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Research Article

Use of bamboo and clove essential oil nanoemulsions to improve shelf-life and enhance meat safety by inactivating multidrug-resistant *Salmonella* Typhimurium

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Abstract

This study evaluated essential oil-based nanoemulsions (EO-NEs), *viz*. clove (CEO-NE) and bamboo (BEO-NE) as natural preservatives against multidrug-resistant (MDR) *Salmonella* Typhimurium in fresh chicken meat during 8 days refrigerated storage. Both CEO and BEO contained high phenolics (33.08 vs. 28.82 mg GAE/mL) and flavonoid (12.74 vs. 19.26 mg QE/mL) contents, contributing to strong antioxidant activity. BEO-NE even outperformed butylated hydroxytoluene (BHT) and Trolox in DPPH assays. Agar well diffusion tests revealed significant antimicrobial effects, with 2.5% CEO-NE showing a larger inhibition zone (24 mm) than BEO-NE (18 mm). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) studies further confirmed their potency against MDR strains. In meat samples, both EO-NEs delayed pH increase, reduced lipid oxidation, and inhibited microbial growth, while maintaining better colour stability and sensory quality (odour, appearance), than controls. The findings highlight CEO and BEO nanoemulsions as effective, natural alternatives in combating MDR pathogens, therefore could be used in improving the shelf-life and ensuring the safety of fresh chicken meat.

Keywords: Bamboo and clove essential oil, Chicken meat, MDR *Salmonella* Typhimurium, Meat safety, Nanoemulsions

Highlights

- Clove and bamboo EO NEs tested against MDR *S*. Typhimurium in chicken meat.
- Both EO NEs had high total phenolics and flavonoid contents, exhibiting strong antioxidant activity.
- BEO NEs outperformed BHT and Trolox in DPPH assays, showing good radical scavenging.
- CEO-NE @ 2.5% exhibited a larger inhibition zone (24 mm) than BEO-NE (18 mm).
- EO NEs delayed pH rise, reduced lipid oxidation, inhibited microbes, and preserved meat colour/odour.

INTRODUCTION

Chicken meat is widely consumed and offers several advantages over red meats. However, its nutrient-rich composition and high-water content make it highly susceptible to microbial contamination throughout production, distribution, and consumption stages. This susceptibility poses significant challenges to meat safety, as microbial contamination can lead to rapid spoilage and potentially severe foodborne illnesses (Das et al., 2019). *Salmonella* Typhimurium is a major foodborne pathogen capable of causing serious foodborne diseases such as salmonellosis (Teklemariam et al., 2023), with poultry meat accounting for nearly 25% of reported outbreaks (Akil & Ahmad, 2019). To prevent bacterial contamination and growth, synthetic preservatives are often used. However, their indiscriminate use raises concerns about toxicity to non-target organisms, the development of microbial resistance, and environmental sustainability degradation (Maurya et al., 2021).

Again, the rise of *Salmonella* infections highlights the urgency for effective antimicrobial strategies in food safety (Nath et al., 2026). As per the World Health Organization (WHO), at least 7, 00, 000 deaths occur on a daily basis due to diseases caused by antibiotic-resistant bacteria. In 2019 alone, nearly 3 million cases of antibiotic-resistant infections were documented in the

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United States (World Health Organization [WHO], 2021). This information highlights the urgent need for new solutions to combat MDR pathogens. Viewing bacterial resistance as an epidemic with deep consequences, antimicrobials derived from natural sources offer effective and often economical alternatives. Further, consumer's demand for meat products free from synthetic additives has spurred research into natural alternatives, including essential oils, recognized for their antioxidant and antibacterial properties (Das et al., 2023).

Essential oils (EOs), derived from various plant parts like leaves, fruits, and stems, contain phenolic compounds and terpenoid fatty acids, and are known for their antioxidant and antimicrobial properties, making them effective in extending the shelf-life of meat products (McClements et al., 2021; Biswas et al., 2023). In recent days, a number of EOs are being utilized to extend the safety and shelf-life of meat and meat products from poultry (Bhattacharya et al., 2025; Das et al., 2025) and pork (Daniloviæ et al., 2021). However, their application faces challenges due to limited water solubility, intense aroma, volatility, and chemical instability. Encapsulating EOs into nanoparticles, particularly nanoemulsions with droplet sizes typically below 200 nm, has emerged as a promising strategy to enhance the stability, bioavailability, and efficacy of bioactive compounds in food preservation (Das et al., 2020). Research findings involving essential oil-based nanoemulsions have shown promise in controlling and inhibiting different spoilage organisms, including MDR pathogens, resulting improvement in shelf-life and safety of foods (Maurya et al., 2021). In a study, nanoemulsion with *Cleome viscose* EO was found to be effective against methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Streptococcus pyogenes*, and drug-resistant extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (Krishnamoorthy et al., 2018). Essential oil-based nanoemulsions have also been reported to inhibit the growth of various MDR pathogens such as *Staphylococcus saprophyticus*, *Enterococcus faecalis*, and *S. aureus* (Amasha, 2024). However, there is limited information on the use of essential oil–based nanoemulsions against multidrug resistant *S.* Typhimurium, the pathogen responsible for salmonellosis, a significant threat to public health. Hence, this study was conducted to investigate the efficacy of bamboo and clove essential oil-based nanoemulsions in inactivating multidrug resistant *S.*Typhimurium inoculated in fresh chicken-meat. Further, this study assesses the impact on microbiological, physicochemical, and sensory attributes of chicken breast fillets during 8 days refrigerated storage study.

MATERIALS AND METHODS

Raw materials: Fresh chicken meat was purchased from the local market. The analytical-grade chemicals, reagents and media used were procured from HiMedia Laboratories, Mumbai, India, and Sigma Aldrich, USA. The food-grade bamboo (*Bambusa vulgaris* L.) and clove (*Syzygium aromaticum*) essential oils were purchased from standard firm (R. V Essential, Delhi, India) and nanoemulsions were prepared from the respective EOs. Based on the ingredients to be encapsulated, food-grade materials such as oil, stabilizers, and emulsifiers were procured from the standard firms. The bacterial strain *viz*., MDR *S.* Typhimurium used in this study was isolated from poultry (Table 1). All the *S.* Typhimurium isolates used in this study were resistant to extended-spectrum β-lactams, tetracycline and quinolones groups of antibiotics in disk diffusion assay, as recommended by the Clinical and Laboratory Standards Institute [CLSI], 2022. Further, *S.* Typhimurium isolates were found ESBL producers in the combination disk assay (De Gheldre et al., 2003). For the aerobic packaging of chicken meat cuts, natural-coloured low-density polyethylene (LDPE) pouches (200 gauges) were bought from the local market.

