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Research Article

Death Kinetics of *E. coli* O157:H7, *E. coli* and Natural Contaminant Coliforms in Minced Beef During Irradiation Treatment and Storage

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Abstract: The death kinetics of *Escherichia coli* O157:H7, *E. coli* type 1 and total coliforms after irradiation treatment in minced beef were evaluated to analyze the effect of increasing irradiation doses. Irradiation doses ranging from 0.0 kGy to 1.5 kGy were evaluated for reducing numbers of *E. coli* during frozen storage conditions at –18 °C for 30 days. D_{10} values of *E. coli* O157:H7, *E. coli* type 1, and total coliforms were 0.245 kGy, 0.552 kGy and 0.293 kGy, respectively. An irradiation dose of 1.5 kGy was shown to inactivate 10^5 MPN/g of serotype O157:H7 and 10^3 MPN/g of *E. coli* type 1. This inactivation level might be considered safe for the consumption of minced beef. Finally, *E. coli* type 1 was found to be a suitable indicator for assessing the impact of irradiation on *E. coli* O157:H7 serotype. There was no significant change in numbers of bacteria during frozen storage.

Key Words: Irradiation, *E. coli* O157:H7, *E. coli* type 1, death rate, storage, meat

E. coli O157:H7, *E. coli* ve Doğal Kontaminant Koliformların Kıymada Işınlama ve Depolama Sırasında Ölüm Kinetiği

Özet: Kıymada 0.0 – 1.5 kGy arasında uygulanan ışınlama sonrasında ve –18 °C'da 30 güne kadar yapılan depolama sırasında *Escherichia coli* O157:H7 ve *E. coli* tip 1'in ölüm kinetiği incelenmiştir. *E. coli* O157:H7, *E. coli* tip 1 ve doğal kontaminant koliformların D_{10} değerleri sırasıyla, 0.245 kGy, 0.552 kGy ve 0.293 kGy olarak belirlenmiştir.

Bulgular, 1.5 kGy ışınlamanın 10^5 EMS/g O157:H7 serotipi ve 10^3 EMS/g *E. coli* tip 1'i inaktive ettiğini ve kıymanın güvenilir bir biçimde tüketilebilmesi için bu inaktivasyon değerinin yeterli olduğunu, *E. coli* tip 1'in, *E. coli* O157:H7 için iyi bir ışınlama indikatörü olduğunu göstermiştir. Donmuş depolama boyunca bakteri sayılarında istatistik açıdan önemli bir değişme olmamıştır.

Anahtar Sözcükler: Işınlama, *E. coli* O157:H7, *E. coli* tip 1, ölüm oranı, depolama, kıyma

Introduction

Most *Escherichia coli* strains are commonly encountered among the normal facultative anaerobic microflora of the human intestinal tract and are assumed to be beneficial as they are involved in vitamin synthesis. However, some serotypes, such as O157:H7, are among the most significant food-borne human pathogens. *E. coli* O157:H7 has been known to be an important pathogen since the early 1980s, when it caused epidemics and sporadic cases of bloody diarrhea (1,2). The infection has been known to develop into severe hemolytic uremic syndrome. Its inter-human transmission as well as animal to human transmission have been documented. The more serious syndrome of hemolytic uremia has mostly affected the immune suppressed, children or elderly, and deaths have occurred particularly in nursing homes (3,4). The prevalence of *E. coli* O157:H7 in 3450 minced meat samples was reported as 0.12% (5).

E. coli O157:H7 is different from other *E. coli* serotypes. This serotype is known to be tolerant to acid and salt, sorbitol negative, MUG negative, unable to grow at 44.5 °C, resistant to cefixime-tellurite and sensitive to heat and irradiation (1,6,7). Most food-borne outbreaks caused by this microorganism have been associated with the consumption of foods of bovine origin such as ground beef and raw milk. Although it is easily destroyed by sufficient cooking of hamburgers and other meat products, in places such as fast-food restaurants, it is

frequently found to be the most common cause of sporadic cases or outbreaks (6,7).

Growth modelling of bacterial food pathogens as affected by pH, temperature and nutrient composition has revolutionized the discipline of predictive microbiology. Data provided by such an approach have been of major significance in the development of new food formulations, the design of food processing operations and the application of risk management systems. Commonly, predictive models are based upon data that are derived from investigations using pure cultures of the test organisms grown in defined media. Predictive models have been produced for *E. coli* O157:H7, defining its growth characteristics. It is essential that before being applied to a specific food product, models are validated by confirming the growth of the respective organisms in the food before use. Although validation studies have demonstrated good agreement between theoretical models and applied situations, in certain cases the growth of organisms in the food tests has differed considerably from that predicted in the modelling studies (8,9).

