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Assessment of Antibacterial Impact of Onion Powder on *Escherichia coli* contaminating broiler chicken cuts

Mohammed, Kamla Adam, Hamad, Randa Mahmoud, Saleh, Abdulsalam, Akwieten, Hana Ealoma, Abd Alaziz, Muhanid Mohammed

Department of Preventive Medicine and Public Health, Faculty of Veterinary Medicine, Omar Al- Mukhtar University, Libya

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ABSTRACT

The present study aimed to evaluate the efficacy of onion powder on Enteropathogenic *E. coli* strains recovered from broiler chicken meat. A total of 50 random samples of freshly slaughtered broiler chicken cuts which were collected from poultry retail shops in a trial for isolation and identification of Enteropathogenic *E. coli*. In addition, an experiment was designed to evaluate the effectiveness of addition of onion powder as natural preservative to control the contamination of broiler chicken cuts with Enteropathogenic *E. coli*. It was found that the total incidence of *E. coli* in broiler chicken cuts was 54% and the incidence was higher in the examined samples of thigh (60 %) than the samples of breast (48 %) with significant difference between the incidences. In addition, serological identification showed that the incidence of *E. coli* O_{867} (42.30 %) was lower than that of *E. coli* O_{125} (57.70 %) by adding of onion powder by different concentrations on artificially inoculated chicken meat cuts with *E. coli* O_{125}, it was observed that addition of onion powder by concentration of 3% scored the highest Reduction % in *E. coli* counting compared to that of 2 and 2.5% concentrations especially at 72 hours after treatment.

1. INTRODUCTION

Chicken meat may be an exceptionally well-known food and is favored by consumers due to decreased production costs, low fat percentage and high dietary value. Also, it provides an animal protein of high biological value for consumers at all ages containing all the essential amino acids required for growth with high proportion of unsaturated fatty acid and low cholesterol value. Moreover, it is easily digestible and acceptable by the most people (Abou Hussein, 2007).

Chicken meat is a common vehicle of pathogenic microorganisms that are considered as the most important causes of foodborne outbreaks in people (Noori and Alwan, 2016). *Escherichia coli* bacteria are one of the most important gastrointestinal inhabitants in most creatures, including humans and poultry. Most *E. coli* are commensal, but small proportions are potentially harmful and cause diseases worldwide (Frye and Jackson, 2013). Pathogenic *E. coli* are classified into classes based on the production of different virulence factors and on the clinical manifestations that they cause (Kim et al., 2020).

Shiga toxins-producing *E. coli* (STEC) are a group of highly pathogenic strains known as enterohemorrhagic *E. coli* (EHEC) or verotoxins-producing *E. coli* (VTEC) (Detzner et al., 2020). It is considered as one of the most emerging foodborne zoonotic bacteria causing various clinical signs as watery or bloody diarrhea and possibly life-threatening disorders such as hemorrhagic colitis (HC), thrombotic thrombocytopenic purpura (TTP), hemolytic uremic syndrome (HUS) and acute renal failure (Karmali et al., 2010).

The pathogenicity of STEC strains is attributed to the production of different virulence factors including two potent phage-encoded cytotoxins as *stx1*, and *stx2*. These toxins are like to those produced by *Shigella dysenteriae* which inhibit protein synthesis in host cell leading to cell death (El Syaed and Mounir, 2020).

Avian strains of *E. coli* show many similarities with human extra intestinal pathogenic *E. coli* (ExPEC) strains, in that most of the virulence genes they possess, and they can be transferred to humans through consumption of contaminated food or food products causing a variety of infections (Ewers et al., 2007).

Pathogens difficult to be eliminated during food processing, as many types of bacteria can adhere to food surface forming biofilms where they can survive even with sufficient methods of decontamination (Mohsenipour and Hassan shahian, 2015).

Investigation of *E. coli* in chicken meat was investigated by several authors; (Zhao et al., 2001, Samaha et al., 2003, Amin 2008, Abd El-Rahman et al., 2010, Eyi-Ayla and Arslan-Seza 2012, Hossam, 2012, Eskander, 2015, Hassan, 2015, Ibrahim et al., 2015, Hamd, 2016, Saad, 2016, Gharib et al., 2017, Rahman et al., 2017, Xuan Binh et al., 2017, Hozayn, 2018 and Mohammad, 2020).