Table 1. Characteristics of ESBL-producing *Salmonella* Typhimurium isolates used in this study

Sl no	Isolates	Characteristics	AST profile	Source
1	ST-IVRIERS-23	ESBL	AK-CPD-TE	Poultry
2	ST-IVRIERS-77	ESBL	CPD-GEN-NAL	Poultry
3	ST-IVRIERS-92	ESBL	CPD-GEN-NAL-FOS	Poultry
4	ST-IVRIERS-430	ESBL	AK-CPD-TE	Poultry

AST=Antimicrobial susceptibility testing; ESBL-Extended spectrum beta lactamase; AK-Amikacin, CPD- Cefpodoxim; TE-Tetracycline; GEN: Gentamicin; NAL: Nalixidic acid; FOS: Fosfomycin

Preparation of clove and bamboo essential oil nanoemulsions: The coarse emulsion was prepared using different concentrations of bamboo and clove essential oil (2.0%, 2.5%, and 3%) by micro-fluidization process and nanoemulsions were prepared after sonication maintaining constant

ultrasound amplitude and frequency of 100 µM and 20 kHz, respectively (Fig. 1). Characterization in terms of droplet size and zeta potential, viscosity, emulsion stability and encapsulation efficiency of selected nanoemulsions (2.5%) was done as per our previously published study (Das et al., 2024). The study developed essential oil (CEO) nanoemulsions, focusing on their clove properties and potential applications. The optimal formulation, containing 2.5% CEO, exhibited a droplet size of 125.3 nm and a zeta potential of around -30 mV, ensuring stability against aggregation. The encapsulation efficiency was high at 83%, indicating effective oil retention within the droplets. Transmission electron microscopy confirmed spherical droplet morphology, and the emulsions showed minimal shear thinning with stable viscosity. Cytotoxicity assays on human lymphocytes demonstrated that the nanoemulsions were relatively non-toxic, with over 81% cell viability even at the highest concentrations (Das et al., 2024).

***In-vitro* antimicrobial analysis of clove and bamboo essential oil nanoemulsions:** To assess the *in-vitro* anti-bacterial activity of nanoemulsions of the EOs, the agar well diffusion assay was carried out as per the procedures outlined by Sriram et al. (2019), with slight modifications. Mueller-Hinton Agar (MHA) plates were inoculated with the test isolate using a standard suspension adjusted to 0.5 McFarland turbidity, equivalent to approximately 1.5×10^8 CFU/mL. Following that, about 120 µL of each culture was spread using a cotton swab on MHA plates. Using a sterile cork borer, 6 mm wells were dug on the agar plates. The base of each well was then sealed with around 25 µL MHA and allowed to set at room temperature. Each row was filled with 100 µL of the appropriate nano-emulsion oils, and positive control (ceftriaxone), followed by incubation at 37°C. The antibacterial activities of the nano-emulsion oils were evaluated by measuring their zone of inhibition (ZOI) in mm against the test bacteria.

Determination of MIC and MBC values: The resazurin-based micro-broth dilution approach was used to estimate the minimum inhibitory concentration (MIC), and the usual spread plate inoculation method was used for minimum Biocidal Concentration (MBC) (Sfeir, 2013). The test bacteria inocula were prepared in Tryptic Soy Broth (TSB) according to the criteria provided by the Clinical and Laboratory Standards Institute (CLSI, 2022). The OD_{600} value (0.08–0.12) was adjusted to obtain ~1 x 10^8 CFU/mL. After that,

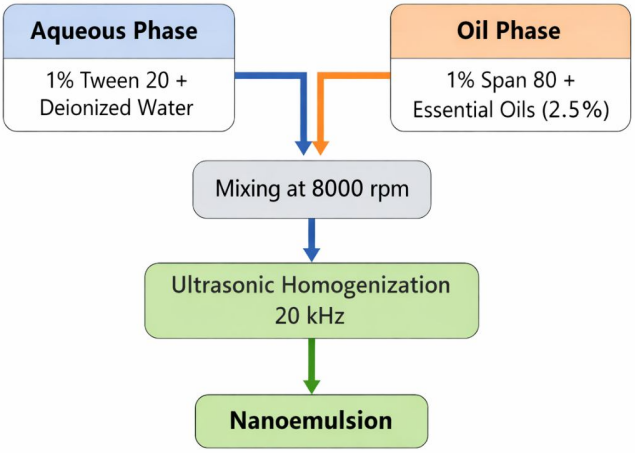


Fig. 1. Flow diagram for preparation of essential oil nanoemulsions

TSB was used to further dilute the modified inocula by 1:100, to yield approximately 1×10^6 CFU/mL. The MIC values of nano-emulsion oils against MDR *S.* Typhimurium were assessed using a 96-well microtiter plate. Each well in column 1 contained 197.5 µL of Mueller-Hinton broth (MHB) and containing 2.5 µL of respective inoculums of *S.* Typhimurium inoculums. Subsequently, columns were filled with varying amounts of MHB (194.5 µL, 191.5 µL, 185.5 µL, 172.5 µL, 147.5 µL, 97.5 µL). Additionally, different volumes (3 µL, 6 µL, 12 µL, 25 µL, 50 µL, 100 µL) of 2.5% concentration of CEO-NE, or 3 µL, 6 µL, 12 µL, 25 µL, 50 µL, 75 µL, 100 µL of 2.5% concentration BEO-NE were added to each well to achieve a final volume of 200 µL. The last column was kept as negative control, where only 100 µL each of nanoemulsion and respective sterile broth was added. In order to determine the antibacterial activity of nanoemulsion oils against MDR *S.* Typhimurium, the microtiter plate was incubated for 24 hours at 37°C in an aerobic environment. Following the incubation period, each well received 20 µL of sterile resazurin dye (0.015% w/v), and the plates were then re-incubated for a further 2 hours to see visible colour changes, if any. The MIC value was determined by analyzing the columns with the lowest concentrations that did not exhibit any colour changes (i.e., the blue resazurin colour stayed intact) during incubation. The MBC was determined by directly plating the contents of wells with concentrations above the MIC on sterile MHA plates and incubating for 24 hours at 35°C to detect the presence of bacteria. The lowest concentration of nanoemulsion oil that did not produce any colonies on inoculated agar plates was considered as MBC and the results were recorded in microlitres.

