



Full Length Article

Assessment of the Impact of Rosemary Chitosan Microemulsion Effect on *Escherichia coli* and *Listeria monocytogenes* Dipping in Chicken Meat Stored at 4°C

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Abstract

This article studies the potential use of the Rosemary (REO) microemulsion with/without chitosan to assess the growth inhibition of Gram-positive and Gram-negative pathogenic bacteria represented by *Listeria monocytogenes* and *Escherichia coli*. The chicken samples were divided into four groups after inoculation of *E. coli* and *L. monocytogenes* separately: control group (without treatment), 1% chitosan nanoparticle treatment group, 0.5% REO microemulsion treatment group, and 0.5% REO + 1% chitosan microemulsion treatment group. Chicken samples were dipped into the treatments for 15 days of refrigerated storage at 4°C. All groups were kept refrigerated, and bacterial counts were taken on days 0, 1, 3, 6, 9, 12 and 15. Chitosan nanoparticle and REO microemulsion with/without chitosan were spherical shape and showed a narrow size distribution of 23.98 ± 0.83 , 34.24 ± 2.2 and 28.01 ± 1.36 nm with a polydispersity index (PDI) of 0.86, 0.33 and 0.54, respectively indicating that greater homogeneity was achieved. REO chitosan microemulsion has 12 components detected by GC-Mass as follows: pinene (22.21%), borneol (21.32%), 1-(4-methoxyphenyl) ethanoneoxime (3.48%), oxocamphor (0.49%), camphor (2.99%), limonene (0.29%), cis-linalool oxide (0.87), 2-(5-chloromethoxyphenyl) pyrrole (2.19%), homofarnesol (0.27%), levoverbenone (0.45%), peruvial (0.73%) and campesterol (1.22%). The results showed a substantial reduction in *L. monocytogenes* and *E. coli* count in all treatment groups when compared to the control group, with the greatest inhibitory efficacy in the 0.5% REO chitosan microemulsion group. A favorable effect of chitosan treatment on chicken acceptability during refrigerated storage was reported, with an improvement in the sensory qualities of the products. Thus, REO chitosan microemulsion is advised to be used in chicken to enhance resistance to harmful microorganisms. © 2022 Friends Science Publishers

Keywords: Fillet; *L. monocytogenes*; *E. coli*; Chitosan; Rosemary; Microemulsion; GC-Mass

Introduction

The chicken business is growing worldwide as chicken remains one of the least expensive protein sources and white meat is considered healthier than red meat (Souza *et al.* 2018).

Poultry and poultry products are among the foodstuffs that must be produced and stored safely and sanarily. Because of poor sanitary processing and storage procedures, microbial contamination occurs, leading to safety and spoiling issues. Spoiled chicken meat poses an economic hardship for farmers and necessitates new techniques to increase shelf life and overall meat safety/quality, which is the primary issue facing the poultry processing business (Petrou *et al.* 2012).

Food safety is one of the most critical concerns in the

food business. Therefore, new procedures and technologies are being developed in the food industry to improve the quality and safety of poultry meat. In the past few years, consumer demand for nutritious food devoid of chemical preservatives has grown considerably (Petrou *et al.* 2012). Accordingly, there is a growing trend toward using natural ingredients derived from plants and animals, including antimicrobials, oxidants, coloring materials, and sweeteners (Mehdizadeh *et al.* 2020).

Listeria monocytogenes is a Gram-positive foodborne bacterium that results in serious food safety problems, particularly in meat and poultry products (Malhotra *et al.* 2015), because of its capacity to survive and thrive at refrigeration temperatures. Food surface treatments are crucial for food safety and quality (FAO 2013). However, as

people become more aware of the possible dangers of synthetic preservatives, using a combination of natural antimicrobials and antioxidants has received great attention. *Escherichia coli* have been identified as a foodborne pathogen since 1982, which has greatly influenced the food sector (McClure *et al.* 2000).

Rosemary plant and REO are widely used as they are safe with no caveats. It is used as a flavoring agent and prevents microbial growth and rancidity development in meat through the main active compounds, such as rosmarinic acid and carnosic acid (Jongberg *et al.* 2013; JSMO 2016).