Food safety could be an around the world wellbeing objective as foodborne illnesses are a primary wellbeing concern. A few food preservation strategies have been detailed counting; steaming method but it diminished the supplement quality, the freezing and thawing cycles have harmful impact on quality. Moreover, utilize of chemical additives for food preservation is constrained by reduction

* Corresponding author: abdulsalam.abdullah@omu.edu.ly

in its sensory quality. Hence, there's a need to create sound science or innovatively based preservation strategies with the possibility holding food quality.

The utilize of manufactured compounds have critical disadvantages, such as expanding cost, dealing with hazards, concerns around residues on food and danger to human environment. Subsequently, there has been expanding interest to supplant manufactured additives with characteristic, successful and nontoxic compounds.

Considering the reality that poultry meat has a place to perishable foods, the most concern of industries is the shelf-life expansion of the poultry meat. Present day patterns to realize this target incorporate the application of the hurdle technology concept and utilize of natural food preservatives so as to maintain negligible processing and also to provide protection from both spoilage and pathogenic microorganisms (Khiari et al., 2014).

Understanding of plant biochemistry, physiology and chemistry of natural products have appeared that the secondary metabolites may be utilized to control pathogenic bacteria to overcome the prior specified issues related with manufactured chemicals (Delaquis and Mazza, 2008).

Essential oils are considered great normal additives rather than chemical ones and their application in food meets the requests of consumers for gently prepared of normal products (Burt, 2004). Onion is the generic title of the *Allium cepa* family. It was cultivated 6000 years BC within the Nile valley. It has been respected all through time not only for its culinary utilize, but also for its therapeutic properties. Utilization of Onion is advantageous to human wellbeing as scientific studies show that Onion contains polyphenol molecules or phytonutrients which includes flavonoids, tannins as well as allicin which have antioxidant and antimicrobial properties (Skrinjar and Nemet, 2009).

The principal antimicrobial impact of Onion is attributed to quercetin and allicin (thio-2-propene-1-sulfinic acid-5-allyl-esters), quercetin attacks the bacteria DNA gyrase while, allicin prevents certain thiol containing enzymes in bacteria by the rapid reaction of thiosulfinates (Ankri and Mirelman, 1999).

Control of food poisoning bacteria remains an important objective for sectors of food industry; therefore, the main aim of the present study was to evaluate the efficacy of onion powder on Enteropathogenic *E. coli* strains recovered from broiler chicken cuts.

2. MATERIAL AND METHODS

2.1. Samples:

A grand total of 50 random samples of freshly chicken cuts including, 25 breast and 25 thighs were collected from poultry retail shops. Each sample (250 g) was kept in a separate plastic bag and transferred directly to the laboratory under complete aseptic condition to be examined bacteriologically.

2.2. Isolation and identification of E. coli from broiler chicken cuts:

2.2.1. Preparation of samples for bacteriological examinations:

It was done according to APHA (2001). Accurately, 25 g of each sample were aseptically transferred into sterile blender flask containing 225 ml of sterile peptone water (0.1%) and homogenized at 4000 rpm for 2.5 minutes.

2.2.2 Horizontal method for detection and enumeration of Escherichia coli (ISO 7251, 2005):

Accurately, 1 ml from original dilution was inoculated into MacConkey's broth with inverted Durham´s tubes and then incubated at 35 °C for 24 hours. Positive result is indicated by gas production. Loopful from positive MacConkey's broth tubes were separately streaked onto Eosin Methylene Blue agar (EMB) then incubated at 37° C for 24 hours. Suspected colonies were metallic green in color. Suspected colonies were purified and inoculated into slope nutrient agar tubes for biochemical identification of E. coli (Quinn et al., 2002) .

Isolates were preliminary identified biochemically as E. coli then subjected to serological identification according to Kok et al. (1996) by using rapid diagnostic E. coli antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types.

