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Ameliorative effect of cinnamon and its nanoemulsion on quality of beef burger during refrigerated storage

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ABSTRACT

In the present research, cinnamon essential oil (CEO) and it's nanoemulsion was studied to evaluate their antioxidant and antibacterial effect against *Salmonella* Typhimurium artificially inoculated in beef burger stored at 4±1°C. The active phenolic compounds of CEO were studied using GC/MS analyses and the antibacterial activity of CEO and Nano-emulsion (CNE) against *S.* Typhimurium was determined and the result of MIC was 0.3%. The obtained results showed that the sensory properties of different treated burger samples during cold storage (4ºC) were enhanced by using CEO and CNE with different concentrations compared to the untreated (control) samples after 5 days of the storage period with significant difference (P-Value <0.05) by both concentrations of CNE. Moreover, during storage, pH and TBA values increased slightly, and the rise became more obvious for control on the 7th day of the storage then spoiled. For samples incorporated with CNE (MIC2), which maintained accepted pH of meat up to the 12th day of the storage. Regarding various treatments, the antibacterial effectiveness of CEO and CNE demonstrated a potential impact on the APC and overall yeast and mold count. Using natural antibacterial CEO could be the solutions reducing chemical preservation in fresh and refrigerated meat products. Cinnamon NE is a promising cost effective, innovative eco-friendly preservatives in meat production.

Introduction

On one hand, Burgers are considered in the category of junk food that may contain many hazards but on the other hand give consumers a quick, practical, and inexpensive meal (Andreani *et al*., 2023). High moisture content, nutritional levels, and pH values are factors make these meat products susceptible to both microbial and oxidative deterioration. So, these are not suitable for long-term storage (Das *et al*., 2020). One of the problems of raw minced meat is dangerous microorganisms that are already present on the surface of the meat spread and relocate through the core (Kassem *et al*., 2020). Then during handling and processing operations, the risk of contamination raises. Also, growth of microbes like bacteria, molds, and yeast increase as well as encourage oxidation processes, which compromises the safety and quality of beef burger products and results in significant economic losses (Roila *et al*., 2022).

Refrigeration storage of fresh meat and meat products extends shelf life by days or weeks (Schlei *et al*., 2020). So, it is essential to add antimicrobial and antioxidant additives especially those of synthetic origin to extend refrigerated storage time (Fazelipour *et al*., 2020)

Due to the increasing consumer awareness about synthetic chemicals safety and the bigger demand for fresh food; studies are focused on the use of natural ingredients with bioactive compounds and antioxidants to improve the shelf life and food quality that associated to meet consumer wishes (Zapaśnik *et al*., 2022). Essential oils (Eos) are natural complex compounds derived from the secondary metabolism of herbal and medicinal plants. They are made of quite different concentrations of volatile active components including terpenes, terpenoids, phenylpropanoids, and aromatic compounds. EOs are attracting interest as natural additives due to antimicrobial and antioxidant properties (Coté *et al*., 2017). Moreover, EOs are approved by the Food and Drug Administration and "generally recognized as safe". Despite their various advantages, the use of EOs in food industries is limited due to their hydrophobic nature, high volatility, reactivity with food components, as well as their sensitivity to light, oxygen, pressure, and heat (Amiri *et al*., 2020). One of these Eos cinnamon oil which has strong antimicrobial and antioxidant properties and are natural and environmentally safe, have recently received increased attention (Santos *et al*., 2022). Cinnamon oil, consist mainly of cinnamaldehyde, reported as an active volatile ingredient that contributes to the strong cinnamon-like flavor, antimicrobial activities (Chuesiang *et al*., 2023) even against multidrug-resistant *S.* Typhimurium (Alibi *et al*., 2020) and excellent antioxidant activity, with several molecular mechanisms (Huang and Chen, 2022).

Nanotechnology is referred as the technology that deals with the fabrication and application of nanomaterials with sizes from 1–100 nm; the physical and chemical properties of a substance vary with this size, while its water solubility, stability, bioavailability and physiological activities can be enhanced substantially with minimum side effects (Jagtiani, 2022). Furthermore, the use of nano-emulsified EOs is one of the most promising nanotechnologies, considered an alternative strategy to overcome mentioned drawbacks by enhancing the physicochemical properties and oxidative stability of emulsion systems, limiting the possible interaction with food components; the environment, and further masking their strong flavor (Ameur *et al*., 2022).

In the last years, nanoemulsions have been used in a variety of food fortification, due to their large surface area, transparent appearance, and extended shelf life (Yang *et al*., 2023).

In this sense, the paper's main goal was to develop safe, non-toxic and biocompatible nanostructures built from food-grade materials, utilizing easy, cost-effective and environmentally friendly techniques, by fabricating essential oils nanoemulsions to broaden its antimicrobial spectrum including major Gram-negative foodborne pathogens.

Materials and methods

Cinnamon essential oil (CEO) was purchased from the National Research center, Cairo, Egypt. The CEO emulsion was prepared by adding

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Muller-Hinton Broth (MHB) and Tween 80 as a solvent to obtain concentration 10% (100 mg/ml) (Bonou *et al*., 2016). From this first concentration, the other dilutions prepared by a two-fold way using MHB only to obtain concentrations of 5%, 2.5%, 1.25%, 0.625%, 0.312% and 0.156%.

