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Effect of Electron Beam Irradiation on Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Thyphimurium in Minced Camel Meat during Refrigerated Storage

A. Amiri [1], H. Zandi [2,3*], H. Mozaffari Khosravi [4]

1. Department of Food Hygiene and Safety, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Research Center for Food Hygiene and Safety, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Department of Medical Microbiology, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
4. Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

HIGHLIGHTS

- The microbial loads of minced camel meat samples were reduced significantly with increasing the dose of irradiation.
- Dose of 5 kGy highly reduced *Salmonella enterica* serovar Typhimurium and completely destroyed *Escherichia coli*.
- *E. coli* was more sensitive to electron beam irradiation than *S. enterica* serovar Typhimurium.

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Acronyms and abbreviations
CFU=Colony Forming Unit

ABSTRACT

Background: Electron beam irradiation is one of the effective ways to control food-borne pathogens. We evaluated the effect of electron beam irradiation on survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Thyphimurium in minced camel meat during refrigerated storage.

Methods: The meat samples were inoculated with *E. coli* O157:H7 and *S. enterica* serovar Thyphimurium and then irradiated with doses of 0, 1, 2, 3, and 5 kGy. The samples were stored at 4±1 °C and evaluated microbiologically up to 10 days. Data were analyzed using SPSS software version 18.

Results: The microbial loads of minced camel meat samples were significantly reduced (p<0.0001) with increasing the dose of irradiation. The most effective dose was 5 kGy that highly reduced *S. enterica* serovar Typhimurium, and completely destroyed *E. coli* O157:H7. However, *E. coli* O157:H7 was more sensitive to electron beam irradiation than *S. enterica* serovar Typhimurium.

Conclusion: Electron beam irradiation effectively reduced the population of both *E. coli* O157:H7 and *S. enterica* serovar Typhimurium in minced camel meat in a dose dependent manner.

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Introduction

Camel meat is a good source of macro- and micro-nutrients, having fewer calories and cholesterol than the other red meat that are risk factors for heart and cardio-vascular diseases. This feature of camel meat along with healthy protein sources has led to increased demand for camel meat (Al-Owaimer et al., 2014; Kadim et al., 2008).

Meat meals are considered the most consumed foods in different populations, therefore their contamination by pathogenic bacteria can cause food poisoning epidemics

* Corresponding author (H. Zandi)
✉ E-mail: zandi@ssu.ac.ir
ORCID ID: https://orcid.org/0000000283232370

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(Hennekinne et al., 2015). Since minced meat is a proper environment for proliferation of spoilage and pathogenic bacteria, it is ranked among the most perishable foods (Aymerich et al., 2008).

For centuries, many methods have been developed to preserve food, among which, the irradiation method is safer and more affordable (Farkas and Mohácsi-Farkas, 2011; Kanatt et al., 2010; Tauxe, 2001). Currently, ionizing irradiation has been introduced as the most effective method to destroy spoilage and pathogenic microorganisms, without affecting nutritional and sensorial properties of foods. The International Atomic Energy Agency (IAEA), World Health Organization (WHO), and Food and Agriculture Organization (FAO) confirmed that irradiation up to 10 kGy provides health and safety of food without undesirable effects on human health (Lacroix and Follett, 2015; Roberts, 2014). Electron beam irradiation is one of the ionizing irradiations in which high energy is used for pasteurization and sterilization of foods. Electron beam power is electricity in which linear accelerator is used to generate accelerating electron beams close to the speed of light (Cabeza et al., 2009; Lung et al., 2015; Pillai and Shayanfar, 2018; Tahergorabi et al., 2012).

Since camel meat has recently found its status among consumers in Iran, and in addition, scientific studies on this subject are quite limited, the aim of this experimental study was to evaluate the effect of electron beam irradiation on survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Thyphimurium in minced camel meat during refrigerated storage.

Materials and methods

Preparation of the samples

Fresh camel meat was purchased from a slaughterhouse in Yazd, Iran, grounded by using a 3 mm grinder plate and put into a polystyrene bag. The final weight of minced meat was 25 g per bag. Electron beam irradiation was done using electron beam accelerator (TT-200, IBA, Belgium) at the Research Complex of Radiation Application (Taft, Yazd Province, Iran) for sterilization of the minced camel meat samples. The samples were exposed to an irradiation dose of 15 kGy at 10 MeV, beam dimension 1000×1000 mm, beam current 4 mA, and conveyer velocity 2/58 m/min. A cellulose triacetate and standard calorimeter dosimeter were used to determine the absorption dose. After sterilization, the samples were transported to the microbiology laboratory in dry ice.