2.3. Evaluation of onion powder efficacy on Enteropathogenic E. coli strains recovered from broiler chicken cuts:

Commercial onion powder was obtained from a retail store. After sterilization at Autoclave, 3 different concentrations were prepared from onion powder including 2, 2.5 and 3% solutions. Each concentration was added to experimentally inoculated broiler chicken cuts samples with *E. coli* O_{125} (EPEC) previously isolated from broiler chicken cuts with the rate of 1×10^8 CFU/g. *E. coli* count was performed in the following times; 0, 2, 24, 48, 72, 96 and 120 hours, respectively. All samples were stored at 4 °C in the refrigerator. The color, odor and overall acceptability were determined for each sample of chicken meat. Finally, reduction % (R%) was calculated for each after addition of different concentrations of onion powder according to the following equation according to Beginner's Guide, (2017);

$$\text{Reduction (R) \%} = \frac{\text{Initial load} - \text{New count}}{\text{Initial load}} \times 100$$

2.4. Statistical analysis:

The statistical analysis was carried-out using the Chi2-test for detection of the significant incidences of different E. coli isolates among different parts of broiler chicken cuts at different period of experiment and at different concentration of the onion powder, the statistical analysis was carried-out according to SAS, (2004).

3. RESULTS

The recorded results in **Table (1)** showed that the overall isolation rate of *E. coli* from chicken cuts was 54% and the incidence was higher in the examined samples of thigh (60 %) than the samples of breast (48 %) with significant difference between the incidences at $P < 0.01$.

Table 1 Incidence of different strains of isolated pseudomonas species from examined samples

Chicken cuts	No. of examined samples	Positive samples No.	%
Breast	25	12	48.0
Thigh	25	15	60.0
Total	50	27	54.0
$Chi^2 = 8.22$**	** Significant at ($P < 0.01$)		

Results recorded in **Table (2)** cleared that the incidence of *E. coli* O_{867} (42.30 %) was lower than that of *E. coli* O_{125} (57.70 %). In addition, the incidence of *E. coli* O_{867} was higher in the examined samples of thigh (23.08 %) compared to that of breast (19.24 %). Also, the incidence of *E. coli* O_{125} was higher in the examined samples of thigh (34.6 %) compared to that of breast (23.08 %).

Table 2 Serotyping of enteropathogenic E. coli recovered from chicken cuts

E. coli serotypes	Chicken cuts				Total	
	Breast		Thigh		No	%
	No.	%	No.	%		
E. coli O_{867} (EHEC)	5	19.24	6	23.08	11	42.30
E. coli O_{125} (EIEC)	6	23.08	9	34.62	15	57.70
Total	11	42.30	15	57.70	26	100.0

Results observed in **Table (3)** cleared that, the effect of Onion powder on artificially inoculated chicken cuts with *E. coli*, differ significantly ($P < 0.01$) among storage time and concentrations of Onion powder. The findings clarified that 3 % concentration causes a higher reduction % in *E. coli* counting compared to that of the concentration 2 and 2.5 % of Onion powder. Also, by increasing the storage time, the effect of Onion powder decreased in prevention the growth of *E. coli* than the lower period of exposure to Onion powder. The results cleared that; efficacy of Onion powder decreased till reaching the storage period of 120 hours in which the spoilage time of broiler chicken cuts.

Table 3 Additional effect of Onion powder on artificially inoculated broiler chicken cuts with E. coli O125 (1×108 CFU/g) stored at refrigerator (4 °C)

Storage Time	Control group	Concentration of Onion powder					
		2 %		2.5 %		3 %	
		Count	R%	Count	R%	Count	R%
Zero time	1×10^{8}	1×10^{8}	0.00	1×10^{8}	0.00	1×10^{8}	0.00
2 hours	3×10^{9}	4×10^{4}	99.96	2×10^{4}	99.98	1×10^{4}	99.99
24 hours	7×10^{9}	5×10^{3}	99.99	4×10^{3}	99.99	2×10^{1}	99.99
48 hours	6×10^{10}	4×10^{2}	99.99	1×10^{2}	99.99	1×10^{1}	99.99
72 hours	9×10^{10}	6×10^{2}	99.99	5×10^{2}	99.99	3×10^{1}	99.99
96 hours	8×10^{11}	5×10^{5}	99.50	9×10^{4}	99.91	9×10^{3}	99.91
120 hours	Spoilage according to sensory evaluation						
Chi^2 =	10.23**			** = Significant at (P < 0.01)			

The recorded results in **Table (4)** cleared that, the effects of Onion powder (3 %) on grades of sensory traits of chicken meat stored at refrigerator differ significantly ($P < 0.01$) among different period of storage. The results cleared that, the sensory traits (color, odour, appearance, consistency and overall grades showed excellent grades at Zero time, 2 hours and good results observed at 24 hours, acceptable results observed at 48 hours, while, bad results observed at 72 hours, while the spoiled results observed at 96 hours of the storage.

