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Novel approach of using nanotechnology for improving meat product quality

Nahla A. Abo EL-Roos*, Manal A. Esam , Elsayed M. Abd- Elaaty***

*Food Hygiene Department, Animal Health Research Institute, Agriculture Research Center of Egypt, Shebin El koom Branch, Egypt.
** Veterinary Doctor at Directorate of Veterinary Medicine in Giza

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ABSTRACT

A total of 90 random samples of meat products represented by minced fresh beef, beef burger and kofta (30 of each) were collected from various markets in Kalyobia governorate, Egypt to assess their bacteriological profile. The average values of APC were 4.46 ± 0.09, 4.69 ± 0.11 and 4.78 ± 0.26 (log cfu /g) in examined minced fresh beef, beef burger and kofta samples, respectively. Moreover, Coliforms counts ranged from 2.48 to 3.95 with an average 3.41± 0.08 (log cfu/g) in minced fresh beef, 2.6 to 4.81 (3.6 ± 0.12 log cfu /g) in beef burger and 2.69 to 4.89 (3.92 ± 0.28 log cfu /g) in kofta. The mean values of *Staph. aureus* count (log cfu /g) in examined samples of minced, beef burger and kofta were 2.38 ± 0.06, 2.89 ± 0.07 and 2.68 ± 0.11, respectively. Furthermore, the incidence of different enteropathogenic serotypes of *E. coli* isolated from minced fresh beef were, O_{26}: H_{11} EHEC (6.7%), O_{91}: H_{21} EHEC (3.3%) and O_{127}: H_6 ETEC (3.3%). While, O_{91}:H_{21} EHEC (3.33%), O_{111}:H_2 ETEC (6.67%), O_{128}:H_2 ETEC (3.3%), O_{86} EPEC (6.7%) and O_{121}:H_7 ETEC (3.3%) serotypes isolated from beef burger samples. Moreover, O_{26}: H_{11} EHEC (3.3%), O_{114}:H_4 EPEC (3.3%), O_{159} EIEC (3.3%), O_{55}:H_7 EPEC (3.3%), O_{111}:H_2 ETEC (3.3%), O_{146}:H_{21} EPEC (3.3%) and O_{121}:H_7 ETEC (3.3%) isolated from kofta samples. The serological identification of *Salmonella* isolates revealed the detection of (3.3%) *S.Enteritidis* in minced fresh beef and kofta and (3.3%) of *S.Infantis* in minced fresh beef only while, prevalent serotype was *S.Typhimurium*, as (3.3%) isolated from minced fresh beef and beef burger and (6.7%) from kofta samples. Also, (3.3%) *S.Virchow* and *S.Tsevie were* isolated from beef burger samples. The public health importance of the isolated microorganisms and the recommended points were discussed. In addition, the evaluation of the efficacy of zinc oxide nanoparticles in improving minced fresh beef quality, while being in cold storage and determination of the antibacterial and antioxidant activities of ZnO nanoparticles. Using various concentrations (20, 40, and 60 ppm) of Zinc oxide ZnO NPs. Treated groups with ZnO nanoparticles showed a reduction in the inoculated *E. coli.*

*Corresponding author: Nahla A. Abo EL-Roos, Shebin El koom Provincial Lab., Animal Health Research Institute, Agriculture Research Center (ARC), Giza, Egypt
E-mail address: Dr.nahlashawky@yahoo.com
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INTRODUCTION

Meat products are gaining popularity as they represent quick easily prepared meat meals and solve the problem of the shortage in fresh meat at high prices. Although, meat products may be derived as raw materials from a source less in microbial contamination, they could be contaminated in the course of manufacture **(Younes et al. 2019).** The most important bacterial pathogens in meat products that are responsible for food-borne infections including *E. coli,* Salmonellae and *S. aureus* **(Saif-Marwa 2015).** The bacterial contamination and hygienic measures during meat production can be determined through estimation of aerobic plate count (APC) and total coliforms count **(Hamed et al. 2015).** Coliforms are used as a general indicator of sanitary conditions in the food-processing environment (**Feng et al. 2002).** *E.coli* is commonly nonvirulent but some strains have adopted pathogenic or toxigenic virulence factors that make them pathogenic to humans and animals **(Younes et al. 2019).**

Staphylococcus aureus is considered one of the main source of bacterial contamination in cooked meat due to workers handling during its preparation and processing **(FSIS 2013).**

