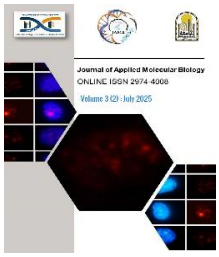
Journal of Applied Molecular Biology (JAMB)
Molecular Biology Research & Studies Institute, Assiut University, Egypt
ISSN 2974 – 4008
Volume 3 (2): July 2025

https://jamb.journals.ekb.eg



Impact of Capsicum Nano-Emulsion on *Salmonella Typhimurium* Isolated from Ready-to-Eat (RTE) Chicken Products

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ARTICLE INFO

Article History:
Received: 03-12-2024
Accepted: 24-03-2025
Oline: 27-07-2025

Keywords:
Salmonella Typhimurium, Shawarma, Chicken breast, Chicken thigh, Capsicum, Nanotechnology.

ABSTRACT

The current investigation was designed to detect *Salmonella* serovars in some of ready-to-eat (RTE) chicken meat products (Shawarma and grilled chicken). Ninety samples were examined, including 30 Shawarma, 30 grilled chicken breasts and 30 grilled chicken thighs, collected randomly from different localities in Assiut city, Egypt, in the period from December 2022 to January 2023. The obtained results showed that 11.11% of the samples were contaminated with *Salmonella* spp.in which *Salmonella Enteritidis* was isolated from 1Shawarma and 1 chicken thigh samples, while *Salmonella Typhimurium* was isolated from 2 Shawarma, 3 chicken thighs and 3 chicken breasts samples. A nano-emulsion was formulated using capsicum oil and subsequently analyzed using Zeta-Sizerand Fourier-transform infrared spectroscopy (FTIR). Along with a sensory examination of the Shawarma during storage, the essential oil' (EO) minimum inhibitory concentration (MIC) was measured. Capsicum essential oil (CEO) and its nano-emulsion (CNE) as 0.0975% exhibited an antibacterial activity against *Salmonella Typhimurium* inoculated in chicken meat with reduction% as 98.8 and 99.4%, respectively, without sensory impairment. In order to increase food protection and prevent food deterioration, this study recommended employing capsicum essential oil and its nano-emulsion as an antibacterial ingredient in food production.

1.INTRODUCTION

Ready-to-eat (RTE) foods are those prepared for immediate consumption, either raw, cooked, heated or refrigerated, without requiring further heat treatment [1]. One advantage of RTE foods is their convenience, allowing for quick preparation and consumption. However, in low-income countries, factors such as unsafe food preparation, poor hygiene, improper production and handling, and insufficient food safety regulations make these populations particularly vulnerable to foodborne illnesses [2]. The safety of RTE street food largely depends

on the quality of the raw ingredients used. Food can become contaminated by microorganisms at any stage of production, from processing to consumption [3].

Common sources of contamination include unsanitary utensils and unhygienic vendor [1]. Significant problems also arise from inadequate infrastructure, lack of clean water, improper food storage temperatures, and exposure to animals & pests [3]. Furthermore, foodborne illnesses claim the lives of the World Health Organization (WHO) estimates that each year 600 million cases of food-borne disease and over 400,000 fatalities occur globally as a result of food tainted with chemicals 56 million people annually and affect 7.7% of the global population [4 & 5]. Pathogens like *Shigella* and *Salmonella* spp. are frequently linked to RTE street food; 38% of the RTE foods that were evaluated contaminated with *Salmonella Typhimurium* and *Salmonella Enteritidis* serovars [1]. These pathogens can cross-contaminate or come from animal and human waste. Globally, one of the biggest causes of bacterial foodborne illnesses is still *Salmonella*, and poultry meat is a major vector for salmonellosis [6].

Chili peppers are the most widely used spice globally. Capsicum plants have been cultivated since ancient times, with over 20 species identified, most native to tropical South America [7]. Capsicums are rich in nutrients like zinc, vitamin C, iron, calcium, potassium and various vitamins. They also possess anti-inflammatory, anti-diabetic and anti-cancer properties [8].

Essential oils (EOs) are becoming more and more popular because of their antibacterial and antioxidant qualities and their potential to replace chemical additives in food [9]. However, controlling EOs can be challenging due to their sensitivity to light, moisture and air. Nano-emulsions (NEs) have gained attention for their ability to enhance the stability and efficacy of EOs in food applications [10]. The transformation of EOs using the nano-emulsification technology can maintain the integrity of the antibacterial agent and improve the antibacterial efficacy without affecting the quality of food [11].

The current study aimed to identify *Salmonella* isolates from fast food items, particularly chicken Shawarma sandwiches, and to assess the risk of food poisoning; in addition to the comparison of antibacterial activities of capsicum essential oil (CEO) and its nano-emulsions (CNE) against the isolated *Salmonella* species.

2. MATERIALS AND METHODS

2.1. Collection of samples:

A total of 90 random samples of chicken meat (30 Shawarma sandwiches, 30 grilled chicken thighs and 30 grilled chicken breasts) were collected from different restaurants in Assiut city, in the period from December 2022 to January 2023. The ready-to-eat (RTE) chicken samples were first removed from their packaging, cut into small pieces using sterilized knives, and then homogenized with a specific enrichment broth for bacteriological examination.