Table 4 Changes in grades of sensory traits of treated chicken meat with Onion powder (2 %) stored at refrigerator (4 °C)

Storage time	Grades of sensory traits					
	Color (5)	Odour (5)	Appearance (5)	Consistency (5)	Overall (5)	Grade
Zero time	5	5	5	5	5	Excellent
2 hours	5	5	5	5	5	Excellent
24 hours	4	3.5	4	4	3.5	Good
48 hours	3	2	3	2.5	2.5	Acceptable
72 hours	3	2.8	3	2	2	Bad
96 hours	2	1	1	1	1	Spoiled

Chi^2 = 15.39** ** = Significant at (P < 0.01)

Results observed in **Table (5)** cleared that, the effects of Onion powder on grades of sensory traits of chicken meat stored at refrigerator differ significantly ($P < 0.01$) among different period of storage. The results cleared that, the sensory traits (color, odour, appearance, consistency and overall grades showed excellent grades at Zero time, 2 hours, and very good results observed at 24 hours, good results observed at 48 and 72 hours, while, acceptable results observed at 96 hours, while the spoiled results observed at 120 hours of the storage.

Table 5 Changes in grades of sensory traits of treated chicken meat with Onion powder (2.5 %) stored at refrigerator (4 °C)

Storage time	Grades of sensory traits					
	Color (5)	Odour (5)	Appearance (5)	Consistency (5)	Overall (5)	Grade
Zero time	5	5	5	5	5	Excellent
2 hours	5	5	5	5	5	Excellent
24 hours	4.7	4.5	4.7	4.8	4.9	Very good
48 hours	4.5	4	4	4	4	Good
72 hours	4	3.5	3.8	3.6	3.5	Good
96 hours	2.5	2.5	2.7	2.5	2.5	Acceptable
120 hours	--	--	--	--	--	Spoiled

Chi^2 = 16.23** ** = Significant at (P < 0.01)

The results presented in **Table (6)** cleared that the effects of Onion powder on grades of sensory traits of chicken meat stored at refrigerator differ significantly ($P < 0.01$) among different period of storage. The results cleared that, the sensory traits (color, odour, appearance, consistency and overall grades) showed excellent grades at Zero-time, 2 hour, very good results observed at 2 hours, good results observed at 24 and 48 hours, while acceptable results observed at 72 hours and the spoiled results observed at 96 hours of the storage

Table 6 Changes in grades of sensory traits of treated chicken meat with Onion powder (3 %) stored at refrigerator (4 °C)

Storage time	Grades of sensory traits					
	Color (5)	Odour (5)	Appearance (5)	Consistency (5)	Overall (5)	Grade
Zero time	5	5	5	5	5	Excellent
2 hours	4.5	4	4.5	4.5	4.5	Very good
24 hours	4	3.5	4	4	3.5	Good
48 hours	3	2	3	2.5	3	Good
72 hours	2.9	2.5	2.6	2.4	2.5	Acceptable
96 hours	1.5	1	1	1.8	1	Spoiled

Chi^2 = 15.22** ** = Significant at (P < 0.01)

4. DISCUSSION

In Egypt consumer´s habit is to purchase freshly slaughtered broiler chicken meat from poultry retail shops to consume it or to preserve it till need. Chicken differ from other food animals as its skin is not removed during cooking so it may be a source of contamination to meat. From the other side, meat acts as a good media for growth of many organisms due to its high nitrogenous compounds.

4.1. Incidence of *E. coli* from broiler chicken cuts:

The presence of *E. coli* in food was considered as an indicator of faults during preparation, handling or storage. It was also, considered as an indicator of fecal contamination, beside. It may induce severe diarrhea in infants and young children, as well as food poisoning and gastroenteritis among the adults (Hassan, 2007).