Detection of antimicrobial activity assay of CEO

The minimum inhibitory concentration (MIC) against *S.* Typhimurium was detected by using agar well diffusion method (Chattopadhyay *et al*., 2009). The bacterial reference strain employed in this study was *S.* Typhimurium-NCTC12023, which obtained from the Animal Health Research Institute, Assiut, Egypt.

Detection of volatile compounds of CEO

The volatile components of CEO were analyzed by Gas chromatography– mass spectrometry (GC–MS) according to Abd El-Kareem *et al*. (2020). The GC-MS (7890A-5975C, Agilent, USA) in Nawah Scientific Inc., (Cairo, Egypt) using a HP-5 capillary column (The column was 30 meters in length with an inner diameter of 0.25 mm and 0.25 µm thickness) with 95% methyl and 5% dimethyl poly siloxane as the stationary phase. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The split ratio was 30:1 and the injected quantity was 0.2 µL. The program oven temperature conditions were maintained at 40°C for 5 min, increased to 260°C at a rate of 3°C/min and held for 10 min, and then increased to 280°C at 10°C/min and maintained for 2 min. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage was adopted at 1823.5 V. The ion source was established at a temperature of 230°C, the maximum temperature was set at 250°C, and the quadrupole rod temperature was employed at 150°C, with the maximum temperature of 200°C. The mass range for this scanning was 50.0- 550.0 amu. All volatile components were identified by matching the recorded mass spectra with the standard mass spectra provided by NIST11.L database.

Preparation and characterization of Cinnamon nanoemulsion,

Preparation of Cinnamon nano-emulsions (CNEs)

The oil-in-water NEs were prepared by dissolving 3.5 v/v% of Tween 80 in deionized water at room temperature. The non-ionic surfactant was preferred due to its favorable oil-in-water (O/W) characteristic. Tween 80 displays efficient solubility with essential oils, and effectively minimizes droplet diameter by adhering to the droplet's surface, improving the overall stability of O/W emulsion system. The mixture was shaken using a magnetic type of stirrer for 10 minutes to obtain a homogenous solution. Then, the CEOs were added slowly and mixed with a direct driven stirrer (Hotplate stirrer, DAIHAN Scientific Co., Ltd, Korea) for 15 min, then sonication of the resulting emulsion using a 25 kHz ultrasonic Homogenizer (USH650, max power: 650 watt) for 20 minutes The CNEs were stored in the laboratory condition at 25°C (Nirmal, 2018).

Characterization of the prepared Cinnamon nanoemulsion

Measurement of particle size and polydispersity index (PDI)

The particle size and PDI of nano-emulsions were measured at 25±0.2°C by Zeta-sizer (3000 HS, Malvern Instruments, Malvern, UK) in the Faculty of Pharmacy, Al-Azhar University, Assiut. For measuring particle size, weighed amounts of formulations were dispersed in double-distilled water (1:20) to avoid multiple scattering effects and obtaining homogenous dispersion and that had to be used instantly for measuring the particle size and PDI (Nirmal *et al*., 2023).

Fourier-transform infrared spectroscopy (FTIR) spectral analysis

FTIR analysis was performed in the analytical chemistry accredited laboratory, Chemistry Department, Faculty of Science, Assiut University, Egypt. It is used for identifying the functional groups with their means of attachment and the fingerprint of the molecule. For performing FTIR, samples were prepared by potassium bromide pellet method and then samples were scanned in FTIR spectrometer in the wave number range of 4000- 500 cm-1 (López-Cano *et al*. 2023).

Morphological study of nanomaterials

Electronic Microscope Unit, Assiut University Assiut, Egypt. Transmission electron microscopy (TEM) was used for studying morphology of NEs through negative-staining electron. For performing TEM, few drops of NE and NNPs were prepared in double-distilled water and filtered through filter measured 200 nm then placed onto nanocarbon-coated copper grids for 1 min., negatively stained with phosphotungstic acid for 10 min at room temperature. Later, the grids were observed in a transmission electron microscope (PHILIPS, model CM10) at an acceleration voltage of 100 kV (Shakeel *et al*., 2009).

Cytotoxicity of the prepared CNEs

Cell culture

Vero: Green monkey kidney was obtained from Nawah Scientific Inc., (Mokatam, Cairo, Egypt). Cells were maintained in DMEM media supplemented with 100 mg/mL of streptomycin, 100 units/ml of penicillin and 10% of heat-inactivated fetal bovine serum in humidified, 5% (v/v) CO2 atmosphere at 37°C (Skehan *et al*., 1990).

Cytotoxicity assay

Cell viability was assessed by Sulforhodamine B (SRB) assay. Aliquots of 100 µl cell suspension ($5x10^3$ cells) were in 96-well plates and incubated incomplete media for 24 h. Cells were treated with another aliquot of 100 µl media containing drugs at various concentrations. After 72 h. of drug exposure, cells were fixed by replacing media with 150 µl of 10% TCA and incubated at 4°C for 1 h. The TCA solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70 µl SRB solution (0.4% w/v) were added and incubated in a dark place at room temperature for 10 min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then, 150 µl of TRIS (10 mm) was added to dissolve protein bound SRB stain; the absorbance was measured at 540 nm using a BMG LABTECH®-FLUOSTAR Omega microplate reader (Ortenberg, Germany) (Allam *et al*., 2018).

