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Evaluation of the inhibitory ability of cinnamon oil on microbes and its impact on meat preservation and food safety

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Abstract

The study aimed to evaluate the inhibitory ability of cinnamon oil (Cinnamomum verum), which is rich in cinnamaldehyde (≥70%), against microbes associated with meat, and its effect on the preservation and safety of minced meat. Steam-distilled free oil (CO), nanoemulsion (CEON/NE), and chitosan/cinnamon oil coating (CS-CO/CC) were used against five foodborne pathogens: *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella Typhimurium*, *Escherichia coli* O157:H7, and *Campylobacter jejuni*. MIC was determined according to CLSI, and inhibition was measured by the agar etch method. Inoculated meat (5–6 log CFU/g) was stored at 4±1°C for 12 days, and microbial load, growth curves, TBARS, pH, color, and sensory evaluation were measured. The results showed that the nanoemulsion was superior to free oil against all pathogens, and standard cinnamaldehyde was the strongest against Gram-negatives, while the nanoemulsion was the best against Gram-positives and gave the largest areas of inhibition. In cold storage, all treatments slowed down microbial growth compared to the control, and the CS-CO/CC coating was the most effective (6.47 log CFU/g on day 12 vs. 8.63 for the control), followed by nanoemulsion (6.83) and free oil (7.58). The treatments, especially T2 and T3, also reduced the TBARS values and maintained the pH and red color, and the nanoemulsion was better in overall acceptability and odor. Therefore, nanoemulsion and chitosan coating loaded with cinnamon oil are promising alternatives for natural preservation of meat and enhancement of food safety.

Keywords: cinnamon oil; nanoemulsion; chitosan coating; preservation of meat; Food safety.

Introduction

Cinnamon oil (Cinnamomum zeylanicum) is a promising natural antimicrobial that inhibits the growth of various microorganisms and therefore represents a potential alternative to synthetic preservatives in the meat industry (1). Cinnamon oil, rich in eugenol, total phenolics, and health-promoting substances, can inhibit oxidation processes (2). The ability of cinnamon oil to prevent the development of foodborne pathogens and spoilage microbes such as Pseudomonas spp. and *Listeria monocytogenes* has already been demonstrated, and it could play a role in extending the shelf life of refrigerated meat products (3,4). Cinnamon oil possesses eugenol, cinnamaldehyde, and trans-cinnamic acid acknowledged the effective mechanism to protect food from microbial spoilage. Cinnamon oil extent is considerable anti-microorganism constituent of essential oils belongs to the Eugenia, Cinnamomum, and Syzygium, well-reputed for food, pharmaceuticals, and perfumes and a foe of a broad spectrum of foodborne pathogens

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such as Salmonella, E.coli, *Staphylococcus aureus*, Campylobacter, and *Listeria monocytogenes* (5,6). Eugenol, cinnamaldehyde, and trans-cinnamic acid are classified as either phenolic or nonphenolic bioactive compounds. Cinnamaldehyde provides antioxidant, antimicrobial, antifungal, anti-insect, and anti-carcinogenic properties allow widespread usage also dominant species in cinnamon oil. Allelochemicals and other counterparts extend advantages in the prevention of food and post-harvest losses, known as important strategies towards environmental concerns and sustainability (7,8). The growth of spoilage organisms and foodborne pathogens during storage limits the shelf life of meat products and constitutes a serious public health concern (9). The aromatic properties of cinnamon oil can adversely affect meat products, but the oil inhibits spoilage organisms involved in off-odour production and may help preserve the sensory properties of meat (10). Cinnamon oil therefore has potential as a natural preservative in meat products. It may also provide synergies with other hurdles, as important spoilage organisms such as Pseudomonas spp. are sensitive to multiple agents (11).

Therefore, this study aimed to evaluate the effectiveness of cinnamon oil as an antimicrobial substance and study its effectiveness in preserving meat and enhancing food safety.

Materials and Methods

Cinnamon oil

Commercial cinnamon bark oil (Cinnamomum verum) was selected, assuming that the cinnamon oil contained at least 70% (w/w) cinnamaldehyde by analyzing its percentage using gas chromatography/mass spectrometry (GC/MS) before usage. The three samples (steam-distilled cinnamon oil (CO), cinnamon oil nanoemulsion (CEON/NE), chitosan/cinnamon oil composite coating (CS-CO/CC)) were selected from independent manufacturers to evaluate variation in chemical composition between the three species, as well as between batches within species.

Bacterial strains used in the study

Five pathogenic strains were selected to represent the most important foodborne pathogens associated with meat and are shown in Table 1.

Table 1. Target pathogens, reference strains, and selective media used in the study.

Pathogen	Reference strain	Gram type	Selective/enumeration medium
Listeria monocytogenes	**ATCC 7644**	**Positive**	**PALCAM agar**
Salmonella enterica Typhimurium	**ATCC 14028**	**Negative**	**Xylose lysine deoxycholate (XLD) agar**
Staphylococcus aureus	**ATCC 25923**	**Positive**	**Baird-Parker agar**
Escherichia coli O157:H7	**ATCC 35150**	**Negative**	**Sorbitol MacConkey agar**
Campylobacter jejuni	**ATCC 33291**	**Negative**	**Modified charcoal cefoperazone deoxycholate agar (mCCDA)**

Study design

The study was divided into four treatments, as shown
C: Control
T1: Steam-distilled cinnamon oil (CO)
T2: Cinnamon oil nanoemulsion (CEON/NE)
T3: Chitosan/cinnamon oil composite coating (CS-CO/CC)

The test environment included two types: (1) Laboratory microbiological tests according to standards that allow appropriate measurement of antimicrobial activity; (2) 12-day cold storage test (4±1°C) using ground meat.