Rosemary oleoresin, extract, and essential oils have been identified as possible antioxidants, which are frequently utilized in the food sector (Hussain *et al.* 2010). The rosemary extract effectively delayed lipid oxidation in meat. Furthermore, REO has potent antibacterial action against Gram-negative and Gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Listeria monocytogenes* (Keokammerd *et al.* 2008; Kahraman *et al.* 2015).

Chitosan is one of the natural additives originating from animals with a wide range of applications in food bio-preservation due to its biodegradability, biocompatibility, nontoxicity, and antibacterial activity (No *et al.* 2007; Paparella *et al.* 2011; Grande-Tovar *et al.* 2018). Edible coatings and films have attracted further attention because of their ability to carry food additives, including antimicrobials, antioxidants, flavors, and colors and preserve the functionality of such agents on the food surface (Ricci *et al.* 2018).

Microemulsions are colloid solutions and thermodynamically stable, single optically isotropic dispersions composed of a water phase, oil phase, surfactant, and cosurfactant, with a droplet size of 10–100 nm (Zhang *et al.* 2015). Microemulsions can be specifically used in food products because of their unique features, including ease of preparation, high-grade functions, and fine particle size; these advantages facilitate the transfer of active compounds and enhance their interactions with biomembranes (Moghimi *et al.* 2016). The work aimed to evaluate the effect of REO and its microemulsion with/without chitosan on inhibiting the growth of the pathogenic *E. coli* and *L. monocytogenes* inoculated in chicken fillet stored for 15 days at 4°C.

Materials and Methods

Preparation and extraction of REO

The fresh herb of *Rosmarinus officinalis* was brought from the experimental farm at the Medicinal and Aromatic Plants Research Department in Gezert El-Shaer, El Qanatir El Khayriyah, Egypt. The essential oil was extracted using the water-steam distillation method (Cliventer system) on the whole fresh herb samples for 3 h in the Medicinal and

Aromatic Plants Lab, Dokki, Giza, according to Miller method (1963).

Preparation and Treatments of Chitosan Nanoparticles

The 1% chitosan nanoparticle was spontaneously obtained upon adding 1 and 0.5% acetic acid chitosan acidic solutions, respectively, to 0.7 mg/mL solutions of TPP aqueous basic solution. The ratio of TPP to chitosan was 1:3 under magnetic stirring at room temperature for 1 h according to Youssef and El-Masry (2018).

Preparation of Rosemary Microemulsion with or without Chitosan

Rosemary oil (0.5%) was mixed with tween 80 (4.5%) as an emulsifier and was stirred for 30 min. The 1% chitosan solution was added to form and obtain a uniform, stable and clear emulsion, according to Rao and McClements (2011). A 1% sodium chloride solution is used to replace chitosan in the other treatment.

Characterization of Chitosan Nanoparticle and Microemulsion

Zetasizer Malvern Instrument (Corp, Malvern, UK) was used to measure surface charge (zeta potential), droplet size, size distribution (polydispersity indexes) and electrical conductivity of the microemulsion. High-resolution transmission electron microscopy (HRTEM) observations were performed using a JEM 1400F HRTEM at beam energy of 300 keV.

The examined samples determined using direct capillary column (30 m x 0.25 mm x 0.25 µm film thickness) of Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) for chemical composition. The components were identified by comparing their mass spectra with those of WILEY 09 and NIST 14 mass spectral database according to El-Kareem *et al.* (2016).

Cell Culture

Green monkey cell line (Vero cell) purchasing from Nawah Scientific, Inc. (Cairo, Egypt), was maintained in media supplemented with 10% of fetal bovine heat-inactivated serum, streptomycin (100 mg/mL) and penicillin (100 units/mL) at 37°C, 5% humidity and CO₂ atmosphere. Cytotoxicity and cell viability assays were performed using the SRB assay at different solution concentrations (0.01, 0.1, 1, 10 and 100 µg/mL), according to Allam *et al.* (2018).

