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Research Article

Nanoemulsification of Rose (*Rosa damascena*) Essential Oil: Characterization, Anti-*Salmonella*, In Vitro Cytotoxicity to Cancer Cells, and Advantages in Sheep Meat Application

Iman Saffari[1], **Abbasali Motallebi Moghanjoghi**[1], **Reza Sharafati Chaleshtori**[2], **Maryam Ataee**[1], and **Azad Khaledi**[3]

[1]*Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran*
[3]*Infectious Diseases Research Center, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran*

Correspondence should be addressed to Abbasali Motallebi Moghanjoghi; abbasalimotallebi@gmail.com and Reza Sharafati Chaleshtori; sharafati.reza@gmail.com

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Nowadays, consumers are pushing for the use of natural preservatives such as essential oils (EOs) in the fresh and processed foods, including meat. However, this action is related to a variety of challenges that are associated with essential oils, including low solubility in water, high volatility, and limited long-term stability. These factors collectively reduce their effective utilization in this application. Therefore, the objective of this research was to investigate the morphology and stability of the *Rosa damascena* essential oil nanoemulsion (RDNE), as well as its antibacterial and anticancer properties. Also, we examined its effects on the quality characteristics of ground sheep meat during the refrigerated storage. The zeta potential and particle size of RDNE were −47.5 mV and 100 nm, respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the RDNE against *Salmonella typhimurium* were 375 and 750 μg/mL, respectively. The most inhibition of biofilm formation (80%) was observed for RDNE at 2 MIC. The RDNE had an inhibitory effect on the expression of the *Salmonella* enterotoxin gene (*stn*). The obtained IC_{50} of RDNE against colon and cervical cancer cell lines (HCT 116 and HeLa cells) were 3.31 and 4.6 μg/mL, respectively. Adding RDNE at a concentration of 250 μg/g to fresh ground meat resulted in the total bacterial count (TBC) remaining below 8 log CFU/g during storage. Moreover, the samples coated with RDNE (250 μg/g) had the fewest coliforms, lactic acid bacteria (LAB), *S. typhimurium*, pH, and total volatile basic nitrogen (TVB-N) content. RDNE coating improved the flavor and overall acceptability of the samples. Therefore, RDNE is recommended as a natural preservative agent to enhance the bacterial and chemical stability of sheep meat.

1. Introduction

Lately, there has been a surge of interest in colloidal nanoparticles due to their promising range of potential applications. The distinctive physicochemical properties of colloidal nanoparticles make them suitable candidates for design and application as pharmaceuticals and foods preservative agents. The advancement of nanotechnology has led to the emergence of various colloidal nanocarriers within the particle size range of 1–to 100 nm. Among these, polymeric nanoparticles, solid lipid nanoparticles, liposomes, and micelles have established themselves as nanocarriers across diverse industries. However, in contemporary times, even more advanced and innovative nanosystems, including dendrimers, nanoemulsions, nanogels, nanosuspensions, and nanotubes, have demonstrated even greater potential than their predecessors, thanks to the progress achieved through nanotechnological approaches [1, 2]. A colloidal particle system, known as a nanoemulsion, is made up of two heterogeneous liquids [3]. Due to their

small particle size, ease of preparation, increased bioavailability, biological effectiveness, and kinetic stability, nanoemulsions are known as a perfect carrier for transferring lipophilic materials. They are also referred to as a self-preserving antimicrobial composition due to the lack of enough water in their structure for microorganism growth [4]. Furthermore, nanoemulsions show the stable thermodynamic and kinetic properties that could be characterized by droplet sizes ranging from 20 to 400 nm and a uniform size distribution, setting them apart from other emulsions [5]. Due to their numerous physiological, chemical, and biological attributes, nanoemulsions find applications in diverse industries, including oil and gas [6], agriculture, chemistry, food, and cosmetics [7].

Rose (*Rosa damascena*) essential oil (REO) is an agricultural product cultivated in various Northern Hemisphere nations, such as Morocco, Iran, Egypt, Turkey, Bulgaria, France, China, and India. Iran, specifically Azerbaijan, Kerman, Fars, and Kashan, stands out as the leading global producer of this item [8]. Previous studies have documented a diverse range of physiological activities associated with REO, including antimicrobial, antioxidant, analgesic, and anti-inflammatory properties [9–13].

Foodborne pathogens, i.e., bacteria, fungi, and viruses, pose a global threat by causing food poisoning and intestinal infections. Among these pathogens, *S. typhimurium* is recognized as a highly dangerous bacterium in the terms of human pathogenicity [14]. Gastroenteritis, predominantly caused by *Salmonella* serotypes, particularly *S. typhimurium*, is the most common form of *Salmonella* infection in humans. This leads to an annual incidence of 1.4 million cases of nontyphoidal salmonellosis reported in the United States alone [15, 16]. *S. typhimurium* is responsible for causing various illnesses, including acute gastroenteritis, typhoid or paratyphoid enteric fever, and systemic infections. Human infections typically occur through the consumption of raw foods, such as meat and eggs, with food contamination from animal sources being the primary route of transmission [17, 18]. Given that genes play a crucial role in determining the virulence of *S. typhimurium*, the key genes include *spvC*, *invA*, *stn*, and *gyrB*. The *spvC* gene, located on a plasmid, is essential for the bacterium's survival within the host cell. The *invA* gene is responsible for bacterial invasion of epithelial cells, while *gyrB* encodes the DNA gyrase subunit involved in invasion. In addition, the *stn* gene produces an enterotoxin and codes for a protein that causes diarrhea [19, 20].

In previous studies, various essential oils have been investigated for their biological properties both in vitro and in food models. Dini et al. found that the shelf life of refrigerated meat could be extended more effectively using cumin essential oil nanoemulsion compared to chitosan [21]. Keykhosravy et al. demonstrated that chitosan can increase the shelf life of turkey meat by up to 9 days, while nanoemulsions containing a combination of *Bunium persicum* and *Zataria multiflora* showed an even greater increase of up to 20 days [22].

Therefore, the objective of the present study was to assess the morphology and stability of RDNE, as well as its antibacterial and anticancer properties through in vitro evaluations. Furthermore, the study aimed to investigate the effects of RDNE on the quality, physicochemical characteristics, and sensory attributes of sheep meat during refrigerated storage at a temperature of $4 \pm 1^{\circ}$C.

2. Materials and Methods

2.1. REO and RDNE Preparation. The REO was purchased from Zarin Golab company (Kashan, Iran). To prepare RDNE, 93 μg/mL of REO, 115 μg/mL of Tween 80 (Sigma Aldrich, USA), and 115 μg/mL of Span80 (Sigma Aldrich, USA) were mixed for nanoemulsion formulation. By slowly and continuously adding REO and surfactants to water while shaking at 3000 rpm, we created a coarse emulsion. Then, a 20 kHz sonicator was utilized to perform ultrasonic emulsification on the coarse emulsion (Heshler, Germany). A sonotrode with a 13 mm of diameter piezoelectric crystal was used, and the operation power was changed to 400 W. Finally, 7 mL of the mixture was placed in the sonicator, and ultrasonic waves were delivered for 10 min [23].

