein putrefaction, followed by deamination of amino acids by microbes as a source of energy (Shukla et al., 2015). At the end of storage, the pH of the active-packaged chicken meat was lower than that of the gelatin-nanochitosan-coated sample. Lower pH values in the active-packaged samples compared to the gelatin-nanochitosan coated sample during refrigerated storage could have a greater role of CEO in the shelf life increasing

of chicken samples through inhibiting the microbial growth and activity of endogenous proteases (Shukla et al., 2015). The pH changes in the coated samples containing essential oil (active-packaged) were lower than those in the gelatin-nanochitosan-coated sample ( $P < 0.05$ ) after 12 days of storage, which may be due to the effect of CEO in inhibiting the growth of microorganisms and reducing the accumulation of lactic acid produced by bacteria (Shukla et al., 2015). A similar result was reported by a study that used chitosan active coatings for fresh chicken meat stored at 4 °C (Ghaderi et al., 2024).



**Figure 1.** Changes in pH, TBRS, TVN and peroxide value in meat with films containing gelatin/nanochitosan/cumin essential oil (0, 0.3, 0.6 and 0.9%).

#### Oxidation indices

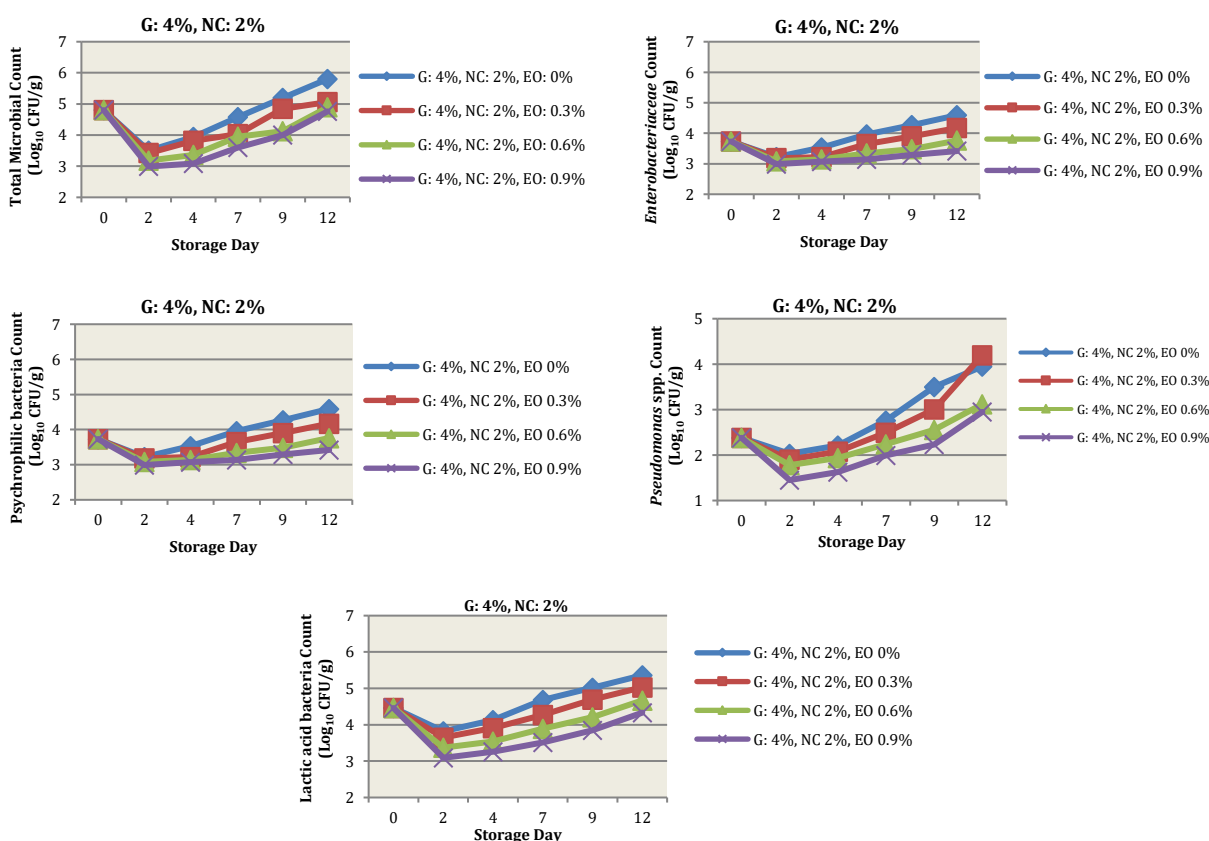
Oxidation is the main process by which unsaturated fatty acids and oxygen interact, resulting in the oxidative deterioration of meat and meat products (Huang & Ahn, 2019). Primary lipid oxidation was determined by measuring the peroxide value. TVN is often used as a biomarker of protein and amine degradation, which is related to the spoilage of meat products (Moosavi-Nasab et al., 2021). The measured PV, TVN, and TBA values are shown in **Figure 1**. PV and TVN values in all treatments