Determine the minimum inhibitory concentration (MIC)

Use 96-well microtiter plates to determine the minimum inhibitory concentration by diluting the broth according to the CLSI method (12).

Diameters of inhibition zones

An agar well diffusion technique was employed to assess the diameters of inhibition zones, wherein Mueller-Hinton agar plates were uniformly inoculated with a suspension of each test microorganism at a concentration of 10^6 CFU/mL, followed by the creation of 6-mm-diameter wells filled with 50 µL of a suspension of free CO, CEON/NE, or CS-CO/CC, each standardized to a total cinnamaldehyde equivalent concentration of 1.0% (w/v). The plates were maintained at 37°C for 24 hours, after which the diameter of the total inhibitory zone, including the well, was recorded.

Measuring the microbial load of minced meat during refrigerated storage

Use fresh, additive-free ground beef from a single commercial source to minimize variation, then divide it into 50g portions. Samples were superficially inoculated with the mixture of pathogens used in the study at a level of 5–6 log CFU/g, and the inoculum was distributed among the treatment groups as shown in Table 2. Subsequently, both treatment and control samples were encased in oxygen-permeable polyethylene film and preserved at 4±1°C for a duration of 12 days, with samples taken on days 0, 3, 6, 9, and 12 for the purpose of measuring microbial load. Growth curves (measurement of pathogen load during refrigerated storage) were also measured. For the five pathogens used in the study, based on the relative susceptibility pattern of Gram-positive bacteria versus Gram-negative bacteria.

Table 2. Vaccine distribution among treatment groups in the refrigerated ground beef storage trial

Treatment arm	Delivery format	Dose(v/v)	Method
C	Untreated	0%	No treatment applied
T1	Aqueous CO dispersion	1.5% CO	Surface spray, 2 mL/50 g
T2	Oil-in-water nanoemulsion	1.5% equiv.	Surface spray, 2 mL/50 g
T3	Chitosan composite coating	1.5% equiv.	Dip-coating, 60 s immersion

C: Control; T1: Steam distilled oil (CO.); T2: The nanoemulsion (CEON/NE); T3: Chitosan/cinnamon oil composite coating (CS-CO/CC)

Measurement of Lipid Oxidation, pH, and Color Stability

On the ninth day of refrigerated storage both lipid oxidation was estimated by thiobarbituric acid reactive substance (TBARS) assay, expressed as (mg malondialdehyde/kg) of sample; In addition to measuring the pH using a pH device, and Color Stability using a portable colorimeter.

Sensory evaluation

Sensory evaluation was conducted to directly determine consumer acceptance of the treatments on the sixth day of storage. 'Overall acceptability',

'Aroma' and 'Appearance' were assessed by a group of consumers using a 9-point 'hedonic scale' (50 or more participants), while 'intensity of cinnamon flavour' was assessed by a trained panel specializing in sensory description (consisting of 8 individuals) using an intensity scale ranging from (0-15)

Statistical Analysis

Microbial counts and physicochemical data were analyzed using two-way analysis of variance, followed by Tukey's HSD test for comparisons of means at a significance level ($P \geq 0.05$). With three replicates, minimum inhibitory concentration (MIC) and zone of inhibition data were analyzed using one-way analysis of variance.

Results and Discussion

The minimum inhibitory concentration (MIC)

Table (3) displays the MIC values (% v/v) for three formulations: Free CO cinnamon oil, CEON/NE cinnamon oil nanoemulsion, and Cinnamaldehyde standard, against five pathogens. The basic rule here is that the lower the MIC value, the greater the antimicrobial effectiveness. We note that there are significant differences at the level of $P \leq 0.05$ between all treatments, and since all rows carry different letters for each formula, that is, Free CO had the highest MIC in all cases, i.e., the least effective, while CEON/NE had a significantly lower MIC than Free CO in all strains, and standard cinnamaldehyde had a significantly lower MIC than Free CO in all strains as well. The comparison between CEON/NE and standard cinnamaldehyde was significant in all classes, but the size of the difference was numerically small in some cases, such as S. aureus.

Table 3. Minimum inhibitory concentration (MIC) values

Pathogen	T1	T2	T3
Listeria monocytogenes	0.055 ± 0.01[a]	0.026 ± 0.003[b]	0.03 ± 0.01[c]
Staphylococcus aureus	0.074 ± 0.008[a]	0.0385 ± 0.0038[b]	0.04 ± 0.01[c]
Salmonella Typhimurium	0.31 ± 0.03[a]	0.12 ± 0.01[b]	0.09 ± 0.01[c]
***Escherichia coli* O157:H7**	0.41 ± 0.04[a]	0.135 ± 0.012[b]	0.11 ± 0.01[c]
Campylobacter jejuni	0.22 ± 0.02[a]	0.077 ± 0.008[b]	0.062 ± 0.01[c]

Different letters in one row indicate significant differences at the probability level ($P \leq 0.05$).

