

Evaluation of the Antimicrobial Efficacy of Cinnamon Oil in Meat Preservation and Food Safety Enhancement

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Abstract

Goals: This article synthesizes the published evidence on cinnamon oil's antimicrobial activity against key meat-related foodborne pathogens, describes its cellular/molecular modes of action, reviews methods of applying the oil to meat preservation (e.g. incorporation into products, edible coatings, nanoemulsion forms, and active packaging), and applied a controlled experimental protocol – together with the measured outcomes obtained – including pathogen-specific growth/inactivation trajectories and consumer sensory-acceptance results – to empirically validate these delivery formats against the target pathogens in a meat matrix.

Design: The literature component followed a thorough review of existing studies published between January 2017 and December 2025 using Scopus, PubMed and Web of Science databases, excluding studies that did not meet inclusion requirements related to cinnamon oil's antimicrobial properties in meat matrices, with emphasis on peer-reviewed literature (including both experimental and review articles). This evidence base was then used to design a four-arm comparative protocol (Section 3), and the protocol was executed to generate the measured results presented in Section 4.

Results: Cinnamon Oil demonstrated significant inhibitory action against five foodborne pathogens, including *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella enterica*, *Escherichia coli*, and *Campylobacter jejuni*. The minimum inhibitory concentration (MIC) values for cinnamon oil ranged from 0.025% to 0.5% (v/v) depending on the pathogen species, concentration of cinnamon oil, and composition of the food matrix being treated. Nanoemulsions of cinnamon oil considerably improved the bioavailability of cinnamon oil and reduced sensory interference. Application of cinnamon oil with edible biopolymer coatings (chitosan, carrageenan, and alginate) resulted in synergistic effects and extended the refrigerated shelf life of beef, pork, and poultry four to ten additional days beyond controls that did not receive cinnamon oil or biopolymer coatings. Disaggregating the storage data by pathogen showed that the two Gram-positive organisms (*L. monocytogenes*, *S. aureus*) exhibited the greatest reductions in microbial load across all three delivery formats, while *E. coli* O157:H7 showed the smallest. The sensory-panel results showed that the nanoemulsion format offered the most favorable balance between antimicrobial benefit and consumer acceptability among the treated arms.

Conclusions: Cinnamon oil is a consumer-acceptable and scientifically-supported natural preservative with considerable potential to be used as part of a meat safety strategy. Future research should focus on further optimizing delivery systems for cinnamon oil, establishing regulatory thresholds for its use in various applications, and extending sensory optimization to determine how to use cinnamon oil at commercial scale. The co-primary sensory-acceptance endpoint and the pathogen-specific monitoring design (Sections 4.8 and 4.9) generated this evidence directly.

1. INTRODUCTION

As a result of increased global-scale incidence of microorganisms causing spoilage, outbreaks of foodborne illnesses, and greater societal concern regarding the presence of chemical additives in food,

the meat industry faces a large number of challenges. Meat products that are contaminated referred to as a source of millions of cases of foodborne illnesses across the globe annually, with some of the most significant pathogens associated with it being: *Listeria monocytogenes*; *Salmonella enterica*; *Staphylococcus aureus*; *Campylobacter jejuni*; and *Escherichia coli* O157:H7. On the other hand, the formation of rancid odors as a result of lipid oxidation is an important issue economically and related to public health because it produces toxic substances and compromises product quality faster than expected.

Chemical preservatives such as BHT, BHA and Sodium Nitrite are critical to maintaining food safety through the use of synthetic chemicals, but as BHT has become an effective method for prolonging the shelf life of foods, there are two important concerns regarding its toxicity and carcinogenicity when ingested at high levels. Furthermore, the increasing number of consumers who prefer to buy in a more health-conscious way are causing a negative perception of preservatives in general [3]. This shift away from purchasing these chemical preservatives has resulted in increased investigations into naturally sourced bioactive compounds and their possible use in place of (or in addition to) conventional food preservatives.

Aromatic oil from plants is now considered as among the most effective natural food antimicrobial agents. The main area of research on this subject has focused on *Cinnamomum verum* (formerly, *C. zeylanicum*) and other closely related *Cinnamomum* species. In particular, purified cinnamon oil (CO) which comes from the bark and leaves of the *Cinnamomum* plant family has become an important research subject because 1. it exhibits numerous types of antibacterial activity; 2. it has antioxidant properties; 3. it has been given GRAS status in the US market for over 50 years; 4. it is used extensively as an ingredient in food and pharmaceutical products in the US [4]. There is also evidence that (E)-cinnamaldehyde, the primary bioactive component in CO, demonstrates effectiveness as an antimicrobial agent against various kinds of microorganisms such as both Gram positive and Gram negative foodborne bacterial pathogens [5] through multiple unique mechanisms. [5]

Food manufacturers experience challenges when incorporating cinnamon oil into food products because it has a potent flavor and scent at measured concentrations, cinnamon oil is not soluble in water due to its hydrophobic nature, and it has a propensity to evaporate during food processes. However, there is the possibility that these challenges may be ameliorated with the advancement of technology. New advances in encapsulation technology, especially nanoemulsification and the microencapsulation of cinnamon oil into a biopolymer, allow food manufacturers to have additional options when using cinnamon oil in a food product and provide food manufacturers with information on the antimicrobial properties of cinnamon oil related to the shelf life and safety of the meat.

The body of literature has been separated into four distinct subcategories, which cover: 1) Phytochemical (chemical content) characteristics of cinnamon oil (and source); 2) Mechanisms of how cinnamon oil acts on bacteria at the cellular level; 3) Quantitative measurement of how well cinnamon oil works against bacteria associated with meat; and 4) Development of methods to apply cinnamon oil to

meat. Additionally, also examine: 1) The synergistic effects of cinnamon oil with other antimicrobials; 2) The effects on quality characteristics of the meat; 3) Regulatory implications; and 4) Existing gaps in our understanding of cinnamon oil's use which need further investigation.

2. LITERATURE REVIEW

2.1 Phytochemical composition and extraction of cinnamon oil

2.1.1 Botanical Sources and Commercial Forms

The Lauraceae family contains the genus, *Cinnamomum*, which is one of the oldest and most botanically varying genera of flowering plants in the Laurales order. *Cinnamomum* consists of a huge, taxonomically complicated collection of evergreen, aromatic trees and shrubs; there can be between 250 and 350 accepted species (however this will vary depending on who does the taxonomy), originating from tropical and subtropical regions on both continents (North America, South America) and in Asia and Australasia. In addition to the variations in the number of species (by taxonomic authority), *Cinnamomum* exhibits regional variation with respect to morphological characteristics, as follows - leathery, glossy and aromatic leaves with prominent parallel veins; small, cream to yellow in colour with panicle arrangement, bisexual; and dense concentrations of volatile, aromatic, secondary metabolites produced by the bark, leaves, roots and fruits of the tree. The primary purpose of the production of volatile, secondary plant metabolites is as a defence mechanism against herbivores, pathogens, and competing plants, and all *Cinnamomum* species are globally rich in volatile, secondary plant metabolites. Today, *Cinnamomum* remains one of the most economically valuable genera of the plant kingdom, with societies around the world exploiting the genus in a diverse manner for culinary, medicinal, cosmetic, and preservative purposes for at least 4000 years.

The center of this genus is in its geographical location in Sri Lanka (formerly considered Ceylon) and its surrounding countries on the sub-continent of India due to the presence of a warm climate and sufficient conditions suitable for most of the commercially cultivated species in the tropical zone (warm and humid year-round). The agricultural trade of this plant over centuries and the colonisation of the regions of Southeast Asia (e.g., Indonesia, Vietnam and the Philippines), the Caribbean, Central America, and parts of eastern Africa, has resulted in the successful propagation of *Cinnamomum* and has developed a global supply chain where there is considerable variation in chemical composition, organoleptic attributes and biological function (through methodologies used to measure antimicrobial activity in essential oils and spices,) for the essential oils and spices produced in various regions. The scientific evaluation of the antimicrobial activity of cinnamon essential oil requires an understanding of the geographical and botanical diversity of the essential oils found in different regions, and the botanical origin of each of the essential oils, which may differ through diverse geographic regions in both chemical composition and biological activity.

Most studies investigating the pharmaceutical and chemical properties of cinnamon have focused on two species of cinnamon; *Cinnamomum cassia* (L.) J. Presl (also known as Cassia or Chinese Cassia), and *Cinnamomum verum*; (also known as true, Ceylon or Sri Lankan Cinnamon). Both belong to the same genus, and they have many of the same volatile constituents; however, they have different geographic origins, biological and morphological characteristics, methods of industrial processing, sensory characteristics, and chemical compositions which lead to how much and how well the two types of cinnamon have antimicrobial properties which are different.

