

## Effect of Cinnamon and Rosemary Nano-Emulsions against *Escherichia coli* O157:H7 Isolated from Shawarma Sandwiches

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### Abstract

The study was conducted to determine the incidence of *E. coli* serotypes in beef and chicken shawarma sandwiches, particularly *E. coli* O157:H7. The antibacterial activity of nano-emulsions (NEs) was evaluated, such as cinnamon and rosemary, against *E. coli* O157:H7. A total of 100 samples from ready-to-eat beef and chicken meat shawarma sandwiches (50 each) were isolated and identified as *E. coli* using a Sorbitol MacConkey (SMAC) agar assay. The results were confirmed by serology and polymerase chain reaction PCR using the *phoA* gene, which is specific for *E. coli* and the *fliCH7* gene, which is specific for *E. coli* O157:H7. Cinnamon and rosemary NEs were prepared, characterized, and evaluated in vitro to determine the minimum inhibitory concentration (MIC) using a well diffusion method. The incidence of *E. coli* species isolated from beef and chicken shawarma sandwiches samples was 58% and 10%, respectively. While, *E. coli* O157:H7 was detected in 6% of beef shawarma sandwiches only. Both cinnamon and rosemary NEs exhibited antimicrobial activity against *E. coli* O157:H7, and the cinnamon NE was more effective compared with that of rosemary with a mean inhibition zone of  $7.67 \pm 1.202$  mm and  $7 \pm 0.5774$  mm at MIC 0.78% and 3.125%. Further studies are required to detect the safety of effectiveness of natural NEs in the food industry.

### Keywords:

*E. coli* O157:H7, Nano-emulsions, PCR, Shawarma.

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## Introduction

Shawarma is a type of grilled meat loaf known for its delectability and relatively inexpensive cost. Shawarma sandwiches are the most popular ready to eat (RTE) dishes in Egypt made of beef or chicken meat and sold at many fast-food restaurants (Rodríguez-Cavallini et al., 2010).

The most prevalent pathogens detected in RTE meat are *E. coli*. *E. coli* O157:H7 infections are becoming more pathogenic, resulting in numerous foodborne outbreaks. The majority of RTE food-related illnesses may due to bad sanitation measurement during different parts of manufacturing, storage and manipulation (McCown and Grzeszak, 2010, Adzitey et al., 2019, Adzitey et al., 2020).

The natural essential oil and their nano-emulsions are gaining traction as new food emerging technique by using them in different food industry to prolong the storage duration, manage food safety concerns, and perhaps replace synthetic preservatives (Amin, 2013).

Cinnamon (*Cinnamomum zeylanicum*) and rosemary (*Rosmarinus officinalis L.*) have wide anti-bacterial range (El Bayomi et al., 2021). They are added to food frequently because it is palatable and safe to consume. The primary active ingredients include rosmarinic and carnosic acid, which are used extensively in the food sector. In meat Cinnamon is used as a carminative, flavoring agent, culinary herb, antioxidant, and prevents microbial growth and delayed lipid oxidation (rancidity) (Jongberg et al., 2013).

Nano-emulsions (NEs) are essential oils (EOs) extracted from natural plants, such as cinnamon and rosemary, to render them more hydrophilic and improve oil solubility. NE formulations have a larger surface area and they are effective against a wider range of germs, it protects the active

ingredients from physicochemical modification. Compared with free EOs, they are considerably more stable. Because of the very small droplet diameter of NEs, Brownian motion effects dominate gravitational forces, and the steric repulsion range is less affected by particle size, which prevents the aggregation or agglomeration (Meneses et al., 2019).

The hygienic conditions of RTE meat manufacturing were indicated by the microbiological quality, so they must be checked routinely. Exploring novel alternatives to manage foodborne diseases, with a focus on strategies that minimize the public health hazards, is needed (Elsherif and Elhabtey, 2020).

