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# ***Thymus daenensis* L. essential oil nanoemulsion-enriched chitosan/carboxymethyl cellulose edible coating: Impact on chemical, microbial, and sensory characteristics of the coated beef during refrigerated storage**

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Article Info	Abstract
<p><b>Article history:</b> Received: 15 November 2024 Accepted: 11 December 2024</p> <p><b>Keywords:</b> Carboxymethyl cellulose Chitosan Coating Essential oil Nanoemulsion</p>	<p>The study was aimed at extending the shelf life of beef using chitosan/carboxymethyl cellulose (CS/CMC) edible coating containing <i>Thymus daenensis</i> L. essential oil nanoemulsion (TDON). The coated beef with CS/CMC composite incorporated with 1% and 2% TDON was evaluated for microbial and chemical changes as well as sensory properties during 15 days under refrigeration storage conditions. It was found that CS/CMC coating significantly controlled the microbial population in the samples (<math>P &lt; 0.05</math>). <i>Enterobacteriaceae</i> counts in the TDON treatment showed the greatest reduction compared to other bacterial groups. The shelf life of the coated treatment without TDON was not significantly different from that of the uncoated samples (<math>P &gt; 0.05</math>). The results of the chemical analysis and sensory evaluations indicated that the TDON treatments (especially at 2%) exhibited lower peroxide and TBARS values, along with the highest sensory scores compared to the control samples (<math>p &lt; 0.05</math>). According to the results, CS/CMC composite coating enriched with TDON could be a promising approach to enhance the shelf life and quality of refrigerated beef.</p> <p>©2025 Published by Amol University of Special Modern Technologies Press. This is an open-access article under the CC-BY4.0 license (<a href="https://creativecommons.org/licenses/by/4.0/">https://creativecommons.org/licenses/by/4.0/</a>).</p>

## Introduction

Meat, being a high-protein food sourced from animals, is highly prone to spoilage and the growth of microorganisms, resulting in a relatively short shelf life under refrigeration (Quintavalla and Vicini, 2002). The packaging and coating of food is a crucial aspect of maintaining the quality of both fresh and processed products. There has been a growing emphasis on developing methods to prolong the shelf life of food products without relying on synthetic preservatives, which poses a significant challenge in the food science

(Ur Rahman *et al.*, 2018).

Plastic-based packaging and coatings are mainly made of petroleum derivatives and synthetic polymers to increase the shelf life of food. Increasing consumer concerns, environmental hazards, and very slow degradability have challenged the use of these compounds for food coatings. Hence, extensive studies are being conducted to find and use natural and environmentally friendly alternatives for covering and

packaging food (How *et al.*, 2024).

Chitosan (CS), a polysaccharide derived from the deacetylation of chitin under alkaline conditions, is recognized as a significant natural biodegradable polymer for food packaging due to its antimicrobial, antioxidant, and safe properties (Rajaei *et al.*, 2017). Research has focused on enhancing the performance of this cationic polysaccharide through combinations with other natural polymers, nanomaterials, antimicrobial agents, and natural antioxidants to control spoilage and pathogenic microorganisms in food (Rajaei *et al.*, 2017; Dordevic *et al.*, 2022). Sodium carboxymethyl cellulose (CMC), a widely used cellulose polysaccharide in the food industry, has garnered considerable attention for its application in food coatings. This anionic biopolymer is suitable for coatings and packaging due to its favorable mechanical properties, which include flexibility, compatibility with other polymers, gas resistance, moisture retention, and complete surface coverage of food (Gutierrez *et al.*, 2012). The ongoing development of active edible coatings that integrate CMC and CS with other polymers, along with the incorporation of antimicrobial compounds into these composites, has substantially improved their efficacy in extending the shelf life of food (How *et al.*, 2024).

The adoption of natural antimicrobial and antioxidant compounds, such as plants-derived volatile essential oils (EO), as alternatives to chemical preservatives in food processing and packaging has garnered considerable attention (Izadi *et al.*, 2024). *Thymus daenensis* L. essential oil (TDO) has significant antimicrobial and antioxidant effects on enhancing of food shelf life due to the presence of effective compounds such as carvacrol and thymol. The antimicrobial potential of EOs for enhancing the efficacy of active films and coatings has been demonstrated in various studies (Bamian *et al.*, 2023; Izadi *et al.*, 2023; Motalebinejad *et al.*, 2023). However, their effectiveness remains constrained due to certain characteristics, including poor solubility in water, high volatility, and a notably strong odor. These limitations present significant challenges in the application of EOs, necessitating further research to improve their functional properties and broaden their utilization in active packaging technologies (Dima *et al.*, 2014).