Determination of MLCs Cinnamon nanoemulsion against S. Typhimurium

Effect of CEO and CNE on growth of *S.* Typhimurium in experimentally manufactured beef burger (food model) during refrigerated storage

Minimum inhibitory concentration was selected according to the obtained results in vitro, the selected MIC against *S.* Typhimurium was 0.6% for CEO and 0.3% for CNE. Each concentration was used for burger manufacture separately.

Preparation of Burgers according to Ghaderi-Ghahfarokhi et al. (2017)

Burgers meat samples were divided into 5 groups with different treatments: Treat 1 (Control -ve free from preservatives), Treat 2 (Control +ve inoculated by *S.* Typhimurium (10^5) strain only), Treat 3 (Free CEO MIC1), Treat 4 (Free CEO MIC2), Treat 5 (CNEs MIC1) and Treat 6 (CNEs

MIC2). Then samples packed in polyethylene bags and stored at 4°C with specific testing at 0, 1, 3, 5, 7, ...days.

Sensory evaluation

Organoleptic evaluation of beef burger samples was assessed by 5-7 members of Food Hygiene Department, Assiut University, (with experience in burger processing and evaluation). The panelists were instructed to evaluate color, appearance, odor and overall acceptability by using a 9-point hedonic scale. Attribute scales according to Fourati *et al*. (2020) varied from 1 to 9 with 9 being excellent, 5 being the lower limit of acceptability and 1 being very bad. A score below 5 indicated the sample being unacceptable.

Effect of CEO and CNE on shelf life of experimentally manufactured beef burger (food model) during refrigerated storage

Preparation of burgers and experimental application of preservative's MICs as previously mentioned without inoculation of any bacteria according to International Organization for Standardization ISO-6579, 2002 (ISO, 2002).

Sensory evaluation Attribute scales according to Fourati et al. (2020)

Physico-Chemical examination

Determination of pH

The value of pH measured using AD 1030 bench meter (Adwa Instruments, Woonsocket, RI, USA), as detailed by Garavito *et al*. (2020).

Determination of Thiobarbituric acid (TBA)

The Thiobarbituric acid value (TBA) was determined according to Egyptian Organization for Standardization and Quality Control (EOS, 2006). The amount of TBRAS were expressed as mg of malondialdehyde per kg of meat. TBA standard curve was constructed using TEP solution.

Microbiological examination was performed by using Aerobic Plate Count (APC) (ISO-4833, 2003) and Total Yeast and mold count (Acharya and Hare, 2022)

Statistical Analysis

All experiments were carried out in triplicate. One-way analysis of variance was performed using the SPSS program (SPSS Inc., Chicago, IL, USA) to determine the statistical significance of differences within the samples.

Results and Discussion

Based on recent literature, synthetic food additives can be associated with the development of human diseases, and concretely, the consumer demand for healthier food products with a clean label increased over the last few years (Flórez-Méndez and López, 2022). Among natural preservatives, EOs from spice and medicinal plants have aroused interest for their antimicrobial potential, inhibiting growth of spoilage and pathogenic microorganisms.

Antibacterial activity of CEO

The well diffusion test method could be considered as a useful tool to discriminate among EOs activity (Koeth *et al*., 2017), although EOs viscosity as well as a polar nature could hamper diffusion, thus resulting in activity underestimation. MIC determination was instead useful to determine the antibacterial effect of the CEO (Mazzarrino *et al*., 2015) and to identify the concentrations that did not allow microbial replication.

The inhibition zones of different concentrations of CEO (10%, 5%, 2.5%, 1.25%, and 0.625) against *S.* Typhimurium, which were 35, 30, 26, 21, 15 and 10 mm, respectively.

Lower antibacterial activity against *S.* Typhimurium obtained from previous Egyptian research, where the inhibition zones were 8 mm with pure CEO (Zaghloul *et al*., 2017) and 7 and 18 mm with concentrations of 10 and 20 µl/ml (Elgammal *et al*., 2020).

Regarding MIC values of CEO were similar 0.6% in Italy by Ebani *et al*. (2019), 5 µl/ml (Rachkeeree *et al*., 2014)., 6.25 mg/ml of cinnamaldehyde (main component of CEO) (Klangpetch and Noma, 2018). Much higher activities with lower MICs were obtained in other studies, against *S.* Typhimurium (ATCC 14028); 0.0625% in Korea by Park *et al*. (2018) and 0.0312 % in Iran (Mortazavi and Aliakbarlu, 2019). The mode of action of CEO is complex, it has a potential membrane permeabilizing activity that causes the weakening of the outer membrane by molecules that disintegrate the lipopolysaccharides layer in Gram-negative bacteria (Yap *et al*., 2015). However, the primary mechanisms of action of cinnamaldehyde are to impede the enzymes that form cell walls, rupture cell membranes, and restrict cell growth (Jha *et al*., 2022).

Based on our results, CEO was highly effective and promising natural antimicrobial against the tested pathogen. It is noteworthy to mention that it's safe to be used in food; however, in large doses or when used over long periods, it could lead to adverse effects in addition to present of several obstacles in their use in food (Valdivieso-Ugarte *et al*., 2021).