Therefore, the goal of study was to use bacteriological and serological methods for isolation of *E. coli* and its different serotypes in beef and chicken shawarma sandwiches, which was subsequently verified by PCR. Also, the effect of cinnamon and rosemary NEs antibacterial activity against *E. coli* O157:H7 isolated from beef and chicken shawarma sandwiches available from the fast-food restaurants at Assiut City, Egypt.

## Materials and methods

### Ethical approval:

All experimental procedures in the present study were performed and approved in accordance with the Ethics Committee of the Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt.

## Materials and methods

### 1. Collection of meat samples

A total of 100 samples from RTE beef and chicken shawarma sandwiches (50 each) were obtained from fast-food restaurants at Assiut City, Egypt.

## 2. Isolation and Identification of *E. coli*

The isolation of the *E. coli* was done according to Sancak et al., (2015) and Elsherif and Ali, (2020) using Sorbitol MacConkey (SMAC) agar plates. Suspected colonies were morphologically and biochemically identified (MacFaddin, 2000).

### 2.1. Serological typing of the isolated *E. coli*

The isolates were serotyped at the Food Analysis Center, Faculty of Veterinary Medicine, Benha University, Egypt, according to Kok et al., (1996) using a rapid diagnostic Anti-sera kit for *E. coli* (Denka Seiken Co., Japan) to diagnose enteropathogenic types.

### 2.2. Detection of *E. coli* and *E. coli* O157:H7 using PCR.

DNA was extracted using QIAamp DNA Mini kit (Qiagen, Germany) following the manufacturer's instructions. The primers used were *phoA* for *E. coli* detection and *fliC* for *E. coli* O157:H7. The PCR reaction was done according to Hu et al. (2011) and Fratamico et al., (2000) using Applied Biosystems 2720 thermal cycler. The products of PCR were separated on 1.5% agarose gels and detected with ethidium bromide and photographed by gel documentation system (Alpha Innotech, Biometra. 100 bp ladder (Fermentas, Germany) was used.

## 3. Preparation of cinnamon and rosemary NE

The cinnamon EO was added to Tween 80 in volume 1:5 (v/v) to fabricate their NE (Simge et al., 2017). Nano-emulsion of rosemary was fabricated by dissolving 2:1 (v/v%) of Tween 80 and Span 20 in deionized water at room temperature and the oil concentration was adjusted to 10% in the NE (Mossa et al., 2019). The mixture was shaken for 10 min using a magnetic stirrer till a homogenous solution was

obtained. Then, slowly added the essential oil and mixed with direct driven stirrer (DAIHAN Scientific Co., Ltd, Korea) for 15 min followed by sonication using a 25 kHz ultrasonic homogenizer (USH650, max power: 650 watt) for 20 min. A 0.22 µm (200 nm) filter was used for the filtration of all NEs. The characterization of NEs was done in Nanotechnology Unit, Al-Azhar University, Assiut using Zeta-Sizer (3000 HS, Malvern UK).

## 4. The effect of cinnamon and rosemary NEs on *E. coli* O157:H7

The antibacterial activity of cinnamon and rosemary NEs at various concentrations was assessed using the agar well diffusion method. The isolates were grown in BHI broth and incubated for 24 h at 37°C. By adding sterile saline to the growing culture, which was adjusted to the turbidity of a 0.5 McFarland standard then diluted triple to reach (10<sup>5</sup> CFU/ml) (Gupta et al., 1992).

A sterile cork pourer was used to make 3–4 mm diameter agar wells on the solidified Mueller Hinton agar dishes with spreading the prepared bacterial suspension (100 µl). Each well filled with 80 µL in the following order: double fold serial dilutions from NEs (100 to 0.78%), one well in each plate represented as positive control. After 45 min at room temperature inside laminar flow, the plates were incubated and the inhibitory zone sizes were measured (Elsherif and Ali, 2020).

## 5. Statistical analysis

SPSS (SPSS Inc., Chicago, IL, USA) was used to calculate the least significant difference for the mean and standard error values at  $p < 0.01$ .

## Results

### 1. Incidence of *E. coli*

*E. coli* fermented sorbitol was recovered from 17 (34%) of the 50 beef and 5 (10%) of the 50 chicken shawarma sandwich samples examined (Table 1).