Staph. aureus is an important cause of food intoxication throughout the world. This bacterium can contaminate several foods, including minimally processed meat products and produce several types of enterotoxins **(Balaban & Rasooly 2000).** Also, contamination of minced fresh beef with Salmonella is still considered a major problem in food hygiene **(Vipham et al. 2012).** Humans become infected with *Salmonella* primarily through fecal contamination of food products or water **(Wells et al. 2001).**

Salmonellosis is still one of the major global causes of gastroenteritis in humans and animals (**Grimont & Weil 2007). Abd-Elhafeez et al. (2022)** revealed that minced fresh beef, kofta and beef burger collected from low price sources have inferior quality. Therefore, the aim of the present study was designed to evaluate the bacteriological status of minced fresh beef, beef burger and kofta. Furthermore, studying the antibacterial effect of ZnO nanoparticles on *E. coli* that inculated experimentally in minced meat.

MATERIAL AND METHODS

1. Collection of samples:

A total of 90 samples of minced fresh beef, beef burger and kofta (30 of each) were collected from various markets in Kalyobia governorate, Egypt to assess their bacteriological profile. Each sample was kept in a separate sterile plastic bag in an ice box and then transferred to the laboratory under complete aseptic conditions without undue delay. The collected samples were subjected to bacteriological examination to determine the potential health hazard associated with their contamination and subsequently their validity for human consumption.

2. Bacteriological examination:

Preparation of samples (ISO 4833-1, 2013):

Twenty five grams of the sample, 225 ml of sterile peptone water were added and thoroughly mixed using a sterile blender for 1.5 minutes, from which tenth-fold serial dilutions were prepared. The prepared samples were subjected to the following examinations.

2.1. Aerobic Plate Count (ISO 4833-1, 2013):

One ml from previously prepared serial dilution was separately inoculated on sterile duplicate of plate count agar and plate incubated at 30^{o}C for 3 days. Aerobic Plate Count (APC) per gram was calculated on plates containing 30-300 colonies and each count was recorded separately.

2.2. Coliform count (ISO 4832, 2006):

Using Violet Red Bile agar medium at 37^{o}C for 24 hours. All dark red colonies measuring 0.5 mm in diameter on the plates were then counted and the average number of colonies was determined.

2.3. Screening for Enteropathogenic *Escherichia coli*

2.3.1Enrichment broth:

One ml from previously prepared serial dilution was inoculated into MacConkey broth

tube supplemented with inverted Durhams tubes and incubated at 44°C for 24 hours.

2.3.2 Plating media:

Loopfuls from positive MacConkey broth tubes were separately streaked onto Eosin Methylene Blue agar medium (E.M.B.), which was then incubated at 37°C for 24 hours. Suspected colonies were metallic green in color, purified and inoculated into slope nutrient agar tubes for further identification.

2.3.3 Identification of Enteropathogenic *E. coli* (MacFaddin, 2000).

2.3.3.1. Morphological identification:

2.3.3.1.1. Microscopical examination

Films of pure suspected cultures were stained with Gram's stain and examined microscopically. Gram negative, medium size, stained evenly coccobacilli were suspected to be *E. coli*.

2.3.3.1.2. Motility test:

Motility medium was inoculated by the stabbing technique in semisolid nutrient agar to a depth of 5 mm and then incubated at 37°C for 24 hours. A circular growth from the line of stabbing represented a positive test.

2.3.4. Biochemical identification according to Cruickshank et al. (1975) and Quinn et al. (2002).

2.3.5. Serological identification of *E. coli*:

The isolates were serologically identified according to Kok et al. (1996) by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co. Japan) for diagnosis of the Enteropathogenic types.

The diagnostic *E. coli* antisera sets used for identification include:

Set 1 : O- antisera:

Polyvalent antisera 1: O1, O4, O26, O86a, O111, O119, O127a and O128.

Polyvalent antisera 2: O44, O55, O113, O125, O126, O146 and O166.

Polyvalent antisera 3: O18, O114, O142, O151, O157 and O158.

Polyvalent antisera 4: O2, O6, O7, O27, O78, O148, O159 and O168.

Polyvalent antisera 5: O20, O25, O63, O91, O153, O163 and O167.

Polyvalent antisera 6: O8, O15, O17, O115, O169 and O171.