2.2. Isolation of *Salmonella* spp.:

225 ml of buffered peptone water (BPW) were used to pre-enrich 25 g of each sample, which were then incubated for 24 h at 37° C. One ml of the pre-enriched culture was added to 10ml of selective enrichment broth(Rappaport Vasiliadis broth, RV,HIMEDIA, M1491) and incubated for an additional 24h at 37°C. Xylose Lysine Deoxycholate (XLD) (HIMEDIA, M031F) agar was inoculated with a loopful of the selective enrichment culture, and incubated for 24 h at 37°C. The characteristic *Salmonella* colonies were red with or without black centers. These colonies were cultured onto nutritional agar (HIMEDIA, M1269S) slants and incubated for 24 h at 37°C since they were thought to be *Salmonella*. The isolates' slants were stored in a refrigerator for further confirmation and identification [12].

2.3. Biochemical reactions:

The following biochemical tests were used for confirmation, triple sugar iron (TSI) [13], urease test [13], indole test [14] and lysine iron agar [15].

2.4. Molecular detection of *Salmonella*:

Oligonucleotide primers used for the molecular detection of *Salmonella.*

Target Bacteria	Gene	Sequence	Amplified Product	Reference
Salmonella Typhimurium	*STM4495*	GGT GGC AAG GGA ATG AA CGC AGC GTA AAG CAA CT	915 bp	**Liu *et al.* [16]**
Salmonella Enteritidis	*sefA*	GCAGCGGTTACTATTGCAGC TGTGACAGGGACATTTAGCG	310 bp	**Akbarmehr *et al.* [17]**

2.4.1. DNA Molecular Weight Marker:

The DNA ladder used was the Gene Ruler 100 bp DNA ladder (Cat. no. SM0243) from fragment which has 10 bands with a size range of 100-1000 bp.

2.4.2. Cycling Conditions for PCR Primers:

The PCR conditions for the different primers were outlined below:

Cycling conditions of the different primers during cPCR

Target bacteria	Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
Salmonella Typhimurium	*STM4495*	94˚C 5 min.	94˚C 30 sec.	50˚C 1 min.	72˚C 1 min.	35	72˚C 10 min.
Salmonella Enteritidis	*sefA*	94˚C 5 min.	94˚C 30 sec.	52˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.

2.4.3. Agarose Gel Electrophoresis:

Agarose gel electrophoresis was used with molecular weight markers to check the PCR results for evidence of positive amplification. The ladder was mixed by pipetting, and 6 µl were directly loaded [18].

2.5. Preparation of Nano-emulsions:

Oil-in-water NEs of capsicum oil was prepared by dissolving 2 v/v% of tween 80 in deionized water at room temperature. Thenon-ionic surfactant was preferred due to its favorable oil-in-water (O/W) characteristic. Tween 80 displayed efficient solubility with essential oils, and also effectively minimized droplet diameter by adhering to the droplet's surface, improving the overall stability of O/W emulsion system. The mixture was shaken using a magnetic type stirrer for 10 min to obtain a homogenous solution. Then, the EO was added slowly and mixed with a direct driven stirrer (Hotplate stirrer, DAIHAN Scientific Co., Ltd, Korea) for 15 min followed by sonication of the resulting emulsion using a 25 kHz ultrasonic homogenizer (USH650, max power 650 watt) for 20 min. The end products of NEs were stored in the laboratory condition at 25° C. The nano-emulsion was prepared in the Animal Health Research Institute's Nanotechnology Research Unit in Assiut, Egypt [19].

2.5.1. Characterization of the prepared Nano-emulsions:

The mean droplet size and polydispersity index (PDI) was measured using a Zeta-Sizer (Malvern Instruments, UK). The measurements were conducted at 25°C, with each sample measured 3 times [20]. The data were processed using Zeta-Sizer® software (version 7.03).

Fourier-transform infrared spectroscopy (FTIR) was used to detect functional groups in the nano-emulsion, using a scanning range of 4000-500 cm^{-1} [21].

2.6. Determination of Minimum Inhibitory Concentration (MIC) of Capsicum Nano-emulsion:

The MIC was determined by the agar well diffusion method. Two-fold serial dilutions of the capsicum nano-emulsion were used. Zones of inhibition were measured to determine the MIC [22, 23].

2.7. Detection of antibacterial activity of CEO and CNE in the prepared chicken meat (Shawarma) against *Salmonella* during refrigerated storage:

2.7.1. Preparation of Shawarma samples:

One kg of chicken meat was used for Shawarma preparation. The prepared Shawarma was divided into 10portions: each part contained 100 g chicken meat. The 1stpart is a negative control (chicken meat only), 3 parts contained chicken meat inoculated with *Salmonella*, 3 sacs contained chicken meat with *Salmonella* and 0.0975%capsicum oil (0.0975ml/100g), and the last 3 sacs contained chicken meat with *salmonella* and 0.0975%capsicum nano-emulsion (0.0975 ml/100g). All the prepared samples were stored at 4°C±1°C and analyzed at zero time, after 1h, 2 h, 4 h, 24 h and 30 h.