Analysis of antioxidant activities of clove and bamboo essential oil nanoemulsions

Estimation of total phenolics content (TPC)- For estimation of total phenolics content of clove and bamboo essential oil, the Folin–Ciocalteu technique was used as per the standard method. Briefly, various concentrations of CEO and BEO were prepared by dissolving them in methanol. From these solutions, 0.5 mL of each concentration was combined with 2.5 mL of 0.2N Folin–Ciocalteu reagent and incubated in the dark for 5 minutes. Subsequently, 2 mL of 7.5% (w/v) sodium carbonate was added to the mixture, which was then kept in the dark for 2 hours. During this incubation, the solution developed a blue colour. The optical density was measured using a spectrophotometer at a wavelength of 760 nm. The phenolic content in the CEO and BEO samples was quantified using a gallic acid calibration curve as the standard reference for polyphenols. Results were expressed as gallic acid equivalents (GAE).

Estimation of total flavonoid content- For estimation of total flavonoid content of clove and bamboo essential oil, the aluminium chloride ($AlCl_3$) colorimetric method was used as per the method of Pathak and Niraula (2019), with slight modifications. For the analysis of flavonoid content, different concentrations of 1 mL CEO and BEO were placed in separate test tubes. To each tube, 4 mL of double-distilled water and 0.3 mL of sodium nitrite were added and mixed thoroughly, then allowed to stand in the dark for 6 minutes. Thereafter, 0.3 mL of 10% $AlCl_3$ was introduced into the solution, and the mixture was kept in the dark for an additional 5 minutes to ensure complete reaction. Following this, 2 mL of 1 M sodium hydroxide was added, and the final volume was adjusted to 10 mL with double-distilled water. After a 15-minute of incubation period, the samples turned pink, and the absorbance was measured using a spectrophotometer at 510 nm. The flavonoid content in the CEO and BEO samples was quantified and expressed as milligrams of quercetin equivalents (QE) per milliliter.

Assessment of *in-vitro* antioxidant activities of CEO and BEO

Radical scavenging activity using DPPH (2,2-diphenyl-2-picrylhydrazyl) method- The radical scavenging activity of CEO and BEO was assessed using the DPPH assay with some modifications (Hossain et al., 2011). In this procedure, test tubes were prepared with 1 mL of the sample, which had been diluted in methanol to various concentrations, and mixed with 2 mL of a 0.1 mM DPPH methanolic solution. The test tubes were then incubated in the dark for 30 minutes. The scavenging activity was indicated by the colour change from deep violet to light yellow. Following incubation, the absorbance was recorded at 510 nm using a Spectra Max® M5 spectrophotometer (USA). Methanol served as the blank for absorbance measurements, while BHT, BHA, and Trolox were used as positive controls. The percentage of inhibition or radical scavenging activity was calculated using the following formula:

% Radical scavenging activity = (Control OD - Sample OD/ Control OD) ×100.

Hydrogen Peroxide (H_2O_2) scavenging activity- The capacity of clove and bamboo essential oils to neutralize hydrogen peroxide was evaluated using a modified method (Keser et al., 2012). A 40 mM H_2O_2 solution was prepared in phosphate-buffered saline (PBS, pH 7.4). Different concentrations of 0.1 mL CEO and BEO were combined with 0.6 mL of the 40 mM H_2O_2 solution in Eppendorf tubes and incubated for 10 minutes. The absorbance of the reaction mixture was then measured at 230 nm using a Spectra Max® M5 spectrophotometer (USA). Methanol served as the blank, while BHT, and Trolox were used as positive controls. The percentage of H_2O_2 scavenged by CEO, BEO, and the standard compounds was calculated using the following formula:

$$\% \text{ Scavenged } H_2O_2 = [(T_C - T_S)/T_C] \times 100$$

(Where T_C is the absorbance of the control and T_S is the absorbance standards and samples).

Ferrous ion chelating activity- The ability of clove and bamboo essential oils to chelate ferrous ions was assessed using a slightly modified method (Patial & Cannoo, 2020). To start, 25 µL of a 2 mM $FeCl_2$ solution was combined with 1.5 mL of water and 2 mL of test samples in methanol at various concentrations. Following this, 50 µL of a 5 mM ferrozine solution was added to the mixture, which was then shaken vigorously and incubated at room temperature for 10 minutes. The absorbance of the resulting solution was measured at 562 nm using a Spectra Max®M5 spectrophotometer (USA). Methanol was used as the blank, while BHA, BHT, and Trolox served as positive controls. The extent of inhibition of ferrozine-Fe_{2+} complex formation was calculated using the following formula:

$$\text{Ferrous ion chelating} = [(T_C - T_S)/T_C] \times 100$$

(Where T_C is the absorbance of the control and T_S is the absorbance standards and samples).

Results from the above antioxidant assays were expressed in µL/mL from percentage antioxidant capacity to allow direct comparison of the potency of different oils regardless of their maximum % activity, and to present the data in terms of the quantity/concentration of oil required to produce a given antioxidant effect, which is more informative for potential applications.

Assessment of *in-situ* preservative effects in fresh meat cuts

Preparation of fresh chicken meat- The sample preparation for fresh chicken meat cuts was carried out with minor modifications following the procedure (Hien & Dao, 2022). Briefly, fresh chicken meat was separated into six equal portions, weighing 20 g each. After that, each meat piece was sterilized by first rinsing in 70% (v/v) ethanol solution, then cleaned with distilled water, and dried for 15 minutes at room temperature. The ethanol rinse was applied solely to decontaminate the meat surface, ensuring that any observed effects could be attributed exclusively to the inoculated MDR organisms, rather than to microorganisms naturally present on the meat. It should be emphasized that the meat samples were used strictly as experimental model systems under controlled laboratory conditions and were not intended for human consumption.

Each meat piece was subsequently dipped in either the BEO-NE or CEO-NE solution for one minute, except for the control group, which was treated with a sterile distilled water dip. Thereafter, the BEO-NE and CEO-NE treated samples were inoculated with 1 mL (10^6 CFU/mL) of surface-cultured MDR *S.* Typhimurium. After that, all the samples were packaged aerobically in LDPE pouches and stored 8 days at 4±1°C. The chicken meat cuts were subjected to analysis of various parameters such as pH, TBARS, instrumental colour (*L**, *a** and *b** values), bacterial counts, and sensory attributes, that were carried out on 0, 2nd, 4th, 6th, and 8th day of refrigerated (4±1°C) storage.