increased during the 12 d of refrigeration. From 4<sup>th</sup>-12<sup>th</sup> days, there was a significant difference between the gelatin-nanochitosan-coated sample and the active-packaged samples. The gelatin-nanochitosan-coated sample had the highest PV, TVN, and TBA values compared to the active-packaged samples ( $P < 0.05$ ). In this study, the application of higher concentrations (0.6% and 0.9%) of Cumin EO was more effective in maintaining the TVN values within the allowable amount in samples stored until the 12<sup>th</sup> day. The antioxidant and antimicrobial properties of the CEO incorporated in gelatin-

nanochitosan film might be the main reason for the low TVN values in active-packaged samples (Safari et al., 2023).

The lower TBA values in the active-packaged samples can be attributed to the inhibition of oxygen permeation by the coating. It can also be due to the antioxidant effect of CEO, as well as the synergistic

effect of gelatin-nanochitosan film and CEO in delaying the oxidation process (Emir Coban & Pelin Can, 2013). Overall, PV, TBA, and TVN values of the active-packaged samples were lower than those of the gelatin-nanochitosan-coated sample ( $P < 0.05$ ) after 21 d of storage, which may be due to the inhibitory effect of CEO on the growth of microorganisms and lipid oxidation.



**Figure 2.** Changes in total bacterial counts, *Enterobacteriaceae*, lactic acid bacteria, and *Pseudomonas* in meat with films containing gelatin/nanochitosan/cumin essential oil (0, 0.3, 0.6 and 0.9%).

In the 4<sup>th</sup> day, PV, TVN, and TBARS values in all treatments increased significantly and continued until the last day (Fig. 1). However, in the active-packaged samples containing the highest EO level of 0.9%, this process advanced with the slightest slope. The lowest peroxide value was observed in the active-packaged sample containing 0.9% CEO. This might be related to the simultaneous effect of the oxygen/gas barrier function of the gelatin-nanochitosan film, which directly limited the exposure of meat to oxygen, and the high antioxidant activity of CEO incorporated in the film (Jacinto-Valderrama et al., 2023). Various studies have shown

that the application of essential oils can delay meat chemical spoilage. In this regard, Lin et al. (2023) studied the effects of a gelatin-active packaging film loaded with other natural additives and reported similar TBA results for chicken meat. Da Rocha et al. (2018) observed that film functionalized with 0.5% of clove EO to increase the shelf life of fillets had a greater effect on lipid oxidation and microbiological spoilage than similar functionalized with 0.5% of fish protein hydrolysate during the storage for 15 days. Lekjing (2016) reported that cooked pork sausages wrapped with films containing 2% chitosan and 1.5% clove oil had the lowest TBA values compared



to other treatments (Song et al., 2021). Mohamed and Mansour (2012) evaluated chicken burgers frozen for 3 months and indicated the potential use of natural herbs and essential oils to protect burgers against lipid oxidation.

#### Microbial analysis

Antimicrobial packaging is the best alternative technique to reduce or inhibit the growth of spoilage and pathogenic microorganisms (Bonilla et al., 2014). Essential oils have good antimicrobial properties against a wide range of Gram-negative and Gram-positive bacteria, which may be due to their phenolic compounds (Gaba et al., 2022). The spoilage processes can cause the production growth of aerobic spoilage organisms, shrinkage, oxidation, and color deterioration of chicken meat (Tooryan & Amiri, 2020). As demonstrated by the results of the present study, over the storage period under cold conditions, microbial counts (total bacterial count, psychrophilic and lactic acid bacteria, *Pseudomonas*, and *Enterobacteriaceae*) significantly increased in all samples ( $P < 0.05$ ), and this increase was higher in the GNC-coated sample than in the active-packaged samples. The changes in total bacterial count, psychrophilic bacteria, and lactic acid bacteria were almost consistent in active-packaged samples compared to gelatin-nanochitosan-coated samples over the storage period. At 12<sup>th</sup> days of storage, the number of aerobic mesophilic bacteria in the gelatin-nanochitosan-coated sample reached  $5.80 \log_{10}$  CFU/g, while the number of aerobic mesophilic bacteria in the active-packaged samples reached  $4.76\text{--}5.06 \log_{10}$  CFU/g. As shown in **Figure 2**, the gelatin-nanochitosan containing CEO effectively inhibited microbiological growth on the surface of chilled chicken meat, and the total bacterial count exceeded the limit value of  $6.7 \log_{10}$  CFU/g on the 12<sup>th</sup> day. As shown in **Figure 2**, on the first day, the population of *Pseudomonas* in the gelatin-nanochitosan-coated sample was  $3.73 \log_{10}$  CFU/g, which increased to  $4.59 \log_{10}$  CFU/g at the end of the storage period. *Pseudomonas* counts were significantly lower in the active-packaged samples than in the gelatin-nanochitosan-coated samples during cold storage. At the end of storage, the highest decrease in bacterial counts was observed in the sample coated with active packaging containing 0.9% CEO. In this study, the application of CEO to a