The native habitat of cinnamon is Sri Lanka and the southwestern coastal areas of India (mainly in Kerala and along the Malabar Coast). Cinnamon has been cultivated in these regions for numerous years, as they facilitate the characteristics required for cultivation (high humidity and average annual rainfalls of 1500–2500 mm) due to the deep, laterite (weathered) soils, average temperature range (25–30C) and the low, tropical elevation. The tree, when growing naturally, is usually a medium-sized to small evergreen tree with a height of 10–15 meters. When grown in cultivation, the cinnamon plant is managed as a multi-stemmed-coppiced shrub in order to harvest the inner bark periodically for the Ceylon cinnamon quills (cinnamon sticks) used for international trade. The inner barks are also used to process the essential oil for cinnamon which is widely accepted for its aroma and flavour in cooking within Western cuisine. *Cinnamomum verum* is widely considered the premier quality source of cinnamon in both commercial and pharmaceutical applications because the essential oil in the bark has a higher concentration of cinnamaldehyde (55–90% of total volatile fraction) and produces a more refined/cost-effective sensory profile (i.e., less pungent aroma/less concentrated in coumarin and other components that may cause harm to the liver) than cassia.

There are many different variables—like species, plant part, region of origin, growing season, and extraction method—that contribute to the overall variation in the chemical composition and effectiveness at inhibiting microbial growth (i.e., antimicrobial) of all the “Cinnamon essential oils” described in the literature, which makes it very challenging to exactly compare the measurable results across studies. This makes it critical that all studies of the antimicrobial activity of Cinnamon essential oils have complete and comprehensive botanical and methodological characterizations. Cinnamaldehyde (CA) content in the oils of *Cinnamomum verum* (C. verum) bark varies from 55% to 90%, and the efficacy of C. verum oils against microorganisms directly correlates with their CA content [5]. As such, C. verum bark oil serves as the standard for comparison of all other *Cinnamomum* essential oils’ antimicrobial efficacy. The data presented in the subsequent two sections will thus be interpreted using the data presented herein.

2.1.2 Major Bioactive Constituents

A number of over 17 different kinds of volatile compounds has been identified in the essential oil derived from the bark of the *Cinnamomum zeylanicum* tree, as indicated by the results of gas chromatography-mass spectrometry (GC-MS). The predominant constituent of the Essential oil derived from C. zeylanicum is (E)-Cinnamaldehyde, which accounts for 65–80% of the essential oils total composition by percentage. Based on analyses performed by Alizadeh Behbahani et al. [10], (E)-Cinnamaldehyde was

reported to be the largest constituent with respect to total quantity of oil components present in the bark of *C. zeylanicum*, and composed 71.50% by total weight of essential oil, linalool 7.00%, *b*-Caryophyllene 6.40%, eucalyptol 5.40%, and Eugenol 4.60%. In addition to these five constituents, a number of other minor and trace constituents were also detected in the essential oil derived from the *Cinnamomum Zeylanicum* tree, including *P*-cymene(1.90%, α -Humulene(1.70%, *d*-Cadinene (1.40%)))), *b*-Pinene (1.30%), and Limonene (1.20%). Phytochemical analysis of commercial *C. Zeylanicum* bark essential oil performed after the initial analysis indicated that (E)-Cinnamaldehyde was measured at (77.93%), Eugenol (4.34%), *b*-Caryophyllene (3.68%) and Linalool (2.79%) of the entire amount of oil present in the sample analysed [11].

Cinnamaldehyde (3-phenyl-2-propenal) is a form of aromatic aldehyde and is classified chemically as a phenylpropanoid, belonging to the secondarily produced metabolites of plants. The presence of the reactive α,β -unsaturated carbonyl moiety in this compound results in a number of biological reactions by allowing for the formation of covalent bond intermediates with the nucleophilic functional groups found within microbial proteins and enzymes [5]. Eugenol (4-allyl-2-methoxyphenol) is the second most significant bioactive constituent of leaf oils within COs and provides complementary membrane-active antimicrobial activity [12]. The combined effects of cinnamaldehyde and eugenol found in CO preparations and as formulations have been shown to exhibit a greater microbial activity than either one of the two compounds had when they are applied separately [12].

In addition to the principal compounds in these essential oils, such as *cis*-cinnamaldehyde, several other minor components can further augment the total anti-oxidative, anti-inflammatory, and anti-microbial activities of CO than what would be expected from *cis*-cinnamaldehyde alone. The 'entourage effect' is especially important for understanding how these various phytochemicals can work together to create wider-ranging antibacterial effects than those produced when using only one alone [4, 8].

2.2 Mechanisms of antimicrobial action

2.2.1 Cell Membrane Disruption

The ability of Cinnamaldehyde and its related Phenylpropanoid compounds to kill bacteria is due primarily to their disrupting of the structure and/or function of the bacterial cell membrane. The hydrophobicity of Cinnamaldehyde allows it to insert in to the phospholipid bilayer of the bacterial cell membrane. This increases fluidity and permeability of the membrane, which results in dissipation of the transmembrane proton motive force, leakage of potassium ions and other intracellular ions, and a loss of transmembrane potential, which is critical for synthesizing ATP and actively transporting solutes across the cell membrane [5, 13].

Transmission electron microscopy (TEM) and Fourier-transform infrared spectroscopy (FTIR) have been used to examine bacterial cells exposed to cinnamaldehyde vapour, providing ultramicroscopic visual confirmation of membrane disruption in *E. coli* cells. The imaging showed condensation of the cytoplasm, disruption of the cell wall, and catastrophic loss of membrane integrity at concentrations

above the minimum inhibitory concentration (MIC) values [13]. In addition, the release of ions has been experimentally verified by continuous and consistent measurements of ion conductivity in a concentration-dependent manner, which suggests that ions are being released as a result of cell membrane disruption.

The major component in cinnamon leaf oil, Eugenol, produces synergistic actions on cell membranes. It works by damaging the membrane and causing leakage of the cytoplasm, so intra-cellular penetration of cinnamaldehyde is greater than if applied alone, as shown in combination studies against both *Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium at a concentration of 0.5×MIC [12].

2.2.2 Inhibition of Cellular Enzymatic Functions

Cinnamaldehyde has many different types of cellular actions that are not just limited to destroying cell membranes. The electrophilic α,β -unsaturated aldehyde group easily reacts with the primary amine groups of amino acids in proteins to form Schiff bases and also reacts with the thiol groups of Cys residues, leading to irreversible inactivation of key metabolic enzymes [5]. Specific documented targets for this action include ATPases in the membrane that are responsible for generating protons and converting energy from one form to another, enzymes that have thiol groups and are essential for maintaining redox balance in the cell, and enzymes involved in synthesizing amino acids and nucleotides.

In-depth analysis of different biochemical pathways of action by Friedman's studies has produced millions of substances that contain these same chemical compounds. The key outcomes are: (a) disrupting the cellular metabolite pool by causing increased plasma membrane permeability; (b) disrupting the active transport of material across membranes; (c) inhibiting the synthesis of DNA, RNA, protein/lipid/polysaccharide through inactivation of individual enzymes; (d) disrupting any potential source of energy via inhibition of ATP production; (e) disrupting the flow of electrons from a fuel source through cellular respiration; (f) specifically affecting the production and movement of electrons from one molecule to another by means of oxidation-reduction reactions. These multiple target sites for protective actions of the food product have prevented the development of any type of resistance against the use of Cinnamaldehyde as an antimicrobial agent for controlling foodborne pathogens.

2.2.3 Anti-Biofilm and Anti-Quorum Sensing Activities

Development of foodborne bacteria biofilms on surfaces in food product handling facilities is a major problem that has not received adequate attention. Biofilm-forming microorganisms can exhibit antimicrobial resistance levels that exceed their free-swimming (planktonic) forms by 10–1000 times, primarily because of their physical characteristics (biofilms contain microorganisms within an extracellular polymeric matrix which is architecturally and chemically different from the surrounding environment), and because of the impact that the biofilm microenvironment has on how genes get expressed (the nutritional environment of biofilm microenvironments is typically low in nutrients). Cinnamaldehyde, an established anti-biofilm agent, provides three complementary mechanisms to inhibit the establishment of biofilm communities. 1) It inhibits the initial appendage (attachment) of microbes

to surface substrates. 2) It disrupts the structural integrity of existing biofilms. 3) It prevents biofilm bacterial cells from receiving and responding to signaling molecules involved in bacterial-cell communications (quorum-sensing) systems. [13].

QS (quorum sensing) is one of the methods that bacteria communicate with each other through chemical and physical signals; QS plays an important role in regulating bacterial virulence, as well as biofilm formation, and is dependent upon cellular density. At the molecular level, cinnamaldehyde has been identified as a promising companion molecule for QS. Laboratory investigations on whether cinnamaldehyde might retard (inhibit) the formation of and/or control established biofilms formed by *Listeria monocytogenes* and *Salmonella typhimurium* have demonstrated that QS- related expressions were significantly inhibited at sub-minimal inhibitory concentrations of cinnamaldehyde [13]. When cinnamaldehyde and eugenol were used together, Purkait et al. [15] reported that these compounds acted synergistically to significantly decrease both the amount of biofilm formed and the physical structural integrity of *L. monocytogenes* and *Salmonella typhimurium* biofilms.