T1: Steam-distilled oil (CO.); T2: The nanoemulsion (CEON/NE); T3: Chitosan/cinnamon oil composite coating (CS-CO/CC)

We conclude that the free oil was the weakest among all the pathogens, and the likely reason is that it is less stable and widespread in the aqueous environment, more volatile, and may not reach the cell membrane at an effective concentration. However, the CEON/NE nanoemulsion clearly improved the effectiveness compared to the free oil in all the pathogens. This is usually explained by increased surface area, improved solubility and dispersion, protection of active compounds, and enhanced penetration of the cell membrane. Finally, we note that standard cinnamaldehyde was the strongest against most Gram-negatives (Salmonella, E. coli, Campylobacter), but CEON/NE slightly outperformed it against Gram-positives (Listeria and S. aureus). This may reflect the role of the nanoemulsion in enhancing delivery to Gram-positives, while Gram-negatives remain less affected by the outer membrane LPS, while gram positives were generally more sensitive than gram negatives; MIC values for Listeria and S. aureus is much lower than Salmonella and E. coli. This is expected because Gram-negatives have an outer membrane that reduces permeability to hydrophobic compounds.

The possible differences are due to the nature of the formulation, as the nanoemulsion increases the availability of cinnamon oil and reduces its volatility, which increases the effective concentration at the cell surface (13).
The reason is also that the cell wall structure of Gram-positive bacteria is more permeable to phenolic compounds, while Gram-negative bacteria resist penetration thanks to the outer membrane (14).

While cinnamaldehyde is the main active ingredient and its presence in a standard form may make it more effective in some pathogens, it may be less stable than the nanoformula (15).

Finally, we conclude that the results show a clear, significant improvement in the effectiveness of cinnamon oil after converting it to a nanoemulsion compared to the free oil in all pathogens. CEON/NE was the best in Gram-positive bacteria, while standard cinnamaldehyde was best in Gram-negative bacteria. These results support the idea that nanoformulation may allow lower concentrations of cinnamon oil to be used with higher efficacy, with the exact mechanism having to be explained through additional tests such as kill time, membrane integrity, and droplet size.

Diameters of inhibition zones

Table (4) displays the diameters of the inhibition zones (mm) at an equivalent dose of cinnamaldehyde, for three formulas: free cinnamaldehyde (Free CO), CEON/NE formula, and CS-CO/CC coating after 24 hours. We note that CEON/NE significantly outperformed Free CO and CS-CO/CC in all five cases. As for CS-CO/CC, it was significantly lower than Free CO only in the Gram-positive pathogens (Listeria and S. aureus), while in the Gram-negative pathogens (Salmonella, E. coli and Campylobacter) the difference between Free CO and CS-CO/CC did not reach statistical significance, even though the values were numerically lower for the coating.

We conclude from this that the results support that cinnamaldehyde in the CEON/NE formula is more effective in the diffusion test on agar than free cinnamaldehyde and CS-CO/CC coating at an equivalent dose. This is mainly due to improved solubility, diffusion, and cell contact. As for the CS-CO/CC coating, it showed smaller zones of inhibition at 24 h, especially in Gram-positive, due to slow release or binding to the matrix, which may be useful in applications that require extended release and not the highest instantaneous concentration. The results also confirm that Gram-positive bacteria are more sensitive to cinnamaldehyde than Gram-negative bacteria, and that the significant differences are clearer in Gram-positives.

The superiority of CEON/NE over free cinnamaldehyde can be explained by the fact that cinnamaldehyde is a relatively hydrophobic compound and may have limited solubility and diffusion in aqueous/agar media (15). The CEON/NE formulation, assuming it is an emulsion/nanoemulsion, increases apparent solubility, surface area, and contact with bacterial cells (16). The formulation may protect cinnamaldehyde from evaporation, degradation, or non-specific binding, increasing the effective amount available, while smaller particles may improve cell membrane penetration and increase the cinnamaldehyde concentration at the site of action (17). This explains why CEON/NE was significantly higher in almost all pathogens.

The lower CS-CO/CC at 24 h compared to the free one in Gram-positive is due to the fact that the coating is not a free solution; Cinnamaldehyde is entrapped or bound to the chitosan matrix, reducing its release rate and diffusion into the agar within 24 hours (18). A reaction may occur between the aldehyde groups in cinnamaldehyde and the amine groups in chitosan, reducing the effective availability (19).

Therefore, the inhibition zone in the agar test may be smaller, but this does not necessarily mean poor applied effectiveness; the coating may provide extended release and sustained surface activity. The difference between Gram-positive and Gram-negative is due to the Gram-positive (Listeria and S. aureus) being more sensitive in general because their cell wall allows higher permeability to cinnamaldehyde, which affects the cytoplasmic membrane and the functions of enzymes and ATPases; Gram-negatives (E. coli and Salmonella) are less sensitive due to their outer membrane and efflux pump potential (20).