2.7.2. Sensory Analysis of the prepared Chicken Meat samples before and during storage:

Sensory evaluation was conducted by 10employeesaged from 30 to 60 years old as 5males and 5 females working at Animal Health Research Institutein Assiut (AHRI). They evaluated Shawarma sandwiches (*Salmonella* free) based on appearance, odor, flavor and overall acceptability. The scoring scale ranged from excellent (5) to poor (1) [24].

2.7.3. *Salmonellacountin* the prepared samples; 10g chicken meat were subjected to a series of ten-fold serial dilutions by adding 90 ml of 0.1% sterilized peptone water, then 0.1 ml was spread out on XLD agar plates according to **ISO 6579-1** [12]. The plates were incubated at 37 °C for 24 h.

The microbial reduction percentages were calculated according to the following formula:

Microbial reduction% = (control count-test count)/control count × 100

2.8. Statistical analysis

Every experiment was run in triplicate (each sample repeated 3 times). Analysis of variance in one way was carried out utilizing Graphpad prism 8.0.2.to determine whether the variations within the samples are statistically significant. Origin Lab 2021 was used to construct the FTIR results for analysis and visualization.

3. RESULTS

The obtained curves highlighted the peak strength of phenols, as identified through FTIR spectroscopy (curves between T% 'transmission%' & wave number 'cm^{-1}'). In the reference spectrum for CEO, the peak ranged from 3282.64 to 3385.31 cm^{-1}, whereas in CNE, this peak appeared at 3421.54 cm^{-1}. The CNE expressed significant 2 C–H peak 2924.06 and 2856.32

cm^{-1}, but in CEO was shifted and observed at 2925.51 and 2854.42 cm^{-1} **(Figure 3)**. Aromatic rings had distinctive peaks between 1400 and 1600 cm^{-1}, while CEO was found at various peaks between 1465.77–1640.66 and from 1459.00–1642.52 cm^{-1}in its NE.

Table 1. Incidence of *Salmonella* spp. in the examined chicken meat samples.

Samples	No. of the examined samples	Positive samples	
		No.	%
Shawarma	30	3	10
Chicken breast	30	3	10
Chicken thigh	30	4	13.33
Total	90	10	11.11

Table 2. Frequency distribution of different *Salmonella* spp. from the examined samples.

Salmonella spp.	Shawarma		Chicken breast		Chicken thigh	
	No.	%	No.	%	No.	%
Salmonella Typhimurium	2	6.67	3	10	3	10
Salmonella Enteritidis	1	3.33	-	-	1	3.33

Table 3. Measurement of particle size and polydispersity index of CNE.

CNE	Average dynamic size	(PDI) Polydispersity index
	253±75.56	0.175

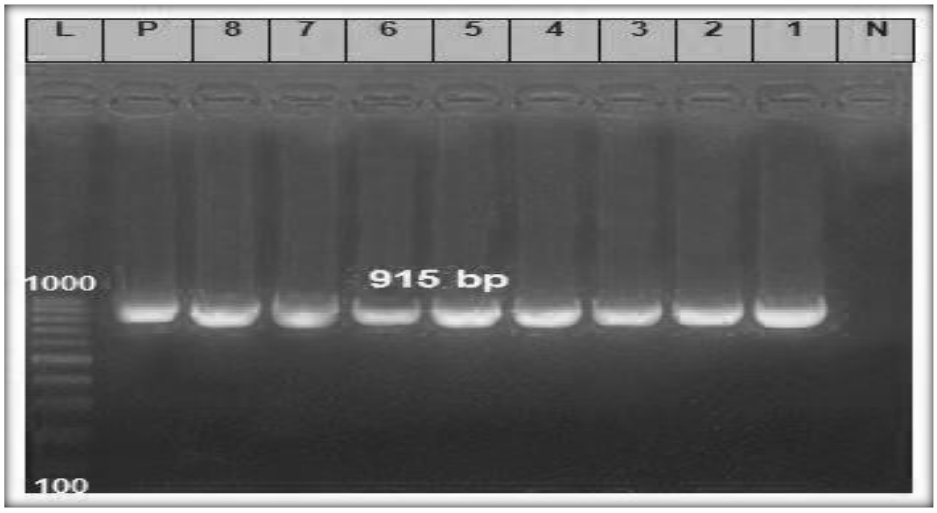

Figure 1. Agarose-gel electrophoresis of the PCR product for the isolated *Salmonella* spp.
Lanes 1 to 8. Positive amplification of 915bp fragment of *Salmonella Typhimurium.*
L. ladder, **P.** positive control, **N.** negative control