2.2. Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis of REO. By using GC-MS analysis, we determined the constituents of the necessary oil. A moderate nonpolar capillary column was integrated with GC-MS (Agilent 6890, USA) (BPX5, 30 m length, 0.25 micrometer film thickness, 0.25 mm internal diameter). The injector port temperature was 260°C. The sample was injected by splitting, and the split ratio was 1 : 10. The temperature was programmed from 50°C to 220°C at 10°C per minute and held at 220°C for 15 min with helium as the carrier gas (flow rate 0.5 mL·min^{-1}). Electronic impact, a 70 eV ionization potential, a 220°C ion source, and a mass range of 30 Da–500 Da included all characteristics of the mass spectra. NIST (2010) used mass spectra and Kováts indexes (KIs) to identify compounds in relation to n-alkanes (Wiley spectral library collection and authentic chemicals) [24, 25].

2.3. Measurement of RDNE Droplet Size. After being properly diluted with distilled water, the nanoemulsion was adsorbed in a copper grid covered in carbon. After that, using a Zeiss EM900 transmission electron microscope (TEM) with an 80 kV accelerating voltage, we analyzed the particles' morphology and size and captured them with a camera. For size analysis of RDNE, the dynamic light scattering (DLS) technique was employed using the Zetasizer Nano ZS, model ZEN3500 from Malvern Instruments, UK [26].

2.4. Zeta Potential of RDNE. Through a Zetasizer, we used electrophoretic light scattering and estimated the zeta potential (mV) of RDNE (SZ-100-Z, Horbia, Japan). 20 μL of RDNE was diluted in 1 mL milli-Q water before being deposited in capillary cells with two electrodes. Then, 30 continuous readings were used to acquire particle charge information [27].

2.5. Antibacterial Activities of RDNE

2.5.1. Agar Diffusion Assay. We prepared a half McFarland standard suspension (1.5×10^8 CFU/mL) from an instantaneous culture in Tryptic Soy Broth (TSB; Merck, Germany) of the *S. typhimurium* (ATCC: 14028). For the disc diffusion method, the Muller–Hinton Agar (MHA; Merck, Germany) media was coated with 100 μL of bacteria suspension at a concentration of half McFarland (1.5×10^8 CFU/mL). Individually impregnated with 50 μL of diluted RDNE (1.87, 3.75, 7.5, and 15 mg/mL DMSO 10%), sterile filter paper discs (6 mm in diameter) were then allowed to dry in an exposed, sterile Petri dish in the vertical laminar flow of a biological cabinet. Gentamicin (10 mg/mL), as an antibiotic, was used as a positive control, and DMSO (10%) was used as a negative control. Next, the plates underwent a 24-hour incubation period at 37°C. The discs' surrounding inhibition zones were measured in millimeters for their diameters [28].

2.5.2. Minimum Inhibition and Bactericidal Concentration. The MIC and MBC of RDNE against *S. typhimurium* were determined using the microdilution method. To achieve final concentrations and volumes of 1.46–375 μg/mL and 200 μL, respectively, prepared stock solutions of individual RDNE (750 μg/mL) were serially diluted in 96-well plates with 15 μL of the inoculum (half McFarland) added to the wells. To check for cross-contamination from one well to another during plate handling, a negative control without the appropriate bacterium and RDNE was added. After that, the plate was incubated for 24 hours at 37°C. Samples in aliquot of 100 microliter, from the wells with no discernible growth were placed onto Nutrient Agar (NA; Merck, Germany) and cultured for 24 hours at 37°C in order to determine MBC. The lowest concentration in the sample at which the complete initial bacterial population could be eradicated was found to be the MBC [29, 30].

2.5.3. Antibiofilm Activity. A bacterial suspension was prepared at a concentration of 1.5×10^8 CFU/mL (equivalent to 0.5 McFarland). The achievement of a 0.5 McFarland suspension was confirmed by spectrophotometric measurements, where the absorbance was at a wavelength of 625 nm fell within the range of 0.08–0.13. Subsequently, 10-fold dilutions were prepared by transferring 1.0 mL of the suspension into 9.0 mL of sterile saline. This process resulted in a final dilution of 1.5×10^6 CFU/mL for the bacteria. Several sublethal doses of RDNE (0.5, 1, and 2 MICs) were inoculated into Brain Heart Infusion (BHI; Merck, Germany) media using the bacterial culture. After 24 hours of incubation, the supernatant from each well was withdrawn, and each well's remaining intact biofilm was dyed with a 1% solution of crystal violet. The excess crystal violet was removed following 30 minutes at 37°C of incubation. The 96-well plate was cleaned twice with sterile distilled water and went through an hour of air drying at that temperature. 200 microliters of 95% ethanol were used to dissolve the labeled biofilm cells, and the samples' optical density (OD) was measured at 570 nm. To minimize the effect of background effects, the positive control wells contained only bacterial cells and BHI (without RDNE), while the negative control wells contained BHI without bacterial cells and RDNE. The antibiofilm index was calculated using the following formula [31, 32]:

$$\text{Antibiofilm activity}\,(\%) = \left(1 - \frac{(\text{OD570 sample} - \text{OD570 negative control})}{(\text{OD570 positive control} - \text{OD570 negative control})}\right) \times 100. \tag{1}$$

2.6. Expression of stn Gene. Junior et al. [33] proposed the extraction of *S. typhimurium* genomic DNA by boiling. All DNA samples were stored at −20°C, until the *S. typhimurium stn* gene was detected in them. The PCR and DNA amplification processes were carried out in 25 μL volumes in microtest tubes (PCR tubes), with 5 μL of DNA template, 12.5 μL of 2X PCR Master Mix (Kiagene, Iran), 1 μL of each primer (20 pmol/μL), and nuclease-free water up to 25 μL. PCR amplifications were carried out using the Mastercycler (Eppendorf, Germany). Table 1 lists the primer sequence, target gene (*stn*), and PCR conditions. PCR products were analyzed by electrophoresis on 1% agarose gel in 1x TBE buffer at room temperature. They were stained with ethidium bromide and visualized using a UV transilluminator (Denazist Asia, Iran) (Ingenious, L. Cambridge, UK) [34].

For target gene expression analysis, first, *S. typhimurium* cells in TSB with different concentrations (0, 75, 125, and 250 μg/mL) of RDNE were cultured overnight at 37°C. Then, we extracted RNAs using an RNA isolation kit (Cinaclon, Iran) following the manufacturer's instructions. cDNA was produced from the total extracted RNA using a special kit (Addbio, Korea). Finally, a StepOnePlus instrument was used to amplify the *stn* gene. The fold change in gene expression due to RDNE treatment was calculated using the $2^{-\Delta\Delta CT}$ method, and the gene expression level was reported in relative units [34].