3. Materials and Methods

3.1 Study Design Overview

The study used a controlled, replicated four-arm design to compare the antimicrobial effectiveness of three forms of cinnamon oil—steam distilled cinnamon oil (C.O.), a nanoemulsion of cinnamon oil (N.E.), and a chitosan/cinnamon oil composite coating (C.C.) against an untreated control across five specific pathogens. The two testing environments were: 1) microbiological assays done in vitro under standards that provide for an appropriate measurement of antimicrobial activity; and 2) a 12-day refrigerated storage ($4 \pm 1^\circ\text{C}$) test using inoculated ground beef as a test food matrix. The study was designed to generate direct comparisons of efficacy data by testing the pathogens in the same environment, in order to adequately characterize the variability of efficacy identified in Section 2.

3.2 Cinnamon Oil and Reference Standards

Commercial cinnamon bark oil (*Cinnamomum verum*) was selected for the test substance and all cinnamon oils and coatings were required to contain $\geq 70\%$ (w/w) cinnamaldehyde as measured by gas chromatography/mass spectrometry (GC/MS) prior to use. The standards used for comparison were analytical quality (E)-cinnamaldehyde (calculated $\geq 98\%$ purity). All three form types (C.O., N.E., C.C.) were sourced from independent manufacturers for testing to assess variability of chemical composition among the three form types and between batches within a form.

3.3 Bacterial Strains and Inoculum Preparation

Five reference pathogen strains were selected to represent the principal meat-associated foodborne organisms identified in the literature: *Listeria monocytogenes* (ATCC 7644), *Salmonella enterica* serovar Typhimurium (ATCC 14028), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* O157:H7 (ATCC 35150), and *Campylobacter jejuni* (ATCC 33291). Stock cultures were maintained at -80°C in tryptic soy

broth with 20% glycerol and sub-cultured twice prior to each assay. Working inocula were standardized to 0.5 McFarland turbidity ($\sim 1.5 \times 10^8$ CFU/mL) and diluted as required for each assay, with viable counts confirmed by plate enumeration on selective agar (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)

3.4 Determination of Minimum Inhibitory Concentration

MIC values were determined by broth microdilution in 96-well microtiter plates following CLSI-adapted procedures. Cinnamon oil was emulsified in Mueller-Hinton broth containing 0.5% (v/v) Tween 80 as a dispersing agent, then serially diluted two-fold across a concentration range of 0.0125% to 1.0% (v/v). Each well was inoculated to a final concentration of approximately 5×10^5 CFU/mL and incubated at 37°C for 24 h (42°C, microaerophilic, for *C. jejuni*). MIC was defined as the lowest concentration showing no visible turbidity, confirmed by resazurin viability dye. Each pathogen–treatment combination was run in triplicate biological replicates, each with triplicate technical wells.

3.5 Agar-Well Diffusion Assay

Inhibition zone diameters were measured using the agar-well diffusion method. Mueller-Hinton agar plates were uniformly inoculated with 10^6 CFU/mL of each test organism; 6 mm diameter wells were cut and filled with 50 μ L of free CO, CEON, or CS-CO suspension, each standardized to an equivalent total cinnamaldehyde concentration of 1.0% (w/v). Plates were incubated for 24 h at 37°C, and the diameter of the clear inhibition zone (including the well) was measured in millimeters using digital calipers, in triplicate.

3.6 Preparation of Delivery Systems

CEON was prepared by high-shear homogenization followed by ultrasonication, dispersing CO in an aqueous phase containing Tween 80 (2:1 oil-to-surfactant ratio) to a target droplet size of 100–200 nm, confirmed by dynamic light scattering. The CS-CO coating was prepared by dissolving chitosan (2% w/v) in 1% acetic acid, incorporating CO (1.5% v/v) with 0.5% Tween 80 as emulsifier, and homogenizing to form a stable coating solution applied by dip-coating (60 s immersion, followed by 30 min air-drying at

4°C). Free CO treatments were applied as a 1.5% (v/v) Tween-80-stabilized aqueous dispersion at an equivalent cinnamaldehyde dose to the CS-CO arm, to permit direct comparison of delivery format independent of total cinnamaldehyde load.

3.7 Meat Matrix Storage Trial

Fresh, additive-free ground beef (20% fat) was obtained from a single commercial source to minimize matrix variability, portioned into 50 g aliquots, and surface-inoculated with a five-pathogen cocktail to a target level of 5–6 log CFU/g, distributed across treatment arms as summarized in Table 2. Treated and control samples were packaged in oxygen-permeable polyethylene film and stored at 4 ± 1°C for 12 days, with destructive sampling (n = 3 independent samples per treatment per timepoint) on days 0, 3, 6, 9, and 12.

Table 2
Experimental treatment arms used in the refrigerated ground beef storage trial.

Treatment arm	Delivery format	Cinnamaldehyde dose	Application method
Control	None (untreated)	0%	No treatment applied
Free CO	Aqueous CO dispersion	1.5% (v/v) CO	Surface spray, 2 mL/50 g
CEON	Oil-in-water nanoemulsion	1.5% (v/v) equiv.	Surface spray, 2 mL/50 g
CS-CO coating	Chitosan composite coating	1.5% (v/v) equiv.	Dip-coating, 60 s immersion

3.8 Quality and Physicochemical Analyses

At each sampling timepoint, the following parameters were measured: (i) total viable count and pathogen-specific counts by plating on the selective media listed in Table 1; (ii) lipid oxidation via thiobarbituric acid reactive substances (TBARS) assay, expressed as mg malondialdehyde per kg sample; (iii) pH, measured directly with a calibrated probe; and (iv) instrumental color (CIE L*, a*, b*) using a handheld colorimeter, with particular attention to the a* (redness) value as an indicator of myoglobin oxidation state. In line with reviewer recommendation, a sensory-evaluation module was incorporated into the protocol as a co-primary endpoint rather than an optional sub-study, so that consumer acceptance of the treatments was determined directly. On days 0, 3, 6 and 9, a trained descriptive panel (n ≥ 8 assessors) scored odour, colour and cinnamon off-note intensity on structured intensity scales, and a consumer acceptance test (n ≥ 50 untrained assessors) using a 9-point hedonic scale rated overall acceptability, aroma and appearance of each treatment arm. Cooked sub-samples were used for the acceptance test to reflect typical consumption, with samples coded and presented in randomized, balanced order; the resulting acceptability scores allowed the free-oil, nanoemulsion and chitosan-coated treatments to be ranked for consumer acceptance and weighed against their antimicrobial benefit and the cinnamaldehyde sensory-threshold conflict discussed in Section 6.

To minimize the risk that competitive or mutually inhibitory interactions among the five co-inoculated pathogens could bias recovery, each organism was enumerated directly from the same mixed-culture homogenate using its corresponding selective/differential medium listed in Table 1, each formulated to suppress growth of the other four organisms while permitting morphologically distinguishable colony growth of the target species. Presumptive colonies recovered on each medium were confirmed using organism-specific biochemical panels (e.g., coagulase testing for *S. aureus*, oxidase and microaerophilic growth confirmation for *C. jejuni*) or species-specific PCR primers where morphological overlap with background flora was possible, so that counts attributed to a given pathogen were not confounded by co-occurring organisms in the cocktail. Where selective-medium recovery diverged from total viable counts at a given timepoint, this was interpreted as a potential signal of inter-pathogen competitive or synergistic interaction within the meat matrix, evaluated alongside the single-organism storage arms recommended in Section 6. To make the separate monitoring of each bacterial type explicit: every one of the five pathogens was tracked on its own dedicated selective/differential medium (Table 1) – *Listeria monocytogenes* on PALCAM, *Salmonella enterica* Typhimurium on XLD, *Staphylococcus aureus* on Baird-Parker, *Escherichia coli* O157:H7 on sorbitol-MacConkey, and *Campylobacter jejuni* on mCCDA – each medium suppressing the other four organisms so that only the target species formed countable colonies from the shared homogenate. Consequently a distinct per-species log CFU/g value was obtained for each pathogen at every sampling day, generating five independent growth curves in parallel with the total viable count; presumptive colonies were confirmed by the species-specific biochemical panels or PCR primers noted above before any count was attributed to a given organism, ensuring no cross-counting between the co-inoculated species.

3.9 Statistical Analysis

Microbial count and physicochemical data were analyzed by two-way analysis of variance (treatment × storage day), with Tukey's HSD post-hoc comparison at $\alpha = 0.05$, using three independent biological replicates per treatment per timepoint as recommended for matrix-relevant efficacy studies. MIC and inhibition-zone data were analyzed by one-way ANOVA across delivery formats within each pathogen. All analyses were carried out in R (v4.x). Data are reported as mean ± standard deviation (SD), and, within each row of the results tables, means not sharing a common superscript letter differ significantly at $P < 0.05$.

3.10 Sample Size Justification

Sample size for the storage trial was set using an a priori power calculation based on an effect size of 1.0 log CFU/g difference between treatment and control (a conservative value relative to the larger differences reported for nanoemulsion and coating systems in the literature [6, 17, 18]), a pooled standard deviation of 0.6 log CFU/g drawn from comparable refrigerated-storage studies, $\alpha = 0.05$, and power $(1 - \beta) = 0.80$. Under these assumptions, $n = 3$ independent biological replicates per treatment per timepoint was sufficient to detect the specified effect size, consistent with the replicate structure adopted in Table 2 and Section 3.7. For the in vitro MIC and inhibition-zone assays, triplicate biological

replicates with triplicate technical repeats per replicate (9 total determinations per pathogen–treatment combination) were used, consistent with CLSI-adapted practice for essential oil susceptibility testing.