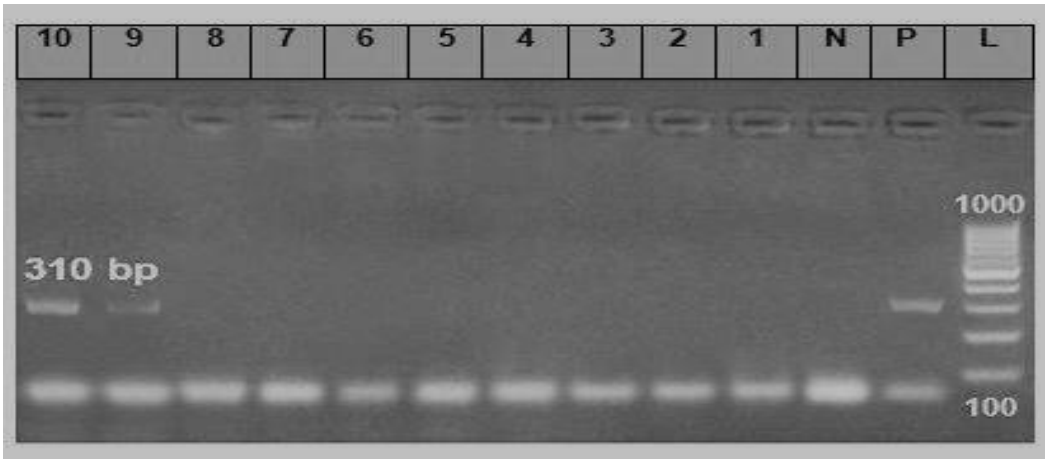

Figure 2. Agarose-gel electrophoresis of the PCR product for the isolated *Salmonella* spp.
Lanes 9 & 10. Positive amplifications of 310bp fragment of *Salmonella Entritidis.*
L. ladder, **P.** positive control, N. negative control

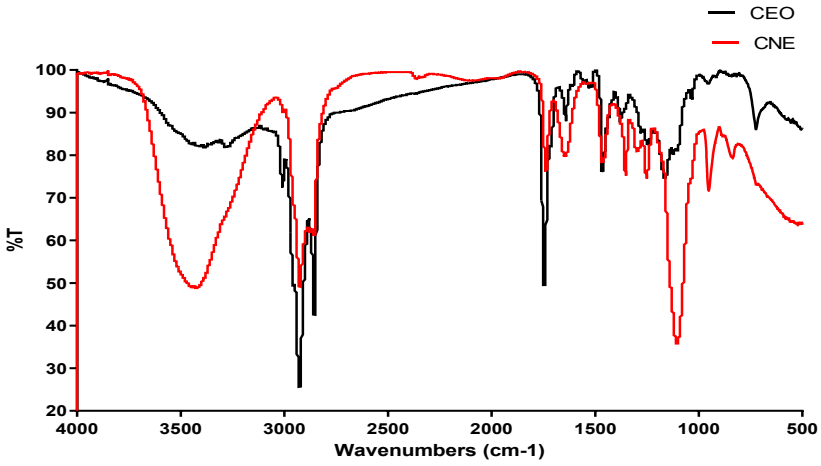

Figure 3. FTIR of capsicum and its nano-emulsion.

Table 4.MIC of CEO and CNE on *Salmonella Typhimurium* by agar well diffusion method.

Conc./ well	Zone of inhibition (mm)	
	Capsicum EO	Capsicum NE
100%	37	35
50%	35	33
25%	35	30
12.5%	34	25
6.25%	33	25
3.125%	31	24
1.56%	28	18
0.78%	26	17
0.39%	27	16
0.195%	20	14
0.0975%*	13	10

*The chosen concentration to be MIC for CEO and CNE against *Salmonella Typhimurium.*

Table 5. Sensory evaluation of capsicum NE in the prepared Shawarma.

Sensory parameter	Control		CEO		CNE	
Appearance	4.25±0.48	Excellent	3.7±0.2*	Very Good	3.48±.02*	Very Good
Odor	4.62±0.01	Excellent	3.9±0.01*	Very Good	3.99±.03*	Very Good
Flavor	4.9±0.01	Excellent	3.93±0.03*	Very Good	2.9±0.01*	Good
Overall Acceptability	4.59±0.01	Very Good	3.84±0.3*	Good	3.45±0.01*	Good

*Significant difference ($p< 0.05$) between treated (with CEO, CNE) and untreated (control) samples.

Table 6. The antibacterial effect of different CEOs and their NEs against *Salmonella Typhimurium* inoculated in the prepared Shawarma.

Storage Period	Control	CEO 0.0975%		CNE 0.0975%	
	Average count (cfu/g)	Average count (cfu/g)	Reduction%	Average count (cfu/g)	Reduction%
Zero time	$2.6x10^4$	$2.3x10^4$	11.5	$2.2x10^4$	15.3
1 h	$2x10^4$	$1.8x10^4$	10	$1.3x10^4$	35
2 h	$1.6x10^4$	$9.8x10^3$	38.75	$7.3x10^3$	54.3
4 h	$9x10^3$	$1.5x10^3$	83.3	$1.1x10^3$	87.7
24 h	$1.2x10^4$	$1.3x10^3$	89	$8x10^2$	93.3
30 h	$1.8x10^4$	$2x10^2$	98.8	$1x10^2$	99.4