Nanoparticles have received significant attention worldwide because of their antibacterial activity and their great physical and chemical stability. These properties are predominated in the food science for enhancing the overall quality, shelf life, taste, flavor, process-ability, etc., of the food (Kumar *et al*., 2020). Also, the strong antimicrobial activity of nano-emulsified version of essential oils has been demonstrated against a broad spectrum of pathogens and spoilage Mos in various food systems (McClements *et al*., 2021).

Chemical composition of CEO

It was found that CEO contained 39 components (Table 1) while the main four ones were (E) Cinnamaldehyde (19.85%), Eugenol 19.25%, Cinnamaldehyde propylene glycol acetal (16.5%) and Benzyl alcohol (13.44%). Cinnamaldehyde is the main component of cinnamon oil. The obtained results agree with these literatures (Wang *et al*. 2022; Cuchet *et al*., 2023). Eugenol, the main component of cinnamon leaves and clove, showed lower antimicrobial activity than cinnamaldehyde, the main component of cinnamon bark (Cava-Roda *et al*., 2021).

Characterization of CNE

Characterization of nanomaterials involves the assessments of their droplet size and PDI by Zeta sizer, their constituents by FTIR and morphology by TEM.

Polydispersity index (PDI) is an important parameter, which represents the particle size distribution of the droplets. The value of PDI is calculated by the ratio of the mean standard deviation of the droplet size to the average droplet diameter (Schober *et al*., 2024) the mean droplet diameter of CNE was 81.35±18.75 nm with PDI of 0.399. The PDI results indicated stability and good homogeneity of the prepared nanoemulsion, as it was lower than 0.5, as a ratio of surfactant used in CNE was used prevent the coalescence at room temperature and for a long period of storage. In addition, the greater value of PDI showed the lower uniformity of droplet size, as PDI represents the homogeneity of droplet size in a nano-emulsion (Yuliani *et al*., 2018). Moreover, samples with a very wide size distribution with a PDI value higher than 0.7 are not suitable (Elsherif

and Shrief, 2021).

FTIR analysis determines the material molecular composition and structure and has been considered as a fingerprint (Nandiyanto *et al*., 2019). FTIR results for analysis of CEO and its NE were shown in Fig. 1, It revealed that strength of the phenols peak, which in the FTIR spectroscopy reference was ranged from 3400 to 3670 cm^{-1} in CNEs. In addition, a significant C–H peak stretching to 3100.54 and 2758.98 cm^{-1}, which in CEO was shifted and observed at 3015.22 and 2929.29 cm^{-1}, also C–H stretching peaks of CEO at 2952.50 and 2923.98 cm^{-1} that were detected at 2925.30 cm^{-1} for its NE. This outcome is consistent with contact angle measurements herein and agrees with that reported elsewhere (Moustafa *et al*., 2022). Interestingly, it was also found that characteristic band at ~ 1731 cm^{-1} observed for -C = O group of CNE and its intensity increased with increasing the content of cinnamaldehyde in the fabricated emulsion, in addition to a strong band at ~ 1670 cm^{-1} for -C = C- group in case of CEO while increase in CNE. Furthermore, the characteristic peaks for aromatic rings ranged from 1400 to 1600 cm^{-1}, and those detected in very strength for EO than its NE. which indicate the powerful flavor (odor and taste) of EO than NE. The difference in peaks and presence of additional functional groups in NEs may act as the main factors in its nano-properties, stability, and antibacterial activity (Elsherif and Shrief, 2021).

The morphology and size by TEM of CNE in Fig. 2 showed separate nano-micelles with average size 56.74±12.33 nm. The size of the CNE measured by TEM was smaller than the results measured by Zeta sizer (refer to the hydrodynamic diameters of nanoparticles in a solution); this difference was attributed to the nanoparticles shrinking during the drying process of the TEM sample (Mittal *et al*., 2014).

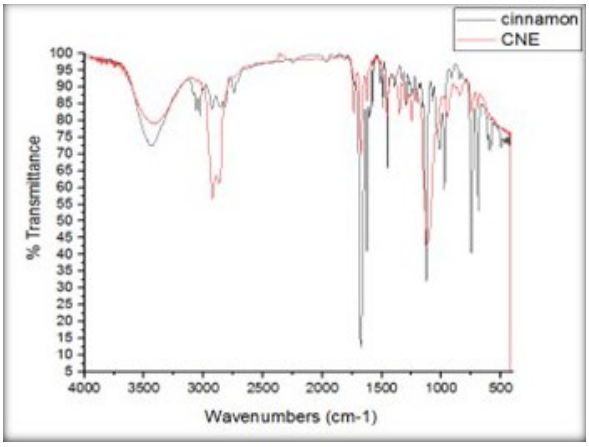

Fig. 1. FTIR of cinnamon and its nano-emulsion.

Cytotoxicity effect of CNE

Cytotoxicity effect of CNE on cell line, namely the Vero SF cells (monkey epithelial cells from kidney) was recommended by Kourmentza *et al*. (2021) (as a normal cell). The cytotoxicity effect of CNE that was determined by SRB assay, the cell viability was located in the range of 85.15–

Table 1. GC-MS analysis of volatile components in CEO.

