COMPARATIVE STUDY BETWEEN THE EFFECT OF GAMMA IRRADIATION AND OZONE GAS ON THE INCIDENCE OF *E. COLI* O_{157}:H_7 IN BEEF BURGER SOLD IN ASSIUT GOVERNORATE

LUBNA M. EBRAHEEM; GHADA M. MOHAMED and MAHMOUD A.M. AMMAR
Animal Health Research Institute, Assiut Laboratory
Email: mahmoud2014eg@yahoo.com

Received at: 29/9/2014

Accepted: 11/11/2014

ABSTRACT

This study aimed to determine the prevalence of *E. coli* O_{157}:H_7 in beef burger, evaluate the effectiveness of gamma irradiation and ozone gas as an antimicrobial intervention on beef burger and evaluate the effect of both on sensory quality of the product. A total of 125 samples of beef burger were collected from different supermarkets in Assiut City, *E .coli* was isolated from 24 samples (19.2%), further examination using polymemerase chain reaction (PCR) revealed that only one confirmed to be *E. coli* O_{157}:H_7 with a percentage of 0.8%. Beef burgers inoculated with *E. coli* O_{157}:H_7 at initial level of 10^6 CFU/g were exposed to different doses of ɣ- radiation (2,4 and 6 KGy) at dose rate 2.32 KGy/h at a constant exposure time. The survival of *E. coli* O_{157}:H_7 was examined post treatments. Irradiation at doses (4 and 6 KGy) significantly decreased the count proportionally to the applied dose without any sensory changes. To explore the effect of ozone on *E. coli* O_{157}:H_7, inculcated samples of beef burger were subjected to 3 different gaseous ozone treatments, 20, 40 and 70 PPM with a time of contact of approximately 3 minutes. It was found that all concentrations significantly reduced the pathogen count without any color or flavor change of beef burger. Gamma irradiation at dose 6KGy is more effective in reduction of *E. coli* O_{157}:H_7 population (a reduction % of 56.o8) than ozone gas at concentration of 70 PPM (a reduction % of 21.14).The public health importance of the organism was discussed and the suggestive measures for safe healthful products were discussed.

Key words: *Incidence, E. coli O_{157}:H_7, Beef burger, Gamma, irradiation, ozone gas, sensory.*

INTRODUCTION

Beef burger is a raw food of animal origin which forms a significant portion of the diet in many countries. Consumers expect meat products to be safe for consumption when handled and cooked properly. Beef burger occasionally poses a high risk to the consumer due to their potential for carrying disease causing bacteria. *Escherichia coli* O_{157}:H_7 is one of the bacteria that have been identified as the cause of several food borne outbreaks (food and Nutrition 1999). *E. coli* bacteria are members of the family *Enterobactreriaceae*, facultative, anaerobic, Gram-negative short-rods and considered a common inhabitant of the gut of worm-blooded animals, including man. Most strains of *E. coli* are harmless, however, some strains, such as *E. coli* O_{157}:H_7, can cause severe food-borne disease (WHO, 1996).

E. coli O_{157}:H_7 attracted attention not only because food-borne transmission is more common, but also because it can cause life-threatening conditions such as hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Buchanan and Doyle, 1997). The pathogenicity of *E. coli* O_{157}:H_7 is attributed to the production of Shiga toxins1and 2, previously known as Verocytotoxins because of their toxicity on Vero cells (OÕBrien, 1992). Approximately half the HUS patients require kidney dialysis and their illness may last from several days to many months or years with a mortality rate of 3-5% (Food and Nutrition, 1999). People of all ages can get *E. coli* O_{157}:H_7 gastroenteritis, however, young children and the elderly tend to develop severe symptoms such as HUS (Koutkia *et al.*, 1997 and Stewart *et al.*, 1997).

The reservoir of *E. coli* O_{157}:H_7 appeared to be mainly cattle, which is present in the intestines in approximately 1 percent of healthy cattle. Meat from cattle may become contaminated with this strain of bacteria during the slaughtering (McEvoy *et al.*, 2003) and grinding process of the beef (Le Saux *et al.*, 1993).
The majority of outbreaks have resulted from the transmission of the organism through the

consumption of beef, most commonly, under-cooked contaminated ground beef (especially hamburger) thus, the term "Hamburger Disease" (Le Saux *et al*., 1993 and Dolye *et al*., 1997).

Public attention has continued to focus on the detection of pathogenic *E. coli* O_{157}:H_7 in beef burger. Since its introduction in the mid-1980, polymerase chain reaction (PCR) technology has proved to be a valuable method for detection of pathogens in food. In the last few years, several papers have been published reporting the development of PCR for the detection of pathogenic *E. coli* O_{157}:H_7 and to identify the H serogroup and the type of shiga toxin produced by this bacterium (Fratamico *et al*., 2000).

Due to the increased resistance of bacterial pathogens to commercially used antibiotics, there has been an increasing interest in the development of new types of effective and non-toxic antimicrobial compounds such as food preservation methods.

Among these methods, food irradiation which considered the most versatile treatment, nowadays. Microorganisms can be inactivated by impairment of important molecules or organelles, such as DNA and the cytoplasmic membrane (Diehl, 1995).

Hence, gamma radiation became an important tool to be used by the food industry not only as a method of preserving food but also to improve food safety (Mulder, 1988). Irradiation by gamma rays with specific doses reduces or eliminates pathogenic and spoilage microorganisms as *E. coli* O_{157}:H_7 in food. The process has little effect on the nutritional value and organoleptic qualities of food where it remains almost unchanged, but Irradiation can minimally affect some very sensitive vitamins, such as thiamin. Beef burger can be treated with a two-sided approach, penetrating 1.5 inches (Elaine, 1998).

Consumers cannot recognize irradiated food by sight, smell, taste, or feel. Irradiated foods can be recognized by the presence of the international symbol for irradiation on the packaging along with the words "Treated with Radiation," or "Treated by Irradiation". The overall retained nutritional quality of irradiated food depends on a number of factors, including irradiation dose, temperature, food composition and the presence or absence of oxygen (Pmiipf, 2000).