2.7. The Effect of RDNE on Cancer Cell Lines. RDNE's antitumor effects on specific and cervical cancer cell lines (colon HCT 116 and cervix HeLa) were analyzed using an MTT assay. 96-well plate was used with a cell-seeding density of 1×10^4 cells per well. Cells were cultured for 24 hours in DMEM (Himedia, Bombay, India) supplemented with 10% FBS (HiMedia, Mumbai, India) and allowed to attach. Medium was replaced with suspensions of RDNE that

Table 1: Primer sequence used for *Salmonella* enterotoxin (*stn*) gene amplification and PCR conditions.

Target gene	Primers sequencing	Amplified segment	Primary denaturation	Amplification (30 cycles)			Final extension
				Secondary denaturation	Annealing	Extension	
stn	AGC GTT CAG GTA CAG ATT CAA CAG GTC AGT CAG GAT GCC CAA AGC	617 bp	94°C 3 min	94°C 45 sec	47°C 30 sec	72°C 30 sec	72°C 5 min

ranged in concentration from 2.9 to 187 μg/mL (each concentration was seeded into at least three wells) and cells were incubated for 48 hours. Then, MTT reagent (10 μl per well) was added. Cells were kept in the incubator for additional four hours at 37°C. After removing the medium, 50 μl of DMSO was added to each well. The OD of the formazan product was measured at 570 nm using a multiwell spectrophotometer. The OD readouts were then analyzed, and the cell viability was calculated using the following formula [35]:

$$\text{Cell viability}\,(\%) = \frac{\text{OD of sample}}{\text{OD of control}} \times 100. \quad (2)$$

$Y = Mx + C$, a linear regression equation, was used to determine the IC_{50} value.

In this case, $Y = 50$ and the values of M and C were obtained from the viability graph.

2.8. Effect of RDNE on Sheep Meat Shelf Life

2.8.1. Preparation of Ground Meat Samples and Treatments. The RDNE samples used in this study were obtained from a butcher shop in Kashan and immediately transported on ice to the laboratory for food hygiene at Kashan University of Medical Sciences in Iran. They were randomly assigned into four groups of ground meat samples (control: without any coating RDNE, treatment 1: ground meat coating with 75 μg/g of RDNE, treatment 2: ground meat coating with 125 μg/g of RDNE, treatment 3: ground meat coating with 250 μg/g of RDNE). After being kept at 4 ± 1°C for 12 days, all samples were examined for their microbiological and biochemical characteristics (days 1, 4, 8, and 12). On the other hand, the ground meat samples (10 ± 0.5 g) were gamma irradiated at the Atomic Energy Organization of Iran at a dose rate of 4 KGy/s. Then, four groups of meat samples were randomly assigned into four groups (control: without any coating RDNE plus 10^5 CFU/mL of *S. typhimurium*; treatment 1: ground meat coating with 75 μg/g of RDNE plus 10^5 CFU/mL of *S. typhimurium*; treatment 2: ground meat coating with 125 μg/g of RDNE plus 10^5 CFU/mL of *S. typhimurium*; treatment 3: ground meat coating with 250 μg/g of RDNE plus 10^5 CFU/mL of *S. typhimurium*). All samples were kept in storage at 4 ± 1°C and analyzed on the mentioned days [22, 36].

2.8.2. Microbial Analyses. Microbial analysis was performed using standard methods, including the enumeration of TBC, coliforms, LAB, and *S. typhimurium*. The TBC, coliforms, and LAB enumeration were performed using pour plate standard method, while the enumeration of *S. typhimurium* was carried out using the spread plate method. Incubation of the plates was conducted at 37°C for 48 hours. Results were shown as log CFU/g [36, 37].

2.8.3. Chemical Analyses. TVB-N and pH parameters were determined using the methods described by Khoshbouy Lahidjani et al. [38], Zhou et al., [39] and other researchers, respectively.

2.8.4. Sensory Analysis. Every sample was fried in soy oil in the oven for ten minutes. Ten evaluators were selected from the food hygiene and quality control laboratory at Kashan University of Medical Sciences according to their prior experience in initial evaluation trials. Panel members were first given information about ground meat and its characteristics (taste, odor, color, and overall acceptability). Before the test, they attended a prep session, allowing each panelist to fully discuss and elaborate on each aspect of the fried ground meat. Three replicates of each sample (0, 75, 125, and 250 μg/g of RDNE) were used for evaluation, and the samples were distributed to panelists in randomized order. All characteristics were scored using a 5-point hedonic scale that was anchored by very nice, 5; good, 4; acceptable, 3; poor, 2; and very poor, 1. A sample of fried ground meat without RDNE was used as the control in the experiment [40].

2.9. Statistical analysis. There were three repetitions of each experiment in this study, and mean values were recorded. The resulting values were then transferred into SPSS software version 21 and compared using one-way ANOVA and the least significant differences (LSD) method. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Chemical Composition of REO. Thirty-three compounds were detected in the REO through GC-MS analysis, the active principle with their retention time (RT), and KI (Table 2). Accordingly, the major compounds accounting for 57.36% of the REO were nonadecane (33.55%), heneicosane (16.03%), and hexadecanal (7.78%) values. The most predominant constituents were other components (66.6%), sesquiterpene hydrocarbons (8.77%), monoterpene hydrocarbons (4.34%), oxygenated monoterpenes (3.16%), and oxygenated sesquiterpenes (1.14%).

3.2. RDNE Characterization. Different morphological sizes were detected for the RDNE in the TEM and DLS analysis. Despite the fact that some different droplets were seen, the size of the emulsion droplets in the image and the findings of the particle size analyzer were in good agreement (ranging from 30 to 50 nm). Oil droplets are distinguished by their clearly defined boundaries and smooth surfaces (Figure 1).

The RDNE's stability was confirmed by zeta potential analysis. Based on the observed zeta potential of −47.5 mV (Figure 2), the RDNE has sufficient electrostatic repulsion to keep itself stable in the solution.

3.3. Anti-Salmonella Properties of the RDNE. In the present study, RDNE was shown to have an inhibition effect on *S. typhimurium* using the Agar disk diffusion method, as depicted in Table 3. The results revealed the anti-Salmonella effects of RDNE on *S. typhimurium* were lower than that of gentamicin. In addition, the MIC and MBC rates were 375 and 750 μg/mL, respectively. In Figure 3, the biofilm

Table 2: Chemical composition of *R. damascena* essential oil.