No.	Compound Name	Area %	Molecular Formula
1	Cinnamaldehyde, (E)	19.85	C_9H_8O
2	Eugenol	19.25	$C_{10}H_{12}O_2$
3	Cinnamaldehyde propylene glycol acetal	16.5	$C_{12}H_{14}O_2$
4	Benzyl alcohol	13.44	C_7H_8O
5	Caryophyllene	3.75	$C_{15}H_{24}$
6	Epiglobulol	1.92	$C_{15}H_{26}O$
7	Quinindoline	1.79	$C_{18}H_{14}N_2$
8	12,15-Octadecadienoic acid, methyl ester	1.68	$C_{19}H_{34}O_2$
9	16-Octadecenoic acid, methyl ester	1.63	$C_{19}H_{36}O_2$
10	Humulene	1.58	$C_{15}H_{24}$
11	3- (O-Azido phenyl) propanol	1.42	$C_9H_{11}N_3O$
12	1-(4-Isopropylphenyl)-2-methylpropyl, acetate	1.37	$C_{15}H_{22}O_2$
13	Heptadecanoic acid, 16-methyl-, methyl ester	1.04	$C_{19}H_{38}O_2$
14	17-Octadecynoic acid	0.97	$C_{18}H_{32}O_2$
15-39	Others (<0.9)	12.7	
39		98.89	

Table 2. Effect of CEO and CNE on S. Typhimurium count (log10 cfu/g) artificially inoculated into burger samples during refrigerated storage.

Treatment	Days				
	Zero	1st	3rd	5th	7th
1	ND	ND	ND	ND	ND
2	4.3	4.93	4.54	4.7	spoiled
3	3.98	3.85**	2.60**	2.72**	spoiled
4	3.91	2.49**	ND	ND	ND
5	3.97	3.54**	2.48**	ND	ND
6	3.93	2.95**	ND	ND	ND

Highly significant** P-Value <0.01, Significant* P-Value <0.05 ND = not detected

(1) Control (-ve) free from preservatives and bacteria, (2) Control (+ve) inoculated by S. Typhimurium strain only, (3) Free CEO MIC1 (0.6%), (4) Free CEO MIC2 (1.25), (5) CNE MIC 1 (0.3%) (6) CNE MIC2 (0.6)

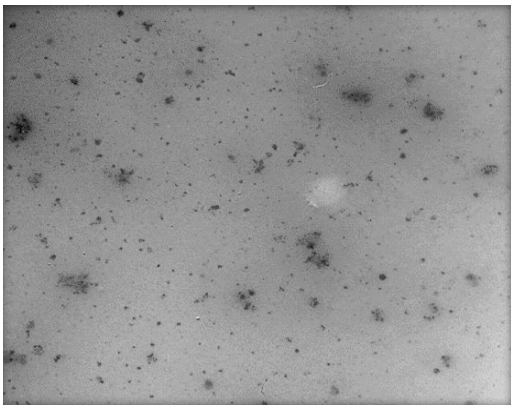

Fig. 2. TEM of cinnamon nano-emulsion (56.74±12.33 nm, separate nano-micelles)

99.7% even at all studied concentrations. The percentages of cell viability were 85.15±0.041, 88.94±0.66, 92.04±1.83. 95.09±1.05 and 98.8±0.096 at concentrations of 100, 10, 1, 0.1 and 0.01 (µg/ml) respectively. This finding of CNE is consistent with Moustafa *et al.* (2022) who confirmed that the material can be exhibited a significant toxicity when cell viability value was less than 50% compared to the control cells. In light of this, our prepared nanomaterials did not exhibit cytotoxicity to the cells, demonstrating the high safety of these biomaterials and their potential as candidates for smart food preservative.

Evaluation of antimicrobial activity of CNE

Antibacterial activity of CNE against *S.* Typhimurium by agar well diffusion method revealed that concentrations of 5, 2.5, 1.25, 0.6 and 0.3% gave clear inhibition zones 42, 34, 28, 19, and 12 mm, respectively.

The result of MIC of CNE were 3 (µl/ml), equivalent to 0.3%. MLCs of CNE were 6 (µl/ml) equivalent to 0.6. It was concluded that CNE had restricted the growth and multiplication of all tested food born bacteria due to its killing action on initial bacterial inoculum exposed to it, this could be correlated with its monodispersed nature and lower droplet diameter along with antibacterial substance content present in CEO. Lower MIC values were obtained 0.0625% (Kang and Song, 2018), 1.6 mg/ml, (Chuesiang *et al.*, 2019) and 0.039% with higher MLC 0.78% (Paudel *et al.*, 2019). Results of this study are in harmony with other studies (Pimple *et al.*, 2019).

Antimicrobial effect of CEO and CNE on burger samples artificially inoculated by S. Typhimurium *during refrigerated storage*

This part of our study aimed at highlighting the importance of using natural preservatives objective assessment tools and consumer/sensory evaluation in determining the quality and acceptability of new food products.