Analysis of meat samples

pH- The pH of each meat piece was measured using a combined electrode digital pH meter (Model CP 901, Century Instruments Ltd., India). About 50 mL of distilled water was used to homogenize 10 g of meat sample for around 1 minute using an Omni homogenizer, Germany. After shaking for 5 minutes, pH was measured by submerging the electrode straight into the meat suspension, and the average of three readings was recorded as the pH of the sample.

Thiobarbituric acid reactive substances (TBARS) value- The extent of lipid oxidation, in terms of TBARS value of the meat samples, was determined by the standard procedure (Witte et al., 1970), with slight modifications. In each case, 25 mL of chilled 20% trichloroacetic acid (TCA) was used to homogenize 10 g of meat sample. Filter paper was used to obtain TCA filtrate from the homogenized meat. Thereafter, a suitable volume of filtrate (3 mL) was mixed with an equal volume of chilled TBA (5 mM) reagent and heated to 70°C in a water bath for 35 minutes, and after that the samples were cooled under running tap water. Using a UV-VIS spectrophotometer (Spectra Max® M5, USA), the absorbance was measured at 530 nm. A factor of 5.2 was used to compute the TBARS value, which was then represented as milligrams of malondialdehyde per kilogram (mg MDA/kg) of the sample.

Microbial counts- To assess the antibacterial activity of clove and bamboo nano-emulsion oils in the MDR *S.* T*yphimurium* bacteria inoculated raw chicken meat samples, determination of microbial counts was performed using the method (Hien & Dao, 2022) with minor modifications. About 10 g of each sample was measured aseptically and homogenized in a pestle and mortar using Buffer Peptone Water (BPW). Subsequently, serial dilution was carried out for every sample, and 120 µL of the diluted samples were spread out on Salmonella-Shigella agar (SSA) plates for respective organisms from 10^{-5} dilution. The plates were then incubated for 24 hours at 37°C. The total number of colonies was counted, compared to the groups of inoculated and non-inoculated samples, and expressed as $\log_{10}$ CFU/g of samples.

Instrumental colour- After cutting the meat samples into slices, three separate areas on the slices were immediately measured for colour parameters. The MINOLTA Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan) was used to assess the CIE colour coordinates, such as lightness (*L**), redness (*a**), and yellowness (*b**) values.

Sensory evaluation- At the specified storage days, assessment of changes in the sensory attributes was carried out for every sample in the treatment and control groups. To judge the odour and colour of the raw meat cut samples, 5-point descriptive scale was used by 10 trained panellists as per the standard method (Das et al., 2006), where score-1 was denoted as very undesirable/ unattractive/dull, and score-5 as very desirable.

Statistical analysis: In the current study, every parameter was measured twice, and each experiment was replicated three times. The data (n=6, except for sensory evaluation, where n=30) obtained were

analysed using SPSS software (Version 20, IBM, USA). One-way and two-way ANOVA were applied to the data in order to analyse the quality and storage parameters, respectively at 95% confidence interval ($p<0.05$). The means of the treatments, the storage time, and their interactions for different parameters were compared using Duncan's multiple range test.

RESULTS

Total phenolics and flavonoid contents: The bamboo essential oil exhibited total phenolics content of 33.08±3.14 mg GAE/mL and a flavonoid content of 12.74±4.12 mg QE/mL. In contrast, clove essential oil showed slightly lower phenolic content (28.82±2.46 mg GAE/mL) but higher flavonoid levels (19.26±3.68 mg QE/mL). These values confirm that both oils are rich in bioactive compounds with potential antioxidant activity.

Antioxidant capacity of CEO NE and BEO NE: The antioxidant potential of CEO NE and BEO NE was assessed using the DPPH radical scavenging assay (Table 2). Both nanoemulsions demonstrated strong activity at low concentrations. The IC_{50} values were 5.37 µL/mL for CEO NE and 1.24 µL/mL for BEO NE. In comparison, synthetic antioxidants showed weaker activity, with IC_{50} values of 13.24 µL/mL for BHT and 6.38 µL/mL for Trolox. CEO NE was approximately twice as effective as BHT and 1.2 times more effective than Trolox, while BEO NE was 10 times more effective than BHT and 5 times more effective than Trolox.

The hydrogen peroxide scavenging activity of CEO NE and BEO NE was evaluated (Table 2). The IC_{50} values were 17.04 µL/mL for CEO NE and 1.76 µL/mL for BEO NE. Both essential oils effectively neutralized 50% of free radicals. In comparison, synthetic antioxidants showed weaker activity, with IC_{50} values of 83.87 µL/mL for BHT and 50.86 µL/mL for Trolox.

The ferrous ion chelating activity of CEO NE and BEO NE was evaluated (Table 2). Both nanoemulsions demonstrated strong chelating ability compared to synthetic antioxidants. The IC_{50} values were 8.54 µL/mL for CEO NE and 0.48 µL/mL for BEO NE. In contrast, BHT and Trolox required much higher concentrations, with IC_{50} values of 59.62 µL/mL and 45.49 µL/mL, respectively.

Table 2. *In-vitro* antioxidant potential of different essential oil-based nanoemulsions in terms of free radical scavenging activities

Nanoemulsions, BHT and Trolox	DPPH scavenging activity (µL/mL)	H_2O_2 scavenging activity (µL/mL)	Ferrous ion chelating activity (µL/mL)
CEO-NE	5.37±0.08	17.04±0.04	8.54±0.02
BHT	13.24±0.26	83.87±1.22	59.62±0.84
Trolox	6.38±0.18	50.86±0.08	45.49±0.22
BEO-NE	1.24±0.08	1.76±0.03	0.48±0.01

[n=6; BEO-NE=Bamboo essential oil nanoemulsion; CEO-NE=Clove essential oil nanoemulsion; BHT-Butylated hydroxytoluene)]

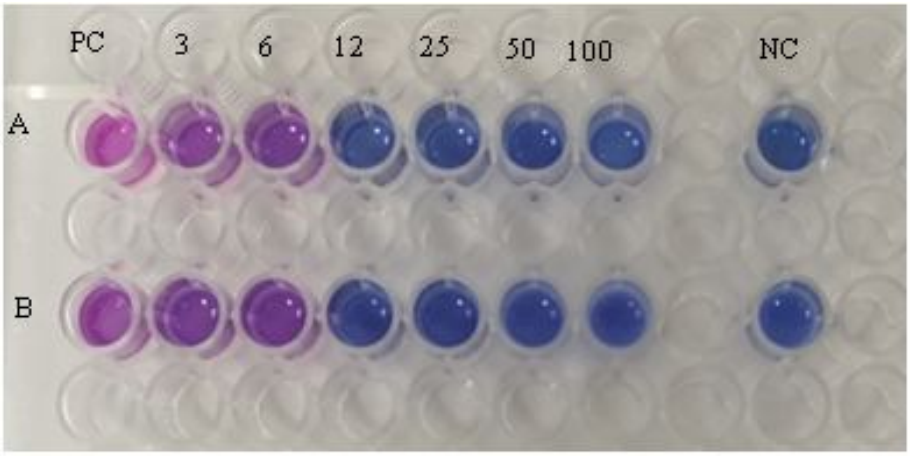


Fig. 2. Minimum inhibitory concentration (MIC) values of clove essential oil nanoemulsion (A) and bamboo essential oil nanoemulsion (B) against *Salmonella* Typhimurium, where NC=negative control and PC=Positive control