Emulsification is an essential encapsulation technique that forms colloidal suspensions of immiscible liquids, typically water and oil, stabilized by an emulsifier. Nanoemulsions, which are particularly effective for encapsulating volatile

compounds like EOs, are characterized by droplet sizes under 100 nm and offer numerous advantages, including high transparency, greater long-term stability, flavor masking of the encapsulated substances, and less impact on the organoleptic properties of food (Mansouri *et al.*, 2021). The small droplet size and high specific surface area lead to enhanced biological activity of EOs and significantly increase penetration ability. The production technology of nanoemulsions is widely utilized in the food industry for controlling release and encapsulating beneficial compounds, such as aromatic EOs, edible oils, and various vitamins, thereby increasing their overall effectiveness (Mirsharifi *et al.*, 2023).

The ongoing challenges of beef shelf life and the limitations of traditional synthetic packaging methods underscore the need for innovative solutions in food preservation. This study specifically aimed to investigate the application of an edible coating based on CS/CMC composite, incorporating nanoemulsion of *T. daenensis* L. essential oil (TDON), to improve the microbiological, chemical, and sensory properties of beef during refrigerated storage.

## Materials and Methods

### Materials

Dried *T. daenensis* L. leaves were purchased from a medicinal plant center in Amol, Iran. Chitosan and carboxymethyl cellulose were supplied from Sigma-Aldrich Co. (USA). Chitosan and Carboxymethyl cellulose were obtained from Sigma-Aldrich company (USA). Fresh beef was aseptically purchased from a local butcher shop (Amol, Iran). Culture media at analytical grade were supplied by Quelab Laboratories (Montreal, QC, Canada). All chemical reagents were obtained from Merck Company (Darmstadt, Germany).

### The EO extraction

The EO was extracted from the dry leaves of *T. daenensis* L. through hydro-distillation employing a Clevenger apparatus. The extraction process lasted for 3 hours, after which the resulting oily liquid was dehydrated with sodium sulfate and subsequently stored in a dark container under cold conditions (Bamian *et al.*, 2023).

### Preparation and analysis of TDON

To produce the nanoemulsion, the EO and Tween 80

(as a surfactant) were first homogenized with a ratio of 1:1 (w/w) for 10 minutes. Subsequently, soy lecithin (as inhibitor of the Ostwald ripening) was added at 1% (w/w) to gradually prepare a coarse emulsion. The resulting oily phase was then combined with a sterile phosphate buffer solution (aqueous phase), and homogenization was continued at 30,000 rpm using a homogenizer (Ultra Turrax T10 basic, IKA, Germany) for three minutes. Ultimately, high-energy emulsification was conducted by subjecting the resulting emulsion to sonication with an ultrasonic homogenizer for 15 minutes, operating at a power of 400 W, a frequency of 20 kHz, and featuring a probe diameter of 13 mm (Bandelin Sonopuls HD 3400, Berlin, Germany) (Ghosh *et al.*, 2013).

The average droplet size (nm), Zeta potential (mV), and the polydispersity index (PDI) of TDON were evaluated employing the dynamic light scattering (DLS) method utilizing a Zetasizer Nano ZS device (Malvern Instruments Ltd., Malvern, UK).

### Preparation of coating treatment

To prepare the composite coating solution, CMC (1%) was dissolved in sterile distilled water over a period of three hours. Concurrently, CS (1%, pre-dissolved in a 2% acetic acid solution) and glycerol as a plasticizer (0.075%) were added to the base solution. The mixture was then thoroughly homogenized using a magnetic stirrer set at 45°C and 1000 rpm to achieve a uniform consistency. As the temperature of the composite solution gradually decreased, TDON (1% and 2%) was separately incorporated into the mixture simultaneously during the homogenization process (Keykhosravy *et al.*, 2020). The coating treatments included control (C, dipped into sterile distilled water), CS/CMC, CS/CMC+TDON 1%, CS/CMC+TDON 2%.