Only 12 (24%) of the 50 samples were tested positive for *E. coli* non fermented sorbitol, which was found in beef shawarma sandwiches.

## 2. Serological typing of the isolated *E. coli*

The data in Table 2 showed that different *E. coli* serotypes in beef shawarma sandwiches such as O125: H21 (4%), O44: H18(4%), O26: H11(8%), O128: H2(2%), O91: H21 (2%), and O159 (2%). O55: H7 (8%), O55: H6 (2%), O11 (2%). The colonies which non-fermented sorbitol (O157:H7 serotypes) identified in 6% of the beef shawarma sandwich samples. On the other hand, the serologically detected *E. coli* fermented sorbitol isolates from the

chicken shawarma sandwich samples were O2: H6 (2%) and O78 (6%).

## 3. Characterization of the prepared NEs

To characterize the NE droplets, a Zeta-Sizer was used to measure the dynamic diameter of fabricated nanomaterial. Table 3 summarizes the findings. The DPs for the cinnamon and rosemary NEs were  $65.13 \pm 31.13$  nm and  $70.54 \pm 45.6$  nm, whereas poly-dispersing indexes were 0.201 and 0.312, respectively that indicate the stability of NEs.

The PCR results are shown in Fig. 1 and Fig. 2.

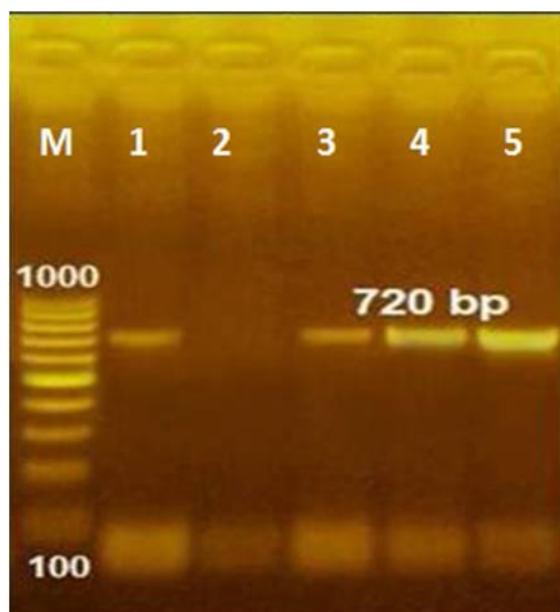
**Table 1. Incidence of *E. coli* in beef and chicken shawarma sandwich samples on SMAC agar.**

Sample type	<i>E. coli</i> fermented sorbitol (FS)		<i>E. coli</i> not fermented sorbitol (NFS)		Total	
	+ve samples (N)	%	+ve samples (N)	%	+ve samples (N)	%
Beef Shawarma sandwich (No.=50)	17	34	12	24	29	58
Chicken Shawarma sandwich (No.=50)	5	10	0	0	5	10
Total (No.=100)	22	44	12	12	34	34

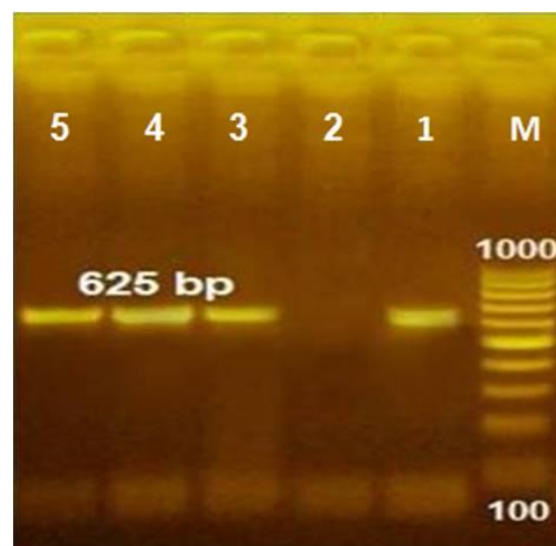
**Table 2. *E. coli* serotypes in beef and chicken shawarma sandwich samples.**