No.	Components	Type	KI	RT	%
1	α-Pinene	MH	939	11.25	2.56
2	Sabinene	MH	975	13.32	0.14
3	β-Pinene	MH	979	13.58	0.52
4	Myrcene	MH	991	14.15	0.72
5	α-Terpinene	MH	1017	15.63	0.07
6	ρ-Cymene	MH	1025	16.13	0.06
7	Limonene	MH	1029	16.28	0.08
8	ϒ-Terpinene	MH	1060	17.82	0.16
9	Rose oxide(cis)	MO	1108	20.52	0.10
10	Nerol	MO	1230	26.25	0.47
11	Geranial	MO	1267	28.45	0.16
12	Citronellyl acetate	MO	1353	31.84	0.46
13	Neryl acetate	MO	1362	33.15	0.61
14	Methyl eugenol	MO	1404	34.46	0.12
15	Geraniol	MO	1253	27.49	1.24
16	β-Bourbonene	SH	1388	33.34	0.27
17	β-Elemene	SH	1391	33.58	0.31
18	E-Caryophyllene	SH	1419	34.94	0.95
19	α-Guaiene	SH	1440	35.52	1.39
20	α-Humulene	SH	1455	36.50	1.21
21	Muurola-4(14),5-diene (cis)	SH	1467	37.57	3.23
22	α-Selinene	SH	1498	38.12	0.31
23	α-Bulnesene	SH	1510	38.36	1.32
24	δ-Cadinene	SH	1523	39.05	0.09
25	Farnezol (2z, 6z)	SO	1718	46.60	1.14
26	Pentadecane	Other	1500	38.00	0.86
27	Hexadecane	Other	1600	41.95	0.30
28	Heptadecane	Other	1700	45.71	4.20
29	Hexadecanal	Other	1876	51.82	7.78
30	Nonadecane	Other	1900	52.72	33.55
31	Eicosane	Other	2000	55.91	3.39
32	Heneicosane	Other	2100	59.03	16.03
33	Octadecane	Other	1800	49.27	0.49
	MH = monoterpene hydrocarbons				4.34
	SH = sesquiterpene hydrocarbons				8.77
	MO = oxygenated monoterpenes				3.16
	SO = oxygenated sesquiterpenes				1.14
	Others				66.6
	Total identified				84.01

inhibitory activity of RDNE was evaluated at different concentrations (0.5, 1, and 2 MICs).

3.4. Molecular Analyses. The *stn* gene was successfully amplified, as the ~617 bp product was observed with 1% agarose gel (Figure 4). The RDNE effect on *stn* gene expression of *S. typhimurium* was also observed (Figure 5). The *S. typhimurium stn* gene showed a greatest decrease in the expression (21.2%) at a dose of 250 μg/mL RDNE.

3.5. Cytotoxicity Analysis of RDNE on HCT 116 and HeLa Cell Lines. The cytotoxicity of RDNE was evaluated on two cancer cell lines. HCT 116 and HeLa cells were exposed to various concentrations of the drug, and a dose-dependent cytotoxicity was observed as shown in Figure 6. With increasing RDNE concentrations, the growth of cancer cells was significantly inhibited. The concentration of RDNE that inhibits cell proliferation by 50% (IC_{50}) was found to be 3.31 μg/mL for HCT 116 and 4.6 μg/mL for HeLa cells.

3.6. Sheep Meat Shelf Life

3.6.1. Microbial Changes. At the beginning (Figure 7(a)), the TBC of fresh ground meat samples was 6.4 log CFU/g and slowly raised to 9.4 log CFU/g throughout the 8th day. Compared to the control group, the TBC in ground meat samples containing RDNE (125 and 250 μg/g) was lower at all-time points. A significant difference was found between the RDNE with 250 μg/g concentration and other treatments on days 4 and 8 regarding the inhibitory growth of bacteria. Also, adding RDNE at a concentration of 250 μg/g into fresh ground meat resulted in a TBC below 8 log CFU/g during the storage.

On the first day, LAB populations were detected at levels ranging from 2.7 log CFU/g in the treated samples to 3.5 log CFU/g, eventually peaking at approximately 4.8 log CFU/g by the 12th day. Throughout the monitoring period, LAB exhibited a nearly uniform growth pattern across all treatments, except for the samples with a 75 ppm RDNE concentration, which had significantly lower final counts (3.6 log CFU/g) compared to the other groups (Figure 7(b)).

The population of coliforms in different types of samples after a 12-day storage period is shown in Figure 7(c). At all intervals of time, the control group's coliform count was the higher. As the figure shows, except for 75 μg/g of RDNE concentration, a steady decrease in the growth of coliforms in treatments was noted from day four to day eight. On the 12th day of the storage period, the control group had the highest population (~5 log CFU/g), while samples with a 250 μg/g RDNE concentration had the lowest population (~4.2 log CFU/g).

Figure 8 depicts how three different treatments affected *S. typhimurium* development over a 12-day storage period. *S. typhimurium*'s initial count was 7.12 log CFU/g; during storage, it rose in all samples. However, in RDNE of 250 μg/g concentration samples (~6.46 log CFU/g), we found the lowest trend.

3.6.2. Chemical Analysis. The comparison of TVB-N and pH during a 12-day period is shown in Table 4. Up to the fourth day, there was no difference between the control and other treatment groups in terms of the TVB-N. As compared to the control at the storage time, TVB-N levels in several treated samples, including samples with RDNE of 250 μg/g concentration, were significantly lower after the fourth day (12 days). In all samples, the amount of TVB-N significantly increased during the storage period. However, the increase was more prominent in the control sample, raising from 12.5 ± 0.14 mg/100 g to 36.7 ± 0.42 mg/100 g in the refrigerated storage.

The initial pH values ranged from 4.65 ± 0.07 to 5.40 ± 0.14 among the samples. Throughout the storage period, the pH values increased; however, a slower trend of pH increase was observed in the ground meat samples wrapped with RDNE as compared to the control group. Notably, the treatment with RDNE at a concentration of 250 μg/g demonstrated the most favorable effect on the pH of the ground meat when compared to other treatments ($P < 0.05$).