Polyvalent antisera 7: O28ac, O112ac, O124, O136 and O144.

Polyvalent antisera 8: O29, O45, O121, O143, O152 and O164.

Set 2 : H- sera.

H2, H4, H6, H7, H11, H18 and H21.

2.4. Screening for salmonellae:

*** Pre-enrichment broth:**

From the original dilution, one ml was inoculated into sterile buffer peptone water and incubated at 37°C for 18 hours.

2.4.1 Enrichment broth:

One ml of the original dilution was inoculated into 9 ml Rappaport Vassilidis broth tube, and then the tube was incubated at 41.5°C for 24 hours (Harvey & Price 1981).

2.4.2 Selective Plating:

Xylose lysine desoxychoclate agar (XLD) was used. Loopfuls from the inoculated tubes were separately streaked onto XLD agar and incubated at 37°C for 24 hours. Suspected colonies were red with or without black centers. Suspected colonies were purified onto nutrient agar plate and incubated at 37°C for 24 hours. Separate colonies were selected and streaked onto slope nutrient agar for further identification. The purified isolates were identified morphologically, biochemically and serologically.

2.4.3 Identification of salmonellae

2.4.3.1. Morphological identification:

2.4.3.1.1. Microscopical examination

Films of pure suspected cultures were stained with Gram's stain and examined microgram Gram-negative, medium size, stained evenly bacilli were suspected to be salmonellae.

2.4.3.1.2. Motility test:

Motility medium was inoculated using stabbing technique to a depth of 5 mm and then

incubated at 37°C for 24 hours. A circular growth from the line of stabbing represented a positive test.

2.4.3.2. Biochemical identification according to Cruickshank et al. (1975) and Quinn et al. (2002).

2.4.3.3. Serological identification of salmonellae:

Serological identification of salmonellae was carried out according to Kauffman – White scheme for the determination of Somatic (O) and flagellar (H) antigens using *salmonella* antiserum **(DENKA SEIKEN Co. Japan).**

2.4.3.3.1. Identification of Somatic (O) antigen "Slide agglutination test":

A dense suspension of the organism was prepared by suspending growth in 0.5 ml of saline solution.

* Using a wax pencil, 2 circles about 1 cm in diameter on a microscopic slide were marked.
* One drop of *Salmonella* Polyvalent "O" antiserum was put in one of the marked circles and one drop of the saline solution was put in the other circle (negative control).
* Using a clean dropper, one drop of bacterial suspension (0.05 ml) was transferred into each of the circle and mix thoroughly by gently racking for 1- 2 minutes (excessive evaporation was avoided).
* Positive reaction was adopted by rapid and complete agglutination. A delayed or partial agglutination should be considered negative.
- *Salmonella* group and the other somatic components of the group were also identified using by using separate "O" antiserum factors.

2.4.3.3.2. Identification of Flagellar (H) antigen "Tube agglutination test"

Determination of Flagellar (H) antigens was carried out by using Polyvalent H antiserum for both phase 1 and phase 2 in order to determine the complete antigenic formula of the isolates. A loopful of H antiserum was added to one drop of the bacterial suspension in the small agglutinating tube and mixed gently by a sterile loop. The agglutination tube was gently agitated for one minute and observed for agglutination under normal lighting conditions.

2.5. Determination of *Staph. aureus* count (FDA, 2001):

One ml from each of the previously prepared serial dilutions was spread over Baired Parker agar plate using a sterile bent glass spreader. The plates were retained in upright position until the inoculums is absorbed by agar for about 10 min. The inoculated and control plates were inverted and incubated at 35°C for 48 hours. After which they were examined for colony character. The developed colonies (shiny black colonies) were enumerated and calculated as presumptive *S. aureus* count/g. Also, the colonies were picked up and purified on nutrient agar slopes for further identification.

*** Identification of Staphylococci species:**

2.5.1. Morphological examination (ISO, 1995)

Films were prepared from a pure culture of the isolated microorganism stained with Gram's stain and then examined microscopically. Staphylococci appeared as Gram positive cocci resembling grape like clusters.

2.5.2. Biochemical identification (MacFaddin, 2000)

2.6 Assessment of antibacterial activity of nanomaterials in minced meat

Minced beef

Fresh minced beef in butcher's shop was purchased and immediately transported to the laboratory in an icebox and stored at 4 °C until use. Thin sheets of minced beef were treated with ultraviolet light (wavelength 385 nm) for 30 min, 15 min per side to eliminate background microflora (**Morsy et al. 2018**).