The recorded results in Table (1) revealed that the overall isolation rate of *E. coli* in broiler chicken cuts was 54% and the incidence was higher in the examined samples of thigh (60 %) than the samples of breast (48 %) with significant difference between the incidence at ($P < 0.01$). Occurrence of *E. coli* in chicken cuts in the current work was nearly similar to that obtained by Rahman et al., (2017) (49.02%). In addition, the incidence was higher than that recorded by Abd El-Rahman et al., (2010) (10.6%), Zhao et al., (2001) (38.7 %), Samaha et al., (2003) (22.86 %), Amin (2008) (34%), Hossam, (2012) (10%), Mohamed, (2013) (40 %), Ibrahim et al., (2015) (4 %), Saad, (2016) (24 %), Gharib et al., (2017) (46.12%), Xuan Binh et al., (2017) (43.3%) and Hozayn, (2018) (46%). On contrary, it was lower than that recorded by and Eyi-Ayla and Arslan-Seza (2012) (87.5%),

Eskander, (2015) (66%), Hassan, (2015) (80%) and Hamd, (2016) (82%).

High incidence of *E. coli* is good marker to fecal contamination, water pollution and lack of sanitary condition during hand evisceration and washing of chicken carcasses.

Also, the recorded data in Table (1) showed that the prevalence of *E. coli* in the examined samples of chicken thigh (60%) was higher than that in breast samples (48%). These findings agreed with those of Eskander, (2015) who recorded that the prevalence of *E. coli* in breast (66%) was lower than that in thigh (76%), while it disagreed with that of Al-Dughaym and Al-Tabari, (2010) who found that the prevalence of *E. coli* in breast cut (70%) was higher than in thigh samples (60%). Lastly, results of broiler chicken cuts samples are exceeded the standard limit (free from *E. coli*) established by EOS, (2005).

Serotyping of the obtained isolates of Enteropathogenic *E. coli* from broiler chicken cuts was recorded in Table (1). It was found that the incidence of *E. coli* O_{867} (42.30 %) was lower than that of *E. coli* O_{125} (57.70 %). In addition, the incidence of *E. coli* O_{867} was higher in the examined samples of thigh (23.08 %) compared to that of breast (19.24 %). Also, the incidence of *E. coli* O_{125} was higher in thigh samples (34.6 %) compared to that of breast (23.08 %). This finding was in harmony with that of Mohammad (2020) who found that occurrence of *E. coli* was higher in thigh samples (43%) compared to that of breast (27%). In addition, serotyping of *E. coli* isolates revealed the presence of O_{26} (EPEC), O_{86} (EHEC), O_{119} (EPEC), O_{126} (EIEC) and O_{124} (ETEC) strains.

Generally, the presence of *E. coli* in broiler chicken cuts was considered as a sign for mishandling or contaminated environmental conditions which agreed with Frazier and Westhoff, (1998) and Hashim, (2003). *E. coli* related coliform bacteria predominant among aerobic commensal flora in gut of man, animal and poultry, so their presence in poultry carcasses is an indicator of fecal contamination.

4.2. Effect of adding Onion powder on artificially inoculated broiler chicken cuts with *E. coli*:

Sulphur compounds in Onions have also been shown to be anti-inflammatory both by inhibiting formation of thromboxanes and by inhibiting the action of platelet-activating factor (PAF). Thiosulfinates condition anti-thrombotic benefits, including antioxidant activity reduced serum cholesterol and enhance invitro platelet activity (Ying and Chang, 1998).

As shown in Table (3), it was noticed that the inoculated *E. coli* showed an increasing count by time passing in the control group that did not treat with Onion powder (1×10^8 cfu/g at zero time till reach 8×10^{11} cfu/g after 96 hours) and spoiled after 120 hours.

Concerning the treated groups, the tabulated data showed that addition of Onion powder (2%) resulted in decrease of *E. coli* count (R% = 99.96%) after 2 hours (4×10^4 cfu/g), then R% increased to 99.99% after 24 hours (5×10^3 cfu/g), 99.99% after 48 hours (4×10^2 cfu/g) and 99.99% after 72 hours (6×10^2 cfu/g). On contrary, R% of *E. coli* count slightly decreased to 99.5% after 96 hours (5×10^5 cfu/g). Finally, spoilage occurred after 120 hours.