On the other hand ozone stands out for its antimicrobial properties and its high oxidative compound (Tiwari *et al*., 2008 and 2010). The biocidal effect of ozone is caused by a combination of its high oxidation potential, and its ability to diffuse through biological cell membranes (Kim *et al*., 1999). It is able to inactivate bacterial vegetative cells and spores, yeast, molds, and viruses with no waste/toxic products (Tiwari *et al.,* 2010). Thus it is Generally Recognized As Safe (GRAS) for food applications (Dhillon, 2009 and Tiwari *et al.,* 2010). An additional advantage is the lower cost of ozone equipment (Guzel-Seydim *et al.,* 2004). Ozone has proven to be a very effective in many food processing applications (Joel and George, 2011), including red meat and beef applications against several microorganisms (Akbas and Ozdemir 2006) specifically *E. coli* O_{157}:H_7 (Kim and Yousef 2000).

However, ozone is traditionally used in the aqueous phase for surface sanitation and general disinfection. Ozone gas may be an alternative to aqueous ozone in ground beef processing as an antimicrobial intervention (Rice *et al,* 1982). Before ozone can be applied successfully in food processing, patterns of microbial inactivation by ozone should be elucidated (Kim and Yousef 2000). Therefore, several factors affect the ozone efficacy including the strain of the microorganism, age of the culture, density of the treated population, and presence of ozone-demanding medium components and method of applying ozone (Khadre, *et al.,* 2001).

As there is little information about the occurrence of *E. coli* O_{157}:H_7 in meat products of ground beef specially beef burger in Egypt, therefore, the purpose of the present investigation was designed to evaluate the following: prevalence of *E. coli* O_{157}:H_7 in beef burger, confirmation of the isolated strains by PCR assay and, determine the gamma radiation dose which reduce the level of this microorganism. Also, evaluate if there is a possibility that ozone gas may be an effective on this microorganism intervention in beef burger as well on the sensory attributes.

MATERIALS and METHODS

1. Isolation of *E. coli* O_{157}:H_7 from beef burger:

1.1. Collection of samples:

A total of 125 random samples of beef burger were collected from different supermarkets, and groceries in Assiut Governorate. The samples were obtained in their casing as sold to the consumers and collected in presterilized polyethylene containers. The collected samples were transferred directly to the laboratory in an ice box with a minimum of delay, where they prepared for bacteriological examination.

1.2. Preparation of samples:

At the laboratory, frozen samples were thawed by overnight refrigeration. Each sample was aseptically and carefully freed from its casing.

1.3. Selective enrichment broth: (Tarr *et al.,* 1999):
Twenty-five grams of beef burger was aseptically weighed and placed in sterile stomacher bag containing 225 ml of modified vancomycin-trypticase soy broth (m-VTSB) supplemented with vancomycin

(40mg/litre), and stomached at low speed for 2 min. The stomached material was transferred to sterile flask and incubated aerobically at 37°C for overnight.

1.4. Isolation on selective plating media: (De Boer and Heuvelink, 2000)
One hundred µL of incubated broth was pipeted onto dried surface of MacConkey sorbitol agar (MSA) plates and incubated at 37°C for 24h. Colorless (pale) colonies (sorbitol-negative colonies) were picked up and purified for further confirmation.

1.5. Identification of presumptive colonies:
The presumptive colonies were confirmed to be *E. coli* following the protocol described by (Varnam and Evans,1991) using the microscopical examination and the biochemical identification: indole production test, methyl red test, voges-proskauer test, simmon's citrate test, urease test and triple sugar iron reaction.

1.6. Serological identification of *E. coli* by latex agglutination test: This was done according to the technique adopted by Krishnan *et al.*, 1997.

1.7. Detection of *E. coli* O_{157}:H_7 by PCR:
Total genomic DNA and PCR amplifications for the three strains (*E. coli* O_{157} confirmed isolates) was done as described by (Toma *et al.*, 2003). For *E. coli* O_{157}:H_7 specific identification, two primers pairs were used. The primer's sequence, the target, the PCR products size and the references, are listed in table (1).

DNA amplification reaction:
The bacterial genomic DNA samples were amplified by PCR in a reaction mixture(25µl) containing 13.25 sterile distilled H2O, 2.5ml 10 x buffer, 0.63ml 10mMNTPs, 1ml 25Mm Mgcl2, 1.25 µl primer F(20pmol/ml), 1.25 µL primer R(20pmol/ml) and fill up to 25 µl PCR grade water. The PCR protocol consisted of the following steps: An initial denaturation (2 min at 95°C) for 30 cycles, primer denaturation (1 min at 95°C) 1 cycle, primer annealing (1 min at 57°C), primer extension (2 min at 72°C) and a final elongation (5 min at 72°C). The PCR products were electrophoresed in 2.5% agarose gel and stained with ethidium bromide.

Table 1: Oligonucleotide sequences used for identification of *E. coli* O_{157}:H_7 by PCR:

Primer Name	Target gene	Oligonucleotide sequence (5′ → 3′)http://www.ncbi.nlm.nih.gov/pmc/articles/PMC140333/table/t2/ - t2fn1	Product size	Reference
VTcom-u	Stx	GAGCGAAATAATTTATATGTG	518 pb	Yamasaki *et al.* (1996)
VTcom-d	Stx	TGATGATGGCAATTCAGTAT		

2. Effect of gamma radiation or ozone gas on *E. coli* O_{157}:H_7 inoculated in beef burger and their effect on sensory quality of beef burger:

Preparation of bacterial inoculum:
The confirmed isolate of *E. coli* O_{157}:H_7 (a beef burger isolate), was used. The strain was maintained on tryptic soya agar (Biolife). Tryptic soya broth (Difico Laboratories) was inoculated from purified separate colony of the strain and incubated at 37 ºC for 24h. After this period, a 100 µ L of incubated broth was pipeted onto dried surfaces of MacConkey Sorbitol Agar (MSA) plates and surface spread using sterile glass rod then incubated at 37°C for 24h. Colorless (pale) colonies (sorbitol-negative colonies) were counted and the CFU/mL was calculated. Serial dilutions of the known population broth was prepared by diluting 1mL of the suspension with 9mL of sterile peptone water to yield a final inoculum approximately 10^6cfu/mL.