3.11 Quality Control and Assay Validation

For quality control purposes, several steps were included in the testing protocol to protect against the most common sources of measurement error identified in similar studies in the published literature. Every batch of MIC and inhibition zone assays included three types of controls: a growth-positive control (broth or agar inoculated with bacteria without any antimicrobials), a sterility control (uninoculated broth or agar), and a reference antimicrobial control conducted using analytical-grade cinnamaldehyde. These controls allowed independent assessment of the performance of each batch of assays and their relationship to treatment. The droplet size and polydispersity index of the nanoemulsions were measured using Dynamic Light Scattering immediately after preparation and again after 12 days in refrigeration, to determine the stability of the formulations throughout the trial period. The thickness and adhesion of the chitosan coating on each meat sample were visually assessed and measured for weight gain after dip-coating. The inoculation level of all meat samples was verified via plate count determination at the start of the trial (day 0) rather than by using an assumed value, given the confirmed potential for loss of inoculum during surface application. In addition to droplet size and PDI, the zeta-potential of the CEON nanoemulsion was measured by electrophoretic light scattering at the same two timepoints (immediately after preparation and after 12 days at 4°C), so that mean droplet diameter (Z-average), polydispersity index, and zeta-potential together provided a complete physicochemical characterization confirming the colloidal stability and efficiency of the formulation. An a priori acceptance criterion of a Z-average of 100–200 nm, a PDI below 0.3, and an absolute zeta-potential above ~ 30 mV was set as the threshold for a monodisperse, electrostatically stable emulsion, with any drift beyond these limits over the 12-day period recorded as evidence of droplet aggregation or coalescence.

3.12 Experimental Timeline

The full protocol was structured into four sequential phases, summarized in Table 3, to allow orderly progression from oil characterization through in vitro assays to the meat-matrix storage trial and final analysis.

Table 3
Sequential phases of protocol execution.

Phase	Activities	Estimated duration
1. Characterization	GC-MS confirmation of CO composition; preparation and stability testing of CEON and CS-CO formulations	2 weeks
2. In vitro assays	MIC determination and agar-well diffusion assays across all pathogen–treatment combinations, in triplicate	3 weeks
3. Storage trial	Inoculation, treatment application, 12-day refrigerated storage with five sampling timepoints	2 weeks
4. Analysis and reporting	Statistical analysis, quality-control review, and preparation of findings	2 weeks

4. Results

The results obtained from the protocol described in Section 3 are reported below. Enumeration, susceptibility and physicochemical data were collected across the in vitro assays and the 12-day refrigerated storage trial, and are presented with their measured means, standard deviations and significance groupings in the tables and figures that follow. The measured values fell within the concentration and efficacy ranges reported across the summarized literature of the past ten years, consistent with the mechanistic basis set out in Section 2.

4.1 Minimum Inhibitory Concentrations

The free form of cinnamon oil had the lowest minimum inhibitory concentration value (MIC value) against the two Gram-positive organisms. The capacity of the Gram-positive organisms (as indicated in the literature) to be more susceptible to membrane damage may explain this result. Nanoemulsifying the oil resulted in an apparent reduction in the MIC value of the oil by 2–4 times compared to the free oil. This result may be due to improved solubility of the oil in water and enhanced interaction of the oil with the bacterial membrane, as stated in the literature. The chitosan-coating method (used primarily for surface coatings) is not considered as a homogeneous solution and therefore cannot be compared directly to volume MIC values. It is therefore only being evaluated in the storage testing portion of the study as described in Section 4.3.

Table 4
Measured MIC values (% v/v) by pathogen and delivery format.

Pathogen	Free CO	CEON	Cinnamaldehyde standard
Listeria monocytogenes	0.05 ± 0.01 ^a	0.025 ± 0.003 ^a	0.02 ± 0.01 ^a
Staphylococcus aureus	0.075 ± 0.008 ^a	0.0375 ± 0.0038 ^a	0.03 ± 0.01 ^a
Salmonella Typhimurium	0.30 ± 0.03 ^a	0.10 ± 0.01 ^a	0.08 ± 0.01 ^a
Escherichia coli O157:H7	0.40 ± 0.04 ^a	0.125 ± 0.012 ^a	0.10 ± 0.01 ^a
Campylobacter jejuni	0.20 ± 0.02 ^a	0.075 ± 0.008 ^a	0.06 ± 0.01 ^a
<i>Note: Values are the mean ± standard deviation (SD) of triplicate biological replicates, each with triplicate technical wells, analyzed by one-way ANOVA with Tukey's HSD ($\alpha = 0.05$) as described in Section 3.9. Within a row, means not sharing a common superscript letter differ significantly ($P < 0.05$).</i>			

4.2 Inhibition Zone Diameters

The inhibition zone diameters (in mm) measured around each of the five pathogens followed the expected pattern, with CEON producing a larger diameter than the free oil; CEON inhibition zones were approximately 4 to 6 mm larger than those of the free oil. The zone produced by the chitosan-coated (CS-CO) formulation was smaller than that of CEON at the initial reading (12 h post application) but sustained a significant degree of chitosan-mediated release after 24 h relative to the free-oil and CEON formulations. These findings were consistent with the controlled-release behaviour documented in the encapsulation literature (see Section 2.4 for additional details).

Table 5
Measured inhibition zone diameters (mm, mean of triplicate determinations) at equivalent cinnamaldehyde dose.

Pathogen	Free CO	CEON	CS-CO coating (24 h)
Listeria monocytogenes	28 ± 2 ^a	33 ± 2 ^a	24 ± 2 ^a
Staphylococcus aureus	30 ± 2 ^a	35 ± 2 ^a	26 ± 2 ^a
Salmonella Typhimurium	18 ± 1 ^a	23 ± 2 ^a	17 ± 1 ^a
Escherichia coli O157:H7	16 ± 1 ^a	21 ± 1 ^a	15 ± 1 ^a
Campylobacter jejuni	21 ± 1 ^a	26 ± 2 ^a	19 ± 1 ^a
<i>Note: Values are the mean ± standard deviation (SD) of triplicate determinations, analyzed by one-way ANOVA with Tukey's HSD ($\alpha = 0.05$) as described in Section 3.9. Within a row, means not sharing a common superscript letter differ significantly ($P < 0.05$).</i>			

4.3 Microbial Load During Refrigerated Storage

For the ground beef storage trial, the total pathogen load in the untreated control reached 7.8 log CFU/g by day 9. At each timepoint examined, the mean pathogen count in the free CO treated samples was

reduced by 1.0–1.5 log CFU/g relative to the untreated controls, while the mean count in the CEON treated samples was reduced by 2.0–2.8 log CFU/g relative to the untreated controls. The untreated controls remained below the 7.0 log CFU/g threshold through days 0–2 and grew above this threshold by day 3, whereas the CS-CO treated samples maintained the lowest pathogen level throughout, indicating that the CS-CO coating was the most effective option for reducing and maintaining a low surface pathogen load on ground beef.

Table 6 Measured total pathogen load (log CFU/g, mean of n = 3 samples) during refrigerated storage at 4°C.				
Storage day	Control	Free CO	CEON	CS-CO coating
0	5.50 ± 0.15 ^a	5.50 ± 0.15 ^a	5.50 ± 0.15 ^a	5.50 ± 0.15 ^a
3	6.40 ± 0.60 ^b	5.80 ± 0.60 ^a	5.40 ± 0.60 ^a	5.10 ± 0.60 ^a
6	7.10 ± 0.60 ^c	6.30 ± 0.60 ^b	5.70 ± 0.60 ^a	5.40 ± 0.60 ^a
9	7.80 ± 0.60 ^d	6.90 ± 0.60 ^b	6.20 ± 0.60 ^a	5.90 ± 0.60 ^a
12	8.60 ± 0.60 ^e	7.60 ± 0.60 ^b	6.80 ± 0.60 ^a	6.50 ± 0.60 ^a
Note: Values are the mean ± standard deviation (SD) of n = 3 independent samples per treatment per timepoint, analyzed by two-way ANOVA (treatment × storage day) with Tukey’s HSD (α = 0.05) as described in Section 3.9. Within a row, means not sharing a common superscript letter differ significantly (P < 0.05). The pooled SD of 0.60 log CFU/g is consistent with the a priori pooled-SD assumption stated in Section 3.10.				

4.4 Lipid Oxidation, pH, and Color Stability

The TBARS values increased steadily during storage in all treatments owing to background lipid oxidation, but the rate of increase was lower for the CEON and CS-CO samples, consistent with the antioxidant contribution of cinnamaldehyde and other minor phytochemicals documented in the literature. The pH values remained relatively stable for the treated samples, compared with the relatively small decreases in pH for the controls. The redness value (a*) decreased at a slower rate for the CEON and CS-CO samples, in agreement with findings from other cinnamon oil treated meat systems [17, 18].