<i>E. coli</i> serotypes	Beef shawarma Sandwich (No.=50)		Chicken shawarma Sandwich (No.=50)	
	No.	%	No.	%
O125:H21 (ETEC)	2	4	-	-
O44:H18 (EAEC)	2	4	-	-
O26:H11 (EHEC)	4	8	-	-
O128:H2 (EETEC)	1	2	-	-
O91:H21 (EHEC)	1	2	-	-
O159 (EIEC)	1	2	-	-
O <sub>2</sub> :H <sub>6</sub> (EPEC)	-	-	1	2
O <sub>78</sub> (EETEC)	-	-	3	6
O55:H7 (EPEC)	4	8	-	-
O157:H7 (EHEC)	3	6	-	-
O55:H6 (EPEC)	1	2	-	-
O11 (EETEC)	1	2	-	-

%: calculated according to the total No. of samples (50).



**Fig. 1.** Results of *phoA* gene of *E. coli* by PCR .M: Marker; lane 1: positive control; lane 2: negative control; lanes 3–5: positive isolates.



**Fig. 2.** Results of *fliC* gene of *E. coli* O157: H7. M: Marker; lane 1: positive control; lane 2: negative control; lanes 3–5: positive isolates.

**Table 3.** Droplet size distribution of cinnamon and Rosemary nano emulsion (NE).

Type	PDI	z-average(d.nm)	Size $\pm$ SD	% intensity
Cinnamon NEs	0.201	66.78	65.13 $\pm$ 31.13	100%
Rosemary NEs	0.312	76.48	70.54 $\pm$ 45.6	100%

#### 4. The inhibitory effect of cinnamon and rosemary NEs on *E. coli* O157:H7

The anti- *E. coli* O157:H7 effect with minimum inhibitory concentrations were

detected by an inhibition zone of 7.67 $\pm$ 1.202 mm and 7 $\pm$ 0.5774 mm with MIC of 0.78 and 3.125% for the cinnamon and rosemary NEs, respectively (Table 4, Fig. 3).

**Table 4.** Determination of the minimum inhibitory concentration (MIC) of cinnamon and rosemary NEs on *E. coli* O157:H7 isolates by the Mean  $\pm$ SE of the Inhibition zone (mm).

NEs oils dilution (%)	Inhibition zone (mm)	
	Cinnamon NE	Rosemary NE
100	17 $\pm$ 0.5774	15.33 $\pm$ 0.333
50	15 $\pm$ 1.155	13.33 $\pm$ 1.202
25	13 $\pm$ 1.528	11.3 $\pm$ 1.453
12.5	12.67 $\pm$ 1.155	10 $\pm$ 1.000
6.25	10.67 $\pm$ 0.3333	8.3 $\pm$ 0.8819
3.125	10.33 $\pm$ 1.453	7 $\pm$ 0.5774
1.56	9.33 $\pm$ 0.8819	NZ
0.78	7.67 $\pm$ 1.202	NZ

NZ: No inhibitory zone



**Fig. 3.** Effect of cinnamon and rosemary nano-emulsions against *Escherichia coli* O157:H7 Isolates

### Discussion

Table 1 shows that the percentage of *E. coli* isolated from beef shawarma was higher compared with that observed by Al-Mutairi, (2011), Nimri et al., (2014), Hassanin et al., (2014), and Karmi, (2019) who isolated (31%), (20%), (25.8), (33.3%), and (15%) of *E. coli*, respectively, however, the percentage was lower than that (78%) reported by El Gohary, (1993). Regarding, chicken shawarma sandwiches results were lower than that found by Nimri et al., (2014), Hassanin et al., (2014), and Sharaf-Eman and Sabra-Sherifa, (2012), who detected *E. coli* at a percentage of 33%, 33.3%, and 20%, respectively.