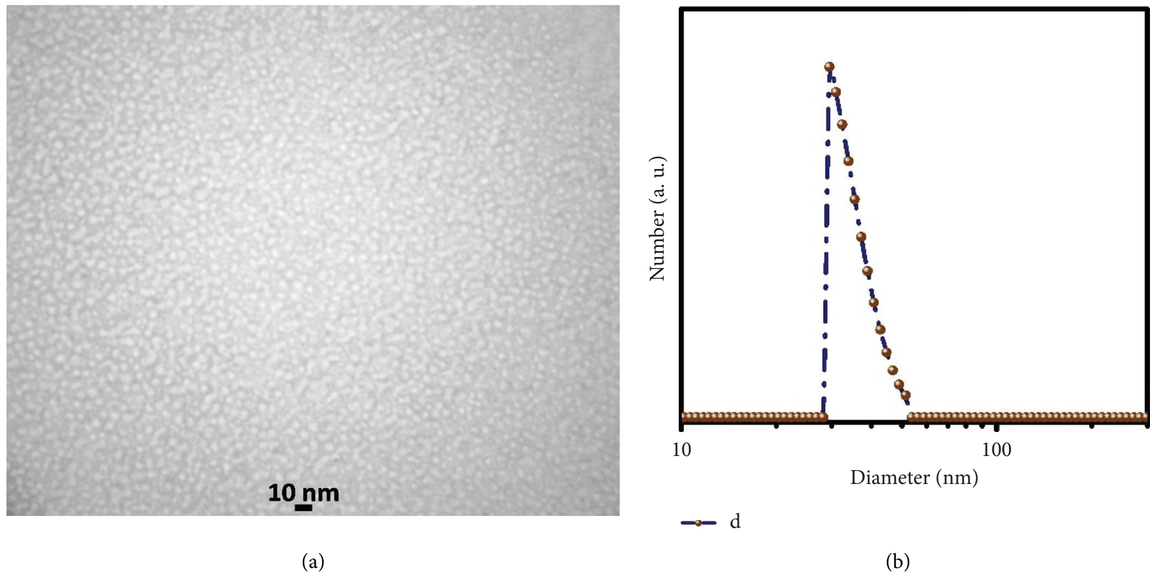

Figure 1: Transmission electron microscopy (TEM) (a) and dynamic light scattering (DLS) (b) images of the RDNE.

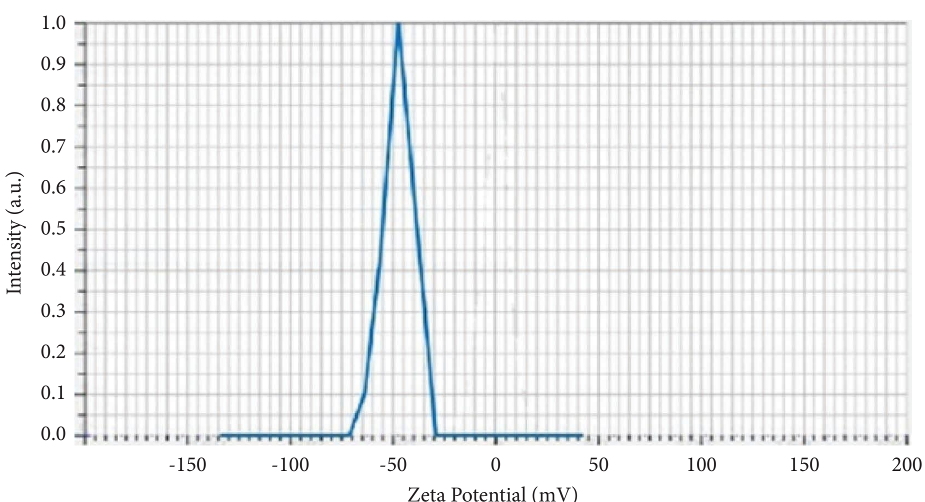

Figure 2: Zeta potential value of the RDNE.

Table 3: Anti-*S. typhimurium* activity of the RDNE by the disk diffusion method.

Treatment	Inhibition zone (mm)
RDNE 15 mg/mL	8.5 ± 1^a
RDNE 7.5 mg/mL	5 ± 0.5^b
RDNE 3.75 mg/mL	3.5 ± 0^c
RDNE 1.87 mg/mL	2.7 ± 0.3^d
Gentamicin 10 μg	10 ± 1^a

Different letters a, b, c, and d in the column represent significant differences ($p < 0.05$).

3.6.3. Sensory Evaluation. Based on the panelist's evaluation, the taste was assessed as satisfactory for the ground meat during the procedures and no detrimental impact on the taste ratings was noted. Hence, greater taste scores were obtained when RDNE content was raised (Figure 9). However, there were no discernible variations in color between the control and RDNE-enriched samples. Moreover, RDNE's red hues indicated that the samples of ground meat did not change in color. All samples that included RDNE showed odor values lower than the control, whereas those without RDNE had more pleasant odors.

4. Discussion

In this study, 84.01% of the total REO was indicated by all the detected chemical compounds. Our results are in agreement with the studies carried out by Alizadeh and Fattahi [41], who identified heneicosane (30.43%) as the primary component of REO. In contrast, another study showed that the essential components included octadecane (4.70%) and nonadecane (0.32%). The discrepancy between the two

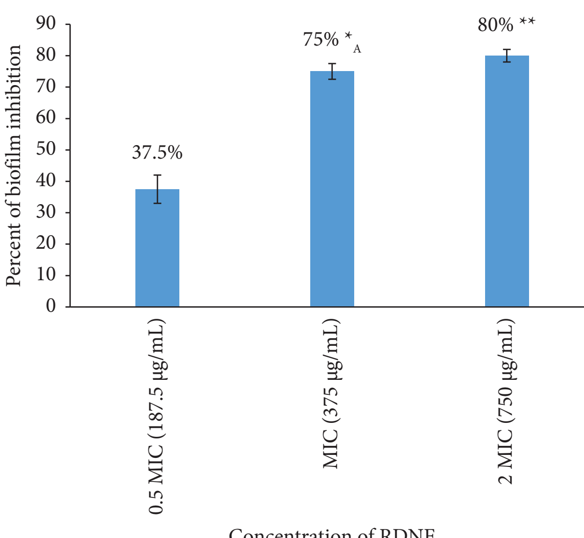

Figure 3: Antibiofilm activity of the RDNE against *S. typhimurium*. Asterisks represent significant differences ($p < 0.05$).

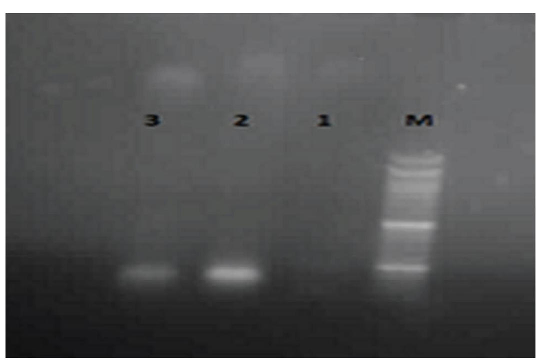

Figure 4: Polymerase chain reaction amplification of virulence gene *stn S. typhimurium* (ATCC 14028) isolate. Lane M: 100 bp DNA ladder; lane 1: negative control; lanes 2 and 3: *stn* gene with twice replicate.

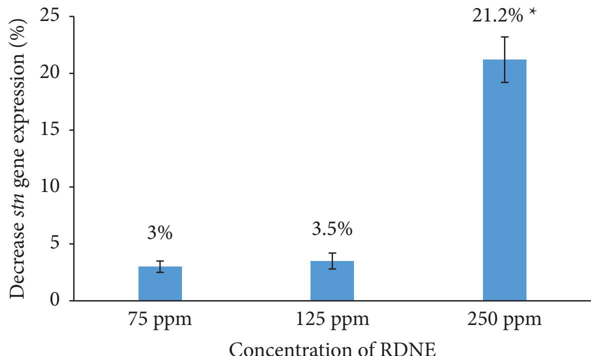

Figure 5: Effect of the RDNE on *stn* gene expression of *S. typhimurium*. The asterisk represents significant differences ($p < 0.05$).