### Beef treatment

The fresh beef was cut into 10 g cubic pieces and, to coat with the treatments, was randomly dipped into four different treatments (two minutes while constantly stirring) and then placed on a sterile grid (20 minutes) to dry the coating solution. Then the samples were drained and packed in sterile plastic bags for storage at 4°C. Each treatment was evaluated against microbial, chemical, and sensory attributes at 0, 3, 6, 9, 12, and 15 days of storage, at 6 intervals (Sedaghat *et al.*, 2015).

### Microbial assay

Ten grams of each beef sample were homogenized with 90 ml of sterile 0.1% peptone water (containing 0.9% NaCl) for one minute. Following the homogenization, appropriate serial dilutions were performed to ensure accurate enumeration of microbial populations. Total viable count (TVC) and psychrotrophic bacteria count (PBC) were evaluated by plating on plate count agar, with incubations carried out at 35°C for 48 hours and at 7°C for 7 days, respectively. To count enterobacteriaceae spp. (EBC) at each sampling interval, Violet Red Bile Glucose (VRBG) agar was utilized, with plates incubated at 37°C for 24 hours. Lactic acid bacteria (LAB) count was assessed via inoculation of de Man Rogosa Sharpe (MRS) agar, which was incubated at 30°C for 48 hours (Sedaghat *et al.*, 2015). The results were expressed as the logarithm of colony-forming units per gram of beef (log CFU/g).

### Chemical assay

#### pH assay

Five grams of the beef samples were homogenized for 30 seconds using 25 ml of distilled water. The pH was measured using a pH meter (Jenway, London, UK), which was calibrated at regular intervals with standard buffer solutions at pH values of 4, 7, and 10 (Fisher Scientific UK Ltd, Loughborough, UK).

#### Thiobarbituric acid reactive substance (TBARS) analysis

TBARS concentration was measured to assess lipid oxidation in the samples. Ten grams of each sample were initially weighed and mixed with one ml of butylated hydroxytoluene (BHT) and 35 ml of 5% trichloroacetic acid. The mixture was filtered using Whatman filter paper No. 4, and 5 ml of the filtrate was combined with 5 ml of 0.02 M thiobarbituric acid. To facilitate the formation of the malondialdehyde-TBA complex, the mixture was heated at 100°C for 60 minutes. After cooling, the absorbance was measured at 532 nm. A standard malondialdehyde solution was used to calculate TBARS. The amount of TBARS in the samples was subsequently calculated as malondialdehyde (MDA) per kilogram of sample (Sedaghat *et al.*, 2015).

#### Sensory analysis

The sensory attributes of coated beef samples (CS/CMC, CS/CMC+TDON 1%, and CS/CMC+TDON

2%) alongside non-coated beef (C) were assessed by a panel of 20 trained individuals. Evaluations focused on aspects such as color, odor, texture, and overall acceptability at six intervals throughout a 15-day storage period. Notably, a taste test was omitted due to rising microbial levels in several treatments late in the storage period, which could jeopardize the health of the panelists. The sensory qualities of the samples were rated using a 9-point hedonic scale, where scores ranged from nine for "extremely liked" to one for "extremely disliked."

## Statistical analysis

The data collected from the evaluation of edible coatings applied to beef samples during storage were analyzed utilizing SPSS software (IBM SPSS Statistics 24). The analysis incorporated ANOVA along with Tukey's test, with a significance level set at  $p < 0.05$ . Each test was conducted with three replicates to ensure reliability.