The most important feature of a new functional product is sensory properties. Because the physical and chemical properties of the product no matter how good if the sensory characteristics of the product are not good this product is not consumed (Świąder and Marczewska, 2021). Table 2 and Figs. 3 and 4, showed, count (log cfu/g) of *S.* Typhimurium, reduction effects (%) and overall acceptability of burger samples with different treatments. In case of Control (+ve) samples the results showed that the initial number of bacteria of (day 0) was 4.3 (log cfu/g) (overall acceptability 8. 33) to reach 4.7 (log10 cfu/g) at day 5 with unaccepted sensory score (4.33) then spoiled on the 7th day (end of experiment). But in case of treated burger samples with CEO MIC2 (1.25%) reduced the initial bacterium counts to 3.91 on day 0 and 2.49 (log cfu/g) on day 1 which was significantly different ($p < 0.05$) with reduction 49.46 % then not detected in the remaining days (highest reduction 100%), while with MIC1 (0.6%) the count on (day 0) was 3.98 to reach 2.72 on day 5 with reduction 42.02% then spoiled (day 7), recorded the highest reduction (42.74%) on the 3rd day. It was noticeable that, on day 0 burger samples treated with CEO (MIC2) were less acceptable than control ones with lower overall acceptability score (7.66). Also, within the days of storage, lower concentration (MIC1) showed better sensory quality than (MIC 2) and control until day 5. Regarding cinnamon nanoemulsions (CNEs), during all days of storage the treated samples were accepted for all panelists and the *S.* Typhimurium count significantly reduced ($p<0.05$) by both concentrations MIC1(0.3%) and MIC2 (0.6%). On zero-day, the count of MIC1 and MIC2 were 3.97 and 3.93 (log cfu/g) with reduction percentage 7.73% and 8.64 % and excellent sensory quality. Complete control of the bacterium occurred approximately after the 1st and 3rd days for MIC1 and MIC2, respectively. In a related study conducted by Chuesiang *et al.* (2021) and Liu *et al.* (2021); they suggested that CNE was more effective in inhibiting the growth of all tested pathogenic bacteria and can be used as an alternative natural antimicrobial agent.

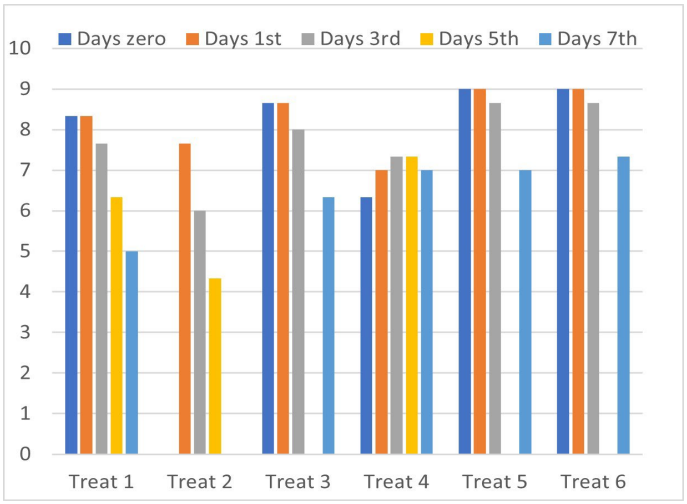

Fig. 3. Effect of CEO and CNE on overall acceptability of burger samples inoculated by S. Typhimurium during storage: Control (-ve) free from preservatives and bacteria, (2) Control (+ve) inoculated by *S.* Typhimurium strain only, (3) Free CEO MIC1 (0.6%),(4) Free CEO MIC2 (1.25) (5) CNE MIC 1 (0.3%) (6) CNE MIC2 (0.6 %) *Score system for sensory evaluation Click or tap here to enter text. 9: Excellent, 8: Very very good, 7: Very good 6: Good; 5: Medium, 4: Fair, 3: Poor 2: Very poor, 1: Very very poor

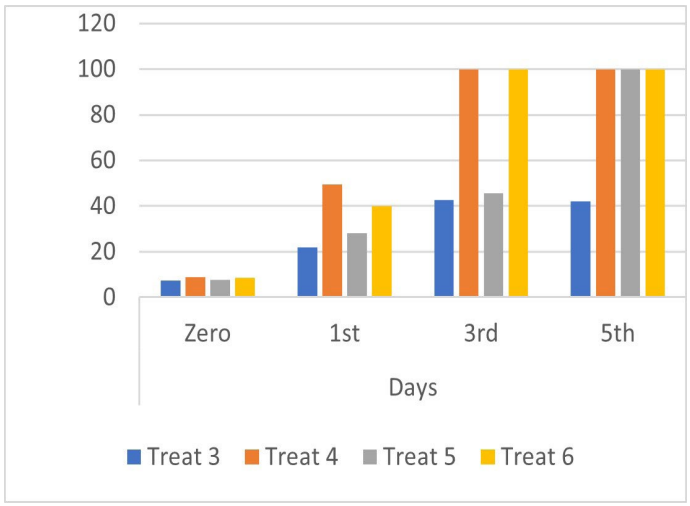

Fig. 4. Reduction effect (%) of CEO and CNE on *S.* Typhimurium count (log10 cfu/g) artificially inoculated into burger samples during refrigerated storage: *Control sample spoiled after day 5; Treat 3: Free CEO MIC1 (0.6%), Treat 4: Free CEO MIC2 (1.25%), Treat 5: CNE MIC 1 (0.3%) Treat 6: CNE MIC2 (0.6%).