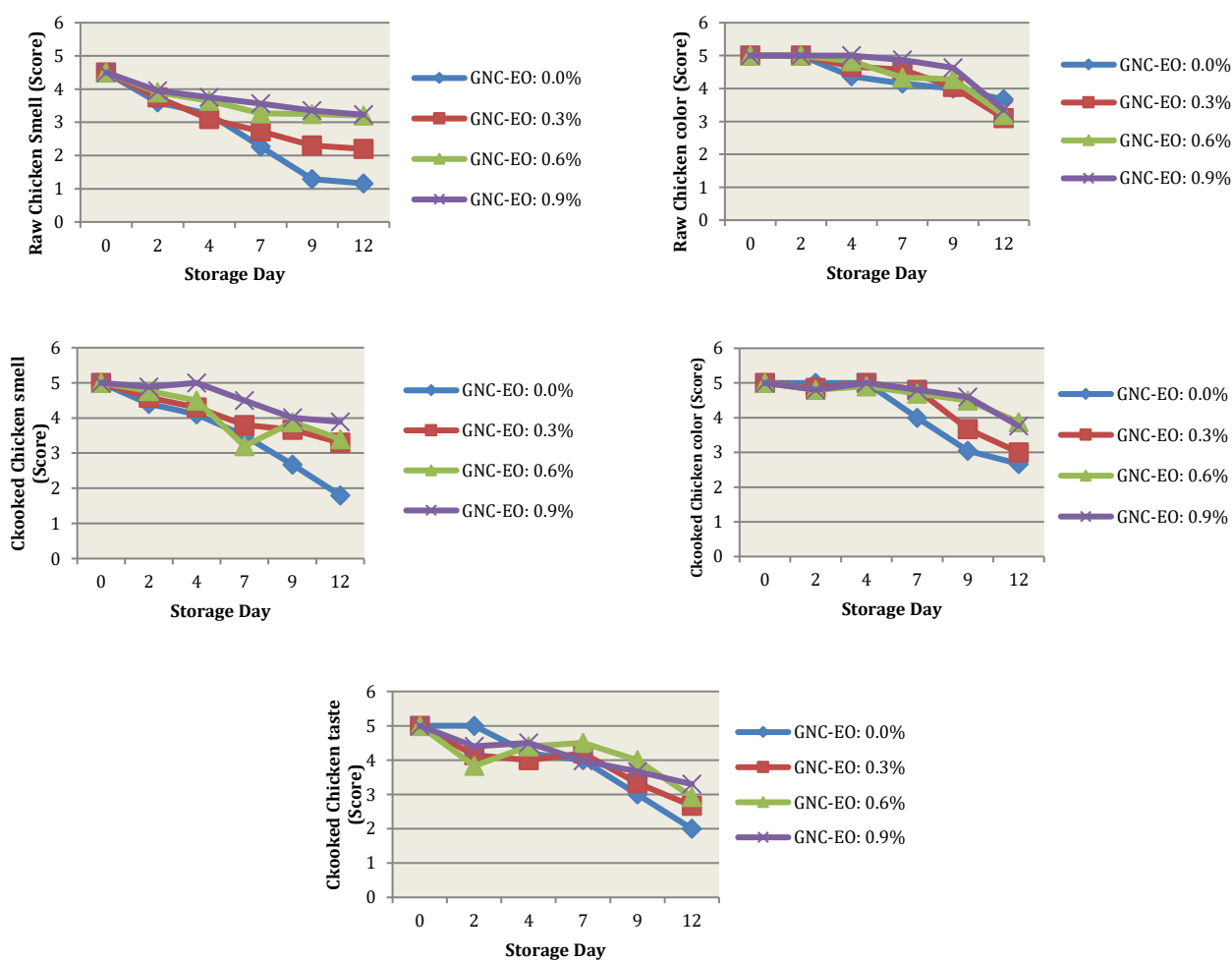
gelatin-nanochitosan film, which naturally contains organic acids and phenolic compounds, enhanced its antimicrobial properties. The antimicrobial activity of CEO includes reducing pH, reaction of phenolic compounds with microbial cell membrane proteins, and inhibition of glycosyltransferases, resulting in the degradation of the microbial cell membrane (Soares et al., 2021). A relatively high concentration of CEO could destroy the bacterial outer wall and increase the amount of oil entering the bacterial cytoplasm, which could cause the leakage of bacterial intracellular material and the termination of DNA synthesis, resulting in the death of bacteria (Nazzaro et al., 2013). Numerous studies have indicated the effects of natural preservatives, such as essential oils, on foodborne pathogens such as *S. aureus*, *S. enteritidis*, *Klebsiella*, *L. monocytogenes*, *E. coli*, and *C. botulinum*, reflecting the researchers' interest in replacing synthetic materials with natural compounds (Yaghoubi et al., 2021). Mahmoudi et al. (2012) showed relatively good antibacterial activity of CEO, with the highest activity observed against *S. aureus*. Choulitoudi et al. (2017) reported the lowest LAB count in smoked eel fillets treated with a carboxymethyl cellulose film incorporated with herbal essential oil. Sirocchi et al. (2017) also studied the effect of rosemary EO in combination with different packaging conditions on increasing the shelf life of red meat stored for 20 days at refrigeration. Their results showed that the growth of psychrophilic bacteria, *Pseudomonas* and *Enterobacteriaceae*, was significantly decreased by using rosemary EO. Maru et al. (2021) showed that samples coated with pullulan in combination with lemon peel extract showed increased bacterial lag phases and growth inhibition in raw poultry meat.