Irradiation can damage and destroy most food-borne bacteria. The biological effects of ionizing radiation on cells can be due both to direct interactions with critical cell components and to indirect actions on these critical targets caused by radiolytic products of other molecules in the cell, particularly free radicals formed from water (10). In ensuring high standards of improved food safety, microorganisms are a major factor that cannot be ignored. In this regard, food and especially meat irradiation is an effective treatment process for the inactivation of bacteria. Low-dose irradiation has been shown to provide adequate inactivation levels to ensure safe consumption of meats even if undercooked (11).

While a number of studies on *E. coli* O157:H7 strains have been carried out in Turkey, studies of the irradiation effects on this particular serotype are inadequate. The aim of this study was to determine the death kinetics and behavior of *E. coli* O157:H7 and as a controlled serotype of *E. coli* type 1 in the irradiation treatment and storage period of minced beef. The determination of the possible use of *E. coli* type 1 as a biological indicator was the second aim of this research.

Materials and Methods

Bacterial cultures and preparation of inocula

A mixture of 5 strains each of *E. coli* type 1 and *E. coli* O157:H7 was used. All strains of *E. coli* type 1 were obtained from the culture collection of the Department of Food Engineering, Ankara University (Ankara, Turkey). The 5 strains of *E. coli* O157:H7 were provided by 5 different culture collection centers: the Department of Food Engineering, Hacettepe University (Ankara, Turkey); the Ministry of Health (Ankara, Turkey); Landbouw University (Holland), the Department of Food Engineering, Ege University (İzmir, Turkey); and the Ministry of Agriculture and Rural Affairs (Inspection Department, İstanbul Turkey).

Each of the 5 strains of *E. coli* type 1 and *E. coli* O157:H7 were inoculated into 10 ml of Tryptic Soy broth (TSB; Merck, Germany) individually and incubated for 16-18 h at 37 °C. These fresh cultures of each strain were then mixed together in equal volumes to generate composite cultures of *E. coli* type 1 or *E. coli* O157:H7.

Minced beef

All minced beef samples used in this study were purchased from retail markets in Ankara. For achieving low-level natural contaminant *E. coli* type 1, the minced meat samples were ground from sliced beef under conditions as hygienic as possible.

Radiation source

Irradiation processes were carried out at room temperature (18-20 ºC) using an Issledovatelj (Gamma-cell; Cobalt 60) irradiator, operated at a dose rate of 2.67 kGy/h, in the Department of Food Irradiation and Sterilization of Ankara Nuclear Research Center in Agriculture and Animal Science of the Turkish Atomic Energy Authority.

Experimental design

The minced beef was first analyzed for natural contamination by total coliforms including *E. coli* type 1 and *E. coli* O157:H7.

Numbers of total coliforms and *E. coli* type 1 were determined using the standard MPN technique with Lauryl Sulfate Tryptose (LST) Broth + MUG (Merck, Germany) medium. Ten grams of minced beef was homogenized for 2 min in 90 ml of Maximum Recovery Diluent (Merck, Germany); after serial dilutions, samples

were incubated in LST broth + MUG for 24 h at 37 °C and then evaluated for the production of gas (for total coliforms) and fluorescence (for *E. coli* type 1). Only gas-positive tubes were evaluated for fluorescence (under 365 nm wavelengths, Merck, Germany) by UV lamp (12).

For the detection of *E. coli* O157:H7, 25 g of minced beef was homogenized for 2 min in 225 ml of TSB supplemented with standard concentrations of vancomycin (8.00 mg/l), cefixime (0.05 mg/l), and cefsulodin (10.00 mg/l) using a stomacher (Laboratory Equipment, London, UK) and then incubated at 37 °C for 24 h. One loop of enriched culture from growth positive flasks was spread onto Sorbitol MacConkey agar supplemented with cefixime – tellurite (Cefixime 0.05 mg/l- Potassium tellurite 2.5 mg/l) (CT-SMAC; Merck, Germany) (12). After the incubation at 37 °C for 24 h, sorbitol-negative colonies were analyzed by O157 latex test (Oxoid). As mentioned in the results section, no positive result was obtained by this test.

Minced beef was divided into 36 portions (10 g each) in sterile polyethylene bags; 12 of these were inoculated with *E. coli* type 1 mixture (approximately 10^5 cfu/g final concentration in minced beef). Twelve of the others were inoculated with *E. coli* O157:H7 serotype mixture (approximately 10^5 cfu/g final concentration in minced beef), while the remaining samples were used as controls for natural contamination with total coliforms. After inoculation, each meat sample was individually hand massaged under sterile conditions for 1 min to evenly distribute the inocula in the meat prior to irradiation at 0.0, 0.5, 1.0 and 1.5 kGy. The irradiated samples were then stored at –18 ºC for 30 days. Microbiological analyses were performed after 0, 15 and 30 days of storage.