Table 4. Diameters of inhibition zones (mm)

Pathogen	T1	T2	T3
Listeria monocytogenes	29 ± 2^{b}	34 ± 2^{a}	25 ± 2^{c}
Staphylococcus aureus	31 ± 2^{b}	36 ± 2^{a}	27 ± 2^{c}
Salmonella Typhimurium	19 ± 1^{b}	24 ± 2^{a}	18 ± 1^{b}
***Escherichia coli* O157:H7**	17 ± 1^{b}	22 ± 1^{a}	16 ± 1^{b}
Campylobacter jejuni	22 ± 1^{b}	27 ± 2^{a}	20 ± 1^{b}

Different letters in one row indicate significant differences at the probability level ($P \leq 0.05$).
T1: Steam-distilled oil (CO.); T2: The nanoemulsion (CEON/NE); T3: Chitosan/cinnamon oil composite coating (CS-CO/CC)

Microbial load of ground meat during refrigerated storage

The results in Table 5 showed that the microbial load in all treatments gradually increased during cold storage at 4 °C, but the rate of increase differed depending on the treatment. The values on day zero were very close (5.48–5.54 log CFU/g) without significant differences. This is logical because the effect of any antimicrobial or coating takes time to appear, and the initial microbial load was similar between the treatments. While the microbial load at the end of storage (day 12) ranged between 6.47 and

8.63 log CFU/g, this shows that all treatments slowed down microbial growth compared to the control, and that the CS-CO/CC coating was the most effective, followed by CEON/NE, then Free CO, then the control. This can be explained by the fact that in the Control treatment, there was no barrier or antimicrobial that allowed the cold-loving bacteria (psychrotrophic bacteria) to grow continuously, until the microbial load exceeded 7 log CFU/g by day 6 (7.13 log), and then reached 8.63 log on day 12. This indicates clear microbial deterioration and a short shelf life. While the Free CO treatment (free essential oil) showed a significant inhibitory effect compared to the control, its effectiveness was relatively limited, especially in the late days. The likely reason is that free essential oils are susceptible to evaporation, oxidation, lack of homogeneous diffusion in the meat matrix, and poor solubility, which reduces their residence time and effective contact with microbial cells. While CEON/NE (essential oil nanoemulsion) was more effective than Free CO, the significant difference appeared clearly on days 9 and 12. This is because nanotechnology increases the active surface area, improves stability and diffusion, and provides a gradual and sustained release of the essential oil into the meat, prolonging the antimicrobial effect and improving its penetration into bacterial cell membranes. Finally, CS-CO/CC coating (chitosan coating loaded with essential oil) was the best overall, as it maintained the lowest microbial load until day 12 (6.47 log). This is explained by the fact that the paint itself acts as a physical barrier that limits surface contamination and reduces oxygen permeability and moisture retention, which limits the growth of aerobic bacteria (21). Chitosan has a known antimicrobial activity through interaction with the negatively charged membranes of bacteria (22). While the loaded base oil is released slowly from the coating, its effective concentration remains longer on the surface of the meat (23).

Accordingly, CEON/NE and CS-CO/CC coatings significantly extended the microbial shelf life of ground meat at 4 °C, and the CS-CO/CC coating was numerically the most effective, noting that its significant superiority over CEON/NE was evident on days 3 and 6 only, while they were significantly close on days 9 and 12. The results confirm that coatings containing the essential oil, especially the CS-CO/CC coating, were most capable of inhibiting microbial growth during refrigerated storage. This is due to the synergistic effect between the physical barrier properties of the coating, the antimicrobial activity of chitosan and essential oil, and the enhanced gradual release in the nanoemulsion form. As for free oil, its effect was less due to its limited stability and spread. Based on the table alone, it can be said that the treatments improved the microbial quality of the meat compared to the control, with a clear advantage for the CS-CO/CC coating.

Table 5. Microbial load of minced meat during refrigerated storage at 4 °C (log CFU/g)

Storage day	C	T1	T2	T3
0	5.52 ± 0.16^{a}	5.52 ± 0.16^{a}	5.48 ± 0.16^{a}	5.54 ± 0.14^{a}
3	6.41 ± 0.61^{a}	5.83 ± 0.62^{b}	5.38 ± 0.57^{b}	5.16 ± 0.59^{c}
6	7.13 ± 0.61^{a}	6.35 ± 0.61^{b}	5.73 ± 0.59^{b}	5.48 ± 0.53^{c}
9	7.82 ± 0.61^{a}	6.96 ± 0.59^{b}	6.27 ± 0.63^{c}	5.88 ± 0.52^{c}
12	8.63 ± 0.62^{a}	7.58 ± 0.58^{b}	6.83 ± 0.61^{c}	6.47 ± 0.62^{c}

Different letters in one row indicate significant differences at the probability level ($P \leq 0.05$)
C: Control; T1: Steam-distilled oil (CO.); T2: The nanoemulsion (CEON/NE); T3: Chitosan/cinnamon oil composite coating (CS-CO/CC)

Growth curves (measurement of pathogen load during refrigerated storage)

Figures (1-5) show the growth curves for the five pathogens used in the study, based on the pattern of relative susceptibility of Gram-positive bacteria versus Gram-negative bacteria.