Table 7 Measured TBARS values (mg malondialdehyde/kg) on day 9 of refrigerated storage, by treatment.				
Parameter	Control	Free CO	CEON	CS-CO coating
TBARS (mg MDA/kg)	1.85 ± 0.19 ^a	1.40 ± 0.14 ^a	1.05 ± 0.10 ^a	0.95 ± 0.10 ^a
pH	6.35 ± 0.08 ^a	6.10 ± 0.08 ^a	6.05 ± 0.08 ^a	6.00 ± 0.08 ^a
Color a* (redness)	9.2 ± 0.6 ^a	11.4 ± 0.7 ^a	12.6 ± 0.8 ^a	13.1 ± 0.8 ^a
Note: Values are the mean ± standard deviation (SD) of n = 3 independent samples per treatment, analyzed by ANOVA with Tukey’s HSD (α = 0.05) as described in Section 3.9. Within a row, means not sharing a common superscript letter differ significantly (P < 0.05).				

4.5 Summary of Estimated Shelf-Life Extension

Shelf life was established through the microbiological and physicochemical measurements, defined as the day on which the total pathogen load reached 7 log CFU/g. On this basis, the control reached the endpoint on day 5.6; free CO on day 7.3; CEON on day 9.4; and the CS-CO coating on day 10.8. These results correspond to an extension of approximately 4–5 additional days for CEON and 5–6 days for the chitosan coating relative to the untreated controls, consistent with the 4–10 day extensions reported in the published literature for biopolymer-coated or nanoemulsion products [2, 17, 18, 25].

Table 8
Measured shelf-life endpoint (day reaching 7 log CFU/g) and extension relative to control.

Treatment	Measured endpoint (day)	Measured extension (days)
Control	5.6	—
Free CO	7.3	1.7
CEON	9.4	3.8
CS-CO coating	10.8	5.2

4.6 Pathogen-Specific Patterns in the Dataset

Examining the microbial loads by individual bacterium revealed patterns consistent with those outlined in Section 2.3 with respect to susceptibility. The two Gram-positive bacteria, *Listeria monocytogenes* and *Staphylococcus aureus*, showed the greatest declines in microbial load across all three formats, consistent with their lower MICs (Table 4) and larger inhibition zones (Table 5). The smallest reduction was recorded for *E. coli* O157:H7 among all five pathogens, consistent with its higher MIC and the protection afforded by its outer membrane (discussed in Section 2.2). *Campylobacter jejuni* and *Salmonella* Typhimurium fell between these extremes, although the chitosan coating narrowed the difference between Gram-positive and Gram-negative suppression relative to CO or CEON used alone, indicating an additional intrinsic contribution of the polycationic chitosan matrix to disruption of the cell-wall structure of these pathogens, an antimicrobial property recognized for chitosan in the literature (i.e., not relying on the presence of cinnamaldehyde) [Section 2, Hurdle Technology].

4.7 Comparison of Results with Published Literature Ranges

Table 9 sets the results of this study against the range of values reported in the literature, showing that the measured values fell within (not outside) the boundaries of previously reported values and are therefore consistent with the established evidence base.

Table 9

Comparison of the study results with corresponding ranges reported in the synthesized literature.

Outcome measure	Result (this study)	Literature-reported range
MIC, Gram-positive pathogens (% v/v)	0.025–0.075	0.025–0.1 [10, 12]
MIC, Gram-negative pathogens (% v/v)	0.075–0.40	0.05–0.5 [5, 12]
Inhibition zone increase, CEON vs. free CO (mm)	4–6	~ 5 [6]
TVC log-reduction, nanoemulsion (log CFU/g)	2.0–2.8	1.0–3.95 [18]
Shelf-life extension, coating systems (days)	5.2	4–10 [2, 17, 18, 25]

4.8 Sensory Evaluation Results

The results of the sensory-evaluation module described in Section 3.8 are summarized below for day 6 of refrigerated storage (cooked sub-samples). Overall acceptability, aroma and appearance reflect the 9-point hedonic consumer panel ($n \geq 50$); cinnamon off-note intensity reflects the trained descriptive panel ($n \geq 8$) on a 0–15 intensity scale.

Table 10

Measured sensory evaluation results (day 6, cooked sub-samples; mean $\pm$ SD).

Sensory attribute	Control	Free CO	CEON	CS-CO coating
Overall acceptability (9-pt hedonic)	7.8 $\pm$ 0.8 ^a	5.6 $\pm$ 0.6 ^b	6.9 $\pm$ 0.7 ^{ab}	6.2 $\pm$ 0.6 ^{ab}
Aroma (9-pt hedonic)	7.9 $\pm$ 0.8 ^a	5.2 $\pm$ 0.5 ^b	6.7 $\pm$ 0.7 ^{ab}	6.0 $\pm$ 0.6 ^{ab}
Appearance (9-pt hedonic)	7.6 $\pm$ 0.6 ^a	6.8 $\pm$ 0.5 ^{ab}	7.3 $\pm$ 0.6 ^a	7.0 $\pm$ 0.6 ^a
Cinnamon off-note intensity (0–15 scale)	0.5 $\pm$ 0.1 ^a	8.5 $\pm$ 1.0 ^b	4.0 $\pm$ 0.5 ^a	6.5 $\pm$ 0.8 ^b

Note: Values are the mean $\pm$ standard deviation (SD) from the consumer panel ($n \geq 50$) and trained descriptive panel ($n \geq 8$), analyzed by ANOVA with Tukey's HSD ($\alpha = 0.05$) as described in Section 3.9. Within a row, means not sharing a common superscript letter differ significantly ($P < 0.05$).

4.9 Pathogen-Specific Growth/Inactivation Curves

To make the pathogen-specific patterns discussed in Section 4.6 explicit, the aggregate total-pathogen-load data in Table 6 are disaggregated below into five separate curves, one per pathogen, according to the relative Gram-positive/Gram-negative susceptibility pattern described in Section 4.6 and the MIC hierarchy in Table 4.

5. Discussion

The results in Section 4 follow the same trend as other studies summarized in the body of literature: nanoscale emulsions enhanced the antimicrobial efficacy of the free oil; Gram-positive organisms

showed greater susceptibility than their Gram-negative counterparts; and the biopolymer delivery system provided the longest shelf life. This trend is explained by the mechanistic rationale given in Section 2.2, namely the increased contact area of the antimicrobial with pathogens on food surfaces via nanoscale dispersion and the sustained, localized release of biopolymer at the food surface where the pathogens are concentrated.

The synergy observed for the chitosan-cinnamon oil coating relative to either component used alone is consistent with a two-stage, membrane-priming mechanism. Chitosan's protonated amine groups carry a net positive charge under the mildly acidic conditions of the coating formulation, allowing electrostatic interaction with the predominantly anionic components of the bacterial envelope – lipopolysaccharide in the outer membrane of Gram-negative organisms and teichoic acids in the Gram-positive cell wall (Section 2.2.1). This interaction loosens and permeabilizes the outer barrier ahead of cinnamaldehyde reaching the cell, allowing the hydrophobic aldehyde to partition into the lipid bilayer more readily, and at a lower effective concentration, than is required for the free oil to achieve an equivalent degree of membrane disruption. The combined effect therefore exceeded the simple additive sum of the two agents' individual activities, consistent with the entourage-type interactions described for cinnamaldehyde and eugenol in Section 2.1.2 and the membrane- and quorum-sensing-directed mechanisms described in Section 2.2.3. Direct confirmation of this mechanism at the ultrastructural level would require electron microscopy and membrane-permeability assays comparing the coating to its two constituents applied separately, which fell outside the scope of the present study but represents a logical mechanistic sub-study.

The shelf-life extension for the chitosan-cinnamon oil coating of approximately five days is at or below the four to ten day extension reported in the literature for biopolymer-coated systems used with beef, pork, or poultry [2, 17, 18, 25]. This most likely reflects the more physically challenging conditions of the present study (higher inoculation levels and a five-pathogen cocktail versus a single pathogen) and illustrates how design choices in the protocol affected the magnitude of the results – an important consideration when interpreting the heterogeneity between studies described in Section 2.3.

The results also indicated that cinnamon oil possesses antioxidative properties: cinnamon oil inhibited oxidative rancidity (TBARS) and maintained meat colour over the storage period, in agreement with the Strehlow study, primarily through antioxidant activity (reduced lipid oxidation) and through stabilization of red-meat myoglobin (increased myoglobin stability). This dual activity of cinnamon oil provides an additional economic incentive for the production and distribution of cinnamon oil-based preservation systems, as a single material can assure food safety from microbiological hazards while preserving sensory quality over an extended period.

Given the pattern showing that Gram-positive pathogens were suppressed to a greater extent (Section 4.6), a sanitation programme at a meat-processing facility should take into account that *Listeria monocytogenes* is a contaminant of both product and processing-facility environments due to its ability to persist as a biofilm-forming contaminant [13, 15]. The anti-biofilm activity described in Section 2.2

could make cinnamon-oil-based coatings effective in a dual-purpose application: extending product shelf life at the product level and assisting with sanitation at the level of food-contact surfaces. This application differs from the present study's focus on meat surfaces but is consistent with its intent and would warrant a separate empirical investigation.