Experimental trials were carried out independently in 4 replicates.

Enumeration of bacteria

Individual 10 g test samples were homogenized for 2 min with 90 ml of Maximum Recovery Diluent. The standard MPN technique was applied. Incubation media for total coliforms, *E. coli* and *E. coli* O157:H7 were standard LST Broth, LST Broth + MUG, and TSB supplemented with the standard concentration of vancomycin (8.00 mg/l), cefixime (0.05 mg/l), and cefsulodin (10.00 mg/l), respectively. Samples were incubated in supplemented TSB for 24 h at 37 ºC. After the incubation of TSB, all growth-positive tubes were streaked on CT-SMAC agar separately. These plates were incubated at 37 ºC for 18-24 h. Sorbitol-negative colonies were confirmed by latex test for the presence of *E. coli* "O157" somatic antigen and positive dishes used for standard MPN determination. Total coliforms and *E. coli* type 1 were analyzed separately by standard MPN (12).

Statistical analysis and mathematics of death kinetics

The simple arithmetical means of individually obtained log values of the numbers of bacteria from 4 replicates were used in the statistical analysis. Minitab Statistical Software Release 13.1 was used for the statistical analyses.

The death curves of *E. coli* O157:H7, *E. coli* type 1 and coliforms were plotted as a function of increasing irradiation dose and the length of storage time. The appropriate linear equation for each death curve was selected before the linear regression analysis was carried out to obtain the negative slope or specific death rate.

Results

Microbiological analyses of the ground beef for natural contaminants resulted in no *E. coli* type 1 (<0.3 MPN/g) or no *E. coli* O157:H7 serotype (none in 25 g) in any replicates.

The results are presented on a logarithmic scale. Mean results for the 4 replicates are given in Table 1. Data from samples of total coliforms irradiated at 1.0 kGy and the results from *E. coli* O157:H7 at 1.5 kGy were not taken into account, as levels of contamination were.

In Table 2, the linear regression equations, the regression coefficients and D_{10} values for the 3 groups of bacteria obtained from the death curves at the irradiation treatment are shown.

Discussion

E. coli O157:H7 has been of particular concern in the USA and other developed countries in recent years. It is not as common a cause of food-borne illness as *Salmonella* or *Campylobacter* but its extreme virulence has caused several outbreaks and mortality rates as high as 30% have been recorded, especially among young people. Most of these illnesses have been associated with the consumption of undercooked, contaminated ground

Table 1. Number of bacteria (log MPN/g) after irradiating at different doses and during subsequent storage at −18 ℃ (n = 4).

Dose	Total Coliforms			*E. coli* O157:H7			*E. coli* type 1		
kGy	day 0	day 15	day 30	day 0	day 15	day 30	day 0	day 15	day 30
0.0	2.86 ± 0.16$^{(a)}$	2.52 ± 0.15$^{(a)}$	2.47 ± 0.19$^{(a)}$	5.51 ± 0.09$^{(a)}$	5.26 ± 0.74$^{(a)}$	5.18 ± 0.06$^{(a)}$	5.75 ± 0.14$^{(a)}$	5.35 ± 0.12$^{(a)}$	4.43 ± 0.08$^{(a)}$
0.5	1.20 ± 0.09$^{(b)}$	1.11 ± 0.04$^{(b)}$	1.06 ± 0.02$^{(b)}$	3.67 ± 0.12$^{(b)}$	3.34 ± 0.11$^{(b)}$	3.19 ± 0.07$^{(b)}$	5.28 ± 0.08$^{(b)}$	5.09 ± 0.08$^{(ab)}$	4.28 ± 0.15$^{(ab)}$
1.0	<0.48	<0.48	<0.48	1.43 ± 0.67$^{(c)}$	1.36 ± 0.01$^{(c)}$	1.18 ± 0.06$^{(c)}$	4.11 ± 0.04$^{(c)}$	4.04 ± 0.05$^{(c)}$	3.67 ± 0.18$^{(bc)}$
1.5	<0.48	<0.48	<0.48	<0.48	<0.48	<0.48	3.11 ± 0.04$^{(d)}$	3.04 ± 0.05$^{(d)}$	2.78 ± 0.16$^{(d)}$

a, b, c, d; Values denoted by the same letters indicate a statistically nonsignificant difference between the data within each column.

Table 2. Linear regression equations, regression coefficients (R^2) and D_{10} values.