Preparation of Microbial Inoculums

Strains from the Animal Health Research Institute of *E. coli* (ATCC 8739) and *L. monocytogenes* (ATCC 14028) were acquired (Food Hygiene Department). Frozen crops were

kept at -80°C and activated in 9 mL of trypticase soy broth (TSB) during incubation at 35°C for 24 h with two successive passes. For individual strains, 1 mL of the inoculum was introduced to 100 mL (TSB) of stock and the Oxoid Incubator Shaker was used to obtain an estimated concentration of about 10^8 CFU/mL as measured by a 0.5 McFarland standard using plating serial dilutions on ALOA and TBX agar. Two serial dilutions of 1 mL of this inoculum have been added to a 9 mL sterile saline to obtain the final concentration of around 10^6 CFU/g.

Dipping of chicken samples

Fresh chicken fillets (10 kg) from a market shop in Cairo, Egypt, without skin were purchased and immediately brought to the refrigerated lab. Then, they were divided into four groups of duplicates and placed under running tap water for two minutes to remove foreign bodies or remains or foreign bodies.

The chicken fillets were contaminated with inoculated soaking solution for one minute and then with the *L. monocytogenes* and *E. coli* strains for 20 min and were dried in a laminar airflow (four groups for each strain) (Olaimit and Holley 2015).

Preparation of treatments

The previously dipped samples were treated using three different immersing solutions: (1) 1% chitosan nanoparticles; (2) 0.5% REO microemulsion; (3) 0.5% REO + 1% chitosan microemulsion for 1 min. Then, the samples for drained for 15 min and stored at 4°C for 16 days. Finally, the analysis was performed on days 0, 1, 3, 6, 9, 12 and 15 of refrigerated storage (Sharifi *et al.* 2017). Two groups were assigned as positive controls for *L. monocytogenes* and *E. coli* (10^6 CFU/mL).

Enumeration of *E. coli*

With 0.1% sterile peptone water, chicken fillet samples (10 g) were brought to a final volume of 90 mL. A stomacher was used to homogenize the materials for 2 min. (Seward Medical, London). Following the production of decimal dilutions, 1 mL of successive homogenate dilutions was cultivated onto TBX agar and incubated at 35°C for 24 h.

Enumeration of *L. monocytogenes*

25 grams of immunized fillets was stomached in 225 mL of Listeria broth and serially diluted using maximal recovery to be counted on selective medium (ALOA agar). Moreover, 1 mL of serial dilutions of homogenates was cultivated onto duplicate plates and incubated at 35°C for 24 h.

All fillets were sealed in plastic bags and stored on a refrigerator shelf for future examination. Each inoculation group was regularly inspected for the inoculated strain count

as detect in the primary count at days 0, 1, 3, 6, 9, 12 and 15 of refrigerated storage to assess the influence of the treatments on the viability of the injected bacterial strains.

Sensory evaluation

For studying the effect of rosemary with/without chitosan microemulsion on the sensory attributes of chicken fillets, four groups of chicken fillet were prepared by dipping in solutions as follows: one group of chicken fillet with 1% chitosan nanoparticles, 0.5% REO+1% chitosan microemulsion and 0.5% REO microemulsion, the last group without treatment.

After 15 min of group treated and then it was permitted to dry for another 15 min in the laminar flow before being kept in the refrigerator at 4°C .

All samples of cooked chicken fillets were organoleptically evaluated by seven panelists from the staff members, according to Petrou *et al.* (2012). Only edible chicken fillets from the control and treatment groups were cooked for 5 min in a microwave oven set to high power (700 W). Using a nine-point hedonic scale, seven panelists were asked to rate the acceptability (total sensory assessment score) in terms of odor, taste, and sight: 9, excellent; 8, very good; 7, good; 6, bad (initial off-odor, off-taste development). A score of 6 was chosen as the bottom limit of acceptability. After the emergence of the first off-odor or undesirable color, the sample was deemed unsuitable.

Statistical analysis

Each test was done thrice, and standard deviation mean value (SD) was given for each occurrence. All data were analyzed using ANOVA on a single-way basis and mean separation was performed using Tukey's multiscopes test (SPSS 19.0). Differences at P 0.05 level were considered significant.