Antimicrobial activity of CEO NE and BEO NE: The antimicrobial activity of CEO NE and BEO NE was evaluated against MDR *S.*Typhimurium using agar well diffusion. At 2.5% concentration, CEO NE produced a larger inhibition zone (24 mm) compared to BEO NE (18 mm). Both nanoemulsions effectively inhibited bacterial growth at this concentration, while lower concentrations (2.0%) showed reduced activity.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays confirmed the potency of both nanoemulsions (Fig. 2). The MIC was recorded at 6 µL, indicating that very small volumes were sufficient to inhibit bacterial growth.

The MBC was determined to be 6-12 µL, confirming bactericidal activity at slightly higher concentrations.

Meat quality parameters

pH: The pH values of treated and control chicken meat groups showed a gradual and significant increase ($p<0.05$) during refrigerated storage (Fig. 3). The control group exhibited the highest rise, with values ranging from 5.66 to 6.96. In comparison, the BEO NE treated samples recorded pH values between 5.47 and 6.22, while CEO NE treated samples ranged from 5.45 to 6.19. Although all groups showed progressive increases in pH over the 8 day storage period; however, the treated samples consistently maintained lower values than the control, indicating a preservative effect of the nanoemulsions.

Thiobarbituric acid reactive substances (TBARS) values: The thiobarbituric acid reactive substances (TBARS) values, which indicate the extent of lipid oxidation, increased progressively in all chicken meat groups during refrigerated storage. The mean TBARS values ranged between 0.31–1.44 mg MDA/kg in the control group, 0.29–0.94 mg MDA/kg in the BEO NE treated group, and 0.30–0.89 mg MDA/kg in the CEO NE treated group (Fig. 4). Although TBARS values rose significantly ($p<0.05$) during storage study in all groups, the treated samples consistently showed lower values compared to the control. Between the two treatment groups, differences were statistically non significant ($p>0.05$), suggesting that both nanoemulsions were equally effective in slowing lipid oxidation.

Instrumental colour: The instrumental colour parameters (L^*, a^*, b^*) of MDR *S.* Typhimurium inoculated chicken meat samples showed progressive changes during refrigerated storage (Table 3). The ranges of the mean L^*, a^* and b^* values of MDR *S.* Typhimurium inoculated control meat samples were 51.60-65.21, 14.93-6.61 and 7.19-12.90, respectively, whereas it was 51.78-63.26, 14.95-8.02, 7.24-10.24 for BEO-NE and 51.88-63.26, 14.95-8.18, 7.19-10.25 in case of CEO-NE treated samples, respectively. Between the treatment and control groups, differences were non-significant ($p>0.05$) on day 0 and day 2; however, from day 4 onward, significant differences ($p<0.05$) were observed. In contrast, comparisons between the treatment groups consistently showed non-significant ($p>0.05$) differences in L^* values across all time points. However, the L^* and b^* values gradually increased and a^* value decreased in all treated and control groups, with the advancement of storage periods.

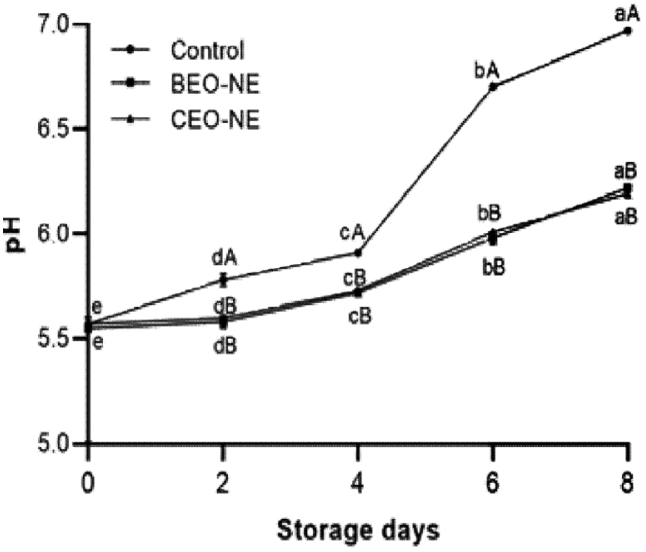


Fig. 3. Effect of essential oil-based nanoemulsions on pH of fresh chicken meat inoculated with *Salmonella* Typhimurium during refrigerated storage. $^{*a-e}$Mean±SE with superscript (between days) are significantly different ($p<0.05$); $^{A-B}$Mean±SE with superscript (between treatments) are significantly different ($p<0.05$). BEO-NE=Bamboo essential oil nanoemulsion; CEO-NE=Clove essential oil nanoemulsion

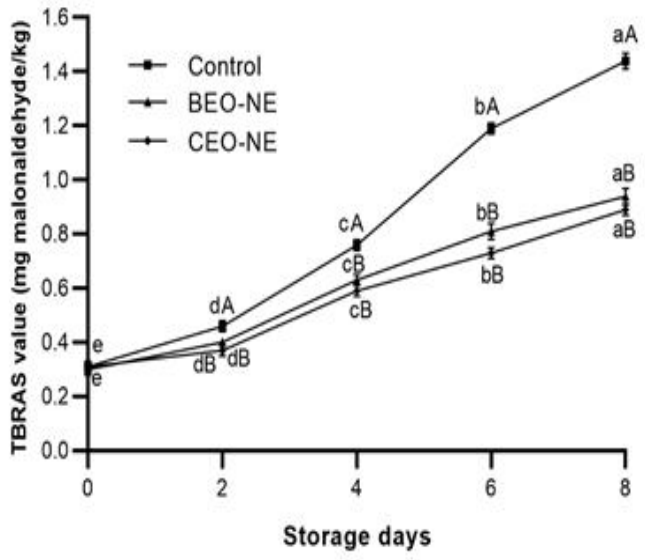


Fig. 4. Effect of essential oil-based nanoemulsions on TBARS of fresh chicken meat inoculated with *Salmonella* Typhimurium during refrigerated storage. $^{*a-e}$Mean±SE with superscript (between days) are significantly different ($p<0.05$); $^{A-B}$Mean±SE with superscript (between treatments) are significantly different ($p<0.05$). BEO-NE=Bamboo essential oil nanoemulsion; CEO-NE=Clove essential oil nanoemulsion

Table 3. Instrumental colour parameter of bamboo and clove nanoemulsion oils incorporated fresh chicken meat cuts inoculated with *Salmonella* Typhimurium during refrigerated (4±1°C) storage.