A lack of sufficient sanitation, cross-contamination during preparation, use of unsuitable, inexpensive ingredients, poor storage, or production conditions may all contribute to the contamination of both beef and chicken shawarma samples by *E. coli* (Farrokh et al., 2013; Elsherif and Ali, 2020). In addition, large amounts of beef and chicken prepared and kept for long time with no proper control promotes the multiplication of bacteria that can taint foods from a variety of sources (Saad et al., 2018).

*E. coli* O157:H7 could be detected in percentage of 6% of beef shawarma (Table 2). This percentage in Table II was higher than that obtained by Karmi, (2019) who reported a value of 5% in beef shawarma; however, the results were lower than that of Nimri et al., (2014) who reported a value of 14.6%.

*E. coli* species and *E. coli* O157:H7 were confirmed by detection of *phoA* gene (Fig. 1) and *fliC* gene (Fig. 2) using PCR, respectively. The *E. coli* O157:H7 is responsible for different outbreaks in human (Wang et al., 2002; Myataza et al., 2017).

Chicken is usually less contaminated with pathogens than beef because of the method of slaughtering, hygienic measures applied in slaughterhouse and production conditions. The results were proved that beef shawarma was only contaminated by *E. coli* O157:H7, whereas it was not detected in chicken shawarma. Post-cooking contamination from handlers during preparation, undercooked cone during slicing, surrounding raw meat juices, or partially cooked cones that are stored for a long time to cool and reheat that lead to fluctuation in temperature may permit bacterial growth can cause an increase in the contamination of the end product (Government of Canada, 2008; Nimri et al., 2014).

### Characterization of NEs

The perfect stability of the fabricated NEs was proved by lower PDI (> 0.5) because of the ratio of surfactants, which are used to prevent coalescence at ambient temperature and maintain stability for period. Most PDI indicates lower uniformity of droplet size for the cinnamon and rosemary NEs, which was  $65.13 \pm 31.13$  and  $70.54 \pm 45.6$ , respectively (Table 3).

This result for rosemary NEs was lower compared with that of Mossa et al., (2019) and Restrepo et al., (2018) who studied average particle of rosemary NE size of 139.9 nm and a range from  $164 \pm 9$  to  $676 \pm 26$  nm. This indicates that the surfactant-to-oil ratio (SOR) had a significant effect on the mean particle size, which dropped as SOR increased. Martin Piñero et al., (2019) demonstrated that particle sizes smaller than 200 nm may be obtained is with a 30% increase in surfactant and a reduction of the EO concentration. For a NE, the PDI represents droplet size homogeneity.

Both rosemary and cinnamon NEs have inhibitory effect on *E. coli* O157:H7 (Table 4, Fig 3); however, cinnamon NEs were more active because of the effects of cinnamon on Gram-negative bacteria, which inhibits bacterial efflux pumps and restores intracellular concentrations. Cinnamaldehyde and eugenol are the primary active compounds present in cinnamon. Cinnamaldehyde inhibits N3-oxohexanoyl-L-homoserine lactone (3-oxo-C6-HSL) and AI-2, which may affect bacterial QS-regulated activities (Niu et al., 2006; El Bayomi et al., 2021). Eugenol inhibits the production of some enzymes required for microbial growth, thus limiting their proliferation (Parasa et al., 2012). The antibacterial effect of rosemary NE may be attributed to its main component,  $\alpha$ -pinene, as well as other components that exhibit antimicrobial effect by invasion of cell walls and cytoplasmic membranes and destructing their structure similar to lipophilic substance (Stojanović-Radić et al., 2010). However, rosemary NE shows little effect on Gram-negative bacteria.

## Conclusion

Our study indicates that beef and chicken shawarma sandwiches are

contaminated by several *E. coli* serotypes, but only beef shawarma sandwiches contain *E. coli* O157:H7. In addition to demonstrating an increase in *E. coli*, which is concern for consumers, we determined the antimicrobial activity of two natural compounds, cinnamon and rosemary NEs, in vitro against *E. coli* O157:H7. Based on MIC results, the cinnamon NE was more effective than rosemary NE with percentage of 0.78% and 3.125%, respectively.

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## Conflict of interest statement

The authors declare that they have no conflict of interest.

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