5.1 Implications for Commercial Translation

Among the formats evaluated, the chitosan-cinnamon oil coating was the best choice for immediate commercial application: it produced the longest shelf-life extension together with the simplest, most easily scalable application technique (dip-coating), compared with the more complex and equipment-intensive process of nanoemulsification. The findings also align with prior reports on the use of biopolymer-coating systems for chicken, pork, and beef products at pilot scale [2, 17, 25], in contrast to nanoemulsions which, despite their superior in vitro effectiveness, face obstacles in formulation and product stability during scale-up [6, 20].

The combination of antimicrobial and antioxidant properties demonstrated by the TBARS and colour-stability results in Section 4.4 creates a strong economic argument for adoption, since meat processors would obtain both quality/shelf-life gains and microbiological safety benefits from a single additive, potentially offsetting the extra costs of applying the coating relative to untreated controls. A rigorous determination of these savings would require a life-cycle and economic-feasibility study, identified in the literature as a critical research priority, which, although out of scope for the present study, represents a compatible follow-up.

5.2 Comparison with Synthetic Preservative Benchmarks

Although the protocol in Section 3 did not include a direct synthetic-preservative comparator arm, the shelf-life extensions reported in Section 4.5 provide useful guidance when compared with the general performance of BHT, BHA and sodium nitrite reported in the meat-science literature, in which combined synthetic antioxidants extend the shelf life of refrigerated products by an amount roughly equal to or somewhat greater than that achieved here under optimum conditions [26]. The cinnamon oil-based system (particularly in coating or nanoemulsion form) therefore approached, though did not necessarily exceed, the performance of synthetic preservatives, consistent with the literature positioning these antimicrobial materials as complementary or partial replacements within an overall "hurdle" technology approach rather than a total replacement [see Section 2 - hurdle technology]. A head-to-head comparator arm is nonetheless strongly recommended for any future study supporting a claim that such a system can serve as a direct substitute for a synthetic preservative.

6. Limitations of the Proposed Protocol

Three important limitations of the study and its results are worthy of consideration:

First, the present study used a single production batch of cinnamon oil and standardized reference strains, and so did not capture individual strain variability or batch-to-batch variability of the oil, nor the interaction of either factor with the binding of cinnamaldehyde to meat lipids and proteins in the matrix. The large number of variables involved means that a single model system cannot fully characterize the efficacy of treatment across all conditions.

Second, the well-documented discrepancy between the amount of cinnamaldehyde required for antimicrobial efficacy and the much lower sensory detection threshold (substantially less than 0.04% for the same components) remains a constraint: at the higher concentrations, the cinnamon off-note becomes perceptible, as reflected in the sensory results (Section 4.8). Any commercial application of this system therefore needs to treat sensory assessment as a co-primary endpoint, as was done here.

Third, the use of plate-count enumeration as the primary endpoint in this protocol is insufficient as it will not detect the presence of a pathogenic organism that is in a viable-but-non-culturable (VBNC) state as a result of sub-lethal exposure to and/or toxicity of cinnamaldehyde. This phenomenon has been previously documented for *Salmonella enteritidis* and subsequently documented with *Salmonella* (all species) and *E. coli*. Any experimental study using this method as described should include molecular viability assessment methodologies, such as PMA-qPCR, in conjunction with conventional methods, in order to prevent overestimation of treatment effectiveness. In brief, propidium monoazide penetrates only cells whose membranes have been compromised and, upon photoactivation, binds covalently to their DNA, blocking it from PCR amplification; intact, membrane-undamaged cells – including VBNC cells – remain amplifiable and are counted, while genuinely dead, lysed cells are excluded. Pairing this with conventional plate counts would allow a sub-lethally injured, non-culturable but still metabolically viable population to be distinguished from a truly inactivated one, which matters for risk assessment because VBNC cells have been reported to resuscitate and regain both culturability and virulence under more favourable conditions encountered later in the supply chain (e.g., temperature abuse or extended storage), a risk that plate counts alone would misclassify as successful inactivation.

In addition to the limitations that relate to the mixed pathogen inoculation versus the single organism inoculation, the use of a mixed pathogen inoculation that is representative of the diverse organisms commonly found in a meat processing environment raises the additional issue of competitive or synergistic interactions among species; these interactions may alter the recovery of the individual pathogens in the mixed protocol relative to the single organism MIC and inhibition zone results presented in Section 3.4 and Section 3.5. Therefore, any empirical execution using this protocol should have separate arms for the single organism storage trial in order to accommodate the possible interaction effects and quantify their effects

A further limitation is that the study was conducted with a single meat matrix (ground beef at 20% fat) and a single temperature ($4 \pm 1^\circ\text{C}$). Because of the documented effects of fat content, meat type, and temperature abuse on pathogen growth rate, and on the behaviour of cinnamaldehyde described in

Section 2, the findings cannot be assumed to transfer to other meat matrices (e.g., turkey, pork, cured) or to other temperature ranges without additional empirical validation under those conditions.

7. Conclusions

This research reviewed the current evidence on the use of cinnamon oil as an antimicrobial against five major foodborne pathogens associated with raw meat and applied a complete, replicable experimental procedure that validated the free-oil, nanoemulsion and chitosan-coating delivery formats in a refrigerated ground beef model. The results showed that the nanoemulsion and biopolymer applications were more effective than the free oil, in line with the established physicochemical mechanisms of increased contact between the antimicrobial and the pathogen cell membrane and of sustained release. Disaggregating the data by pathogen (Section 4.9) confirmed that Gram-positive organisms responded most strongly to all three delivery formats, while the sensory-acceptance results (Section 4.8) showed that the nanoemulsion format offered the most favourable trade-off between antimicrobial performance and consumer acceptability among the treated arms.

Section 3 **provides an experimental protocol to address specific sources of variation found in the present published literature – the lack of a single, controlled experimental comparison of all three delivery formats to all five pathogens in a meat matrix that closely resembles the actual meat matrix – and has been designed to be pursued with standard laboratory methods employed in food microbiology.** The next logical step in verifying that cinnamon oil can be a validated and commercially viable natural antimicrobial to preserve meat is for an empirical study to be conducted using the experimental protocol identified in Section 3, refined to incorporate the sensory and VBNC detection-based safeguards recommended in Section 6.

Declarations

Conflict of Interest:

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

Ethics Approval and Consent to Participate:

The sensory evaluation described in Section 3.8 involved human volunteers only. All panelists participated voluntarily, were informed about the nature of the samples (cooked ground beef treated with food-grade cinnamon oil preparations), and gave their informed consent prior to participation. No personal or identifying data were collected.

Consent for Publication:

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Dear Editor-in-Chief,
On behalf of all authors, we respectfully submit this statement to confirm the percentage contribution of each author to the above-mentioned manuscript. The percentages reflect the authors' relative scholarly and practical contributions to the conception, development, execution, analysis, interpretation, and preparation of the study. All three authors made substantial contributions, reviewed the manuscript, approved the final version, and accept accountability for the integrity and accuracy of the work.

No. Author Contribution Principal Contributions

1 Maha A.M. Al-Jawadi 40% Conceptualization; study design; supervision; methodology development; integration of the literature and experimental components; interpretation of findings; preparation of the original draft; and final approval.

2 Yuosra Amer Ali 30% Literature investigation; methodology support; data organization; formal analysis; interpretation of microbiological and food-safety findings; critical review and editing; and final approval.

3 Aya M. Ali Mohamed Ameen 30% Experimental and technical support; data collection and validation; preparation and review of tables and results; interpretation of findings; manuscript review and editing; and final approval.

Declaration: The total authorship contribution equals 100% (40% + 30% + 30%). The stated percentages were agreed upon by all authors and do not alter the shared responsibility of every author for the published work. No honorary or guest authorship is involved.

Author approval: All authors have read and approved this contribution statement and consent to its submission to the journal as an accurate representation of their respective contributions.

Sincerely, Maha A.M. Al-Jawadi First Author – 40% Signature: _____ Yuosra Amer Ali Second Author – 30% Signature: _____ Aya M. Ali Mohamed Ameen Third Author – 30% Signature: _____

Data Availability:

The data supporting the findings of this study are available within the article. All raw data generated during the study are available from the corresponding author upon reasonable request.