Microbial strains

Two standard strains of pathogenic bacteria, including *S. enterica* serovar Typhimurium (ATCC 14028) and *E. coli* O157:H7 (ATCC 43895) were prepared from Pasteur Institute, Tehran, Iran. Each bacterial strain was subcultured in trypticase soy broth (Merck-Darmstadt, Germany) and incubated at 37 °C for 24 h.

Inoculation

To prepare the bacterial suspension, the 24-h cultivation of mentioned bacteria was prepared. Then, one or two colonies of fresh culture was inoculated to a tube containing 5 ml of Mueller-Hinton broth (Merck-Darmstadt, Germany) to create bacterial suspension with turbidity equal to 0.5 McFarland turbidity as 1.5×10^8 Colony Forming Unit (CFU)/ml.

Electron beam irradiation treatments

The desired bacterial suspension were inoculated to meat bags as 0.5 ml of each culture suspension was added to the pouches. Stomacher (Seward Medical, UK) was used in order to mix the bacteria with whole flesh. To study the effects of electron beam in different doses, the inoculated samples were immediately irradiated at doses of 0 (control), 1, 2, 3, and 5 kGy. After irradiation process, the pouches were stored instantly at 4±0.5 °C. Microbial analysis of *E. coli* O157:H7 and *S. enterica* serovar Typhimurium was carried out on days 0, 1, 3, 5, 7, and 10 of storage. It is necessary to mention that before inoculation of bacteria, microbiological confirmatory test was done in order to ensure the sterility of the samples. Three pieces of irradiated samples were randomly homogenized, and after preparation of dilutions, total bacterial count was investigated.

Microbial enumeration

Ten g of samples was homogenized at a medium speed for 2 min with 90 ml of 0.1% (w/v) sterile peptone water (Titanbiotch, India) in sterile stomacher bag. Afterwards 10^{-1}-10^{-4} serial dilutions were prepared and inoculated on sorbitol MacConkey agar (Merck-Darmstadt, Germany) for enumeration of *E. coli* O157:H7 and Xylose Lysine Deoxycholate gar (XLD; Merck-Darmstadt, Germany) for *S. enterica* serovar Typhimurium. After incubation of plates at 37 ºC for 24 h, the microbial count was expressed as CFU/g.

Statistical analysis

The experiments were investigated in triplicate. Data were analyzed using SPSS software v. 18.0. One-Way ANOVA and Repeated Measure ANOVA were used in order to compare the results of bacterial counts between the groups which treated with different doses of electron beam irradiation. The means were compared by Tukey post hoc test.

Results

The microbial load of control group inoculated with both *E. coli* O157:H7 and *S. enterica* serovar Typhimurium was 6.17 log CFU/g at day zero that was gradually increased until the end of storage period. It was shown that doses of 1 and 2 kGy caused about 2 log reduction in population of both bacteria at the day zero while doses of 3 and 5 kGy completely destroyed the bacteria in this day. Exposure to dose of 3 kGy was sufficient to remove bacteria up to 7th day. Although dose of 5 kGy inhibited the growth of *E. coli* O157:H7 by the 10th day, but *S. enterica* serovar Typhimurium was still viable on this day (Tables 1 and 2).

With increasing the irradiation dose, the population of bacteria in the samples inoculated with *E. coli* O157:H7 was significantly (p<0001) decreased up to 7th day. Afterward, the number of bacteria was significantly (p<0001) increased, while dose of 5 kGy resulted in complete elimination of *E. coli* O157:H7 in all days. With increasing irradiation dose, the number of *S. enterica* serovar Typhimurium was reduced till the 7th day, and after that, was significantly (p<0001) increased. In addition, doses of 3 and 5 kGy lead to complete remove of the bacteria only until 7th day.

The microbial loads of minced camel meat samples were reduced significantly (p<0.0001) with increasing the dose of irradiation. The most effective dose was 5 kGy that highly reduced *S. enterica* serovar Typhimurium and completely destroyed *E. coli* O157:H7.

Discussion

Like other kinds of red meats, camel meat may be contaminated with fecal pathogenic microorganisms at slaughterhouse. Thus, there is an important concern if the camel meat is consumed in semi-cooked manner which is common in some regions. So, finding a suitable dose of irradiation could be helpful for inactivation of pathogenic bacteria such as *E. coli* and *Salmonella*.