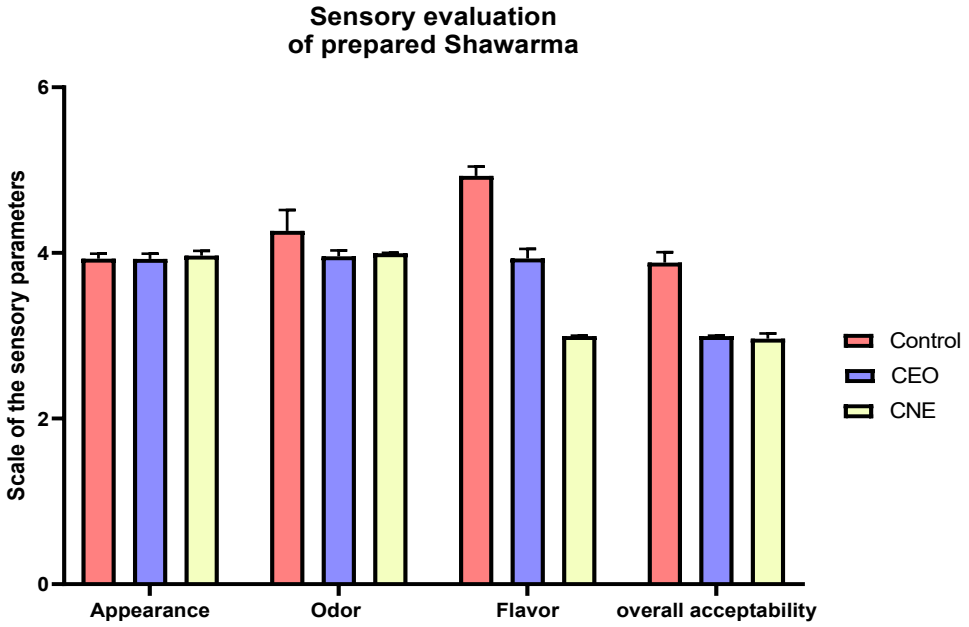

Figure 4 Sensory evaluation of capsicum NE in the prepared Shawarma.

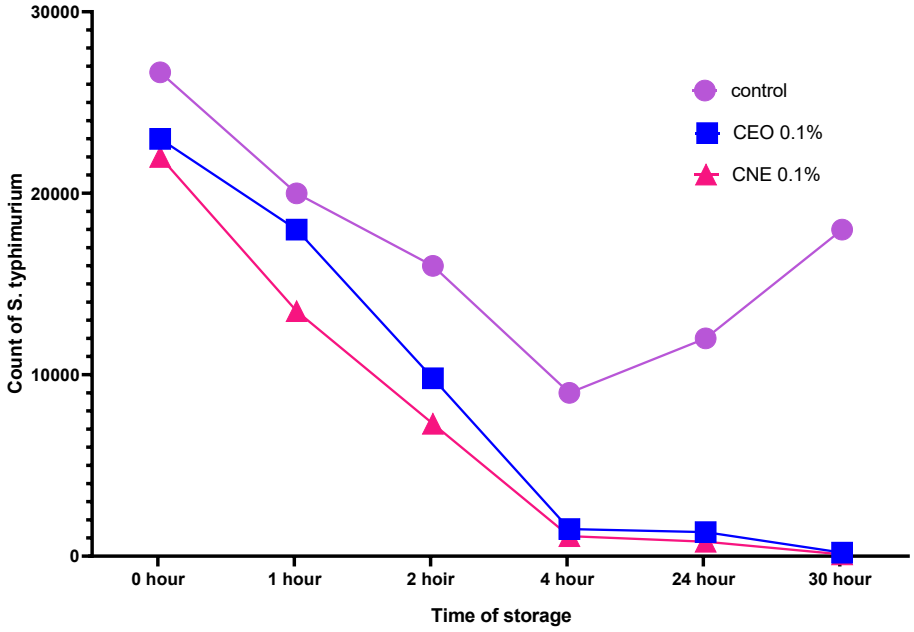

Figure 5 The effect of different CEOs and their NE against *Salmonella Typhimurium* inoculated in the prepared Shawarma.

4. DISCUSSION

Chicken meat is generally regarded healthier option than red meat since it contains lower levels of fat, cholesterol, saturated fats along with higher levels of quality protein compared to meat from other animal sources. Also, *Salmonella spp.* is one of the main reasons for human outbreaks related mainly to consumption of contaminated poultry products [25].

According to the data presented in Table 1, it was clear that 11.11% of the analyzed chicken meat samples were positive with *Salmonella* spp. The obtained result was in agreement with Hasan *et al.* [26] and Rahman *et al.* [27]but higher than results were achieved by Yamaner and Sürücü [28].The incidence of *Salmonella* spp. was distinctly illustrated in the Table 2 in which 2Shawarma (6.67%), 3 chicken breast (10%) and 3 chicken thigh (10%)samples, respectively were positive for *Salmonella Typhimurium* and 1Shawarma sample (3.33%)and 1 chicken thigh(3.33%) were positive for *Salmonella Enteritidis*.

Salmonella contamination in chicken meat is most commonly caused by handling uncooked chicken carcasses, contact with chicken feces and the resulting consumables, as well as cross-contamination from employees' hands, equipment, and utensils [29]. Also, elevated prevalence of *Salmonella* spp. may be attributed to inadequate hygienic practices during the processes of evisceration, scalding, de-feathering, slaughtering and carcass cutting. These procedures cause contamination of healthy and clean birds with those diseased or tainted carcasses, subsequently posing a risk to human health [30]. Mainly, initial contamination of foods is occurred during preparation; however, during storage, transit and packing, they are still at risk of contamination by harmful microbes [31].

The isolated *Salmonella Typhimurium* (Figure 1) and *Salmonella Entritidis* (Figure 2) were confirmed by molecular technique of polymerase chain reaction (PCR). It enables direct pathogen detection and is a quick technique with excellent sensitivity and specificity for particular DNA sequences [29].