Preparation of samples:
Cardboard packages (15 frozen beef burgers each) were acquired at the retail level at Assuit city. Frozen packages were thawed by overnight refrigeration till the temperature reached 2ºC in the center of each beef burger, as measured with a thermometer (HI98509-1, Romania).

Design of the experiment:
Beef burgers were divided into two groups (group A for gamma irradiation) and (group B for ozone gas treatment). Each group was subdivided into seven sub groups (each 7 burgers) where 3 sub groups was subjected individually each for a different dose while another 3 non injected sub groups were treated by the same doses and used for the sensory evaluation of the samples. The seventh (no injected samples) and the eighth (injected samples) sub groups was transported to the place of treatment but not treated (controls).The prepared samples were transported cooled to the place of irradiation or ozone treatment in an insulated ice box.

Inoculation of samples:
Individual beef burgers were aseptically transferred to Styrofoam trays and 1mL of *E. coli* O_{157}:H_7 suspension (10^6 cfu/g) was evenly distributed inside and on the surface of each beef burger, using sterile syringe (the injection occur in Assiut). After 15

minutes, each tray was wrapped with PVC (polyvinyl chloride) film and transported immediately to the place of treatment under cool and hygienic measures.

Irradiation of beef burgers:
Two subgroups (inoculated and non inoculated subgroup) of beef burgers were submitted to the each irradiation dose at the National Center For Radiation and Technology (NCRRT) at Nasr City, Cairo, Egypt using India Gamma cell C060t. The γ- radiation doses were (2,4 and 6 KGY) at dose rate 2.32 KGY/h. Treated inoculated samples and their control were maintained refrigerated until the beginning of microbiological analyses .To study the effect of irradiation on the sensory quality of beef burger, treated non injected subgroups together with non inoculated non treated (control) were separately cooked immediately after treatment and subjected to sensory evaluation. The experiment was repeated 3 times.

Treatment with ozone gas: (Bialk and Demirci, 2007)

This study was performed at EL-Azhar University, Faculty of science. Two subgroups (inoculated and non inoculated subgroup) of beef burgers were submitted to a separate dose. The applied doses were 20, 40 and 70 ppm. The samples were allowed to contact the ozone-containing air for 3 minutes in the container. The whole system was installed in a fume cupboard, and the experiment was completed at room temperature (20°C). After the treatment, the ozone gas was passed through a 2% potassium iodide solution to prevent ozone from being released into the environment. The ozone treatment was performed in a fume hood for safety considerations. The microbiological and sensory analyses were the same as in case of irradiation experiment.

Enumeration of survivors: (Bialk and Demirci, 2007)
For enumeration of *E. coli* O_{157}:H_7 in treated and control beef burgers, Twenty-five grams of beef burger was aseptically weighed and placed in sterile stomacher bag containing 225 ml peptone water stomached at low speed for 2 min. Ten –fold of the stomached material was prepared using peptone water diluents. One hundred µl of each dilution was pipeted onto dried surface of MacConkey sorbitol agar (MSA) plates and incubated at 37°C for 24h. Colorless (pale) colonies (sorbitol-negative colonies) counted and the cfu/g beef burger was calculated. Repesentive colonies were picked for confirmation as *E. coli* using proper biochemical tests and the *E. coli* antiserum O157 assay. Microbiological data were transformed into log10 cfu/g.

Determination of D10-value and reduction %:
(Dickson**,** 2001)
D10value (the dose required to inactivate 90% of a population) were calculated by the formula: D10 value = dose (kGy)/ (log10 count prior to irradiation - log10 count after irradiation).
Reduction % = (log10 count of control - log10 count of treatment) x100/ log10 count of control.

Cooking of beef burger: (Pourkhalili *et al.,* 2013):
The samples were pan fried in sunflower oil for 20 min. The internal temperature during frying was determined as 85°C.

Sensory evaluation of cooked beef burger:
In all sensory tests, the panelists consisted of 5 non-expert members from our laboratory, and scores were obtained by rating the quality attributes using the following scale: 9 – excellent, 8 – very good, 7 – good, 6 – below good/above fair, 5 – fair, 4 – below fair/above poor, 3 – poor, 2 – very poor and 1 – extremely poor. Ratings of 5 and above indicated an acceptable sample, while ratings of less than 5 and more than 3 indicated that the samples were of marginal quality and ratings of 3 and below indicated unacceptable samples (Wierbicki, 1985). Juiciness was defined as the degree to which moisture was released from the sample after seven chews between the molars (Rocha-Garaz and Zayas, 1996).

RESULTS

Table 2: Incidence of *E. coli* and serologically *E. coli* O_{157} in examined samples of beef burger

No. of Examined samples of beef burger	*E. coli*		*E. coli* O_{157}	
	No. of +ve samples	%	No. of +ve	%
125	24	19.2	3	2.4

Table 3: Detection of *E. coli* O_{157}:H_7 and non H_7 strains by PCR assay

Examined samples of beef burger	No. of of *E. coli* O_{157} samples	PCR identification			
	3	*E. coli* O_{157} non H_7		*E. coli* O_{157}:H_7	
		No.	%	No.	%
		2	1.6	1	0.8