Treatments	0 Day	2nd Day	4th Day	6th Day	8th Day
			Lightness *(L*)*		
Control	51.60±0.30^{eA}	55.08±0.56^{dA}	58.17±0.16^{cA}	63.21±0.13^{bA}	65.21±0.59^{aA}
BEO-NE	51.78±0.61^{dA}	52.87±0.81^{dB}	56.93±0.29^{cB}	59.63±0.61^{bB}	63.26±0.55^{aB}
CEO-NE	51.88±0.34^{dA}	52.58±0.63^{dB}	57.04±0.41^{cB}	60.30±0.48^{bB}	63.28±0.43^{aB}
			Redness *(a*)*		
Control	14.93±0.22^{aA}	11.55±0.25^{bB}	10.61±0.27^{cB}	8.38±0.28^{dB}	6.61±0.19^{eB}
BEO-NE	14.95±0.28^{aA}	12.11±0.14^{bA}	11.69±0.17^{bA}	10.16±0.14^{cA}	8.02±0.25^{dA}
CEO-NE	14.95±0.12^{aA}	12.14±0.12^{bA}	11.71±0.10^{cA}	10.19±0.18^{dA}	8.18±0.14^{eA}
			Yellowness *(b*)*		
Control	7.29±0.29^{eA}	8.61±0.29^{dA}	9.80±0.26^{cA}	11.36±0.30^{bA}	12.90±0.16^{aA}
BEO-NE	7.24±0.13^{eA}	8.28±0.11^{dA}	8.78±0.06^{cB}	9.49±0.18^{bB}	10.24±0.15^{aB}
CEO-NE	7.19±0.26^{dA}	8.30±0.13^{cA}	8.69±0.09^{cB}	9.40±0.13^{bB}	10.25±0.09^{aB}

*$^{a-e}$Mean±SE with superscript (a-e) on the same row are significantly different (p<0.05); $^{A-C}$Mean±SE with superscript (A-B) on the same column are significantly different (p<0.05)]. BEO-NE=Bamboo essential oil nanoemulsion; CEO-NE=Clove essential oil nanoemulsion

Table 4. Sensory attributes of bamboo and clove nanoemulsion oils incorporated fresh chicken meat cuts inoculated with *Salmonella* Typhimurium during refrigerated (4±1°C) storage

Treatments	0 Day	2nd Day	4th Day	6th Day	8th Day
			Odour score		
Control	5.00±0.00^{aA}	4.08±0.08^{bB}	3.17±0.17^{cB}	2.58±0.08^{dB}	1.23±0.11^{eB}
BEO-NE	5.00±0.00^{aA}	4.50±0.22^{bAB}	3.67±0.11^{cA}	3.10±0.07^{dA}	2.37±0.12^{eA}
CEO-NE	5.00±0.00^{aA}	4.67±0.21^{aA}	3.75±0.11^{bA}	3.18±0.09^{cA}	2.50±0.11^{dA}
			Colour and appearance score		
Control	5.00±0.00^{aA}	4.50±0.22^{bA}	3.33±0.11^{cB}	2.50±0.23^{dB}	1.40±0.14^{eC}
BEO-NE	5.00±0.00^{aA}	4.67±0.17^{bA}	3.75±0.11^{cA}	3.12±0.07^{dA}	2.25±0.11^{eB}
CEO-NE	5.00±0.00^{aA}	4.75±0.11^{aA}	3.83±0.11^{bA}	3.20±0.09^{cA}	2.60±0.08^{dA}

*$^{a-e}$Mean±SE with superscript (a-e) on the same row are significantly different (p<0.05); $^{A-C}$Mean±SE with superscript (A-C) on the same column are significantly different (p<0.05)]. BEO-NE=Bamboo essential oil nanoemulsion; CEO-NE=Clove essential oil nanoemulsion

Microbial counts: The count of MDR *Salmonella* Typhimurium in chicken meat samples showed progressive increase during refrigerated storage (4±1°C). The mean values ranged between 6.19–7.36 $\log_{10}$ CFU/g in the control group, 5.94–6.32 $\log_{10}$ CFU/g in the BEO NE treated group, and 5.92–6.29 $\log_{10}$ CFU/g in the CEO NE treated group (Table 4). Up to day 2, differences between treated and control groups were statistically non significant (p>0.05). However, from day 4 onwards, significant differences (p<0.05) were observed, with treated samples consistently showing lower microbial counts, compared to controls. Between BEO NE and CEO NE groups, differences remained non significant (p>0.05) throughout storage.

Sensory odour and colour evaluation: Scores for sensory attributes of *S.* Typhimurium inoculated fresh meat cuts are given in Table 5. The sensory evaluation of MDR *S.* Typhimurium inoculated chicken meat cuts revealed a gradual decline in both odour and colour scores during refrigerated storage (day 0 to day 8). Panellists consistently rated the control samples lower than the treated groups as storage progressed. At the end of the storage period, the CEO NE and BEO NE treated samples retained significantly higher (p<0.05) sensory acceptability scores for both odour and colour compared to the control group. This indicates that EO NE treatments were effective in slowing sensory deterioration, although some decline was still observed over time.