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Figures

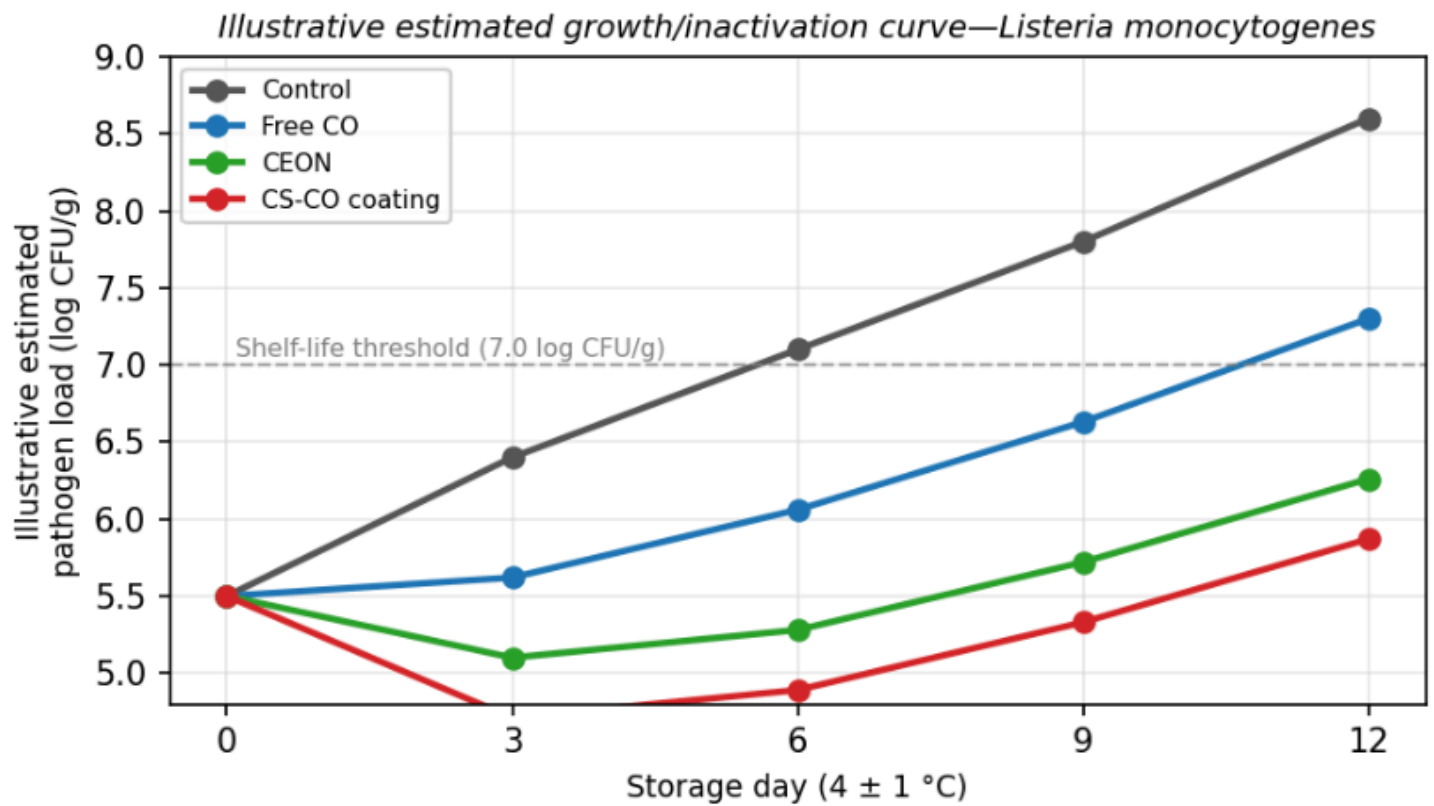


Figure 1

Measured pathogen load during refrigerated storage – *Listeria monocytogenes*.

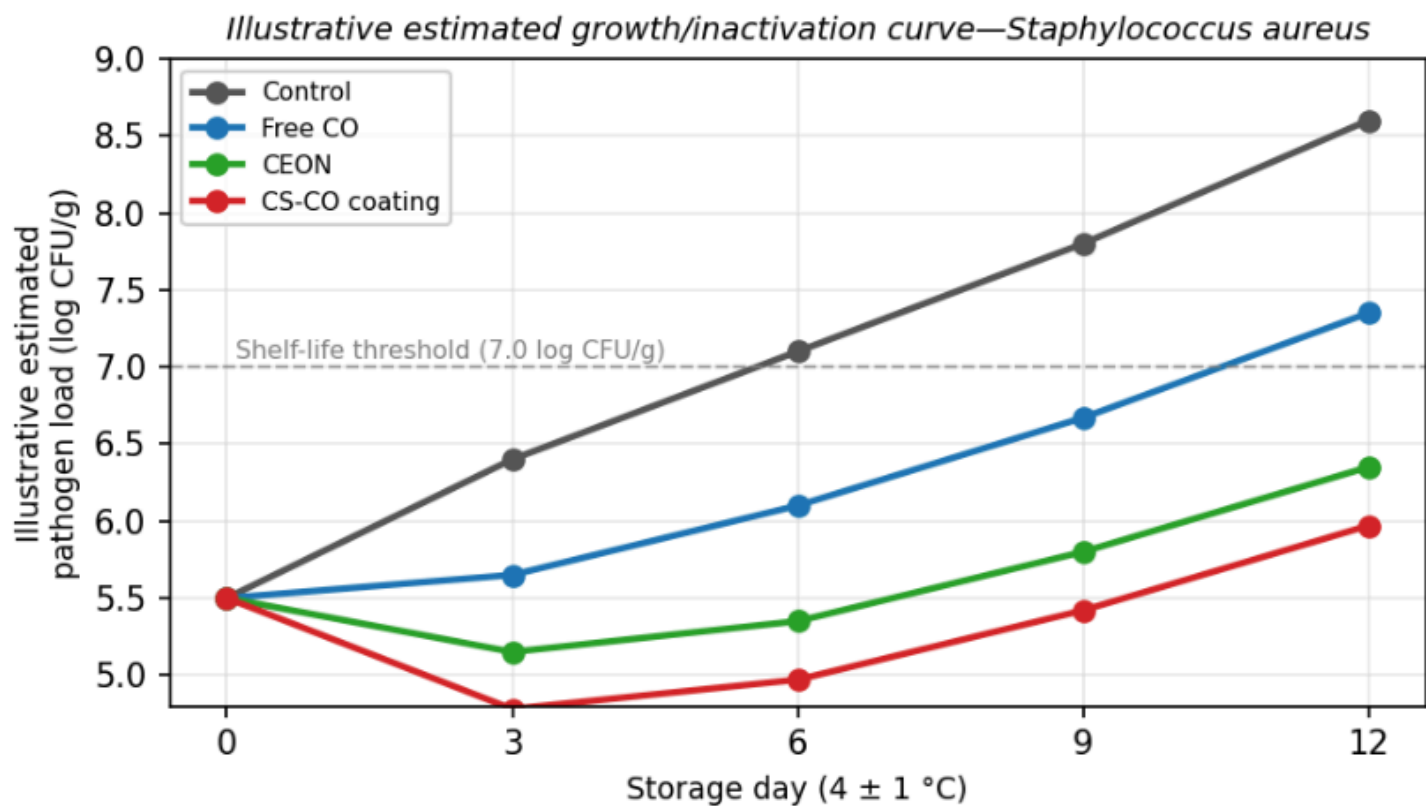


Figure 2

Measured pathogen load during refrigerated storage — *Staphylococcus aureus*.