Table 4: Effect of gamma radiation on the sensory quality of cooked beef burger

Sensory parameters	**Radiation doses**				
	Stat.value	**2kgy**	**4kgy**	**6kgy**	**Control**
Taste	Mean	7.67 N	7.67 N	7.67 N	7.67
	SE	0.33	0.33	0.33	0.33
Odor	Mean	7.67 N	7.67 N	7.67 N	8.00
	SE	0.33	0.33	0.33	0.58
Texture and juiciness	Mean	8.00 N	8.00 N	7.67 N	8.00
	SE	0.01	0.01	0.33	0.01

Mean with the letter N have non-significant differences

Table 5: Effect of ozone gas on the sensory quality of cooked beef burger

Sensory parameters	**Ozone gas doses**				
	Stat. value	20 ppm	40 ppm	70 ppm	Control
Taste	Mean	8.00 N	7.33 N	8.00 N	8.00
	SE	0.01	0.33	0.01	0.01
Odor	Mean	8.00 N	8.00 N	8.00 N	8.00
	SE	0.01	0.01	0.01	0.58
Texture and juiciness	Mean	7.67 N	7.67 N	7.67 N	8.00
	SE	0.33	0.33	0.33	0.58

Means with letter N have non-significant differences

Fig. 1: Effect of gamma radiation on *E. coli* O157: H7 inoculated in beef burger

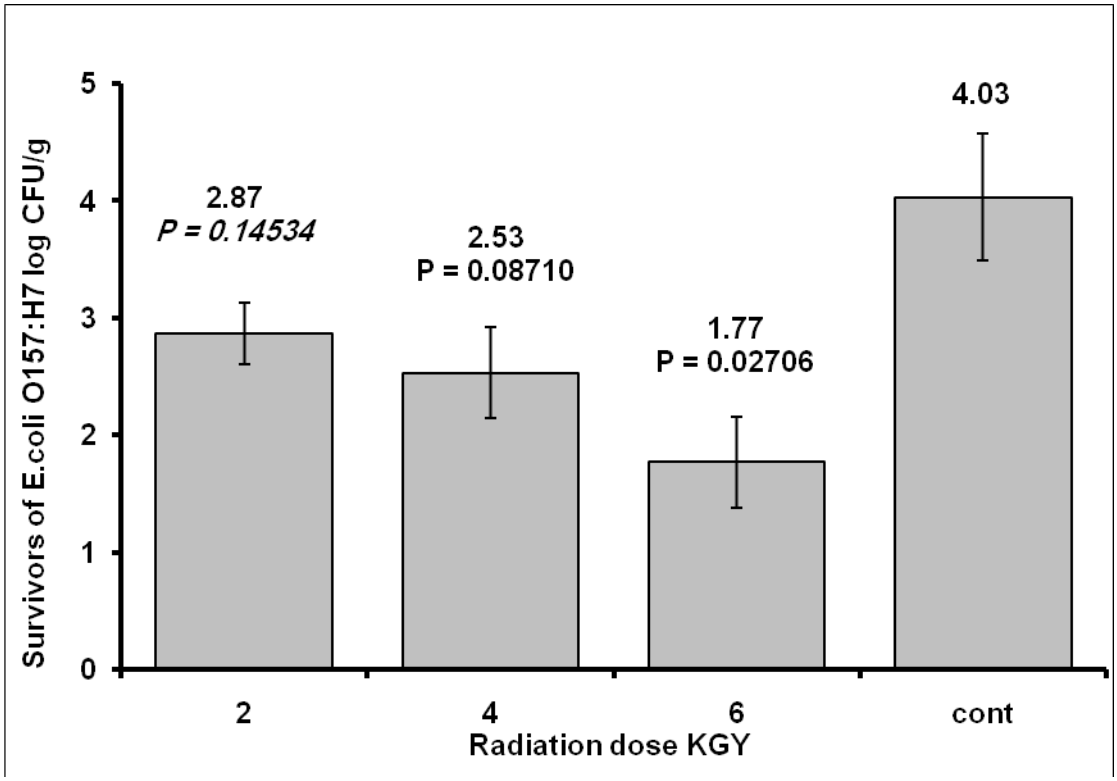
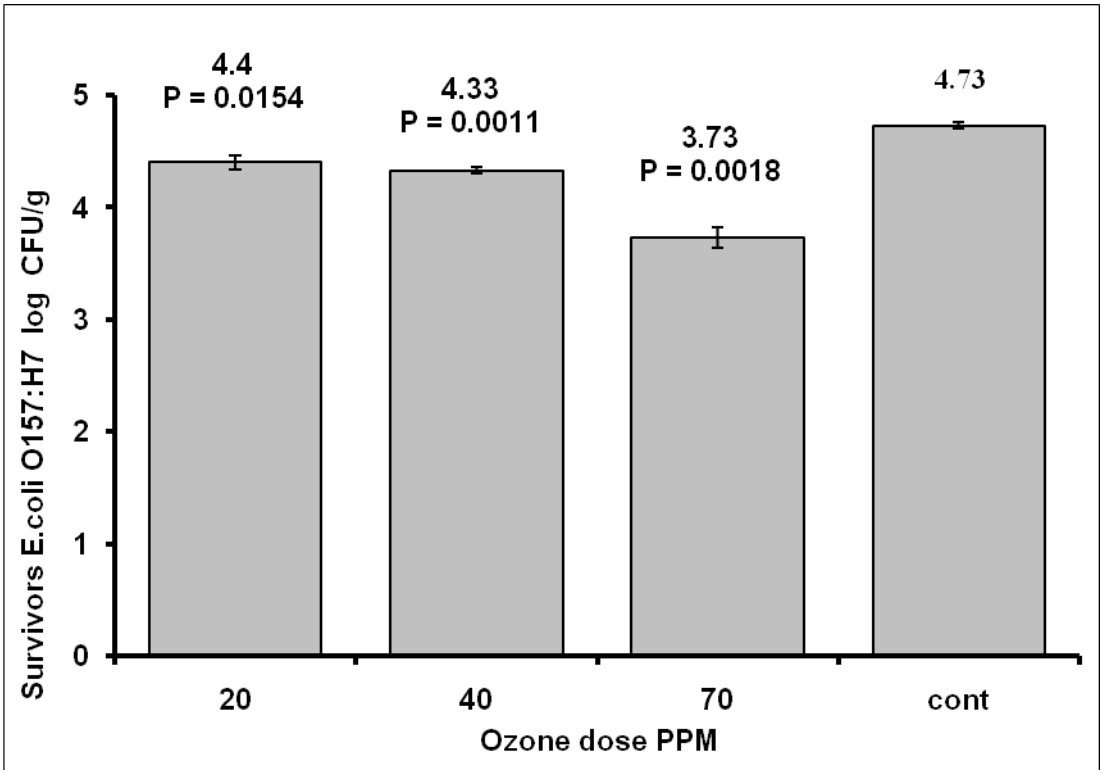
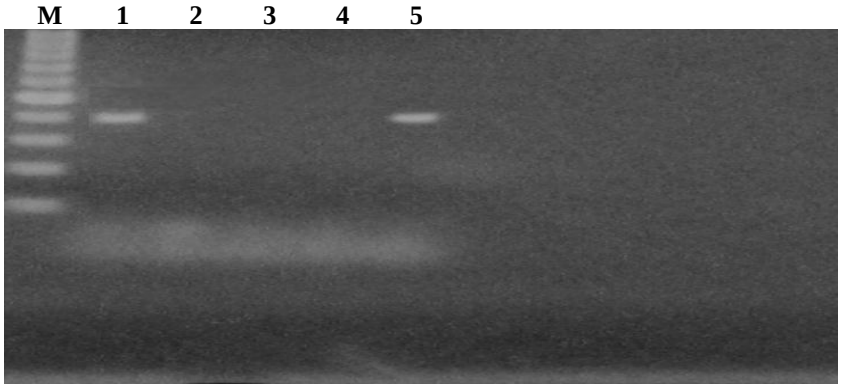