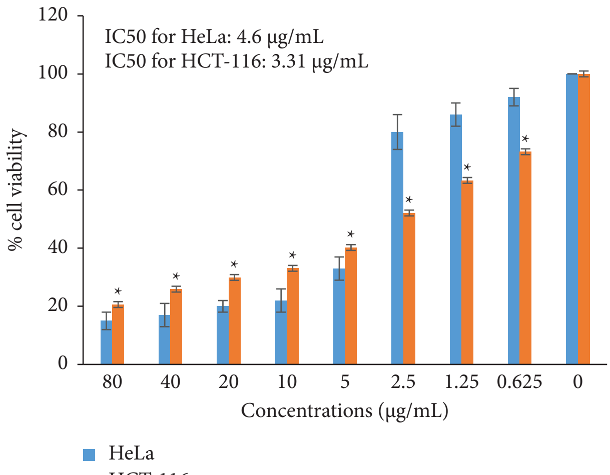

Figure 6: Effect of the RDNE on HCT-116 and HeLa cancer cell lines according to the MTT test. Concentration 0 was the positive control well containing untreated cells, MTT solution, and a solubilizing buffer. The asterisk denotes statistically significant differences between groups for each concentration ($p < 0.05$).

studies may be due to genetic variances, geographic location, collection timing, plant growth phases, and seasonal and environmental conditions [37, 42].

The RDNE was found by visually inspecting the TEM and DLS of the formulation. This study successfully tested the droplet size and physical stability of the chosen formulation. The nanoemulsion's kinetic stability, solubility, and carrier functioning are generally improved with smaller particle sizes [43]. The Ostwald ripening phenomenon, which involves the redeposition of small oil droplets onto larger oil droplets to produce greater sizes, is primarily responsible for the rise in particle size [44].

The effectiveness of RDNE's anti-Salmonella activity was assessed by the disc diffusion, MIC, MBC, and biofilm techniques. Our results revealed that RDNE had anti-Salmonella properties. The use of naturally occurring antimicrobial compounds in plants with lethal and inhibitive effects on infections has grown in popularity due to the increase in bacterial resistance to several antibiotics [22, 45]. Bioactive compounds were present in essential oil (e.g., terpenes). The composition, type, concentration, target microorganism, substrate composition, storage and processing conditions, and antibacterial action of EO show variations across situations [46]. The lipid fraction of the bilayer membrane is disturbed by EO compounds, particularly phenolic compounds, which can interact with cell organelles [47]. This leads to antibacterial activity that was evidenced by the antibacterial actions of EO derived from *R. damascena* against *S. typhimurium*. Nanoemulsions trigger cell death in microorganisms by interacting with their lipids [48]. As nanoemulsions join with the microbe, they release some of their internal contents, causing cell lysis. The likelihood of their interaction with charges on the pathogen surface can be increased by the electrostatic

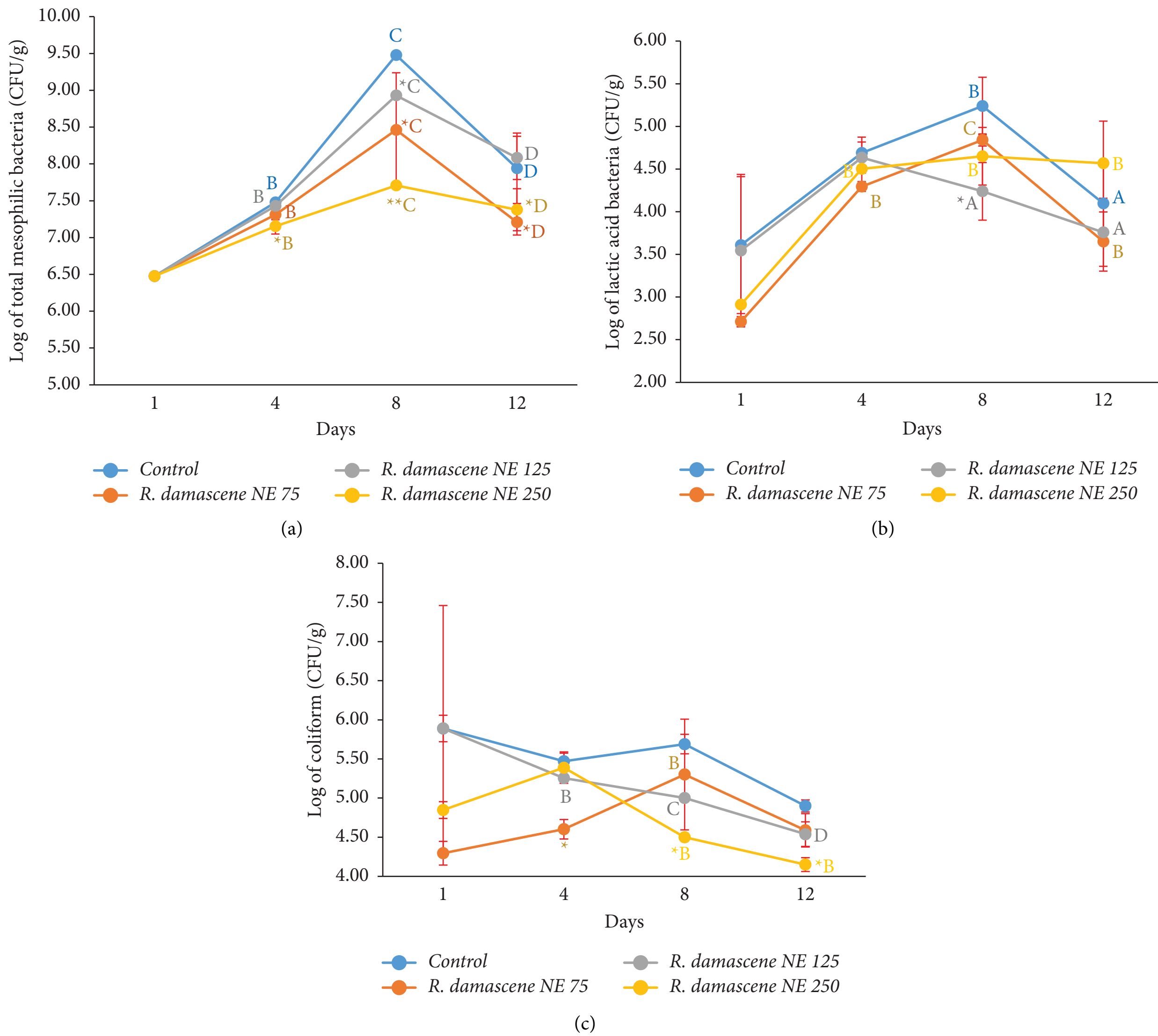

FIGURE 7: Antibacterial activity of *Rosa damascene* nanoemulsion (75, 125, and 250 μg/g concentrations) against total mesophilic bacteria (a), lactic acid bacteria (b), and coliforms (c) in ground sheep meat throughout refrigerated storage (4 ± 1°C). The asterisk denotes statistically significant differences between groups for each time point ($p < 0.05$). Different letters (B, C, and D) in storage time for each group represent significant differences ($p < 0.05$).

attraction [49]. However, it is likely that their major and minor low-molecular-weight (such as terpenes and terpenoids) components, in combination with the currently used nanoemulsion form, cause REO's potent anti-*Salmonella* activity [50].