DISCUSSION

Total phenolics and flavonoid content: The higher total phenolics content in bamboo essential oil and richer flavonoid profile in clove essential oil explain their strong antioxidant potential. Phenolics act as

Table 5. Effect of essential oil-based nanoemulsions on fresh chicken meat inoculated with *Salmonella* Typhimurium (1 X 10^6 CFU/mL) during refrigerated storage

	Microbial count (log CFU/g)				
Treatments	**1st Day**	**2nd Day**	**4th Day**	**6th Day**	**8th Day**
Control	6.19 ± 0.01^{eA}	6.48 ± 0.03^{dA}	6.74 ± 0.02^{cA}	6.96 ± 0.02^{bA}	7.36 ± 0.04^{aA}
BEO-NE(2.5% conc.)	5.94 ± 0.02^{dB}	5.99 ± 0.03^{dB}	6.10 ± 0.02^{cB}	6.23 ± 0.02^{bB}	6.32 ± 0.02^{aB}
CEO-NE(2.5% conc.)	5.92 ± 0.02^{dB}	5.96 ± 0.02^{dB}	6.07 ± 0.02^{cB}	6.17 ± 0.02^{bB}	6.29 ± 0.02^{aB}

*[a-e]Mean±SE with superscript (a-e) on the same row are significantly different ($p<0.05$); [A-C]Mean±SE with superscript (A-B) on the same column are significantly different ($p<0.05$)]. BEO-NE=Bamboo essential oil nanoemulsion; CEO-NE=Clove essential oil nanoemulsion

reducing agents, donating hydrogen ions to neutralize free radicals (Cunha et al., 2018), while flavonoids contribute to metal chelation and radical scavenging. Our findings are in agreement with previous reports on the total phenolics and flavonoid contents of bamboo leaves and bamboo essential oil (Arifani et al., 2021) and on clove essential oil (Saricaoglu & Turhan, 2018), but differ from the results of Tundis et al. (2023) for bamboo and clove essential oils (El-Maati et al., 2016). This could be due to the reason that the antioxidant strength of essential oils depends on their phenolic content and the specific plant parts, cultivars, extraction methods employed and solvents used, etc.(Das et al., 2023).

Antioxidant properties

DPPH Radical Scavenging- The DPPH radical scavenging assay is a widely used method employed for assessing the antioxidant capacity of compounds through their ability to neutralize free radicals. This assay measures how well a substance can reduce the colour of the persistent DPPH radical, where lower values reflect stronger radical scavenging activity (Hasan et al., 2009). The superior performance of BEO NE (IC_{50} 1.24 µL/mL) compared to synthetic antioxidants highlights its potential as a natural preservative. These results are consistent with previous studies (El-Maati et al., 2016), who reported antiradical activities of clove extract ranging from 25.3% to 91.4% and 63.46%, respectively. Additionally, Jin et al. (2011) observed IC_{50} values (µL/mL) of 3.16, 4.94, 5.47, and 4.25 for DPPH scavenging activity in four bamboo species: *Phyllostachys heterocycla* cv. Pubescens, *P. heterocycla* cv. Gracilis, *P. kwangsiensis*, and *P. heterocycla* cv. Heterocycla.

Hydrogen Peroxide Scavenging- Hydrogen peroxide scavenging activity was conducted to assess how best antioxidant compounds in extracts can neutralize hydrogen peroxide. Although H_2O_2 is not highly reactive itself, it can generate harmful hydroxyl radicals in cells. Effectively removing H_2O_2 helps prevent the formation of these radicals, thus protecting biological systems and extending the shelf-life of food products (Dahiru et al., 2022). BEO NE's low IC_{50} (1.76 µL/mL) indicates its efficiency in preventing hydroxyl radical formation, which is critical in food systems prone to oxidative stress. These findings align with studies of others workers, which reported that clove and bamboo shoot extracts had lower H_2O_2 scavenging activity compared to standard synthetic antioxidants (Dahiru et al., 2022).

Ferrous ion chelating activity- Reactive oxygen species (ROS) are crucial intermediates in oxidation reactions driven by metals like iron, which produces free radicals via Fenton reactions. Ferrozine forms complexes with Fe^{2+}, leading to ROS generation. Chelating agents can disrupt this complex formation, reducing the red colour of the complex (Benabdallah et al., 2016). Both nanoemulsions outperformed synthetic controls, with BEO NE showing exceptional chelating ability (0.48 µL/mL). Since transition metals catalyze lipid oxidation via Fenton reactions (Benabdallah et al., 2016), this property is vital for meat preservation. Similarly, 80% ethanolic clove extract (El-Maati et al., 2016) and bamboo oil (Das et al., 2023) found to have significantly lower IC_{50} values for metal ion chelation, compared to standard antioxidants.

Antimicrobial effects: To assess the antimicrobial activity of both nanoemulsion oils against MDR*S.* Typhimurium, the agar well diffusion technique was used. Initially, three concentrations of clove and bamboo EO nanoemulsions (2.0, 2.5% and 3.0%) were evaluated for their antimicrobial activities against the mentioned MDR organism. CEO NE (2.5%) exhibited stronger inhibition (24 mm zone) against MDR *Salmonella* Typhimurium, likely due to eugenol's well documented bactericidal activity. Similar types of findings were also reported by Thanissery et al. (2014) for the antimicrobial activity of clove essential oil against *Salmonella* spp. Recently, Elisha et al. (2022)

also observed similar results for clove plant flower bud extract against MDR *Salmonella typhi*. Likewise, other researchers have also reported about antibacterial activity of bamboo oil against *Salmonella* organisms (Das et al., 2023; Gama et al., 2023).

The MIC is widely recognized as a metric for assessing the effectiveness of antimicrobial agents. Therefore, a lower MIC value is indicative of a more robust and effective antimicrobial activity (Ji et al., 2023). In the present study, both MIC and MBC results confirmed the efficacy of both nanoemulsions, supporting their role as natural antimicrobials. Likewise, Gürbüz et al. (2022) reported in their respective studies that very low concentration of eugenol (active component of clove essential oil) inhibited the growth of *Salmonella* spp. Similarly, Gama et al. (2023) determined the MIC and MBC values of bamboo essential oil against *Salmonella enteritidis*.

Quality changes of fresh chicken meat during storage: This study evaluated the effects of CEO-NE and BEO-NE on MDR *S.* Typhimurium inoculated fresh chicken meat packaged under aerobic conditions and compared with control during 8 days of refrigerated (4±1°C) storage study. A series of physico-chemical analyses, sensory assessments, and antioxidant and antimicrobial assays were used to optimize the amounts of clove and bamboo nanoemulsion oils incorporation in fresh meat cuts.

pH: The observed increase in pH across all groups reflects the natural progression of microbial activity and biochemical changes during storage. As reported by Das et al. (2024), depletion of glucose reserves and subsequent utilization of amino acids from protein degradation contribute to rising pH levels. In untreated controls, the higher rate of increase can be attributed to proteolysis and the accumulation of amino compounds produced by microbial growth (Tomovic′ et al., 2020). In contrast, the significantly lower pH increase in BEO NE and CEO NE treated samples highlights the protective role of essential oil nanoemulsions. Their antioxidant and antimicrobial properties likely suppressed microbial metabolism and delayed proteolytic activity, thereby slowing the accumulation of alkaline compounds. This finding is consistent with earlier studies by Das et al. (2023, 2024), where incorporation of clove and bamboo nanoemulsions into chicken meat products resulted in reduced pH shifts during storage.