Fig. 2: Effect of ozone gas on *E. coli* O157: H7 inoculated in beef burger





Photograph (1): Agarose gel electrophoresis of PCR amplification products using specific primers of O_{157}:H_7 (VTcom-u, Vtcom-d).
Lane M: 518 bp ladder as molecular DNA marker.
Lane 1: Control positive for *E. coli* O_{157}:H_7
Lane 2: Control negative for *E. coli* O_{157}:H_7
Lanes 3 and 4 (1 and 17): -ve *E. coli* O_{157}:H_7
Lane 5 (51): +ve *E. coli* O_{157}:H_7

DISCUSSION

Due to the seriousness of *E. coli* O_{157}:H_7 on public health and its continuous detection in meat products, its prevalence in beef burgers at retail level was explored. As shown in table (2 & 3), *E. coli* was isolated from 24 (19.2%) of 125 samples. Three of positive samples showed negative sorbitol and also presented agglutination with O_{157} antiserum (*E. coli* of serogroup O_{157}), their percentage was 2.4%. The present study gave lower incidence of *E. coli* in examined beef burger than that recorded by Ouf (2001) (30%) and Fathi (2004) (72.5%). On the other hand Mohamed (2001) and Abd-EL-Malek (2005) reported that (40%) and (13.3%) of beef burger were contaminated by *E. coli* which is lower than that obtained in our result. Another study by Tutenel *et al.* (2003) indicated that 0.18% of examined ground beef harbored *E. coli* O_{157}.

Further examination of the three O_{157} isolates (using polymerase chain reaction (PCR) revealed that only one confirmed to be *E. coli* O_{157}:H_7 with a percentage of 0.8%., while the other two samples confirmed to be *E. coli* O_{157} non H_7 with a percentage of 1.6%.

Nearly similar results were recorded by (Jamshidi *et al.,* 2008) who revealed the presence of *E. coli* O_{157}:H_7 in ground beef with a percentage of 1%, also (Ahmed *et al.,* 2013) reported that 1% of beef burger samples were containing *E. coli* O_{157}:H_7. However Cagney *et al.* (2004), Abdel-Sadek (2012) and Jamshidi *et al.* (2012) reported a higher prevalence of the same organism in beef burger (2.91%), (10%) and (4%) respectively. On the other hand, chinen *et al.* (2001) couldn't isolate this pathogen from hamburger patties.

Direct comparison of results of this study with other studies is difficult due to differences in manufacture practices, variation in enrichment and isolation procedures, also differences in sample size. While there is some evidence that *E. coli* O_{157}:H_7 may be increasingly common in beef production systems (McDowell and Sheridan, 2001).

Because of the effectiveness of gamma irradiation in controlling common food-borne pathogens and in treating packaged food, thereby minimizing the possibility of cross-contamination prior to consumer use, most food safety officials and scientists view irradiation as an effective critical point in Hazard Analysis and Critical Control Points (HACCP) established for meat and poultry processing (Satin, 2002).

In view of control measures to prevent or eliminate the hazards of *E. coli* O_{157}:H_7 in beef burger, the results of the present study clearly showed that D10 value 0.64 KGy, (a dose of 2KGy) of gamma irradiation showed an approximated reduction of 1.1 logarithmic cycle on *E. coli* O_{157}:H_7 population compared with the control, while D10 values 1.32 and 1.64 KGy (a doses of 4 and 6 KGy) reduced approximately 1.5 and 2.3 logarithmic cycles of *E. coli* O_{157}:H_7 count respectively in relation to the control (Fig. 1).

The results show that the population of *E. coli* O_{157}:H_7 decreased gradually with increasing irradiation doses, that irradiation of the inoculated samples at doses 4 and 6 kGy significantly ($P < 0.05$) decreased the counts of the inoculated pathogen compared with the control, while irradiation of the inoculated samples at dose 2 KGy showed non significant ($P > 0.05$) effect (Fig. 1). On the counts of

the same pathogen (Rodolfo *et al.*, 2002) reported that the D10 values for *E. coli* O_{157}:H_7 in beef burger ranged from 0.17KGy to 0.27KGy, this result did not agree with those reported in our study.
The broad variation in D10 values for beef burgers can be due to differences in composition of the beef burgers belonging to different brands.