2.6.1 Bacterial strain

Escherichia coli ~ 6 log CFU/ ml was used in this study and obtained from Media Unit, Reference laboratory for safety analysis of food of animal origin, Animal Health Research Institute, Dokki, Giza, Egypt.

2.6.2 Synthesis and preparation of zinc oxide nanoparticles according to **Wang et al. (2007).**

2.6.3 Assessment of antibacterial activity of nanomaterials in minced meat

Minced meat was inoculated with *E. coli* (~ 6 log CFU/ml) to achieve final concentration ~ 4 log CFU/g of minced meat. Then, they were mixed by gently squeezing the bags by hand till homogenous distribution of bacteria occurred, and left for 30 min for complete attachment between minced meat and the inoculum. Minced meat sample was divided into four groups (200 g each); Group 1 (PBS + *E. coli*), Group 2 (20 ppt ZnO + *E. coli*), Group 3 (40 ppt ZnO + *E. coli*), Group 4 (60 ppt ZnO + *E. coli)*. All samples were transferred into sterile (self-closed) polyethylene bag and kept at 4 °C until spoilage. Counting of *E. coli* and sensory evaluation were performed on 0, 3, 6, 9, 12, 15, and 17 days.

2.6.4 Enumeration *E. coli*

Accurately, 1ml from each prepared serial dilution was spread over duplicated plates of EMB agar using a sterile bent glass spreader (FDA, 2001). Suspected colonies of *E. coli* were enumerated and expressed as log CFU/g of sample.

2.6.5 Sensory evaluation

Sensory evaluation was performed under the controlled condition of temperature (25 °c), humidity 55% and light by five well-trained female panelists of 30 to 40 years of age, who were selected according to ISO (2012).

The criteria used as the basis of the organoleptic descriptive assessment and the sample were rated on a continuous hedonic scale (ISO, 2003). The panel received a list of descriptors (odor, color and texture) to score on numerical and continuous scales from 0 (the lowest score for each attribute, very bad) to 10 (the highest score for each attribute, very good).

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using SPSS program for Windows (Version 22) (SPSS Inc. Chicago, IL, USA). Independent T test at the $P \leq 0.05$ were indicated as significant different. Duncan's multiple range test is a post hoc test used for measuring the specific differences between pairs of means. The results expressed as means ± standard error (mean log cfu/g ± SE).

RESULTS

The results recorded in Table (1) showed that the mean values of APC were 4.46 ± 0.09, 4.69 ± 0.11 and 4.87 ± 0.26 (log cfu/g) in minced meat, beef burger and kofta, respectively with significance difference $p < 0.05$.

Table 1. Statistical analysis of APC (log cfu/g) in minced beef, beef burger and kofta (n=30 of each).

Groups	Min.	Max.	Mean± SE
Minced meat	3.71	5.28	4.46 ± 0.09^{a}
Beef burger	3.85	5.69	4.69 ± 0.11^{ab}
Kofta	3.89	5.92	4.87 ± 0.26^{c}

*Mean values with different superscripts in the same columns are significantly different at ($P < 0.05$).

Table (2) showed that *Coliforms* counts in the examined samples significantly differ ($p < 0.05$) as it ranged from 2.48 to 3.95 with an average 3.41± 0.08 log cfu/g in minced meat, 2.6 to 4.81 with an average 3.6 ± 0.12 log cfu / g in beef burger and 2.69 to 4.89 with an average 3.92 ± 0.28 log cfu /g in kofta.

Table 2. Statistical analysis of Coliforms count (log cfu/g) in the examined samples (n=30 of each).

Groups	Min.	Max.	Mean± SE
Minced meat	2.48	3.95	3.41 ± 0.08^{a}
Beef burger	2.60	4.81	3.6 ± 0.12^{ab}
Kofta	2.69	4.89	3.92 ± 0.28^{c}

*Mean values with different superscripts in the same columns are significantly different at ($P<0.05$)

Results in Table (3) demonstrated that the mean values of *S. aureus* count (CFU/g) in the examined samples of minced, beef burger and kofta were 2.38 ± 0.06, 2.89 ± 0.07 and 2.68 ± 0.11, respectively with significance difference $p < 0.05$ between all samples types.