## Results and Discussion

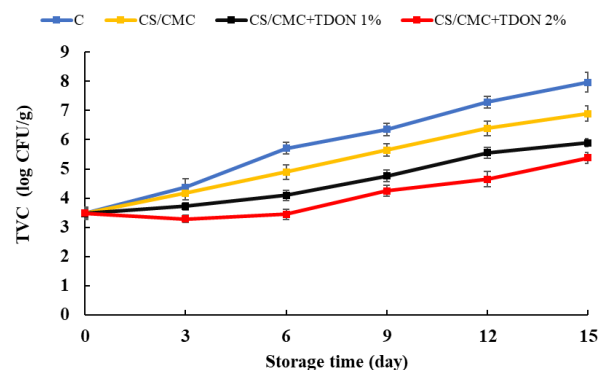
### TDON analysis

The analysis conducted on TDON utilizing Dynamic Light Scattering (DLS) demonstrated that the average size, polydispersity index (PDI), and Zeta potential of the TDON particles were roughly  $5.31 \pm 63.14$  nm, 0.21  $\pm$  0.4, and  $-1.35 \pm 0.03$  mV, respectively. Typically, nanoemulsions of essential oils (EOs) characterized by smaller diameters and lower PDIs are deemed more effective for food preservation, indicating a more homogenous distribution of the oil in the nanoemulsion and, consequently, throughout the food matrix. Furthermore, a lower PDI that approaches zero signifies increased particle stability in the nanoemulsion (Ghosh *et al.*, 2013). A negative Zeta potential signifies the presence of an electric charge around the particles, resulting in broader dispersion, which is influenced by various elements such as ionic strength, the type and concentration of the emulsifiers involved, and their volumetric ratio with the EO (Moghimi *et al.*, 2016). The results highlight the promising efficacy of using Tween 80 in conjunction with lecithin for the ultrasound-assisted synthesis of TDON.

### Microbial analysis

The results of total viable counts (TVC), psychrotrophic bacteria counts (PBC), enterobacteriaceae spp. counts (EBC), and lactic acid

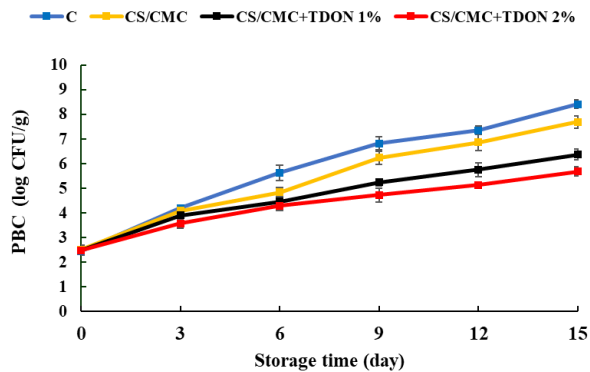
bacteria counts (LAB) in the coated beef samples during cold storage are presented in Figs. 1 to 4. The Total Viable Count (TVC) increased from approximately 3.5 CFU/g on the first day of storage to 7.98 CFU/g by the end of the 15-day storage period in the control group. The treatments coated with the CS/CMC composite did not reach a count of 7 CFU/g until day 12 of storage, whereas the other treatments also did not reach 7 CFU/g until the 12th day (Fig. 1). Treatments containing 1% and 2% TDON significantly controlled TVC during the storage of the beef samples due to the antimicrobial effect of EO. This microbial reduction was more evident in the CS/CMC+TDON 2% ( $p < 0.05$ ). In general, the appearance of spoilage symptoms in meat starts from a TVC higher than 7 CFU/g (Lambert *et al.*, 1991). Due to the slow and controlled release of the active compounds of the encapsulated TDO (mainly nanoemulsion) from the CS/CMC coating, high antimicrobial effects were not observed in the early days of storage. Izadi *et al.* (2024) investigated the effect of encapsulating TDO with beta-cyclodextrin on the shelf life of ground beef. In the early days of treatment, the pure EO's antimicrobial effect was higher than the encapsulated one due to the faster release of the essential oil's effective compounds, which agrees with the results of the present study. The PBC group consists of important aerobic Gram-negative bacteria that can thrive in the cold conditions of a refrigerator, contributing to food spoilage. A rise in the population of this group of bacteria can result in the early spoilage of beef (Sedaghat *et al.*, 2015).



**Fig. 1.** Total viable count (TVC) in the coated beef samples during 15 days of storage at 4°C. Chitosan (CS), Carboxymethyl cellulose (CMC), and TDON (*Thymus daenensis* L. essential oil nanoemulsion).

As illustrated in Fig. 2, the increase in PBC across all treatments was not significantly different after three days of storage. In the control group, the PBC increased from 2.5 CFU/g to 8.42 CFU/g by the end of

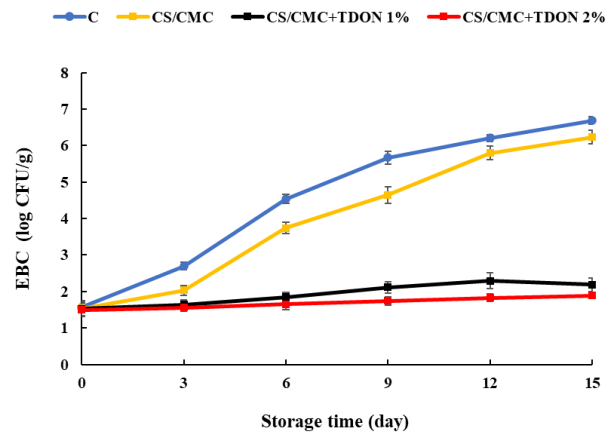
the storage period. However, the samples treated with CS/CMC containing TDON showed significantly better control of PBC compared to the other treatments. Additionally, no significant difference was found between the results of the two concentrations of TDON used in treating the samples.