According to Monk *et al.* (1994), some food preservatives also affect the growth or death of microorganisms when food is submitted to irradiation treatment. There for the presence of these compounds could also have influenced the values obtained.

The effect of gamma irradiation on sensory quality of beef burger is of concern due to the formation of free radicals. To evaluate the effect of irradiation on beef burger, non inculcated cooked samples were submitted to sensory evaluation after being exposed to irradiation doses. In our experiment, the applied doses (2, 4, 6 KGy) did not affect the sensory attributes of the product, (Tab.4). In the same respect (Rodriguez *et al.*, 1993) reported no changes in sensory attributes of beef treated with 2KGy, while (Rodolfo *et al.*, 2002) reported that a dose of 1.2 KGy imparted an unfavorable odor and taste to the beef burger.

Our results indicate that at application doses (2, 4, 6 KGy) of gamma irradiation there is no significant differences between treated samples and control and beef burger will not be adversely affected form a sensory standpoint, (Tab. 4). This information can be used to support the use of gamma irradiation in meat to advance food safety practices.

Also, this research investigates the bacterial action of gaseous ozone for the elimination of *E. coli* O_{157}:H_7 from beef burger by surface exposure technique.

Under identical treatment conditions, (Fig. 2) show that 20 PPM ozone concentration decreased the number of *E. coli* O_{157}:H_7 from 4.7 to 4.4 log10 cfu/g, while 40 PPM ozone concentration reduced the pathogen count to 4.3 log10 cfu/g. At a dose of 70 PPM the greatest reduction in *E. coli* O_{157}:H_7 population was achieved (1 log10 cfu/g).

The results show that all the applied doses of gaseous ozone significantly ($P < 0.05$) decrease the population of *E. coli* O_{157}:H_7 in beef burger comparing with the control. The sensory analysis showed non-significant difference in the treated cooked beef burger with the three doses of ozone gas compared with the control (Tab 5). Joel and George (2011) treated ground beef with gaseous ozone at various levels, it was determined that approximately 73% of the *E. coli* was killed using 50 PPM ozone for 3 minutes with no change in color or flavor at ozone levels while approximately 95.8% of the *E. coli* in the ground beef was killed when the ozone concentration approached 200 PPM. The flavor change (a slight off flavor) occurred in the 200 PPM treated samples while no noticeable color change was present.

Due to the lack of conclusive evidence that ozone gas will be effective on beef burger, and limitation of using only ozone gas as an antimicrobial intervention on beef burger, further research is necessary.

CONCLUSIONS

The application of gamma irradiation at dose 6KGy or ozone gas at concentration of 70 PPM can improve the safety beef burgers through significant reduction of *E. coli* O_{157}:H_7 without any defect in the sensory quality of the product. Gamma irradiation at dose 6KGy is more effective in reduction of *E. coli* O_{157}:H_7 population (a reduction % of 56.o8) than ozone gas at concentration of 70 PPM (a reduction % of 21.14)

REFERENCES

Abdel-Sadek, A. (2012): Characterization of *E. coli* O_{157}:H_7 isolated from meat and meat products. A thesis for the degree of Ph.D in veterinary medical science microbiology, Cairo university faculty of veterinary medicine department of microbiology.

Ahmed, F.; Soodabeh, R.; Mansour, A.; Armaghan, A.; Hamed, G. and Mohammad, H. (2013): Isolation and identification of *E. coli* O_{157}:H_7 from ground beef hamburgers in Khuzestan province, Iran. African Journal of Microbiology Research (7): 413-417.

Akbas, M.Y. and Ozdemir, M. (2006): Effectiveness of Ozone for Inactivation of *Escherichia coli* and *bacillus cereus* in Pistachios, Int. J. of Food Sci. & Technology. 41:(5) 513-519.

Abd-EL-Malek, A.M. (2005): Assessment of some meat products for occurrence of *E. coli* O_{157}:H_7. A thesis for the degree of Ph.D, faculty of veterinary medicine, Assiut University.

Bialk, K.I. and Demirci, A. (2007): Decontamination *E. coli* O_{157}:H_7 and *Salmonella enterica* on blueberries using zone and pulsed UV-light Food Sci.72 (9), 391-396.

Buchanan, R.L. and Doyle, M.P. (1997): Food borne disease significance of *E. coli* O_{157}:H_7 and other enterohemorrhagic E. coli. Food Technol., 51: 69-76.

Cagney, C.; Crowley, H.; Duffy, G.; Sheridan, J.J.; O; Brien, S.; Carney, E.; Anderson, W.; Mcdowell, D.A.; Blair, I.S. and Bishop, R.H. (2004): Prevalence and numbers of *E. coli* O_{157}:H_7 in minced beef and beef burger from butcher shops and super markets in the Republic of Ireland Food Microbiology (21): 203–2012.

Chinen, I; Tanaro, J.D.; Miliwebsky, E.; Lound, L.H.; Chillemi, G.; Ledri, S.; Baschkier, A.; Scarpin,

M.; Manfredi, E. and Rivas, M. (2001): Isolation and characterization of *E. coli O157 : H7* from retail meats in Argentina. J. Food Prot.64, (9): 1346-1351.

De Boer, E. and Heuvelink, A.E. (2000): Methods forthe detection and isolation of STEC. J.Appl. Microbiol. Symposium supplement, 88, 133-143.

Dhillon, B. (2009): Development and evaluation of an ozonated water system for antimicrobial treatment of durum wheat. J. of Food Sci., 74, (7) p: 396-403.

Dickson, J.S. (2001): Food Irradiation: Principles and Applications. Wiley-interscience, 23-35.

Diehl, J.F. (1995): Safety of irradiated foods. Marcel Dekker Inc., New York, p345.