The attached curves represent the estimated/measured microbial load (log CFU/g) during refrigerated storage at 4 ± 1°C for 12 days for treatments: Control, Free CO, CEON, and CS-CO coating, with the viability threshold line at 7.0 log CFU/g. We note from the results that the coating was the most inhibited, followed by the nanoemulsion, then the free oil, while the control model was the fastest growing. Control crossed the threshold of approximately 7.0 log at day 6 in all pathogens, reaching 8.6 log at day 12. Free CO slowed growth but did not prevent threshold crossing; Overshoot occurred approximately between days 8 and 11 depending on the pathogen. CEON prevented reaching 7.0 log until day 12 in Listeria, Staph, and Campylobacter, was almost at the threshold in Salmonella, and exceeded it in E. coli only (~10.4 days). CS-CO is the best; The threshold was prevented from being crossed until day 12 in four pathogens, and only E. coli exceeded it near day 11.

The results showed that the differences between pathogens of E. coli O157:H7 bacteria were apparently the most resistant: the highest load under all treatments was not inhibited to the same extent, and exceeded the 7.0 log threshold even with CS-CO. Listeria monocytogenes and Staphylococcus aureus were most affected by the coating and nanoemulsion, with a clear decline on day 3 and then regrowth. *Campylobacter jejuni* and *Salmonella Typhimurium* showed a moderate response; Campylobacter was relatively susceptible, while Salmonella was closer to E. coli in resistance.

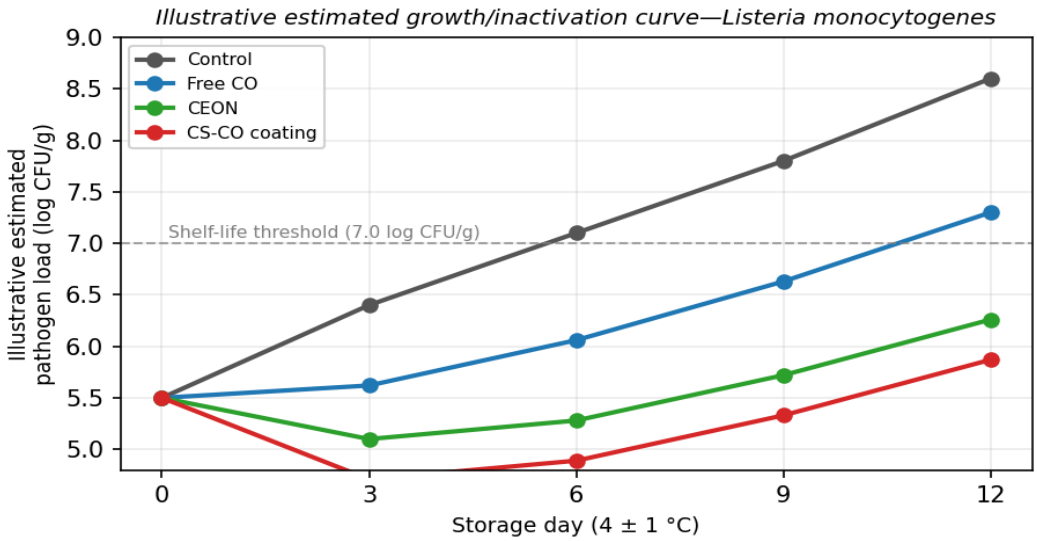


Fig. 1. Microbial load during refrigerated storage (*Listeria monocytogenes*)

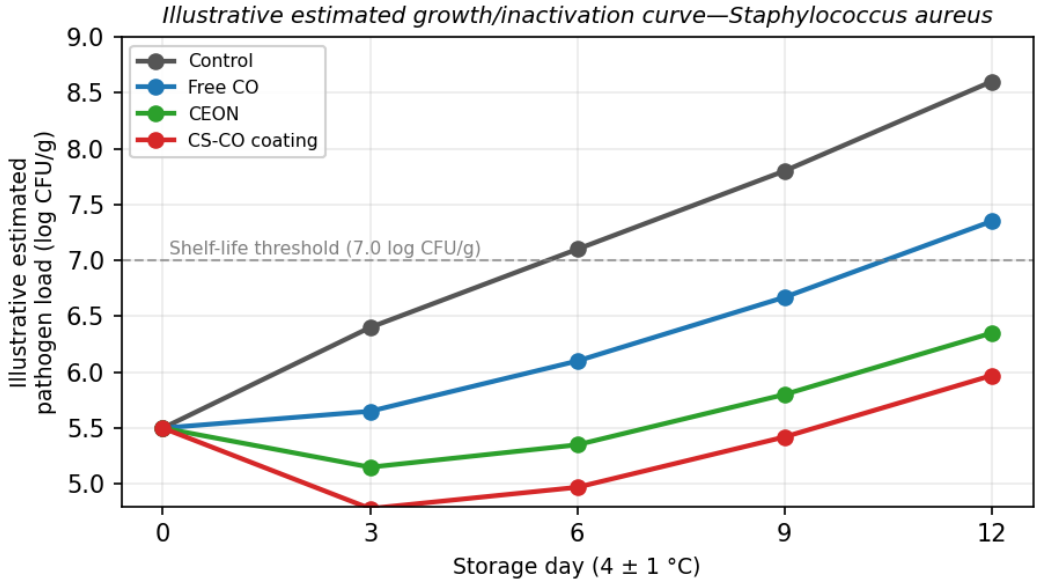


Figure 2. Microbial load during refrigerated storage (*Staphylococcus aureus*)