**Fig. 2.** Psychrotrophic bacteria count (PBC) in the coated beef samples during 15 days of storage at 4°C. Chitosan (CS), Carboxymethyl cellulose (CMC), and TDON (*Thymus daenensis* L. essential oil nanoemulsion).

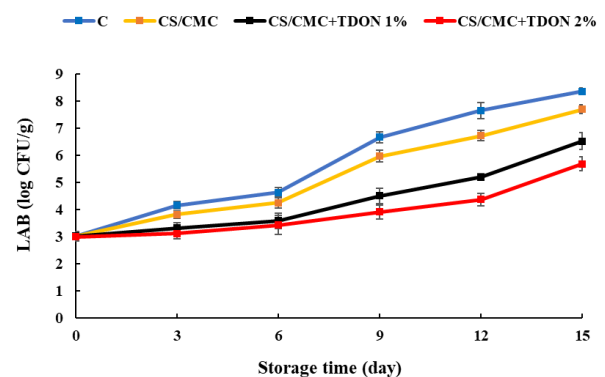
The most significant foodborne pathogenic bacteria belong to the enterobacteriaceae family. As indicator microorganisms, these bacteria can lead to gastroenteritis through the consumption of contaminated food. Therefore, controlling their growth in raw animal-origin foods is crucial (Sani *et al.*, 2017).

The data obtained from the microbial evaluations indicated that, in the control group, the initial EBC increased from 1.5 CFU/g to 6.7 CFU/g by the end of the storage period at 4°C. In contrast, the samples coated with CS/CMC exhibited significantly lower EBC levels compared to the control group (Fig. 3). On the 9th day, treatments containing TDON significantly inhibited the growth of enterobacteriaceae spp. in the beef samples, demonstrating an antimicrobial effect that persisted until the end of the storage period. Furthermore, increasing the concentration of TDON did not have a significant impact on the effectiveness of these treatments. Other studies have confirmed the efficacy of volatile EOs and their nanoemulsions in controlling EBC during food storage, supporting the findings of our study (Keykhosravi *et al.*, 2020).



**Fig. 3.** Enterobacteriaceae spp. (EBC) in the coated beef samples during 15 days of storage at 4°C. Chitosan (CS), Carboxymethyl cellulose (CMC), and TDON (*Thymus daenensis* L. essential oil nanoemulsion).

LAB is a group of gram-positive, microaerophilic bacteria capable of growing in the cold conditions. If their growth conditions are favorable, LAB can cause spoilage of meat and its products (Hernandez-Aquino *et al.*, 2019). In our study the quantity of LAB increased during the storage period across all treatments. In the control treatment, which lacked antimicrobial effects, and in the CS/CMC+TDON 2% treatment, which exhibited the strongest antimicrobial effect, the initial counts of LAB were approximately 2.98 CFU/g and 3 CFU/g, respectively. By the end of the storage period, these counts increased to 8.36 CFU/g and 5.68 CFU/g (Fig. 4).



**Fig. 4.** Lactic acid bacteria (LAB) count in the coated beef samples during 15 days of storage at 4°C. Chitosan (CS), Carboxymethyl cellulose (CMC), and TDON (*Thymus daenensis* L. essential oil nanoemulsion).

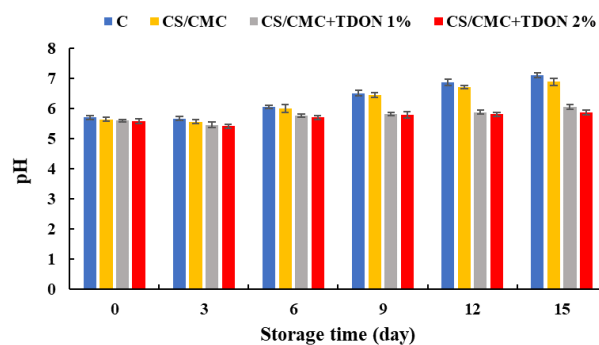
Although the CS/CMC treatment alone showed greater inhibition of LAB compared to the control