Results

Characterization of chitosan nanoparticle and REO microemulsion (with or without coating chitosan)

Particle Size, Morphology, and Size Distribution. TEM was used to determine the size and morphology of the nanoparticles. Three nanomaterials were spherical and showed no aggregation and narrow size distribution of 23.98 ± 0.83 , 34.24 ± 2.2 and 28.01 ± 1.36 nm (Fig. 1a–c) with a polydispersity index (PDI) of 0.86, 0.33 and 0.54, respectively, indicating that greater homogeneity can be realized.

The zeta potential, which indicates unstable and stable suspensions, is often measured *via* dynamic light scattering (DLS). The zeta potential results for chitosan nanoparticle, rosemary chitosan microemulsion, and rosemary microemulsion were 53.5 ± 5.14 mV, 9.69 ± 3.67 mV, 43.3 ± 6.23 mV, respectively, measured at pH 5.

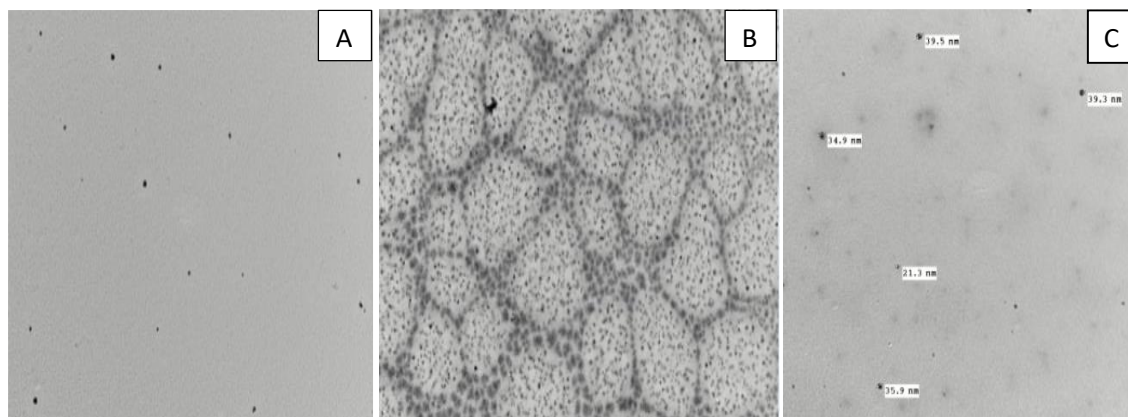


Fig. 1: TEM of (A) chitosan nanoparticle and (B): rosemary chitosan microemulsion and (C) rosemary microemulsion

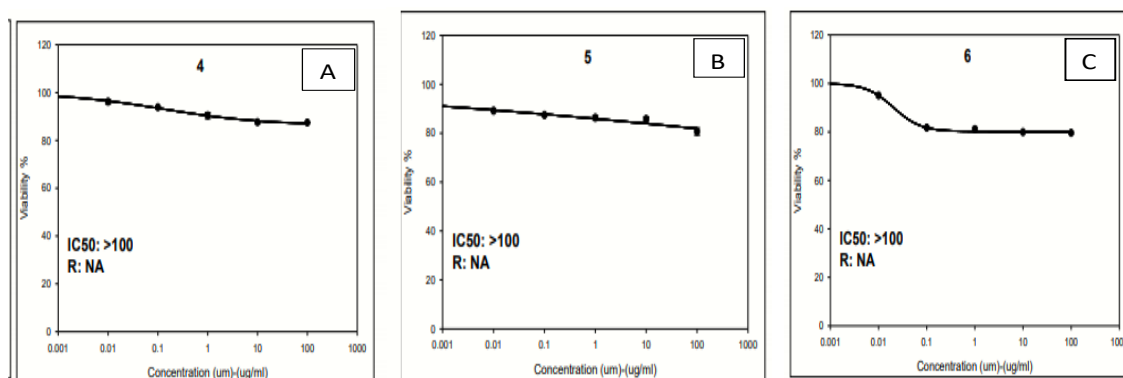


Fig. 2: Cell viability % of (A) chitosan nanoparticle and (B): rosemary chitosan microemulsion and (C) rosemary microemulsion

The analysis of the rosemary oil using GC-Mass showed the presence of terpineol (6.29%), camphor (37.82%), isoborneol (25.96%), levoverbenone (17.92%), citronellol (0.90%), isopulegol (1.53%), bornyl acetate (2.68%), sobroerol 8-acetate (1.09%), and caryophyllene oxide (2.46%). On the other hand, rosemary chitosan microemulsion had 12 components: 1-(4-methoxyphenyl) ethanoneoxime (3.48%), oxocamphor (0.49%), α -pinene (22.21%), camphor (2.99%), limonene (0.29%), borneol (21.32%), cis-linalool oxide (0.87), 2-(5-chloro-methoxyphenyl) pyrrole (2.19%), homofarnesol (0.27%), levoverbenone (0.45%), peruvicol (0.73%) and campesterol (1.22%).