Doyle, M.P.; Zhao, T.; Meng, J. and Zhao, S. (1997): E. coli O_{157}:H_7 in food microbiology, fundamentals and frontiers. PP171-191. American society for Microbiology (ASM) press, Washington, DC.

Elaine, K. (1998): Making Progress in Food Preservation, New Technologies. www. foodproductdesign. com.

Fathi, S.H. (2004): Studies on histamine in some meat products sold in Assiut city. A thesis for the degree of Ph.D, faculty of veterinary medicine, Assiut University.

Food and Nutrition (1999): Guidelines for raw ground beef products found positive for *E. coli* O_{157}:H_7 Guideline no. 10 Issued by Food Directorate. Health Protection Branch Health Canada, March 8.

Fratamico, P.M.; Bagi, L.K. and Pepe, T. (2000): A multiplex PCR assay for rapid detection and identification of *E. coli* O_{157}:H_7 in foods and bovine faces. J.food Prot., 63, 8:1032-1037.

Guzel-Seydim, Z.B.; Greene, A.K. and Seydim, A.C. (2004): Use of ozone in the food industry. LWT-Food Science and Technology, 37, (4): 453-460.

Jamshidi, AB.; Bassami, MR.; Khanzadi, S. and Soltaninejad, V. (2012): Using multiplex-PCR assay in identification of *E.coli* O_{157}:H_7 isolated from hamburger samples in Mashhad, Iran. J. Nutr. Sci. Food Technol. (35): 101-106.

Jamshidi, AB.; Bassami, MR. and Rasooli, M. (2008): Isolation of *E. Coli* O_{157}:H_7 from ground beef samples collected from beef markets, using conventional culture and polymerase chain reaction in Mashhad, northeastern Iran. Iranian J. of veterinary Research, Shiraz University. 9: 72-76.

Joel, L. and George, K. (2011): Efficacy of gaseous ozone gainst Generic *E. coli* in Ground Beef. Ozone Journal, www.ozonesolutions.com. ozone

Khadre, M.A.; Yousef, A.E. and Kim, J.G. (2001): Microbiological aspects of ozone applications in food: a review. J. of Food Sci. 66, (9): 1242-1252.

Kim, J.; Yousef, A. and Dave, S. (1999): Application of ozone for enhancing the microbiological safety and quality of foods: A review. J. of Food Prot., 62, 1071–1087.

Kim, J.G. and Yousef, A.F. (2000): Inactivation Kinetics of Food borne Spoilage and Pathogenic Bacteria by Ozone. J. Food Sci., Vol.65, No.3, 521-528.

Koutkia, P.; Mylonakis, E. and Flanigan, T. (1997): EHE O_{157}:H_7 and emerging pathogen. Am. Fam. Physican, 56, 3: 853-856.

Krishnan, C.; Fitzgerald, V.; Dakin, S. and Behme, R. (1997): Laboratory investigation of outbreak of HCcaused by *E. coli* O_{157}:H_7 J. Clin. Microbiol., 25, 1043-1047.

Le Suax, N.; Spika, J.S. and Friesen, B. (1993): Ground beef consumption in non-commerical settings is a risk factor for sporadic *E. coli* O_{157}:H_7 infection in Canada. J. Infect. Dis., 167: 500-502.

McDowell, D.A. and Sheridan, J.J. (2001): Survival and growth of VTEC in the environmental. In: Duffy, G, Garvey, P., McDowell, D, (Eds.) verocytotoxigenic E.coli, food and Nutrition press, INC. trumbell, CT, USA, PP. 279-304.

McEvoy, J.M.; Doherty, A.M.; Sheridan, J.J.; Thomson-Carter, F.M.; Garvey, P.; McGuire, L.; Blair, I.S. and McDowell, D.A. (2003): The prevalence and spread of *E. coli* O_{157}:H_7 at A commercial beef abattoir. J. Appl. Microbiol., 95, 2: 256.

Monk, D.G.; Beuchat, L.R. and Doyle, M.P. (1994): Irradiation inactivation of food borne microorganisms. J. Food Protect. 58: 197-208.

Mohamed, G.M. (2001): Ready To-Eat-Meat Sandwiches as source of potential pathogens in Assiut city. A thesis for the degree of M.V.Sc., faculty of veterinary medicine, Assiut University.

Mulder, R.W.A. (1988): Salmonella radicidation of poultry carcasses. Beekbergen: Spelderholt Institute for poultry research, Report 363. In: Mayer- Miebach, E. Review Article, Food Irradiation. A means of controlling pathogenic microorganisms in food. Food Sci. Technol., 26: 493-497.

OÕBrien, A.; Tesh, V.L.; Donohue-Rolfe, A.; Jackson, M.P.; Olsnes, S.; Sandvig, K.; Lindberg, A.A. and Keusch, G.T. (1992): Shiga toxin: biochemistry, genetics, mode of action, and role in pathogenesis. Curr. Top. Microbiol. Immunol. 180: 65-94.

Ouf, J.M. (2001): Microorganisms of sanitary important in some meat products and their additives. Ph.D thesis, Faculty of Veterinary Medicine, Cairo University.

Pmiipf (2000): Packaging Materials Issues In Irradiation Processing Of Foods. Society of

Plastics Engineers Polyolefins XII International Conference February 27 – March 1, Houston, TX.

Pourkhalili, A.; Mirlohi, M. and Rahimi, E. (2013): Heme Iron Content in Lamb Meat Is Differentially Altered upon Boiling, Grilling, or Frying as Assessed by Four Distinct Analytical Methods. Sci. world J. vol. 2013: 1-5.

Rice, R.G.; Farquhar, J.W. and Bollyky, L.J. (1982): Review of the applications of ozone for increasing storage times of perishable foods. Ozone Science and Engineering, 4, 147-163.

Rocha-Garaz, A.E. and Zayas, J.F. (1996): Quality of broiled beef patties supplemented with wheat germ protein flour. Journal of Food Science, 61(2), 418–421.