Many virulence genes, including *stn*, have been generated by *Salmonella* spp. to contribute the pathogenesis and colonization of the bacterium in various host tissues [51]. Salmonella's pathogenic symptoms, such as diarrhea, stomach pain, and vomiting, are caused by enterotoxin activity, which is encoded by the enterotoxin gene (*stn*) [52]. Our investigation into the *stn* production by *S. typhimurium* demonstrated that RDNE negatively influenced the inhibition of *stn* expression, which increased with RDNE concentration. The increased antibacterial properties of this nanoemulsion formulation are thought to be the result of a number of factors, including an improvement in the penetration of plant-derived components into bacterial cells and a decrease in their susceptibility to bacterial enzyme degradation [53].

One of the main worldwide causes of death today is cancer [54]. Many studies have demonstrated that nanotechnology could be beneficiary to cancer therapy as an alternative option [55]. Natural substances harmful to cancer cells have also been explored for antitumor therapies [56]. One of the main herbal substances with broad cytotoxicity against cancer cells is *R. damascena* [57]. In our study, HeLa and HCT 116, respectively, cervix and colon cancer cell lines were significantly in a dose-dependent manner responsive to cytotoxic effect of RDNE. The IC_{50} values were approximately 4.6 μg/mL and 3.31 μg/mL for HeLa and HCT 116, respectively. Colon cancer cells were

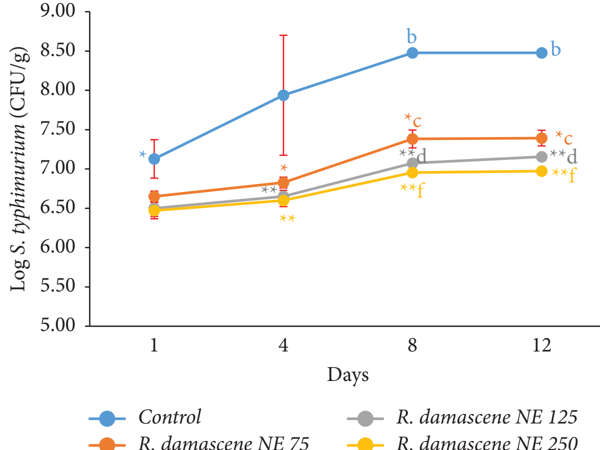

Figure 8: Antibacterial activity of *Rosa damascene* nanoemulsion (75, 125, and 250 μg/g concentrations) against *S. typhimurium* in ground sheep meat throughout refrigerated storage (4 ± 1°C). Asterisks denote statistically significant differences between groups for each time point ($p < 0.05$). Different letters (b, c, d, and f) in storage time for each group represent significant differences ($p < 0.05$).

Table 4: Effect of the RDNE (75, 125, and 250 μg/g concentrations) on chemical properties ground meat throughout refrigerated storage (4 ± 1°C).

Chemical properties	Groups	Days 1	4	8	12
pH	Control	5.40 ± 0.14^{Aa}	5.55 ± 0.21^{Aa}	5.75 ± 0.07^{Ab}	5.75 ± 0.07^{Ab}
	RDNE 75	5.05 ± 0.07^{Ba}	5.40 ± 0.00^{Ab}	5.55 ± 0.07^{Bc}	5.50 ± 0.00^{Bc}
	RDNE 125	4.75 ± 0.07^{Ca}	5.25 ± 0.07^{Bb}	5.45 ± 0.07^{Bc}	5.40 ± 0.00^{Bc}
	RDNE 250	4.65 ± 0.07^{Ca}	5.30 ± 0.00^{Bb}	5.50 ± 0.00^{Bc}	5.45 ± 0.07^{Bc}
TVB-N	Control	12.50 ± 0.14^{Aa}	21.50 ± 0.7^{Ab}	29.40 ± 1.97^{Ac}	36.70 ± 0.42^{Ad}
	RDNE 75	12.50 ± 0.07^{Aa}	20.65 ± 0.5^{Ab}	29.90 ± 1.27^{Ac}	34.00 ± 1.41^{Ad}
	RDNE 125	15.35 ± 0.07^{Ba}	20.65 ± 0.5^{Ab}	25.80 ± 1.13^{Bc}	29.40 ± 1.97^{Bd}
	RDNE 250	15.35 ± 0.14^{Ba}	21.95 ± 0.63^{Ab}	21.20 ± 1.7^{Cc}	24.80 ± 2.55^{Bd}

Different letters A, B, and C in the column represent significant differences ($p < 0.05$). Different letters a, b, and c in the row represent significant differences ($p < 0.05$).

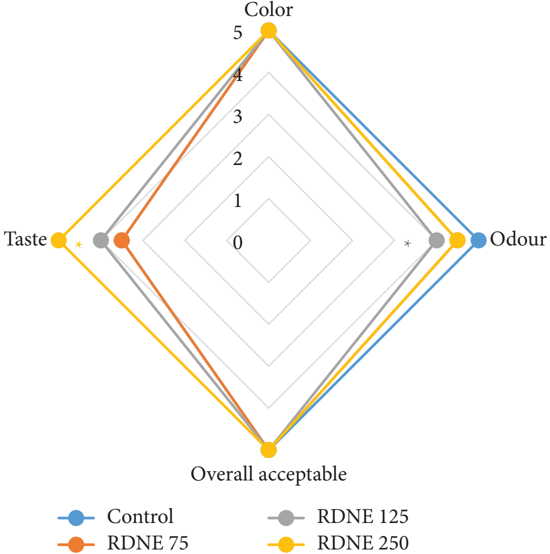

Figure 9: Sensory evaluation scores of ground meat with 75, 125, and 250 μg/g of RDNE concentrations and control sample. The asterisk denotes statistically significant differences between groups for each characteristic ($p < 0.05$).

also found to be more sensitive than HeLa cells. In a study, *R. damascena* extract's cytotoxic effects on the HeLa cell line were assessed. This extract greatly slowed proliferation of HeLa cells with IC_{50} values of 2135, 1540, and 305.1 μg/mL after 24, 48, and 72 hours [58]. Another study examined the cytotoxicity of REO against the lung cancer cell line A549. Using the MTT assay to measure cell viability, the researchers reported that REO had anticancer activity, as it slowed the growth of cancerous cells with an IC_{50} of 36.43 ± 3.373 g/mL [59]. The RDNE demonstrated better cytotoxicity against cancer cells than REO and the extract compared to our findings.