Table 3. Statistical analysis of *S. aureus* count (log cfu/g) in the examined samples (n=30 of each).

groups	Min.	Max.	Mean± SE
Minced meat	2	2.85	2.38 ± 0.06^{a}
Beef burger	2	3.95	2.89 ± 0.07^{b}
Kofta	2	2.90	2.68 ± 0.11^{c}

*Mean values with different superscripts in the same columns are significantly different at ($P<0.05$)

Furthermore, **Table (4)** declared that the incidence of different enteropathogenic serotypes of *E. coli* isolated from the examined samples of minced meat represented by O_{26}: H_{11} EHEC (6.7%), O_{91}: H_{21} EHEC (3.3%) and O_{127}: H_6 ETEC (3.3%) while O_{91}:H_{21} EHEC (3.3%), O111:H_2 ETEC (6.7%), O_{128}:H_2 ETEC (3.3%), O_{86} EPEC (6.7%) and O_{121}:H_7 ETEC (3.3%) serotypes isolated from beef burger samples. Moreover O26: H11 EHEC, O114:H4 EPEC, O159 EIEC, O_{55}:H_7 EPEC, O111:H_2 ETEC, O146:H21 EPEC and O_{121}:H_7 ETEC (3.3%) for each kofta. *E. coli* (EHEC, EPEC, EIEC and, ETEC) involving in poisoning of different meat products.

Table 4. Incidence of Enteropathogenic *E. coli* detected in examined samples (n=30 of each).

E. coli Strains	Minced meat		Beef burger		Kofta		Strain serotype
	NO	%*	NO.	%*	NO.	%*	
O_{26} : H_{11}	2	6.7			1	3.3	EHEC
O_{114} : H_4					1	3.3	EPEC
O_{159}					1	3.3	EIEC
O_{55} :H_7					1	3.3	EPEC
O_{91}: H_{21}	1	3.3	1	3.3			EHEC
O_{127} : H_6	1	3.3					ETEC
O_{111} : H_2			2	6.7	1	3.3	EHEC
O_{146} : H_{21}					1	3.3	EPEC
O_{128} : H_2			1	6.7			ETEC
O_{86}			2	6.7			EPEC
O_{121} : H_7			1	3.3	1	3.3	ETEC

EPEC = Enteropathogenic *E.coli*
ETEC = Enterotoxigenic *E.coli*
EIEC = Enteroinvasive *E.coli*
EHEC = Enterohaemorrhagic *E.coli*
%*: in relation to samples number of each product (30).

Results in **Table (5)** revealed that serological identification of *Salmonella* serovars represented by *S. Enteritidis* (3.3%) from minced meat and kofta, *S. Infantis* (3.3%) in minced meat only, *S. Rissen* (3.3%) from kofta. The most prevalent serotype is *S. Typhimurium* as it isolated from minced meat and beef burger (3.3%) as well as 6.7% from kofta samples. Also, *S. Anatum, S. Virchow* and *S. Tsevie* isolated from beef burger samples (3.3%). Therefore, 11 (33.3%) of the examined samples were unfit for human consumption because of having different *salmonella* species**.**

Table 5. Incidence of *Salmonella* serovars in the examined samples (n=30 of each).

Salmonella serovars	Minced meat		Beef burger		Kofta	
	NO	%	NO.	%*	NO.	%
S. Enteritidis	1	3.3	-	-	1	3.3
S. Infantis	1	3.3	-	-	-	-
S. Anatum	-	-	1	3.3	-	-
S. Rissen	-	-	-	-	1	3.3
S. Virchow	-	-	1	3.3	-	-
S. Tsevie	-	-	1	3.3	-	-
S. Typhimurium	1	3.3	1	3.3	2	6.7

%*: in relation to number of each product (30).

As shown in **Table (6)** *E. coli* counts increased in the control group during cold storage of minced meat which was significantly different ($p < 0.05$) from all treated groups.

Table 6. Antibacterial activity of different concentrations of ZnO NPs against *E. coli* counts on minced beef inoculated with *E. coli* .