Group	Equation	Regression Coefficient	D_{10}
Total Coliforms	y = -3.4220x + 2.8560	R^2 = 1.0000	0.293
E. coli type 1	y = -1.8114x + 5.8893	R^2 = 0.9817	0.552
E. coli O157:H7	y = -4.0720x + 5.5713	R^2 = 0.9968	0.245

x : Irradiation doses
y : Numbers of bacteria

beef used in hamburgers, and several major recalls of this product have been made. In 1997, as a result of *E. coli* O157:H7 problems in the USA, the use of food irradiation brought the debate into the public domain. It has been shown that a relatively low irradiation dose such as 1.5 kGy is sufficient to give a 4 log reduction in the numbers of *E. coli* O157:H7 at 5 °C (13).

There are many factors influencing the resistance of bacteria to irradiation. A few of these are the genus and species (or in some cases serotype), growth phase (log or stationary phase vegetative cell, spore), irradiation temperature, water content, presence of salt, oxygen, and atmospheric pressure. It was found that the radiation resistances of *Bacillus cereus*, *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium significantly increased when the irradiation temperature decreased. These effects can be described by predictive equations relating both radiation dose and temperature. In addition, secondary factors, such as the growth medium upon which the surviving bacteria are enumerated, may greatly influence the results obtained (14).

E. coli O157:H7 and total coliforms were twice as sensitive to the irradiation process when compared with *E. coli* type 1 in the present study. D_{10} values for each group were calculated as 0.245 kGy, 0.552 kGy and 0.293 kGy for *E. coli* O157:H7, *E. coli* type 1 and total coliforms, respectively (Table 2). In another study, the D_{10} value for *E. coli* O157:H7 was calculated as 0.30 kGy (mean of 5 strains) at 5 °C and it was determined that the meat type did not affect the D_{10} value (14).

One log of viable *E. coli* O157:H7 in chicken meat was eliminated by doses of 0.27 kGy at 5 ℃ (15). It was found that the D_{10} values of *E. coli* O157:H7 ranged from 0.21 to 0.307 kGy (6). In a similar study the D_{10} value of *E. coli* O157:H7 at 4 ℃ was 0.39 kGy (15). It was reported that a relatively low irradiation dose of 1.5 kGy was sufficient to induce a 4-log reduction in the numbers of *E. coli* O157:H7 at 5 ℃ (13). Therefore, the radiation dose was required to reduce the population of the microorganisms 10-fold with the increase in irradiation dose. *E. coli* type 1 was twice as resistant to death as *E. coli* O157:H7.

For many microorganisms, the logarithmic phase of the growth curve can be represented as an exponential function. Most microbiological data are not expressed as the ln cfu/ml, but are expressed as the $\log_{10}$ cfu/ml. A similar plot of the $\log_{10}$ of cfu/ml vs. time can also be

used, giving a slightly different formula as t_g = log(2)/slope = 0.301/μ. Mathematically, the difference between the growth and death rates is a matter of sign. Consequently, the exponential growth function can be converted to the death function as t_d = 0.301/μ (16,17).

Linear regression analysis was carried out to obtain the specific death rate for *E. coli* type 1 and *E. coli* O157:H7. Increasing values of specific death rate were observed with increases in irradiation doses. The negative slope of each regression equation obtained from the death curves was named the specific death rate (μ'; $\mu'_{irradiation}$ and $\mu'_{storage}$). Table 2 shows the evaluation of specific death rates according to irradiation dose. The specific death rates of total coliforms, *E. coli* type 1, and *E. coli* O157:H7 were calculated as μ'_{ir} = –3.4220, μ_{ir}' = -1.8114 and μ_{ir}' = -4.0723, respectively. It is important to note that μ_{ir}' values reflect the death process of an exponentially decreasing population.

The numbers of all the tested bacteria irradiated by different doses and stored for up to 30 days at –18 °C in minced beef samples did not vary statistically. Several studies on unirradiated minced beef reported that the *E. coli* O157 serotype is resistant to frozen storage conditions (18,19). Our results showed that irradiation has no negative effect on those bacteria during frozen storage.

In conclusion, during the irradiation process, if *E. coli* type 1 can be reduced to acceptable levels, the O157:H7 serotype of *E. coli* will similarly be reduced to a more acceptable level due to its lower D_{10} value. Hence, the more easily and more quickly determined *E. coli* type 1 may serve as an irradiation indicator for *E. coli* O157:H7. Storage at –18 °C does not affect the viability of the microorganisms when compared to the direct effect of irradiation. Furthermore, probability modelling of death and survival is an important way to achieve a better understanding of how to deal with the complexity of subsequent processing, including irradiation treatment and storage.

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