There is necessity for consistently developing novel antimicrobial agents with distinct mechanisms of action [32]. Shawarma was chosen as a representative product and supplemented it with capsicum oil and capsicum oil nano-emulsion to be added to the food and highlight their effect against *Salmonella Typhimurium* that must not be present in the Shawarma.

Capsicum 'one of carotenoid' has many conjugated double bonds, which give it its deep red color and potent antibacterial qualities. When compared to other often researched carotenoids, capsicum showed better antibacterial action [33]. Herbs and spices are regarded as exceptional antioxidant and antibacterial agents, and they are mostly utilized as bio-preservatives and/or flavoring agents to enhance the organoleptic qualities of food items. Capsicum oil exhibits low water solubility, low stability, and variable oral bioavailability that diminish its usage as a direct food additive. So, the development of nano-emulsion capsicum oil resulting in enhanced capsanthin solubility without reducing its antioxidant activity and chemical stability in addition to improving its sensory qualities [34].

Nanoparticles have received important devotion worldwide as they have a strong antibacterial activity and their great stability physically and chemically. In food science, these qualities are mostly used to improve the product's overall quality, shelf life, flavor, taste, processability, etc [35]. Also, nano-emulsified essential oils have been shown to have potent antibacterial action against a wide range of pathogens and spoilage microorganisms in a variety of food systems [36].

An essential metric that shows the droplets' particle size distribution is the polydispersity index (PDI). The mean standard deviation of the droplet size divided by the average droplet diameter yields the PDI value [37]. The mean droplet diameter of CNE was 253±75.56 nm with PDI of 0.175as shown in Table 3, which indicated the stability of fabricated nano-emulsion [38].

The achieved results were higher than the results of the study of Eid *et al.* [39]. The results of PDI showed constancy and homogeneity of the generated nano-emulsion's, which was less than 0.5, because the surfactant ratio in CNE was employed to stop coalescence at room temperature for an extended storage time. Furthermore, since PDI measured the homogeneity of droplet size in a nano-emulsion, a higher number indicated a lower level of uniformity in droplet size [40].

Antibacterial activity of CEO and CNE against *Salmonella Typhimurium* by agar well diffusion method was shown in Table 4. It's concluded that CEO and CNE restricted the growth and multiplication of *Salmonella Typhimurium* due to its destroying action. Its mono-dispersed nature, reduced droplet width, and antibacterial ingredient content in CEO may have contributed to the initial bacterial inoculum exposure. The same findings were demonstrated in the study of Serio *et al.* [41].

Table 5 and Figure 4 showed that the tested Shawarma appearance, odor, flavor and overall acceptability were of good sensory characters. The selected concentrations of the determined MIC as 0.0975% for both CEO and CNE exhibited a pleasant flavor with good overall acceptability. When comparing the treated samples to the control, there were minor variations in each of the scores for the various sensory characteristics.

The obtained data in Table 6 and illustrated in Figure 5 declared the effectiveness of CEO and CNE(0.0975%) in inhibiting the *Salmonella Typhimurium* growth inoculated in the prepared Shawarma samples. Both CEO and CNE showed significant antimicrobial activity against *Salmonella Typhimurium*.

However, the essential oil showed higher effectiveness, which might be because capsicum is rich in bioactive chemicals that have significant antibacterial agent. The fatty acids found in capsicum, which have been shown to be potent antibacterial substances, may also be connected to its antimicrobial activity [42]. Membrane instability, increased permeability, disruption of the electron transport chain, and inhibition of certain membrane-associated enzymes are some of their modes of action. Fatty acids, such as oleic and linoleic acids, also considerably impede the vital trans-membrane proteins [43].

5. CONCOLUSION

Capsicum essential oil (CEO) and its nano emulsion (CNE) as 0.0975% showed an antibacterial activity against *Salmonella Typhimurium* inoculated in the prepared chicken meat 'Shawarma' with reduction% as 98.8 and 99.4%, respectively, without sensory impairment

Authors' contributions

A.B., A.M.A, and W.M.E contributed to the conceptualization, methodology, practical work, and writing of the original draft. **All authors** have read and approved the final manuscript and agree to its submission.

Funding

This research received no external funding.

Data Availability Statement

Not applicable

Conflicts of Interest

The authors declare no conflict of interest.

Consent to Participate

Not applicable

Consent to Publish

Not applicable

6. REFERENCES

[1] Raza, J., Asmat, T.M., Mustafa, M.Z., Ishtiaq, H., Mumtaz, K., Jalees, M.M. and ur Rehman, Habiba (2021): Contamination of ready-to-eat street food in Pakistan with *Salmonella* spp.: Implications for consumers and food safety. International Journal of Infectious Diseases, 106, 123-127.

[2] Salamandane, C., Lobo, M. L., Afonso, S., Miambo, R. and Matos, O. (2021): Occurrence of intestinal parasites of public health significance in fresh horticultural products sold in Maputo markets and supermarkets, Mozambique. Microorganisms, 9(9):1806.

[3] Andrade, A.A., Paiva, A.D. and Machado, A.B.F. (2023): Microbiology of street food:understanding risks to improve safety. J. Appl.Microbiol.134. https://doi.org/10.1093/jambio/lxad167.