TBARS values: The gradual increase in TBARS values across all groups reflects the natural progression of lipid oxidation during storage, leading to the formation of aldehydes, ketones, and other secondary metabolites that contribute to rancidity and quality deterioration (Jafarinia et al., 2022). The higher TBARS levels in the control group confirm that untreated meat is more susceptible to oxidative damage, which accelerates spoilage and negatively impacts sensory attributes. The significantly lower TBARS values in BEO NE and CEO NE treated samples demonstrate the protective role of essential oil nanoemulsions. Their antioxidant properties, derived from phenolic and flavonoid compounds, likely inhibited free radical formation and slowed the propagation of lipid oxidation.

Although lipid oxidation was not completely prevented, the reduced rate of increase highlights the efficacy of EO NEs in extending meat shelf-life. These findings are consistent with earlier studies. Das et al. (2023, 2024) reported similar trends when clove and bamboo nanoemulsions were incorporated into chicken meatballs, showing reduced TBARS values compared to controls. Likewise, Jafarinia et al. (2022) observed increasing TBARS values in lamb loin cuts treated with virgin olive oil nanoemulsion combined with ajowan essential oil, but at a slower rate than untreated samples.

The ability of EO NEs to mitigate lipid oxidation is particularly important for meat preservation, as oxidative rancidity not only compromises flavour and odour but also reduces nutritional quality. By slowing TBARS accumulation, clove and bamboo nanoemulsions provide a clean label alternative to synthetic antioxidants, aligning with consumer demand for natural preservative strategies.

Microbial counts: The increase in microbial counts across all groups reflects the natural growth of MDR *S.* Typhimurium under refrigerated conditions. The higher counts in the control group highlight the vulnerability of untreated meat to pathogen proliferation, which compromises safety and shelf-life. The significantly lower counts in EO NE treated groups from day 4 onwards demonstrate the bacteriostatic effect of clove and bamboo nanoemulsions. Their antimicrobial activity likely stems from phenolic compounds such as eugenol in clove and bioactive terpenoids in bamboo, which disrupt bacterial cell membranes and interfere with metabolic processes. Although the nanoemulsions did not completely eliminate MDR pathogens, they effectively slowed bacterial growth, thereby reducing the risk of spoilage and foodborne illness.

These findings are consistent with previous studies. Das et al. (2023, 2024) reported similar antimicrobial effects of clove and bamboo nanoemulsions in meat products. Likewise, other researchers have also documented reductions in microbial counts in beef loins and pork cuts following treatment with essential oil–based nanoemulsions. Specifically, cumin nanoemulsions were effective against *S.* Typhimurium (Dini et al., 2020), while black pepper essential oil nanoemulsions demonstrated inhibitory activity against *E. coli* and *S. enterica* (Hien & Dao, 2022). The non significant differences between BEO NE and CEO NE groups suggest that both oils are comparably effective in controlling microbial growth, making them suitable candidates for clean label preservation strategies.

Instrumental colour: Colour is a critical quality attribute influencing consumer acceptance of meat products. The gradual increase in *L** (lightness) and *b** (yellowness), along with the decline in *a** (redness), reflects typical oxidative and structural changes during storage. The slightly higher *L** values in EO NE treated samples may be explained by light scattering from oil droplets within the nanoemulsions, which enhance surface brightness. Jafarinia et al. (2022) similarly reported that *L** values in lamb loin cuts treated with virgin olive oil and ajowan essential oil nanoemulsions were linked to protein structural changes rather than myoglobin oxidation.

Redness (*a**) is particularly important, as consumers associate it with freshness. In this study, CEO NE and BEO NE treated samples retained significantly higher *a** values compared to controls. This preservation of redness can be attributed to phenolic compounds in the nanoemulsions, which interact with haeme proteins and prevent the conversion of myoglobin to ferryl myoglobin, a hypervalent form associated with discolouration (Tesoriere et al., 2007). The decline in *a** values observed in control samples aligns with earlier findings by Keokamnerd et al. (2008), and Zhang et al. (2016) who reported reductions in redness of ground chicken meat, and minced beef meat (Dghais et al., 2023) during storage study. The increase in yellowness (*b**) values across all groups reflects oxidative changes in pigments and lipids. However, EO NE treated samples exhibited lower *b** values compared to controls, suggesting that antioxidant activity of clove and bamboo oils slowed pigment oxidation. Our findings are in agreement with Jafarinia et al. (2022) and Dghais et al. (2023), who observed similar trends in lamb loin cuts treated with ajowan EO nanoemulsions, and nanoemulsions formulated with cinnamon and curcuma EOs in minced beef meat, respectively.

Sensory odour and colour evaluation: Sensory attributes such as odour and colour are critical determinants of consumer acceptance. The significant decline in sensory scores across all groups during storage reflects the inevitable impact of microbial growth and lipid oxidation on meat quality. In the control group, the sharper reduction in sensory quality can be attributed to unchecked microbial proliferation and oxidative rancidity, which produce off odours and discolouration. In contrast, EO NE treated samples maintained higher sensory scores, demonstrating the protective effects of clove and bamboo nanoemulsions. Their antimicrobial activity suppressed microbial spoilage, while antioxidant compounds reduced lipid oxidation, thereby preserving odour and colour. These findings are consistent with the instrumental colour results (Table 3), which showed better retention of redness and reduced yellowness in treated samples. Similar result was also noticed by different researchers when different spice extract, nanoemulsion oil and essential oil was incorporated with fresh raw chicken meat (Zhang et al., 2016), fresh lamb loin (Jafarinia et al., 2022) and minced beef meat (Dghais et al., 2023).

In summary, this study highlights the efficacy of essential oil-based nanoemulsions (EO-NEs), particularly those derived from clove (CEO-NE) or bamboo (BEO-NE), as an innovative alternative to conventional preservation methods for fresh chicken meat. The EO-NEs demonstrated significant antioxidant and antimicrobial properties, effectively inhibiting the growth of multidrug-resistant (MDR) *S.* Typhimurium. Further, the incorporation of EO-NEs in chicken meat during refrigerated storage maintained its microbiological, physicochemical, and sensory qualities, extending shelf-life and enhancing food safety. These findings emphasize the potential of EO-NEs as clean label preservatives in combating MDR pathogens in the food industry, promoting safer and longer-lasting meat products.

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