#### Sensory attributes

The key factors that determine food acceptability are its sensory characteristics. Sensory evaluation scores showed a considerable decrease in all treatments until the end of the storage period (**Fig. 3**). In this study, the gelatin-nanochitosan coated sample received lower scores than active-packaged samples. The sensory scores of chicken meat were unacceptable from the 9<sup>th</sup> day onwards in the gelatin-nanochitosan-coated sample. In addition, the active-packaged sample containing 0.9% CEO showed higher sensory scores than the other

treatments; therefore, so that until the 9<sup>th</sup> day, this was acceptable for the panelists. According to Giatrakou et al. (2010), the addition of chitosan with thyme oil to chicken products resulted in a more acceptable taste and odor compared to untreated samples. Vasilatos and Savvaidis (2013) investigated the effect of chitosan coating alone and in combination with rosemary EO on increasing the shelf life of turkey meat. The evaluation of their samples showed a decrease in sensory scores during 21 d of storage, and the odor and taste scores in the uncoated sample were significantly lower than those

in the treated samples, which is consistent with the results of the present study. findings of this study indicate that the chicken meat with gelatin-nanochitosan film containing CEO had a significant effect ( $p < 0.05$ ) on texture. Honarvar et al. (2017) reported similar results using thyme EO coated with chitosan to preserve chicken meat stored under cold conditions. Zargar et al. (2019) showed that in gelatin films containing *Pimpinella Anisum* essential oil, increasing the EO concentration to 0.6% resulted in increasing acceptance of flavor of minced beef.



**Figure 3.** Sensory evaluation (taste, odor, color and texture) of chicken meat with films containing gelatin/nanochitosan/cumin essential oil (0, 0.3, 0.6 and 0.9%).

## Conclusion

The gelatin–nanochitosan films with the addition of CEO had significant differences in several physicochemical properties such as the thickness,

tensile strength and elongation at break. As that the increase in essential oil concentration increased the thickness, TS and EAB. Results showed that the lowest PV, TVN, and TBA were observed in active-

packaged samples containing 0.6% and 0.9% of CEO. The antibacterial activity evaluation showed that edible film made from gelatin–nanochitosan with the addition of CEO had an inhibitory effect on total bacterial count, coliform, psychrophilic and lactic acid bacteria. According to the finding of this study, it was found that simultaneous use of gelatin–nanochitosan film with CEO 0.6% and 0.9% increases shelf life of chicken meat for 9 days without any undesirable. Results of this study suggested that gelatin–nanochitosan edible film incorporated with CEO, can have functional properties including antioxidant activity and antibacterial activity that indicate its potential as an active packaging application for perishable foods such as chicken meat.

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## Conflicts of interest

The authors declare that they have no conflict of interest.

## Disclaimer

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