Also, the presented data showed that addition of Onion powder (2.5%) resulted in decrease of *E. coli* count (R% = 99.98%) after 2 hours (2×10^4 cfu/g), then R% increased to 99.99% after 24 hours (4×10^3 cfu/g), 99.99% after 48 hours (1×10^2 cfu/g) and 99.99% after 72 hours (5×10^2 cfu/g). On contrary, R% of *E. coli* count slightly decreased to 99.91% after 96 hours (9×10^4 cfu/g). Finally, spoilage occurred after 120 hours.

Finally, the presented data showed that addition of onion powder (3%) resulted in decrease of *E. coli* count (R% = 99.99%) after different times of the experiment; 2 hours (1×10^4 cfu/g), 24 hours (20 cfu/g), 48 hours (10 cfu/g), 72 hours (30 cfu/g). On contrary, R% of *E. coli* count slightly decreased to 99.91% after 96 hours (9×10^3 cfu/g). Finally, spoilage occurred after 120 hours.

The recorded results clarified that addition of Onion powder (3%) scored the highest R% in *E. coli* counting compared to that of 2 and 2.5% concentrations especially at 72 hours after treatment.

Based on the results, the onion powder showed higher antimicrobial activity against *E. coli.* These findings agreed with those recorded by Azu and Onyeagba, (2007) who found that *E. coli* bacteria were less resistant to the extract of onion bulbs, Irkin and Arslan, (2010) who noticed that higher concentrations of onion extract had a significant impact on *E. coli*. In addition, the count of *E. coli* of the samples reached and exceeded the spoilage limit after 9 days at 4°C and Abdel-Salam et al., (2014) who found that red onion essential oils showed a stronger antimicrobial activity for decreasing count of *E. coli* O_{157}:H_7 in sausage. On contrary, this result disagreed with those recorded by Ortiz, (2015) who found that onion was not effective against *E.coli* as no zones of inhibition were gotten and Ziarlarimi et al., (2016) who noticed that *E. coli* was resistant to the aqueous extracts of onion.

4.3. Changes in grades of sensory traits (colour, odour, appearance and consistency) of treated chicken meat with Onion powder:

Data presented in Table (4) clarified that the treated chicken meat with Onion powder (2%) showed an excellent grade of sensory traits at zero and 2 hours after treatment, while the grade decreased to good after 24 hours, then another decrease to acceptable after 48 hours and finally changed to bad after 72 hours while data presented in Table (5) clarified that the treated chicken meat with Onion powder (2.5%) showed an excellent grade of sensory traits at zero and 2 hours after treatment, while the grade decreased to very good after 24 hours, then another decrease to good after 48 and 72 hours and it was acceptable after 96 hours of treatment and finally, data presented in Table (6) clarified that the treated chicken meat with Onion powder (3%) showed an excellent grade of sensory traits at zero time after treatment, while the grade decreased to very good after 2 hours, then another decrease to good after 24 and 48 hours and finally changed to acceptable after 72 hours.

There was a decline of acceptability began after the first day of storage with marked reduction of odor, color, texture and overall acceptability values in the control samples at the 4th day (after 96 hours). Furthermore, the obtained results indicated that the best acceptability quality was attained at Onion powder (3%) treated chicken meat samples, while slight improvement in acceptability of Onion powder (2%) as compared with control samples. These results agreed with those obtained by Sasse et al. (2009) who reported that many herbs and spices as onion contain antioxidant components that improve both color and flavor stability in meat.

5. CONCLUSION

The obtained results in the current work clarified that broiler chicken cuts had a significant incidence of enteropathogenic

E. coli that may represent a threat to human health. At the same time, detection of enteropathogenic *E. coli* reflected unsanitary conditions during the process of retailing and selling of freshly broiler chicken cuts. Application of onion powder as natural preservatives in order to control *E. coli* in fresh retailed chicken cuts clarified that addition of onion powder by concentration of 3% scored the highest reduction % in *E. coli* counting compared to that of 2 and 2.5% concentrations especially at 72 hours after treatment with acceptable sensory traits.

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