Rodolfo, R.O.; Dirceu, M.V.; Maria, T.D.; Bernadette, D.G. and Mariza, L. (2002): Inactivation of *E. coli* O_{157}:H_7 in hamburgers by gamma irradiation. Brazilian J. of Microbiol. 33: 53-56.

Rodriguez, H.R.; Last, J.A.; Mallo, R.A. and Marchevsky, N. (1993): Low –dose gamma irradiation and refrigeration to extend shelf-life of aerobically packed fresh beef round, J. Food Prot. 56: 505-509.

Satin, M. (2002): Use of irradiation for microbial decontamination of meat situation and perspectives. Meat Sci. 62: 277-283.

Stewart, A.I.; Jones, G.A. and McMenamin, J. (1997): Central Scotland *E.coli* O_{157} outbreak: Clinical aspects (Monklands hospital experience). Scottish center for infection and environmental Health Weakly Report, 1 No. 97/13, 9-11.

Tarr, P.I.; Tran, N.T. and Wilson, R.A. (1999): E. coli O_{157}:H_7 in retail ground beef in Seattle: Results of a one-year prospective study. J. Food Prot., 62, 2: 133-139.

Tiwari, B.K. (2008): Effect of ozonation on the rheological and colour characteristics of hydrocolloid dispersions. Food Research International, 41, (10) 1035-1043.

Tiwari, B.K. (2010): Application of ozone in grain processing. J. of Cereal Sci., 51: 248-255.

Toma, C.; Lu, Y.; Higa, N.; Nakasone, N.; Chinen, I.; Baschkier, A.; Rivas, M. and Iwanaga, M. (2003): Multiplex PCR assay for identification of humane dairrhoegenic *Escherichia coli*. J. Clin. Microbiol., 41(6): 2669–2671.

Tutenel, A.V.; Pierard, D.; Vanhoof, J.; Cornelis, M. and Dezutter, L. (2003): Isolation and molecular characterization of *E. coli* O_{157}isolated from cattle, Pigs and chickens at slaughter. Int. J. food Microbiol. (84): 63-69.

Varnam, A.H. and Evans, M.G. (1991): E. coli. in food-borne pathogens. Wolf publishing Ltd., London, England. *E. coli*.

WHO (World Health Organization) (1996): E.coli O_{157}:H_7 Fact Sheets N 125.July.

Wierbicki, E. (1985): Technological and irradiation conditions for radappertization of chicken products used in the United States Army Raltech Toxicology Study. IAEA, Vienna: Food Irradiation Pro-cessing, pp. 79–99.

Yamasaki, S.; Lin, Z.; Shirai, H.; Terai, A.; Oku, Y.; Ito, H.; Ohmura, M.; Karasawa, T.; Tsukamoto, T.; Kurazono, H. and Takeda, Y. (1996): Typing of verotoxins by DNA colony hybridization with poly- and oligonucleotide probes, a bead-enzyme-linked immunosorbent assay and Polymerase Chain Reaction. Microbiol. Immunol., 40: 345–352.

دراسه مقارنه بين تاثير اشعه جاما وغاز الاوزون على تواجد ميكروب الايشيريكية القولونيه O_{157}:H_7 الموجود فى البيف بيرجر المباع فى محافظه اسيوط

لبنى محمد ابراهيم ، غاده محمد محمد ، محمود عمار محمد عمار

Email: mahmoud2014eg@yahoo.com

يهدف هذا البحث إلى تحديد مدى إنتشار ميكروب الايشيريكيه القولونيه O_{157}:H_7 في البيف بيرجر وتقييم فاعلية أشعة جاما اوغاز الأوزون على تواجد الميكروب فى البيف بيرجر كتدخل مضاد للميكروب. حيث جمعت ١٢٥ عينة من البيف بيرجر من متاجر مختلفة في مدينة أسيوط للتحليل الميكروبيولوجي. وقد تم عزل ميكروب الايشيريكيه القولونيه من ٢٤ عينة بنسبة ١٩.٢% . وبإجراء تفاعل البلمرة المتسلسل أكد هذا الاختبار وجود عينة واحدة فقط من الايشيريكيه القولونيه O_{157}:H_7 بنسبة ٠.٨%. لتقييم فاعلية أشعة جاما اوغاز الأوزون على تواجد الميكروب فى البيف بيرجر تم حقن عينات من البيف بيرجر بالايشيريكيه القولونيهO_{157}:H_7 بتركيز 10^6 cfu/g وعرضت هذه العينات لجرعات من أشعة جاما ٢، ٤، ٦ كيلو جراي ثم أجري التحقيق من مدى بقاء هذا الميكروب بعد التعرض، فكان التعرض للجرعتين ٤، ٦ كيلو جراي له تأثير معنوي في تقليل العدد الكلي للميكروب مقارنة بالمجموعة الضابطة للتجربه. بدون أي تغير في الصفات الحسية للمنتج. وأيضاً تم معاملة عينات أخرى من البيف بيرجر المحقونة ب الايشيريكيه القولونيه O_{157}:H_7 بتركيز 10^6 cfu/g وعرضت هذه العينات لتركيزات مختلفة من غاز الأوزون ٢٠، ٤٠، ٧٠ جزأ فى المليون (PPM) ولقد وجد أن جميع التركيزات السابقة أدت إلى اختزال العدد الكلي للميكروب المحقون بصورة معنوية مقارنة بالمجموعة الضابطة للتجربه بدون أي تغير في الصفات الحسية للمنتج. كما اوضحت الدراسة ان أشعة جاما (٦ كيلو جراي) اكثر فاعلية على ميكروب الايشيريكيه القولونيه O_{157}: H_7 من غاز الأوزون (٧٠) جزأ فى المليون حيث كانت النسبة الاختزالية % reduction (56.o8% ، 21.14%) على التوالى. وقد تم مناقشة مدى خطورة هذا الميكروب على صحة المستهلك والطرق المقترحة للحد منها.