Effect of CEO and CNE on shelf life of burger samples during refrigerated storage

Overall acceptability of manufactured burger samples during refrigerated storage was showed in Fig. 5, and showed that at the beginning of the experiment on Day 1, the control samples had highly accepted score (8). While samples treated with CEO recorded the lowest score (6) due to the

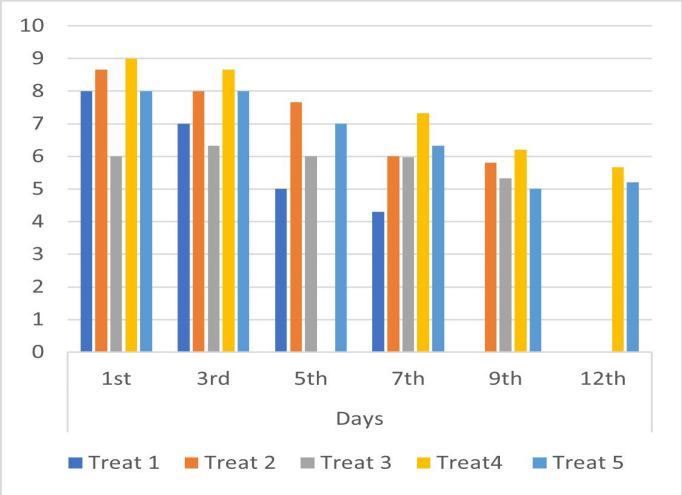

Fig. 5. Effect of CEO and CNE on overall acceptability of manufactured burger samples during refrigerated storage: Treat 1: Control (-ve) free from preservatives, Treat 2: Free CEO MIC1 (0.6%), Treat 3: Free CEO MIC2 (1.25%), Treat 4: CNE MIC1 (0.3 %), Treat 5: CNE MIC2 (0.6 %). Score system for sensory evaluation Click or tap here to enter text.: 9: Excellent, 8: Very very good, 7: Very good 6: Good; 5: Medium, 4: Fair, 3: Poor, 2: Very poor, 1: Very very poor

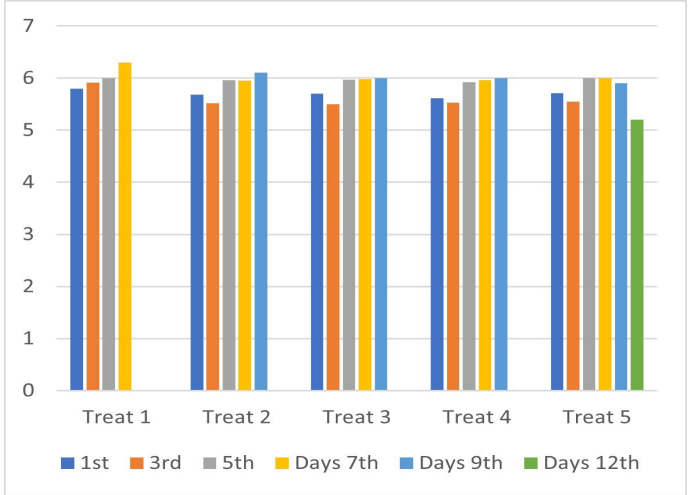

Fig. 6. Effect of CEO and CNE on pH of burger samples during refrigerated storage: Treat 1: Control (-ve) free from preservatives. Initial pH value was 5.71, Treat 2: Free CEO MIC1 (0.6%), Treat 3: Free CEO MIC2 (1.25%), Treat4: CNE MIC1 (0.3 %), Treat 5: CNE MIC2 (0.6%).

strong odor and greasy (oily) appearance. The intense smell and flavor of CEO interfered negatively with the sensory analysis (Gottardo *et al*., 2022). On the other hand, samples treated with CNE (MIC2) showed no signs of spoilage until the 12th day with accepted sensory evaluation. All sensory attributes of samples were influenced by the storage time, formulation and their interaction. Control samples were started to be unaccepted with appearing rancid odor on the 7th day and spoiled with off odor on the 9th day of storage period, while at both CEO concentrations, the panelist could smell only the strong cinnamon odor until the 12th day of storage. Cinnamaldehyde (main component of CEO) has been reported to contribute to the sweet-spicy, pungent, and cinnamon-like odor (Chuesiang *et al*., 2023). Comparatively, in the previous studies by Ghaderi-Ghahfarokhi *et al.* (2016) and in contrary, Shaltout and Koura (2017) found that cinnamon oil, demonstrated the highest enhancement of sensory attributes.

Effect of CEO and CNE on pH values

The initial pH value of the control beef burger was 5.71 (0-day), while pH values varied on the 1st day from 5.8 to 4.5 as shown in Fig. 6. During storage, pH values increased slightly, and the rise became more obvious for control, reaching to 6.3 on the 7th day of the storage then spoiled. Brilliana *et al.* (2017) stated lowering pH values of ground beef after the addition of CEO during refrigerated storage compared to the control sample, these results because of the active components of the CEO that are cinnamaldehyde, linalool and eugenol. Furthermore, more preservative impact was noted for samples incorporated with CNE (MIC2) which maintained accepted pH (5.9, 5.2, 5.0 and 5.35 on the 12th day, respectively) of meat up to the 12th day of the storage.

Effect of CEO and CNE on TBA values

Interestingly, Table 3, shows gradual increase of TBA values of all examined samples which indicates that oxidative deterioration of beef burger lipid occurred during storage time. So, on the 1st day, no significant difference ($p \leq 0.05$) was observed among treated samples and control which was 0.17 and 0.28 on the 3rd day (last accepted value) to reach 0.76 on the 7th day then spoiled. CEO (MIC1) had less values than control ranged (0.18-0.83), indicating antioxidant effect until the 5th day (maximum limit of acceptance, 0.5 mg/Kg). While CEO (MIC2) showed significant increase (P-Value). In the contrary, TBA values of CNE (especially MIC2) samples were significantly lower when compared to the control, indicating that CNE effectively retarded lipid oxidation. Our results agree with the studies of Ameur *et al.* (2022); Shaltout and Koura (2017); Awaisheh *et al.* (2020) and Dghais *et al.* (2023).