Table 1: Mean±standard error loads (log colony forming unit/g) of *Escherichia coli* O157:H7 in control and irradiated minced camel meat samples during refrigerated storage

Duration of storage (day)	Electron beam irradiation dose (kGy)				
	0 (control)	1	2	3	5
0	6.17±0.00	3.92±0.01	3.52±0.02	ND	ND
1	6.18±0.00	3.89±0.01	3.40±0.01	ND	ND
3	6.22±0.00	3.79±0.01	3.35±0.02	ND	ND
5	6.30±0.03	3.54±0.03	3.27±0.05	ND	ND
7	6.37±0.01	3.50±0.02	3.17±0.04	ND	ND
10	6.45±0.03	3.58±0.01	3.40±0.03	2.94±0.05	ND

ND: Not Detected

Table 2: Mean±standard error loads (log colony forming unit/g) of *Salmonella enterica* serovar Typhimurium in control and irradiated minced camel meat samples during refrigerated storage

Duration of storage (day)	Electron beam irradiation dose (kGy)				
	0 (control)	1	2	3	5
0	6.17±0.00	4.12±0.00	3.92±0.04	ND	ND
1	6.20±0.00	4.11±0.00	3.83±0.03	ND	ND
3	6.23±0.01	4.05±0.02	3.72±0.01	ND	ND
5	6.25±0.01	4.05±0.02	3.64±0.01	ND	ND
7	6.39±0.00	4.05±0.01	3.59±0.01	ND	ND
10	6.47±0.00	4.24±0.00	3.86±0.01	3.46±0.09	3.16±0.08

ND: Not Detected

In the current study, the microbial loads of inoculated meat were reduced by increasing the dose of irradiation. Study of Fallah et al. (2010) indicated that population of aerobic bacteria in barbecued chicken meat irradiated by 1.5 and 3 kGy doses of gamma ray were reduced 2 and 3.4 log cycle, respectively; while in the samples treated with a dose of 4.5 kGy, only a few number of bacteria were identified. These researchers revealed that

S. enterica serovar Typhimurium populations in grilled chicken meat were decreased for 3 and 5 log cycles after gamma irradiation at doses of 1.5 and 3 kGy, respectively; also doses of 3 and 4.5 kGy reduced the *E. coli* population to undetectable levels, which is in accordance with our findings. Similarly, Kanatt et al. (2005) concluded that 2 kGy dose of irradiation can completely eliminate *Staphylococcus* spp. in meat products stored at 0-3 °C. In addition, it was revealed that 3 kGy dose of irradiation can eliminate *E. coli* O157:H7, *S.* Typhimurium, and *Bacillus cereus* in commercial seed sprouts (Waje et al., 2009).

Current study showed that the count of pathogenic bacteria increased in non-irradiated groups (control) during the storage time. The initial population of bacteria in control group were greater than 6 log CFU/g, whereas dose of 5 kGy in minced camel meat samples inoculated by *E. coli* O157:H7 and *S. enterica* serovar Typhimurium decreased about 5 log cycle and 3 log cycle, respectively. Kim et al. (2010) reported that the population of aerobic bacteria in beef treated with gamma rays was gradually reduced at the end of the storage period, that is not in accordance with our findings. This controversy may be due to differences in irradiation source, bacterial species sensitivity, culture medium, and types of foods. Also, it has been declared that this variability could be because of difference in the bacterial responses to stresses such as irradiation (Aguirre et al., 2011).

We found that dose of 5 kGy was more effective than dose of 3 kGy in declining the pathogenic bacteria that is consistent with similar studies carried out on gamma irradiated Iranian barbecued chicken (Fallah et al., 2010) and minced camel meat (Al-Bachir and Zeinou, 2009). The results of present study on minced camel meat showed that *E. coli* O157:H7 was more sensitive to electron beam irradiation than *S. enterica* serovar Typhimurium, that is coincident with the research of Kundu et al. (2014) who showed that *Salmonella* inoculated on beef surfaces was more resistant to electron beam irradiation than *E. coli*.

Conclusion

Electron beam irradiation effectively reduced the population of both *E. coli* O157:H7 and *S. enterica* serovar Typhimurium in minced camel meat in a dose dependent manner. However, discoloration and oxidation may be occurred in irradiated vulnerable foods, e.g. meat and meat products. Thus, choosing and applying the appropriate irradiation dose in camel meat industries may be still a controversial issue that requires more researches. Further investigations should be designed to assess the impact of electron beam irradiation on physicochemical, sensorial, and nutritional properties of the minced camel meat.

Author contributions

H.Z. designed and supervised the study; A.A. conducted the experimental work and wrote the manuscript; H.M.K. analyzed the data. All authors read and approved the final manuscript.

Conflicts of interest

All the authors declared that this is no conflict of interest in the study.

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