On the confluent surface of Vero cells, chitosan nanoparticles and rosemary with or without chitosan microemulsion had different concentrations (0.01, 0.1, 1, 10, and 100 $\mu\text{g/mL}$) after 3 days of inoculation. The cell viability% assessed by SRB assay was 87.43%, 80.69%, and 79.66%, respectively, in 100 $\mu\text{g/mL}$ and $\text{IC}_{50} > 100 \mu\text{g/mL}$ (Fig. 2a–c).

Inhibitory effect of different treatments on *L. monocytogenes* and *E. coli*

Table 1 and Fig. 3 show the effectiveness of different

treatments on the behavior of *L. monocytogenes* during refrigerated storage of chicken fillets samples. By comparing the treated samples with the samples inoculated with the strains in the absence of treatment (positive control) at zero days, the initial count of *L. monocytogenes* was $5.65 \pm 0.55 \log \text{CFU/g}$. At 1 day after treatment, the counts of the pathogens in treated samples with 0.5% REO microemulsion, 1% chitosan nanoparticle, and 0.5% REO + 1% chitosan microemulsion were reduced compared to control, whereas, in the control sample, the count remained $5.77 \pm 0.16 \log \text{CFU/g}$.

The count of treated chicken fillet was reduced to 5.62 ± 0.34 , 4.71 ± 0.27 and $4.18 \pm 0.74 \log \text{CFU/g}$, respectively. Significant differences ($P < 0.05$) were observed between means having different letters in the same row between the three groups. Dipping chicken fillets into 0.5% REO microemulsion, 1% chitosan nanoparticle, and 0.5% REO + 1% chitosan microemulsion reduced the *L. monocytogenes* count to about 1.7, 2 and 3 $\log \text{CFU/g}$, respectively, during refrigerated storage up to 15 days. Chitosan coatings are commonly mixed with essential oils and created in the form of microemulsions during refrigerated storage at 4°C to intensify the impact of chitosan against foodborne bacteria.

Table 2 and Fig. 4 show the effect of different

Table 1: *Listeria monocytogenes* count (Log CFU/g) of inoculated chicken fillet stored at 4°C (Mean ± SD)

Storage days	Control	0.5% REO micro emulsion	1% Chitosan nanoparticle	0.5% REO + 1% Chitosan micro emulsion
Zero day	6.17 ^a ± 0.72	5.58 ^a ± 0.25	5.51 ^a ± 0.44	5.83 ^a ± 0.29
1 st day	5.77 ^a ± 0.16	5.62 ^a ± 0.34	4.71 ^b ± 0.27	4.18 ^b ± 0.74
3 rd day	5.68 ^a ± 0.11	4.52 ^b ± 0.12	4.20 ^{bc} ± 0.69	3.84 ^c ± 0.11
6 th day	6.08 ^a ± 0.81	4.48 ^b ± 0.52	3.46 ^c ± 0.33	3.55 ^{bc} ± 0.42
9 th day	6.76 ^a ± 0.21	3.84 ^b ± 0.09	3.65 ^b ± 0.60	3.30 ^b ± 0.61
12 th day	6.81 ^a ± 0.06	3.54 ^b ± 0.21	3.04 ^b ± 0.92	3.27 ^b ± 0.63
15 th day	7.48 ^a ± 0.52	3.89 ^b ± 0.10	3.62 ^b ± 0.58	2.89 ^c ± 0.08

There are significance differences ($P < 0.05$) between means having different letters in the same raw

Table 2: *E. coli* count (Log CFU/g) of inoculated chicken inoculated chicken fillet stored at 4°C (Mean ± SD)