group, the coatings containing TDON had a significant impact on the control and inhibition of these bacteria. Dordevi *et al.* (2022) studied the effect of chitosan coatings enriched with free and encapsulated *Satureja montana* EO on increasing the shelf life of beef samples. They concluded that encapsulating the EO results in a slow and stable release of its active compounds, contributing to the continuous effectiveness of coatings containing the encapsulated EO in enhancing the shelf life of the samples (Dordevic *et al.*, 2022). Chitosan, recognized as an effective antimicrobial agent, alters the permeability of microbial cytoplasmic membranes, leading to the leakage of electrolytes and proteins, which ultimately results in the death of bacterial cells. Its mechanism of action involves disrupting the lipopolysaccharide layer of bacteria and serving as a barrier against oxygen penetration. The figures clearly illustrate that the most significant reduction in bacterial counts was associated with the CS/CMC+TDON 2% treatment. The antimicrobial agents are gradually released from the composite matrix on the surface of the food, sustaining high concentrations over time, which effectively prevents microbial growth (Yuan *et al.*, 2016).

## Chemical analysis

### pH

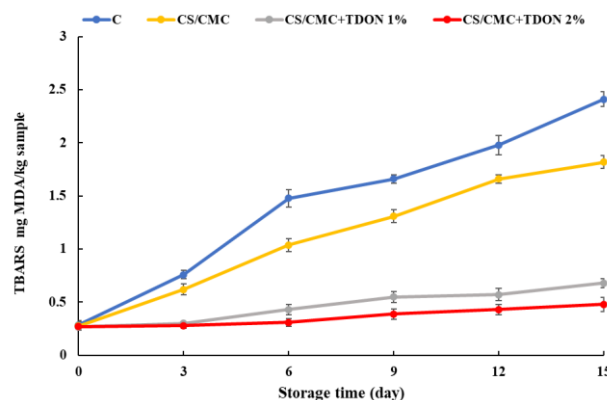
Changes in pH during storage of the treatments are shown in Fig. 5. The pH of all samples increased during storage in the refrigerator, and in the control group, it reached 5.7 to 7.11 at the end of the storage period. The pH level in all samples was relatively constant (even decreased in the treatments containing TDON) until the 3rd day of storage, which may be due to the production of lactic acid, and then increased during the storage period which may be attributed to the activity of bacterial enzymes and endogenous proteases in the meat, leading to proteolysis and the formation of amino acids and free amines (Dordevic *et al.*, 2022). The pH changes in the control and CS/CMC treatments did not have a statistically significant difference during the storage period. However, the increase in pH in the treatments containing TDON was significantly lower than the control and CS/CMC groups ( $p < 0.05$ ).



**Fig. 5.** The changes in pH in the coated beef samples during 15 days of storage at 4°C. Chitosan (CS), Carboxymethyl cellulose (CMC), and TDON (*Thymus daenensis* L. essential oil nanoemulsion).

### Thiobarbituric acid reactive substance (TBARS) analysis

Due to the presence of fat, especially unsaturated fats, beef is very susceptible to oxidative spoilage or fat hydrolysis, which can lead to changes in its taste, color and smell (Pellissery *et al.*, 2020). The malondialdehyde (MDA) level, the primary byproduct of the oxidation of fatty acids, is evaluated to express the degree of lipid oxidation in the beef samples (Ren *et al.*, 2021). The effect of TDON-enriched composite coating on MDA values in terms of milligrams of malondialdehyde per kilogram of beef stored at 4°C is shown in Fig. 6.



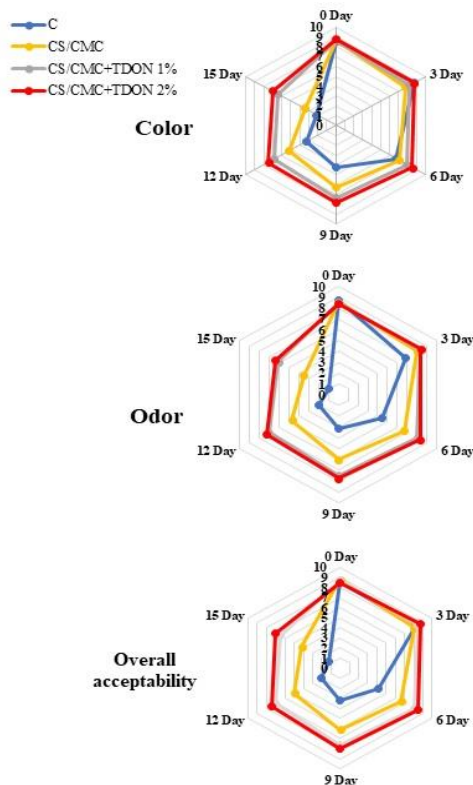
**Fig. 6.** The changes in thiobarbituric acid reactive substance (TBARS) in the coated beef samples during 15 days of storage at 4°C. Chitosan (CS), Carboxymethyl cellulose (CMC), and TDON (*Thymus daenensis* L. essential oil nanoemulsion).