[4] WHO (World Health Organization) (2015): Estimates of the Global Burden of Foodborne Diseases: Foodborne Disease Burden Epidemiology Reference Group 2007–2015; World Health Organization (WHO): Geneva, Switzerland, pp. 1–15.

[5] Lee, H. and Yoon, Y. (2021):Etiological agents implicated in foodborne illness worldwide. Food Sci. Anim. Resour. 41, 1–7.

[6] Lamichhane, B.; Mawad, A.M.M.; Saleh, M.; Kelley, W.G.; Harrington, P.J.; Lovestad, C.W.; Amezcua, J.; Sarhan, M.M.; El Zowalaty, M.E. and Ramadan, H. (2024): Salmonellosis: An Overview of Epidemiology, Pathogenesis, and Innovative Approaches to Mitigate the Antimicrobial Resistant Infections. Antibiotics 2024, 13, 76.

[7] Kim, S., Park, M., Yeom, S.I., Kim, Y.M., Lee, J.M., Lee, H.A. and Choi, D. (2014): Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. Nature genetics, 46(3), 270-278.

[8] Parvez, G.M. (2017): Current advances in pharmacological activity and toxic effects of various Capsicum species. *Int. J. Pharm. Sci. Res*, *8*(5), 1900-1912.

[9] Ibáñez, M.D. and Blázquez, M.A. (2020): Curcuma longa L. Rhizome Essential Oil from Extraction to Its Agri-Food Applications. A Review, Plants 2021, 10, 44. *Plant Essential Oil with Biological Activity*, 223.

[10] Shahavi, M.H., Hosseini, M., Jahanshahi, M., Meyer, R.L. and Darzi, G.N. (2019): Evaluation of critical parameters for preparation of stable clove oil nano-emulsion. Arab. J. Chem. 12, 3225–3230. https://doi.org/10.1016/j.arabjc.2015.08.024.

[11] Noori, S.; Zeynali, F. and Almasi, H. (2018): Antimicrobial and antioxidant efficiency of attributes of chicken breast fillets. Food Control, 84: 312–320. doi:10.1016/j.foodcont.2017.08.nanoemulsion-based edible coating containing ginger(Zingiberofficinale) essential oil and its effect on safety and quality015.

[12] ISO (International Standards Organization) 6579-1 (2020): Microbiology of the food chain. Horizontal method for the detection, enumeration and serotyping of *Salmonella*. Part 1: detection of *Salmonella* spp. ISO norm. International Standardization Organization ed., Geneva, Switzerland.

[13] Ewing, W.H. (1986):Edwards and Ewing's identification of *Enterobacteriaceae* 4th ed. Elsecvier Science Publishing Co. Inc. USA.

[14] MacFaddin, J.E. (1980):Biochemical tests for the identification of medically important bacteria, 2nd ed. Wilkins and Wilkins, Baltimore. M. D.

[15] Koneman, E.W., Schrechenberger, P.C., Allen, S.D., Winn, W.C. and Janda, W.M. (1992):Color Atlas and textbook of diagnostic microbiology. 4th ed. Winters, R. (Ed.) Lippincott Company, Philadelphia.

[16] Liu, B.; Zhou, X.; Zhang, L.; Liu, W.; Dan, X.; Shi, C. and Shi, X. (2012):Development of a novel multiplex PCR assay for the identification of *Salmonella enteric Typhimurium* and *Enteritidis*. Food Control 27 (2012) 8-93.

[17] Akbarmehr, J., Salehi, T.Z. and Brujeni, G.H.N. (2010):Identification of *Salmonella* isolated from poultry by MPCR technique and evaluation of their *hspgro*EL gene diversity based on the PCR-RFLP analysis. African Journal of Microbiology Research, 4(15):1594-1598.

[18] Sambrook, J. Fritscgh, E.F. and Mentiates, T. (1989): Molecular cloning. A laboratory manual, Vol1., Cold spring Harbor Laboratory press, New York.

[19] Ghosh, V.; Mukherjee, A. and Chandrasekaran, N. (2013):Ultrasonic Emulsification of Food-Grade Nanoemulsion Formulation and Evaluation of its Bactericidal Activity. Ultrasonics Sonochemistry,20(1): 338-44.

[20] Hassanien, A.A., Shaker, E.M., El-Sharkawy, E.E. and Elsherif, W.M. (2021):Antifungal and antitoxin effects of propolis and its nano-emulsion formulation against Aspergillus flavus isolated from human sputum and milk powder samples. Veterinary World, 14(9), 2306.

[21] Gurpreet, K. and Singh, S.K. (2018): Review of Nanoemulsion Formulation and Characterization Techniques. Indian Journal of Pharmaceutical Sciences, 80(5):781-789. DOI: 10.4172/pharmaceutical-sciences.1000422.

[22] Ponce, A.G.; Fritz, R.; Del Valle, C.E. and Roura, S.I. (2003): Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. Lebensmittel-Wissenschaftund –Technologie; 36: 679–684

[23] Moreira, M.R.; Ponce, A.G.; De Valle, C.E. and Roura, S.I. (2005): Inhibitory parameters of essential oils to reduce a foodborne pathogen. Lebensmittel-Wissenschaft und-Technologie-LWT; 38: 565–570.