Storage days	Control	0.5% REO micro emulsion	1% Chitosan nanoparticle	0.5% REO + 1% Chitosan micro emulsion
Zero day	6.19 ^a ± 0.55	6.02 ^a ± 0.75	5.70 ^a ± 0.02	4.43 ^b ± 0.70
1 st day	6.26 ^a ± 0.64	5.64 ^{ab} ± 0.42	4.90 ^{bc} ± 0.14	4.54 ^c ± 0.53
3 rd day	6.46 ^a ± 0.45	4.16 ^b ± 0.54	4.06 ^b ± 0.37	3.69 ^b ± 0.36
6 th day	5.49 ^a ± 0.18	4.25 ^b ± 0.71	4.12 ^b ± 0.77	3.53 ^b ± 0.24
9 th day	5.85 ^a ± 0.17	3.72 ^b ± 0.24	3.67 ^b ± 0.27	2.88 ^c ± 0.36
12 th day	6.64 ^a ± 0.33	3.85 ^b ± 0.26	3.00 ^c ± 0.16	-
15 th day	7.30 ^a ± 0.60	3.66 ^b ± 0.35	-	-

There are significance differences ($P < 0.05$) between means having different letters in the same raw

Table 3: Overall Sensory Scores (Mean ± SD) of chicken fillet stored at 4°C

Storage period	Control	0.5% REO microemulsion	1% chitosan nanoparticle	0.5% REO+ 1% chitosan microemulsion
Zero day	9.0 ± 0.0	9.00 ± 0.0	9.00 ± 0.0	9.00 ± 0.0
1 st day	8.8 ^a ± 0.45	8.8 ^a ± 0.45	8.8 ^a ± 0.45	8.8 ^a ± 0.45
3 rd day	8.50 ^a ± 0.45	8.50 ^a ± 0.45	8.50 ^a ± 0.45	8.50 ^a ± 0.45
6 th day	6.20 ^a ± 0.45	8.20 ^b ± 0.45	8.20 ^b ± 0.45	8.20 ^b ± 0.45
9 th day	4.45 ^a ± 0.45	8.20 ^b ± 0.45	8.20 ^b ± 0.45	8.20 ^b ± 0.45
12 th day	2.45 ^a ± 0.45	6.67 ^a ± 0.45	6.20 ^b ± 0.45	6.20 ^b ± 0.45

There are significance differences ($P < 0.05$) between means having different letters in the same raw