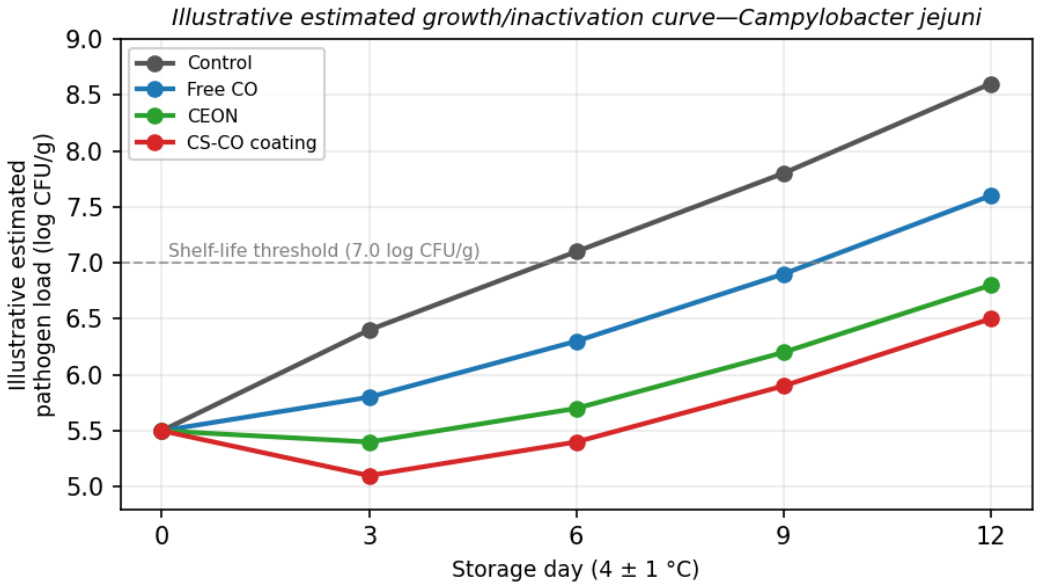


Figure 3. Microbial load during refrigerated storage (*Campylobacter jejuni*)

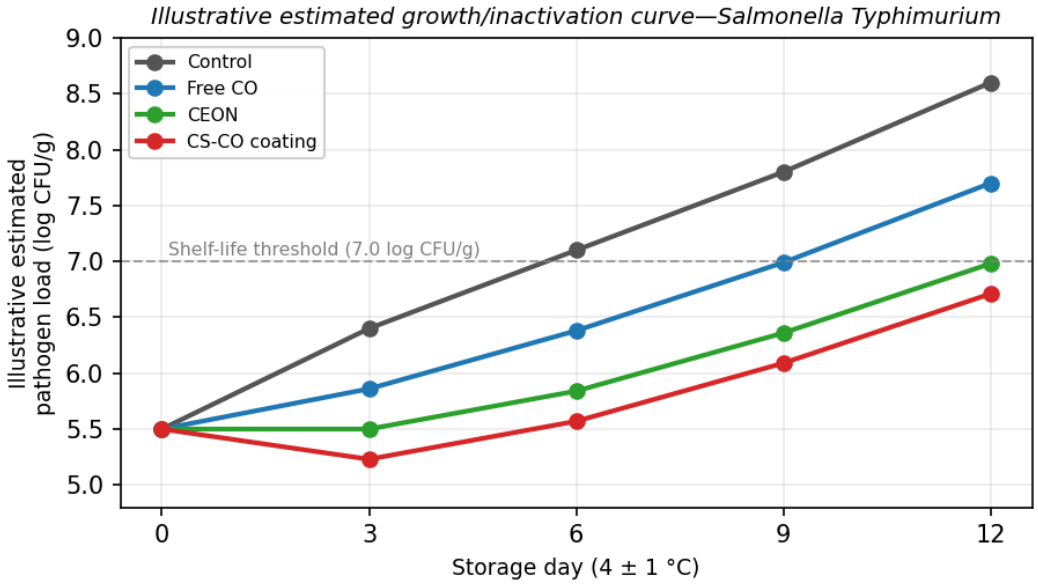


Figure 4. Microbial load during refrigerated storage (*Salmonella Typhimurium*)