The TBARS level on day 0 in the control group varied from 0.24-0.27 mg MDA/kg of the beef samples, which increased to 2.41 mg by the end of the storage period. The MDA changes in the coated samples with

CS/CMC composite were significantly different from those in the control treatment. This difference was more evident from the 6th day of storage in the CMC/CS group. On the other hand, the coatings containing TDON demonstrated more efficacy in controlling the oxidative spoilage process in the samples, thereby extending the meat shelf life. An increase in TBARS levels above two mg of MD per kilogram of meat may exhibit oxidative changes and spoilage in the samples (Mehdizadeh *et al.*, 2023). In the present study, only the control sample showed the highest amount of TBARS, and the CS/CMC coating treatments, particularly those containing TDON, had relatively stable oxidative conditions during storage. The effect of the antioxidant activity of the EOs along with chitosan on TBARS control has also been reported in other studies, which is in line with the present study (Pabast *et al.*, 2018; Dordevic *et al.*, 2022).

### Sensory analysis

Fig. 7 presents the evaluation of color, odor, and overall acceptability of the samples during the 15-day at 4°C.



**Fig. 7.** The changes in sensory attributes in the coated beef samples during 15 days of storage at 4°C. Chitosan (CS), Carboxymethyl cellulose (CMC), and TDON (*Thymus daenensis* L. essential oil nanoemulsion).

Undesirable changes in organoleptic characteristics are significant sensory concerns for meat products, especially when using oxygen-permeable packaging. These changes are caused by bacterial growth, chemical oxidation, and the formation of volatile compounds that can shorten the shelf life of meat (Ruiz-Capillas *et al.*, 2021). Sensory evaluations of the coated beef samples indicated that on day zero, all samples received a high score from the judges. However, during the first days of storage, the TDON treatments received a lower odor score compared to the other treatments, likely due to the release of some components of the encapsulated EO (Zhang *et al.*, 2019). The control and CS/CMC treatments scored less than 5 points for odor properties on days 6 and 12, respectively. Similar results were obtained for color changes, with scores reaching less than 5 on days 5 and 12 for the control and CS/CMC treatments, respectively (Fig. 7). CS/CMC composite coatings containing TDON achieved the highest scores in all sensory characteristics throughout the storage period and better preserved the freshness of the samples. Although no statistically significant differences were observed among the results of TDON treatments with different concentrations, the coated samples containing 2% TDON received the highest odor, color, and overall acceptability scores. Following the results of our study, Dordevic *et al.* (2022) concluded that the characteristics of color, smell, and overall acceptability of the beef coated with chitosan containing nanoencapsulated *Satureja montana* L. EO during 20 days of storage at 4°C received a high score from the evaluators.

As effective food preservatives, plant volatile EOs exhibit significant antimicrobial effects against a wide range of pathogens and spoilage agents in meat and its products. In this study, by nano-emulsification of TDO, its ability to provide sustained antimicrobial effects and continuous expansion in CS/CMC coating improved. Based on the results, CS/CMC composite coating enriched with TDON significantly influenced the chemical, microbiological, and sensory characteristics of the beef samples. Sensory analysis indicated that the samples coated with TDON were preferred over the other samples. Furthermore, the findings revealed that the use of CS/CMC composite coating alone does not extend the shelf life of beef; however, its effectiveness is markedly enhanced when combined with TDON. The CS/CMC+TDON 2% successfully delayed lipid oxidation and inhibited bacterial growth in the coated samples. In conclusion, this biodegradable, natural-based coating demonstrates the potential for extending the shelf life

of beef at 4°C.

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## Conflict of Interest

There are no conflicts to declare.

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