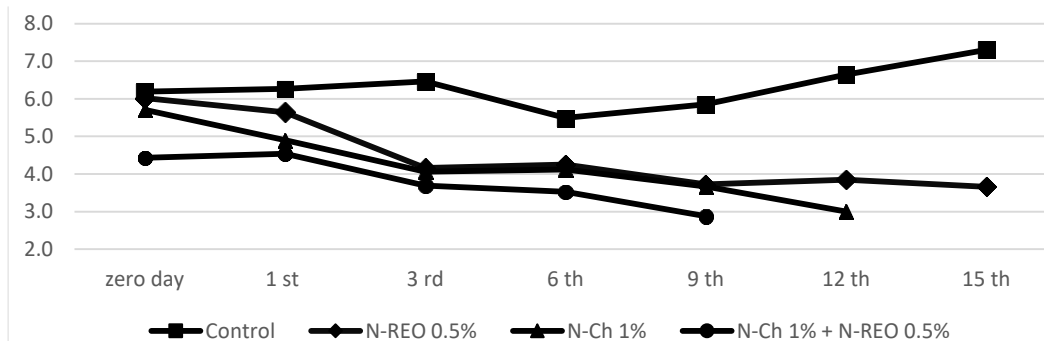


Fig. 3: Mean count of *L. monocytogenes* in different treatments during storage

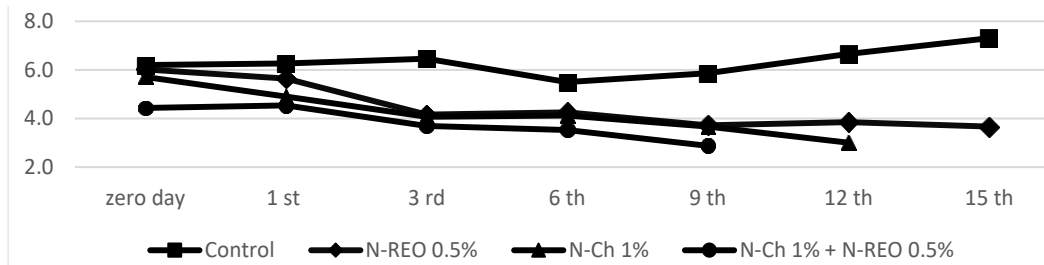


Fig. 4: Mean count of *E. coli* in different treatments during storage

treatments on the growth of *E. coli* during 15 days of refrigerated storage. The initial count of *E. coli* was 6.00 ± 0.05 Log CFU/g in control samples and other treatments. The growth of *E. coli* decreased at 4°C in all treatments. The

maximum bacterial count was observed in control samples on the 15th day of storage (7.45 ± 0.13 Log CFU/g), whereas the minimum count was observed in 0.5% REO + 1% chitosan microemulsion samples (on 8th day of storage: 3.15

± 0.21 log CFU/g; 12th and 15th the counts were less than 3 log CFU/g). When compared to the control group, the results demonstrated a significant drop in *E. coli* count in all treatments with REO chitosan microemulsion having the strongest inhibitory efficacy.

Sensory evaluation

The sensory analysis results are reported in Table 3. The overall acceptability in terms of appearance, color, odor and texture of all samples started at a score of 9. Within 2 days of storage, no significant changes in the samples were found ($P > 0.05$). Three days later, significant changes were observed, as with the scores of the control samples were considerably recorded lower than those of any other treated samples ($P > 0.05$). Based on these sensory scores, especially overall acceptability, the 0.5% REO + 1% chitosan microemulsion mixture yielded the highest acceptability scores between 6 and 9 days of storage.

Discussion

Previous studies have reported that the mean rosemary nanoemulsion particle size ranges from 164 ± 9 to 676 ± 26 nm and the mean PDI was 0.230 ± 0.009 (Restrepo *et al.* 2018). REO is used to make a variety of products. Ultrasound was used for 6 min to create a nanoemulsion with a droplet size of 139.9 nm. The lethal concentration (LC₅₀) of spray application of normal emulsion (EO) was 1,578.50 and 1,829.94 g/mL for juvenile and adult female spider mites, respectively (Mossa *et al.* 2019).

Nanocapsules containing rosemary oil were of average size (145 ± 15 nm) with PDI below 0.3 and negative zeta potential ($-11, 0 \pm 0.5$ mV); they were spherical nanocapsules with regular and homogeneous surfaces. The following key components have been identified in REO using GC-MS: α -pinene (16.07%), 1,8-cineol (13.99%), camphor (10.85%) and cis-verbenone (10.16%) (Khoobdel *et al.* 2017).

α -Pinene is a major constituent in the composition oil, which has antibacterial activity against Gram-negative and Gram-positive bacteria. In the case of REO, major and minor active components, such as borneol, 1,8 cineole, D-limonene, α -pinene, L-linalool, γ -terpinene, D-camphor, p-cymene, α -terpineol, sabinene, α -myrcene, α -thujenol, isocineole, α -phellandrene, α -terpinene, myrtenol, α -terpinolene, 4-terpineol, terpinene-1-ol, γ -terpineol, isopulegol acetate and geraniol have acaricidal activity against several phytophagous mites (Mossa *et al.* 2019). GC/MS analysis of REO showed that γ -terpinene (3.92%), borneol (11.07%), 1,8 cineole (31.45%), D-limonene (9.19%), α -pinene (10.91%), L-linalool (8.86%), D-camphor (7.32%), α -terpineol (3.32%), linalyl acetate (3.37%), and p-cymene (1.82%) were the major components (Gachkar *et al.* 2007; Ebadollahi *et al.* 2014).