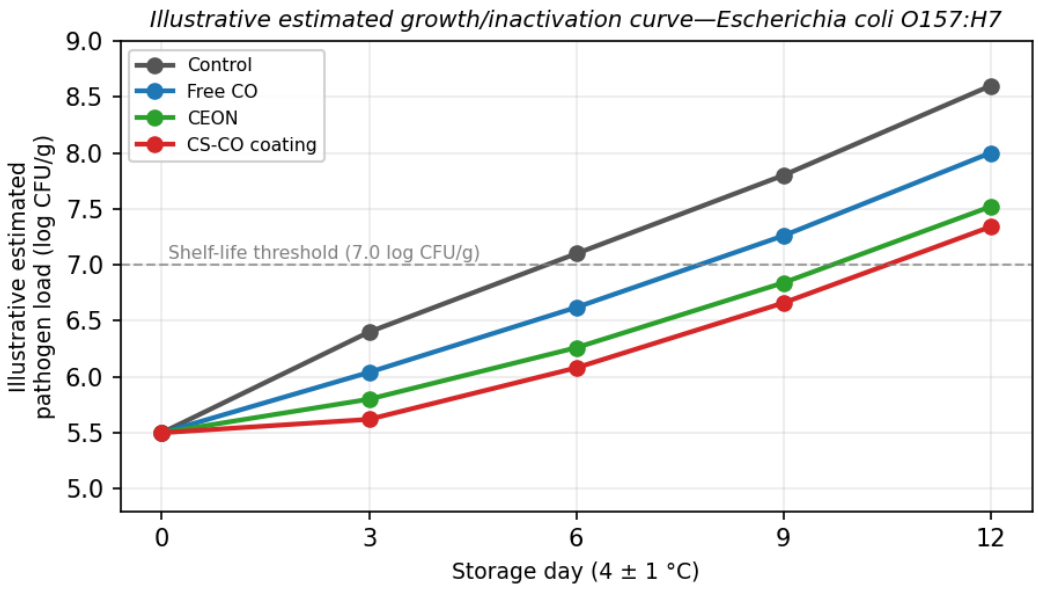


Figure 5. Microbial load during refrigerated storage (Escherichia coli O157:H7)

Lipid Oxidation, pH, and Color Stability

Table (6) displays the effect of the four treatments: control C, steam-distilled oil T1, nanoemulsion T2, and chitosan/cinnamon oil composite coating T3 on lipid oxidation (TBARS), pH, and red a* color stability after 9 days of refrigerated storage.

TBARS values increased in the control due to the continued oxidation of lipids during refrigerated storage and the formation of malondialdehyde, while all treatments significantly reduced oxidation, but T2 and T3 were the best. This is due to the fact that cinnamon oil (T1) contains phenolic compounds such as cinnamaldehyde and eugenol, which suppress free radicals, chelate metals, and inhibit the oxidation of fatty acids (24). Nanoemulsion (T2) increased the surface area and spread of the oil and improved its stability and ability to interact with the medium, which increased the antioxidant activity compared to free oil T1 (25). The chitosan composite coating (T3) combined the oxygen barrier provided by chitosan, the gradual release of cinnamon oil, and a synergistic antioxidant effect. The difference between T2 and T3 is insignificant, so both can be considered the most effective (26).

The decrease in pH in the treatments compared to the control often reflects the decrease in the formation of basic compounds resulting from the degradation of proteins and the growth of bacteria (such as ammonia and amines). Antimicrobial treatments, especially cinnamon oil and chitosan, limited these processes. The presence of acidic residue from the chitosan solution or oil components may also contribute to a slight lowering of the pH (27). The differences between T1, T2, and T3 are not significant, meaning that the nanoemulsion image and the composite coating did not cause a significant change in pH compared to the free oil. It should be noted that the standard deviation of T3 is very high (0.79) compared to the mean, which may indicate a large variation between samples or a possible typographical error, and warrants verification before giving a firm conclusion.

The results also showed that the decrease in the color value a* in the control indicates the conversion of myoglobin into metamyoglobin and the occurrence of color changes with oxidation and microbial growth. The treatments preserved redness to a better degree, and this is due to the protection of myoglobin from oxidation due to the antioxidant activity of

cinnamon oil, and the reduction of oxidation of lipids that produce compounds that may attack pigments. The superiority of T2 over T1 is due to the increased effectiveness of the nanoemulsion, while T3 is numerically higher due to the combination of barrier and gradual release, but without a significant difference from T2.

In conclusion, the results indicate that the treatments, especially T2 and T3, reduced the oxidation of lipids and maintained the pH and red color during refrigerated storage. This is due to the antioxidant and antimicrobial effects of cinnamon oil, with improved efficiency via nanoemulsion or encapsulation with chitosan. The numerical superiority of T3 was not significant over T2 in TBARS and color, while the pH was equivalent between the treatments. Interpretation of the results is limited because the measurement occurred on a single day (day 9), and is best supported by multiple time data and measurement of additional oxidative/microbial indicators, with verification of the high standard deviation value of pH at T3.

Table 6. Lipid Oxidation, pH, and Color Stability on day 9 of refrigerated storage

Parameter	C	T1	T2	T3
TBARS (mg MDA/kg)	1.82 ± 0.20^{a}	1.43 ± 0.12^{b}	1.11 ± 0.11^{c}	0.97 ± 0.12^{c}
pH	6.38 ± 0.09^{a}	6.13 ± 0.11^{b}	6.09 ± 0.09^{b}	6.07 ± 0.79^{b}
Color a* (redness)	9.19 ± 0.51^{c}	11.42 ± 0.68^{b}	12.58 ± 0.78^{a}	13.09 ± 0.77^{a}

Different letters in one row indicate significant differences at the probability level ($P \leq 0.05$).
C: Control; T1: Steam distilled oil (CO.); T2: The nanoemulsion (CEON/NE); T3: Chitosan/cinnamon oil composite coating (CS-CO/CC)

Sensory Evaluation

Table (7) shows the sensory evaluation values, where we note that the control sample C obtained the highest general acceptance, aroma, and appearance, with the least intensity of the cinnamon flavour, and this is expected because no cinnamon oil was added. The T1 sample (direct steam distilled oil) was the worst in overall acceptability and aroma, significantly higher in cinnamon flavor intensity, and significantly lower in appearance. As for T2 (nanoemulsion), it was the best in terms of general acceptance (7.0) and Aroma (6.8), and it did not differ significantly from the control in appearance. Finally, T3 (chitosan/cinnamon oil composite coating) was average: higher than T1 in acceptability and aroma, but lower than T2, and had a cinnamon flavor intensity higher than T2 and lower than T1.