TBC was discovered to be effectively regulated, particularly in 250 μg/g of RDNE concentration. A study suggested that when meat and meat products are refrigerated, the amount of TBC can be reduced using plant extracts, EOs, or a combination of the two [60]. In a study, safflower oil nanoemulsion (SNE) + oxygen absorber (OA) and SNE + 1% cumin essential oil (CEO) were found to be more effective in controlling TBC expansion than the single treatments of SNE and OA. However, the most effective treatment course was SNE + 1% CEO + OA [61].

It is well known that meat with LAB typically has a longer shelf life. LAB might take over as the main bacterial groups after aerobic spoilage bacteria are inhibited. Earlier investigations noted that LAB could develop in a variety of muscle food products both in aerobic and OA packing [62]. When RDNE was used in the meat samples, the population of LAB lowered to its lowest levels (3.6 log CFU/g during the 12th day) compared to the control (4 log CFU/g during the 12th day). In a study, after 10 days of storage at a refrigerator temperature, the amount of LAB was noticeably decreased (3.21–3.71 log) when chicken fillets with PIE (*Polylophium involucratum* essential oil) integrated wrapped samples (0.5, 1, and 1.5%) [63].

The results showed that different concentrations of RDNE reduced the log of coliform, especially 250, 75, and 125 μg/g concentrations had fewer log effects. This finding is due to the fact that meat contains complex nutritional ingredients, such as proteins and fats [64]. The interactions between the target microorganisms and the antimicrobial agent may be hampered by these food components, necessitating a larger concentration of antimicrobial agents [65]. It has also been noted that a high concentration of organic components could be destabilized a nanoemulsion by some mechanisms, including binding impact, depletion, bridging, or electrostatic screening [66].

For *S. typhimurium*, we could add that the phenolic components of the EO of nanoemulsion interact with the Gram-negative cells' outer membrane. Consequently, this alters the structure of the membrane, results in the loss of the functional permeability barrier, and increases the permeability for RDNE [67]. According to our results, different concentrations of RDNE are needed to adequately inhibit *S. typhimurium*.

Over the course of 12 days, the pH values of the control and treated samples increased, and there were no appreciable differences between the treated samples and the control group. However, RDNE treatment at a concentration of 250 μg/g had the best impact on the pH of the ground meat compared to other treatments. The increase in pH in all samples could be attributed to the action of microbial enzymes or endogenous enzymes, such as proteases and lipases, which induce a rise in volatile bases during extended storage [68]. 1% Nacl + lactates and 2% Nacl + lactates treatments in refrigerated and frozen fresh ground pork during 14 days of storage did not significantly differ from the control group, according to Tan and Shelef's report [69].

In TVB-N, samples treated with 250 μg/g of RDNE concentration caused a noticeable decrease in TVB-N in comparison to the control group. With time spent at 4°C, the initial amount of TVB-N in control and coated samples substantially increased, according to our data, which are similar to those of Ojagh et al. [70]. At the end of the trial (16 days), fish samples coated with chitosan-cinnamon had TVB-N that was much lower (14.23 mg/100 g) than fish samples coated with chitosan or the control, which had high values of 22.86 and 42.93 mg/100 g, respectively. Reduced levels of TVB-N in the samples treated with 250 μg/g of RDNE could be the result of a quicker bacterial population decline, a decreased ability of the bacteria to oxidatively deaminate nonprotein nitrogenous compounds, or both [71].

Finally, according to the organoleptic data, there were no appreciable differences in the control and RDNE-enriched samples' color, odor, taste, or acceptability ($p > 0.05$). Samples without RDNE had better scores. This occurs due to the increased nanoemulsion particle size that alters the ground meat matrix's structure, which impacts organoleptic properties. While nanoemulsion may offer a potential mechanism to physically trap the compounds causing these unpleasant tastes or odors, the quality of the raw ingredients and the production process significantly impact the end product's quality and acceptability. Consumers are increasingly interested in meals with the fewest processing steps, the most natural ingredients, and additions, which provide health benefits like antimicrobials and naturally occurring antioxidants. Nanotechnology enables the evolution of numerous ingredients' functionality to satisfy this requirement by reducing the concentration of substances, changing their solubility, and improving or regulating their efficacy [42, 72, 73].

5. Conclusion

The size of the RDNE particles was below 50 nm, indicating good stability. RDNE was observed to be highly effective in controlling *S. typhimurium* in vitro. Our results demonstrated that RDNE exhibited a more pronounced anticancer effect in HCT 116 compared to HeLa cells. In addition, its application in food models resulted in decreased TBC, coliforms, LAB, *S. typhimurium*, pH, and TVB-N content. RDNE coating also enhanced the flavor and overall acceptability of the samples. Considering the biological properties of RDNE, it can be suggested and utilized in the meat industry.

Abbreviations

Eos: Essential oils
RDNE: *Rosa damascena* essential oil nanoemulsion
MIC: Minimum inhibitory concentration

MBC: Minimum bactericidal concentration
stn: Salmonella enterotoxin gene
HCT 116: Colon cancer cell lines
HeLa: Cervix cancer cell lines
IC_{50}: Half maximal inhibitory concentration
TBC: Total bacterial count
TVB-N: Total volatile basic nitrogen
REO: Rose essential oil
gyrB: Gyrase gene B
invA: Invasion protein A
spvC: Salmonella plasmid virulence C
rpm: Revolutions per minute
GC-MS: Gas chromatography-mass spectroscopy
KI: Kováts indexes
TEM: Transmission electron microscope
DLS: Dynamic light scattering
TSB: Tryptic soy broth
MHA: Muller–Hinton agar
DMSO: Dimethyl sulfoxide
NA: Nutrient agar
BHI: Brain heart infusion
OD: Optical density
PCR: Polymerase chain reaction
DMEM: Dulbecco's modified eagle medium
TBE: Tris/Borate/EDTA
FBS: Fetal bovine serum
LSD: Least significant differences
RT: Retention time
SNE: Safflower oil nanoemulsion
OA: Oxygen absorber
CEO: Cumin essential oil.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Iman Saffari, Azad Khaledi, and Reza Sharafati Chaleshtori were responsible for investigation, methodology, formal analysis, and writing of the original draft. Abbasali Motallebi Moghanjoghi, Maryam Ataee, and Reza Sharafati Chaleshtori were responsible for visualization, data curation, and supervision (equal). All of the authors were responsible for writing, reviewing, and editing.

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