Effect of CEO and CNE on APC

APC values over the storage period and the efficacy of different treatments are depicted in Table 4. Initially, the APC in control burger samples was 5.6 (log cfu/g), indicative of acceptable beef meat quality. However, as the storage period increased, the control APC increased more than all treatments to reach 5.71, 5.32, 5.87 and 6.88 (log cfu/g), on the 1st, 3rd, 5th and 7th days respectively, then spoiled. With regard free CEO, MIC1 (0.6%) reduced the initial APC to 5.11 on the 1st day and 4.26 (log cfu/g) on the 3rd day which was significantly different ($p < 0.05$) with reduction 10.44% and 20.01% then increased till 6.30 on the 9th day. However poor sensorial score, CEO MIC2 (1.25%), caused significant gradual decrease in APC till the 5th day (not detected in the first dilution) then increased to reach 4.15 on the 9th day. While, regarding CNE (both concentrations) had significant

Table 3. Effect of CEO and CNE on TBA (mg MDA/kg) of manufactured burger samples during refrigerated storage.

Treatment	Days					
	1st	3rd	5th	7th	9th	12th
1	0.17±.004	0.28±.002	0.53±.008	0.76±.005	Spoiled	spoiled
2	0.18±.01	0.28±.002	0.5±.008*	0.65±.005**	0.83±.01	spoiled
3	0.2±.004*	0.3±.002**	0.52±.008	0.69±.005**	0.73±.002	spoiled
4	0.18±.004	0.27±.002*	0.5±.008*	0.57±.005**	0.69±.008	spoiled
5	0.15±.004*	0.265±.002**	0.43±.008**	0.55±.005**	0.58±.01	0.7±.006

Highly significant** P-Value <0.01, Significant* P-Value <0.05. (1) Control (-ve) free from preservatives. (2) Free CEO MIC1 (0.6%), (3) Free CEO MIC2 (1.25%), (4) CNE MIC1 (0.3 %), (5) CNE MIC2 (0.6 %).

reduction from the 1st to 7th days, 29.04% for MIC1 and 27.20% for MIC2.

Table 4. Effect of CEO and CNE on APC (log10 cfu/g) of manufactured burger samples during refrigerated storage.

Treatment	Days					
	1st	3rd	5th	7th	9th	12th
1*	5.71	5.32	5.78	6.88	spoiled	spoiled
2	5.11	4.26**	4.95**	5.11**	6.3	spoiled
3	4.81*	3.11**	< 2**	2.62**	4.15	spoiled
4	4.85*	4.76	4.79**	4.86**	5.59	4.76
5	4.45*	3.62**	< 2**	< 2**	3.48	5.6

Highly significant** P-Value <0.01, Significant* P-Value <0.05. (1) Control (-ve) free from preservatives, *APC in zero time for control sample was 5.6 (log10 cfu/g), (2) Free CEO MIC1 (0.6%), (3) Free CEO MIC2 (1.25%), (4) CNE MIC 1 (0.3%) (5) CNE MIC2 (0.6 %).

Effect of CEO and CNE on total yeast and mold count

Regarding control samples, low total yeast and mold counts were 2.3, 2.95, 3.52, 3.79 and 4.45 (log cfu/g) on 0, 1st, 3rd, 5th and 7th days. On day 7, CEO MIC1 recorded the highest count 3.95 (reduction only 11.19 %) (Table 5). This was confirmed by Gottardo *et al*. (2022) who mentioned that CNEs had an evident antifungal effect on mold, which may be due to the destruction of the cell wall and membrane structure and inhibition of spore formation.

Table 5. Effect of CEO and CNE on total yeast and mold count (log10 cfu/g) of manufactured burger samples during refrigerated storage.

Treatment	Days					
	1st	3rd	5th	7th	9th	12th
1*	2.95	3.52	3.79	4.45	Spoiled	spoiled
2	< 2	3.54	3.7	3.95	4.58	spoiled
3	< 2	< 2	2.6	2.94	3.15	spoiled
4	< 2	3.38	3.49	3.75	3.85	spoiled
5	2.3	3.4	3.6	3.89	3.96	4.62

Highly significant** P-Value <0.01; Significant* P-Value <0.05. Control (-ve) free from preservatives, *Total yeast and mold count in zero time for control sample was 2.3 (log10 cfu/g). (2) Free CEO MIC1 (0.6%), (3) Free CEO MIC2 (1.25%), (4) CNE MIC 1 (0.3%) (5) CNE MIC2 (0.6 %).

Conclusion

CEO and CNE are promising alternative to chemical preservatives against *S.* Typhimurium both in vitro and in vivo assays. Indeed, CNE can easily be formulated with existing food ingredients and technologies and has unique characterization, high biosafety, rapid onset of action and long-term stability with excellent sensorial scores. It exhibits inhibitory activity against *S.* Typhimurium and extend the shelf life of burger samples with lower concentrations. This substitution may have potential implication for developing green-labeled meat product.

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Conflict of interest

The authors declare that they have no conflict of interest.

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