Therefore, the powerful synergistic antimicrobial

activity of the rosemary chitosan microemulsion against Gram-positive bacteria *L. monocytogenes* resulted in a lower microbial count and inhibition of the microbiological growth of *L. monocytogenes* by 3 log CFU/g. These results agreed with those by Ismail *et al.* (2015), who reported that the number of bacteria decreased in samples wrapped in bionano composites (1.2–2.6 log CFU/g). Moreover, Souza *et al.* (2019) have stated similar results in fresh chicken breast fillets coated with sodium caseinate incorporated with a nanoemulsion of ginger essential oil. Moreover, Noori *et al.* (2018) has shown the reduction of the population of the inoculated meatballs covered with chitosan. The counts of *L. monocytogenes* were around 2 log CFU/g lower, showing the inhibitory effect of *L. monocytogenes* on chitosan growth. On the other hand, Antoniadou *et al.* (2019) have studied edible chitosan film and claimed that the growth of *L. monocytogenes* could not be avoided in RTE beef dissolved at 0.5% (w/v), 0.5% (w/v), or 1% (w/v) and stored at 4°C. However, on day 14, the number of *L. monocytogenes* for all chitosan encapsulated samples was significantly distinct from that of control by 2–3 log CFU/g; On day 15, it was significantly different. This could be attributed to chitosan films having less antibacterial activity as an amino group is less available on chitosan (Coma *et al.* 2002; Cagri *et al.* 2004; Beverly *et al.* 2008).

Several previous studies (Raeisi *et al.* 2012; Shahbazi *et al.* 2015; Ehsani *et al.* 2016) have confirmed the above finding. Nevertheless, chitosan films may have antagonistic, synergistic, or additive effects based on the type of antimicrobial agent and microorganism. The present study confirmed that the application of coating treatments could eliminate the bacterial count to an undetectable (10^3 CFU/g) level. As mentioned, this could be due to the use of coating solutions containing REO as microemulsions.

The effects of 1% chitosan are similar to those found in previous research (Youssef and El-Masry 2018), which indicated the significant antimicrobial activity of 0.5, 1 and 2% chitosan nanoparticles in eradicating foodborne pathogens and maintaining an acceptable sensory quality of chicken meat.

The primary components found in prepared REO microemulsions, such as α -pinene, 1,8-cineol, camphor, myrcene, camphene, borneol and verbenone, significantly contributed to the high antioxidant and antibacterial activities of REO against *L. monocytogenes*, *E. coli*, *Salmonella indiana*, and *Listeria innocua* (Abdullah *et al.* 2015).

Previous research (Hassanzadazar *et al.* 2019) had found that in comparison to Gram-negative bacteria, REO and REO nanoemulsion have more important antibacterial properties and effects on *Shewanella* spp., *L. monocytogenes* x *Staphylococcus aureus*, *S. enteritidis*, *E. coli*, and *P. aeruginosa*. The REO nanoemulsion mechanism of action in Gram-positive bacteria is explained by the fact that (Aminzare *et al.* 2017) the cell membrane was improved by the ion's permeability due to direct

interaction between the phospholipid layer of the cell membrane and the lipophilic components of EO and the lack of an external phospholipid membrane-aided intraexcretion.

Our findings did not agree with those by Ntzimani *et al.* (2010), who found that applying REO (0.2%) to cooked chicken produced an acceptable odor and taste. The obtained results revealed higher sensorial scores in 0.5% REO + 1% chitosan microemulsion samples, which indicate the effects of chitosan coating on preserving sensory characteristics of chicken meat. The results were in line with those of Hassanzadeh *et al.* (2017). Color influences the customer's preferences and choice of food. Food color is determined by the chemical, biochemical, physical, and microbial changes occurring during storage. Accordingly, chitosan's antioxidant qualities and its capacity to function as a metal ion transition chelator that catalyzes myoglobin oxidation can cause redness in muscle food (Yen *et al.* 2008).

Conclusion

This study has demonstrated the counts of *E. coli* and *L. monocytogenes*, which were considerably lowered after treatment for cold chicken fillets with the REO chitosan microemulsion. Compared to the control samples, the sensory characteristics were improved and the storage quality of the chicken breast muscle was retained in the cooling phase. Naturally retaining the storage quality of the chicken breast muscle in conjunction with the microemulsion of chitosan could be a viable technique in the food industry.

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Author Contributions

All authors designed, coordinated, and conducted the experiment, analyzed the data, and wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

All authors declare no conflicts of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable in this paper.

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