Groups	1st day	3rd day	6th day	9th day	12th day	15th day	17th day
Control group	4.22 ± 0.2 [a]	5.60 ± 0.22 [a]	6.25 ± 0.1 [a]	6.95 ± 0.30 [a]	7.11 ± 0.6 [a]	7.96 ± 0.5 [a]	9.22 ± 0.14 [a]
60ppt ZnO	4.22 ± 0.2 [a]	3.38 ± 0.2 [c]	3.35 ± 0.1 [d]	2.30 ± 0.11 [c,d]	1.60 ± 0.2 [d]	ND	ND
40ppt ZnO	4.22 ± 0.2 [a]	3.55 ± 0.3 [c]	3.21 ± 0.1 [d]	2.40 ± 0.20 [d]	2.51 ± 0.11 [c]	2.11 ± 0.3 [c]	ND
20ppt ZnO	4.22 ± 0.2 [a]	3.63 ± 0.1 [b,c]	3.32 ± 0.2 [e]	2.95 ± 0.1 [b,c]	2.80 ± 0.23 [c]	3.02 ± 0.7 [d]	2.92 ± 0.10 [c]

The values are expressed as Mean ± standard error of three experiments. Means within a column and rows followed by different letters are significantly different ($P \leq 0.05$).
ND= Not detected

Sensory evaluation

The nanoparticles effect on overall acceptability (odor, color, and texture) of minced meat during refrigerated storage at 4 °C as illustrated in table (7). Sensory properties were satisfactory for all the samples on the initial day of the storage (1st day), however, they significantly different during the storage period ($P \leq 0.05$). The results proved that all sensory attributes of control samples were acceptable by the 3rd day of the storage period and spoiled at 6th day; while treated samples were acceptable by the 15th day of storage for texture, color, and overall acceptability attributes, by the 12th day of storage for odor attribute .

Table 7. Effects of different concentrations of ZnO NPs on overall acceptability score of minced meat during storage at 4 °C for 17 days.

Groups	1st day	3rd day	6th day	9th day	12th day	15th day	17th day
Control Group	8.5 ± 0.5 [a]	5 ± 0.1 [b]	2 ± 0.1 [b]	S	S	S	S
60 ppt ZnO	8.5 ± 0.5 [a]	8.5 ± 0.1 [a]	7.5 ± 0.3 [a]	7.16 ± 0.10 [a]	6.50 ± 0.2 [a]	5 ± 0.30 [a]	4 ± 0.06 [a]
40 ppt ZnO	8.5 ± 0.5 [a]	8 ± 0.1 [a]	6.5 ± 0.1 [a]	6 ± 0.1 [a]	5.5 ± 0.16 [a]	4.5 ± 0.30 [a]	S
20 ppt ZnO	8.5 ± 0.5 [a]	7.5 ± 0.2 [a]	6 ± 0.30 [a]	5.13 ± 0.4 [b]	4.5 ± 0.15 [b]	4 ± 0.30 [a,b]	S

The values are expressed as mean ± standard error. Means within a column and rows followed by different letters are significantly different ($P \leq 0.05$).
S = Spoiled

DISSCUSION

The highest count was recorded in kofta samples followed by beef burger and minced meat. Similar results demonstrated by Younis et al. (2019) who isolated $9.5\times10^4 \pm 1.6\times10^4$, $1.7\times10^5 \pm 0.39\times10^5$ and $3.3\times10^4 \pm 0.45\times10^4$ from examined minced meat, kofta and burger, respectively. Higher values of APC recorded by Ragab et al. (2016) as they isolated 6.6x 10^8, 4.6 x 10^6 from minced meat and kofta, respectively while, lower results (3.1x 10^5 CFU/ g) from beef burger samples. Younis et al. (2019) also, reported that APC reflect the bacterial contamination and declared the hygienic quality of both meat products. Furthermore, Doyle et al. (2007) reported that fresh minced meat tends to have a short shelf life because the quality of the raw ingredients and re-contaminated through the grinding /handling process.

Coliforms Similar results recorded by Ragab et al. (2016) as they isolated coliforms from minced meat (6 x 10^4 cfu/g) while lower results reported in kofta and beef burger, with average values 6 x 10^2 and 4 x 10^2 cfu/g in the examined samples, respectively. Also, lower results obtained by Younis et al. (2019) as who recorded $0.58\times10^2 \pm 0.21\times10^2$, $0.39\times10^2 \pm 0.10\times10^2$ and $0.34\times10^2 \pm 0.09\times10^2$ for minced meat, kofta and burger, respectively.