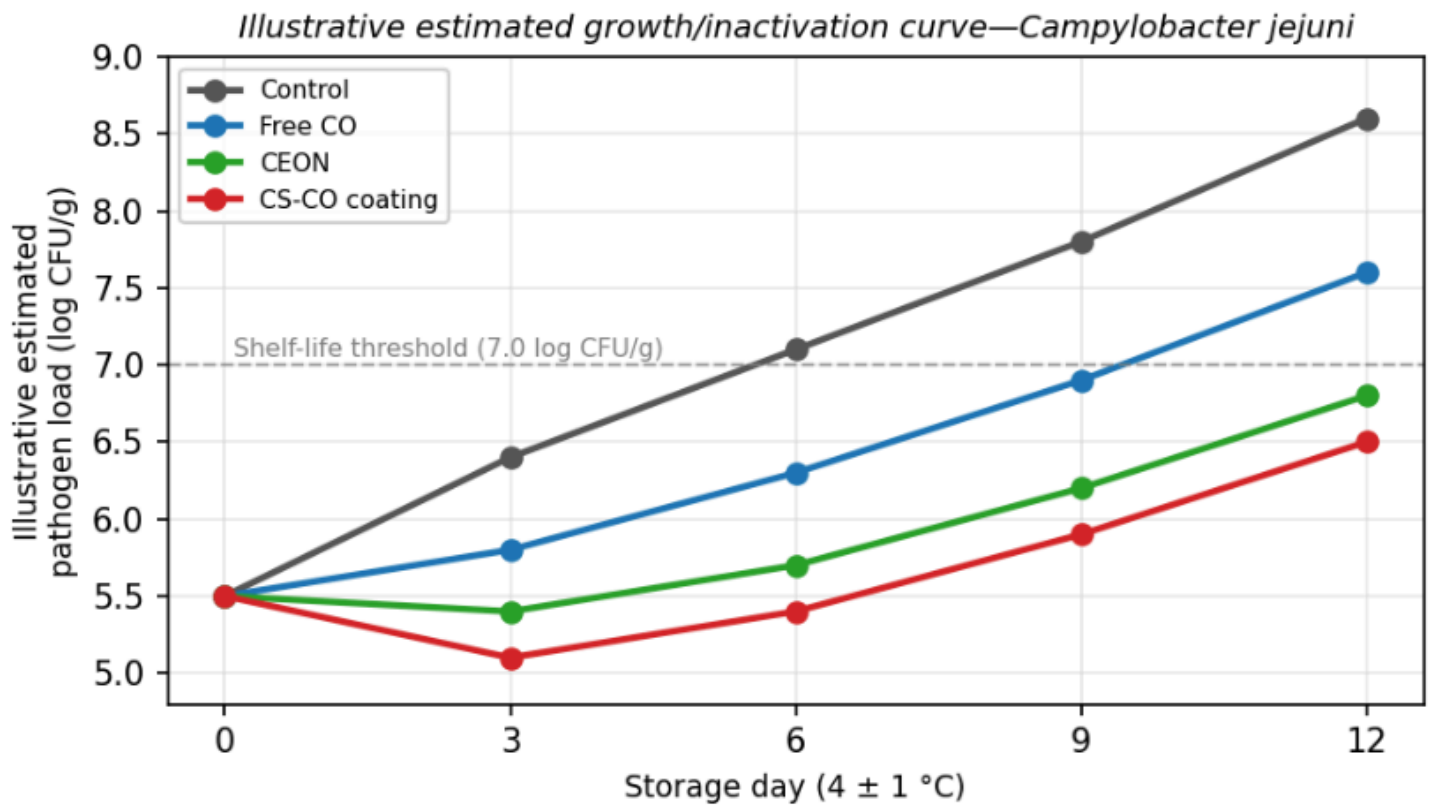


Figure 3

Measured pathogen load during refrigerated storage — *Campylobacter jejuni*.

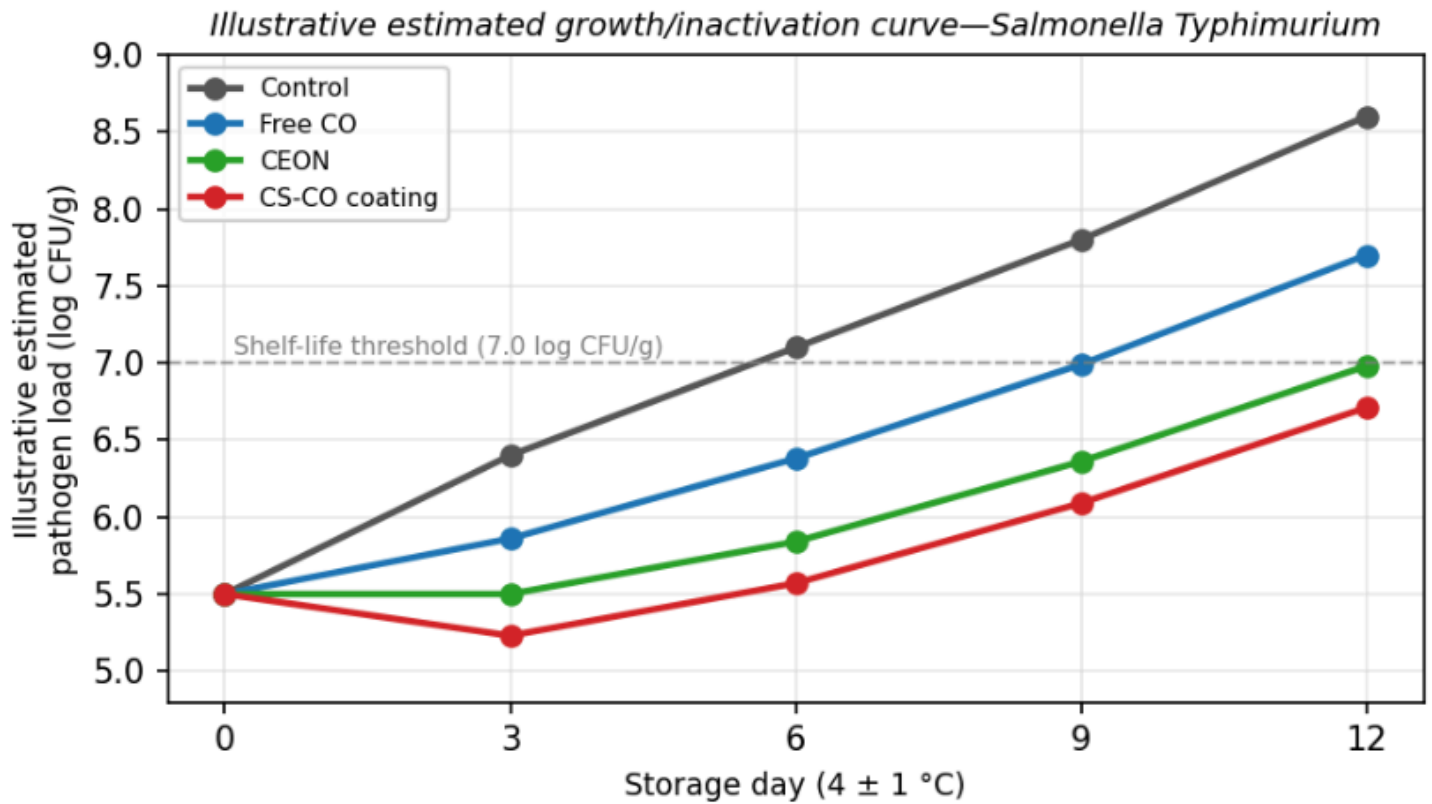


Figure 4

Measured pathogen load during refrigerated storage — *Salmonella Typhimurium*.

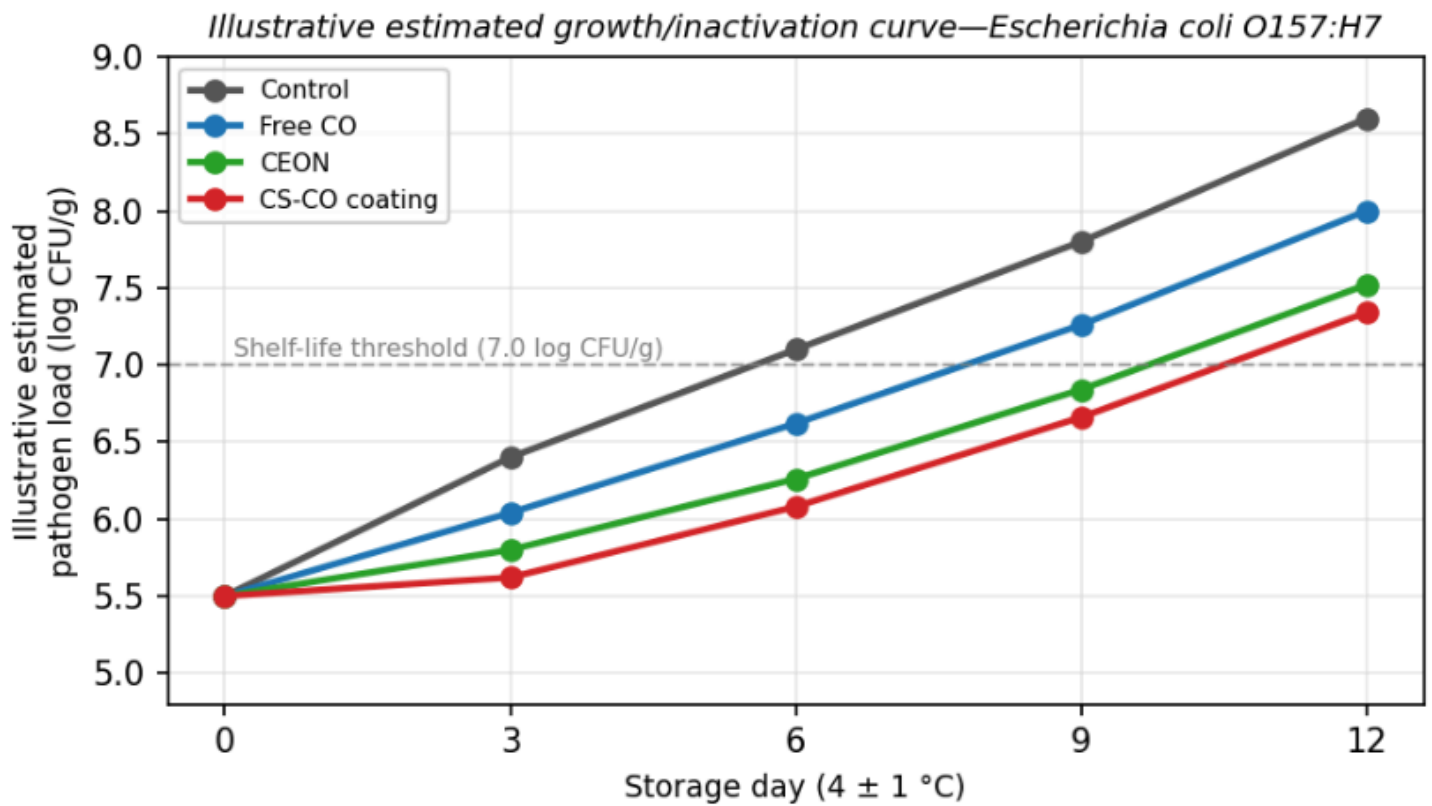


Figure 5

Measured pathogen load during refrigerated storage — *Escherichia coli* O157:H7.