[24] Sahn, M.A., El-Sharnouby, S.A. and Moharram,Y.G. (1995):New rapid salted bouri (Mugilcephalus) fish products. Egypt. J. Food Science. 23: 197-206.

[25] Tarabees, R., Elsayed, M.S., Shawish, R., Basiouni, S. and Shehata, A.A. (2017): Isolation and characterization of *Salmonella Enteritidis* and *Salmonella Typhimurium* from chicken meat in Egypt. The Journal of Infection in Developing Countries, 11(04), 314-319.

[26] Hasan, H.J., Abdulwahid, M.T. and Ayyez, H.N. (2023): Molecular Detection and Phylogenetic Analysis of Citrobacter Freundii Isolates from Broilers in AL-Diwaniyah Province of Iraq. International journal of health sciences, 6(S9), 4736-4752.

[27] Rahman, M.M., Hossain, H., Chowdhury, M.S.R., Hossain, M.M., Saleh, A., Binsuwaidan, R. and El Zowalaty, M.E. (2024): Molecular characterization of multidrug-resistant and extended-spectrum β-lactamases-producing *Salmonella enteric serovars enteritidis* and *Typhimurium* isolated from raw meat in retail markets. Antibiotics, 13(7), 586.

[28] Yamaner, Ç. and Sürücü, N. (2024):Assessment of the Microbiological Quality and Effect of Public Health of Ready-to-Eat Salad Samples in Isparta. Foodborne Pathogens and Disease.

[29] Kanaan, M.H. (2023): Prevalence and antimicrobial resistance of *Salmonella enteric serovars Enteritidis* and *Typhimurium* isolated from retail chicken meat in Was it markets, Iraq. Veterinary World, 16(3), 455-463.

[30] Hassan, A.R.H., Salam, H.S. and Abdel-Latef, G.K. (2016): Serological identification and antimicrobial resistance of *Salmonella* isolates from broiler carcasses and human stools in Beni-Suef, Egypt. Beni-Suef University Journal of Basic and Applied Sciences, 5(2), 202-207.

[31] Klištincová, N., Pin, L., Puškárová, A., Giannino, D., Bučková, M., Lambreva, M.D. and Pinzari, F. (2024): From farm to fork: fungal and bacterial contaminants and their diagnostics in the production steps of ready-to-eat salads. Trends in Food Science & Technology, 104573.

[32] Yu, H.H., Chin, Y.W. and Paik, H.-D. (2021): Application of natural preservatives for meat and meat products against food-borne pathogens and spoilage bacteria: a review. Foods 10, 2418.

[33] Kaulmann, A. and Bohn, T. (2014): Carotenoids, inflammation, and oxidative stress—implications of cellular signaling pathways and relation to chronic disease prevention. Nutrition research, 34(11), 907-929.

[34] Kulkarni, M., Goge, N. and Date, A.A. (2020): Development of nano-emulsion preconcentrate of capsanthin with improved chemical stability. *ASSAY and Drug Development Technologies*, *18*(1), 34-44.

[35] Kumar, P., Mahajan, P., Kaur, R. and Gautam, S. (2020):Nanotechnology and its challenges in the food sector: A review. Materials Today Chemistry, 17, 100332.

[36] McClements, D.J., Das, A.K., Dhar, P., Nanda, P.K., and Chatterjee, N. (2021):Nano-emulsion-based technologies for delivering natural plant-based antimicrobials in foods. Frontiers in Sustainable Food Systems, 5, 643208.

[37] Schober, G.B., Story, S. and Arya, D.P. (2024): A careful look at lipid nanoparticle characterization: analysis of benchmark formulations for encapsulation of RNA cargo size gradient. Scientific Reports, 14(1), 2403.

[38] Ellithy, M.M.A. and Abdrabo, R.A.M. (2024): Plant Based Extract Oil-Based Nano emulsions: Impact on Human Melanoma Cell Line. *Asian Pacific Journal of Cancer Prevention*, *25*(5), 1663-1671.

[40] Eid, A. M., Natsheh, H., Issa, L., Zoabi, M., Amer, M., Mahamid, E. and Mousa, A. (2024): Capsicum annuum Oleoresin Nanoemulgel-Design Characterization and In vitro Investigation of Anticancer and Antimicrobial Activities. Current Pharmaceutical Design, 30(2), 151-160.

[41] Yuliani, S., Muchtadi, T.R. and Syakir, M. (2018): Changes in characteristics of nano-emulsion of cinnamon oil and their relationships with instability mechanisms during storage. Journal of Food Processing and Preservation, 42(10): e13745.

[42] Serio, A., Maggio, F., Ben Hsouna, A., Ben Saad, R., Taiti, C. and Garzoli, S. (2024): Exploring the Metabolome and Antimicrobial Properties of *Capsicum annuum* L. (Baklouti and Paprika) Dried Powders from Tunisia. Molecules, 29(22), 5236.

[43] Yoon, B.K., Jackman, J.A., Valle-González, E.R. and Cho, N.J. (2018): Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. International journal of molecular sciences, 19(4): 1114.

[44] Won, S.R., Hong, M.J., Kim, Y.M., Li, C.Y., Kim, J.W. and Rhee, H.I. (2007): Oleic acid: an efficient inhibitor of glucosyl transferase. FEBS letters, 581(25), 4999-5002.