Higher results of staphylococci recorded by Shaltout (2019) who found that minced meat, kofta, sausage and beef burger were positive for staphylococci with a mean value of $2.11 \times 10^3 \pm 1.45 \times 10^3$, $5.41 \times 10^3 \pm 0.95 \times$ 103, and $6.16 \times 10^3 \pm 0.82 \times 10^3$ (cfu/g) for the examined samples, respectively. Abd El Satter-Alaa (2016) and Badr-Sarah, (2018) mentioned that the presence of *S. aureus* in meat and its products indicates poor hygiene of meat handlers as well as lack of sterilization of utensils and they grow without pronounced change in odour or taste in the products and producing heat stable enterotoxins which lead to food poisoning with severe diarrhea and gastroenteritis among consumers (Plaatjies et al. 2004).

The severity of these serotypes that mainly accompanied with *E. coli* infection in case of haemolytic uraemic syndrome (HUS) also shiga toxin (1&2) causes gastroenteritis (Heyderman, 2001). The results were similar to that obtained for E. coli may be compared with those recorded by Hassan et al. (2016) as they isolated serotypes O_{55}:K_{59}, O_{111}:K_{58}, O_{127}:K_{63} and O_{124}:K_{72} from kofta samples, also, Hassanin et al. (2015) isolated O_{26}:H_{11}, O_{111}:H_4, O_{114}:H_{21} and O_{128}:H_2 serotypes from kofta samples. Furthermore, Gaafar (2020) detected O_{128}:H_2, O_{26}:H_{11} in kofta samples, respectively. Also, Osama et al. (2021) isolated EPEC *E. coli* strains the most common were O_{17}: H_{18}, O_{86}, O_{114}:H_4, O_{146}:H_{21} and O_{55}:H_7; then EHEC (O_{26}:H_{11}, O_{91}: H_{21}, O_{111}:H_2), followed by ETEC (O_{128}:H_2 and O_{125}:H_{21}), and finally EIEC (O_{159}). Nel et al. (2004) reported that the presence of *E. coli* indicated fecal pollution, which occur due to unhygienic slaughtering techniques, contaminated surfaces or handling of meat by contaminated hands of infected person.

Salmonella infections were the reason of 30% of 23,250 notifications of foodborne diseases in Australia Oz Food Net Working Group (2003), with symptoms characterized by dramatic diarrhea, accompanied by abdominal pain, nausea, headaches, vomiting, chills, low grade fever and myalgia (Ziprin and Hume 2001). Elbayoumi et al. (2021) isolated *Salmo-*

nella spp that serologically identified as *S. Enteritidis*, *S. Infantis*, *S. Paratyphi* A and *S. Typhimurium* from minced meat, beef burger and kofta. Also, Hassan et al. (2016) isolated Salmonellae serotypes as *S. enteritidis, S. Typhimurium* and *S. anatum* from kofta samples.

Count of *E. coli* in treated groups decreased throughout storage, indicating antibacterial activity of ZnO NPs. ZnO (60 ppt) exhibited great antibacterial effect against *E. coli*. These results are nearly similar to findings of Marcous et al. (2017) who examined the antibacterial action of ZnO against *E. coli* in calf minced meat and reported that ZnO NPs have effective antimicrobial. ZnO NPs are a novel material controlling foodborne pathogens, thus can be applied for food safety (Ali et al. 2020). Also, Morsy et al. (2018) studied the synergistic antimicrobial effect of ZnO nanoparticle and other compounds as nisin, lysozyme and EDTA nanoparticles on different foodborne pathogens including *E. coli* O157:H7 and proved that ZnO have great antimicrobial effect. As ZnO NPs have great antibacterial activity, it has received significant interest worldwide particularly by the implementation of nanotechnology. The concentrations of ZnO NPs used in this study were less than the permissible limits approved by FDA (2015). ZnO NPs are cheap antibacterial substances that had wide range of antibacterial activity against microbes present in meat, therefore, they help to ensure the quality of meat, increase the shelf life for minced meat, and maintain the health of human.

There was a significant difference ($P \leq 0.05$) for overall acceptability attribute between the treated and control samples on the days 3^{th}, 6^{th}, 9^{th}, 12^{th}, $15^{th,}$ and 17^{th} of the storage time. The concentrations of 60ppt ZnO, enhanced shelf life time of minced meat and delayed its spoilage until 17^{th} day, while minced meat treated with concentrations of 20 ppt and 40ppt (ZnO) spoiled at the 15^{th} day.

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