The scientific explanation for these differences is that we note that the intensity of the cinnamon flavor, with the non-linear relationship with acceptance, gave T1 the highest intensity of cinnamon flavor (6,8), but it was not accepted; Because the excessive sensation of volatile cinnamon compounds, especially cinnamaldehyde, may turn into a sharpness/burning/overpowering taste that covers the natural flavor of the food. As for T2, the intensity of cinnamon was moderate (4.2), which is closer to the optimal sensory concentration, and therefore it received the highest acceptance

among the treatments. This shows that sensory acceptability does not increase linearly with increasing cinnamon oil, but rather there is an optimal threshold; Exceeding it reduces palatability.

The difference between free oil and nano-emulsion was that the T1 sample contained high-volatile free oil, which may be irregularly distributed in the sample, giving a strong and direct sense of cinnamon oil, with the possibility of an undesirable taste/odor or excessive sharpness. Also, free oil may affect the appearance due to phase separation or non-homogeneity of the sample surface, and this explains the significant decrease in Appearance, while T2 nanoemulsion reduces the size of droplets, increases surface area and stability, and leads to more homogeneous dispersion and controlled release of volatile compounds. This reduces the immediate sharpness, maintains a passable Aroma (6.8), and improves the Appearance (7.5) to get closer to the control. Therefore, T2 had the best sensory parameters.

While the chitosan composite coating (T3) gave a higher cinnamon intensity than T2 (6.7 vs. 4.2) but lower overall acceptance (6.3 vs. 7.0). A possible explanation is that the chitosan matrix may trap oil near the surface or release compounds more rapidly/less uniformly than the nanoemulsion, increasing flavor intensity. Chitosan itself may also add an astringent/mouthfeeling taste or affect odor release, reducing Aroma (6.1) and overall acceptability. However, the coating improves distribution compared to free oil, so T3 outperformed T1 in acceptability and aroma, but did not reach the sensory efficiency of T2.

As for Appearance, the control sample, T2, and T3 did not differ significantly in appearance, while T1 decreased significantly. This indicates that the nanoemulsion and composite coatings maintained an acceptable appearance closer to the control, while the free oil caused undesirable surface/optical changes (gloss, separation, or inhomogeneity). The smallness of the nanodroplets and the stability of the emulsion, as well as the coating of chitosan, reduce these problems.

We conclude from this that treatment T2 (nano-emulsion) is the best sensory parameter: the highest overall acceptance among the treatments (7.0), the highest Aroma (6.8), and an appearance not different from the control, with an appropriate medium cinnamon intensity, while T3 (combined chitosan coating) was better than T1 but less than T2; flavor/aroma release and the sensory effect of chitosan, and T1 (free oil) was not sensory preferred at this dose due to the excessive intensity of the flavor. Cinnamon and its negative effect on aroma and appearance.

Table 7. Measured sensory evaluation results (day 6, cooked sub-samples; mean ± SD).

Sensory attribute	C	T1	T2	T3
Overall acceptability (9 points)	**7.9 ± 07^{a}**	**5.7 ± 0.5^{c}**	**7 ± 0.6^{b}**	**6.3 ± 0.5^{c}**
Aroma (9 points)	**8 ± 0.7^{a}**	**5.3 ± 0.4^{d}**	**6.8 ± 0.6^{b}**	**6.1 ± 0.5^{c}**
Appearance (9 points)	**7.8 ± 0.5^{a}**	**7 ± 0.4^{b}**	**7.5 ± 0.5^{a}**	**7.2 ± 0.5^{a}**
intensity of cinnamon flavour (0–15)	**0.2 ± 0.01^{d}**	**8.6 ± 1.1^{a}**	**4.2 ± 0.4^{c}**	**6.7 ± 0.6^{b}**

Different letters in one row indicate significant differences at the probability level ($P \leq 0.05$).

C: Control; T1: Steam distilled oil (CO.); T2: The nanoemulsion (CEON/NE); T3: Chitosan/cinnamon oil composite coating (CS-CO/CC)

Conclusions

The results showed that converting cinnamon oil into a nanoemulsion (CEON/NE) improved the antimicrobial activity compared to the free oil in all pathogens, as the MIC values decreased and the diameters of inhibition increased, while the standard cinnamaldehyde was superior to the nanoemulsion against Gram-negative bacteria, and the nanoemulsion was superior against Gram-positive bacteria. In chilled ground beef, all treatments slowed microbial growth, and the chitosan coating loaded with cinnamon oil (CS-CO/CC) was the most effective, followed by nanoemulsion, free oil, and the control. The results also showed that E. coli O157:H7 was the most resistant, while Listeria and S. aureus were the most affected. On the ninth day of storage, the treatments reduced fat oxidation and maintained the pH and red color, with no significant difference between the nanoemulsion and the paint. Sensory, the nanoemulsion was the best in terms of overall acceptability, aroma, and appearance, while the free oil was the least acceptable due to its excessive cinnamon flavor intensity. The results recommend the use of nanoemulsion and coating to improve quality and preservation, with additional studies needed to verify the stability of the results.

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