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).

Death Kinetics of *E. coli* 0157:H7, *E. coli* type 1 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
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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 (Izmir, Turkey); and the Ministry of Agriculture and Rural Affairs (Inspection Department, Istanbul 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 °C) 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 °C 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 °C. After the incubation of TSB, all growth-positive tubes were streaked on CT-SMAC agar separately. These plates were incubated at 37 °C 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 beef.
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. D10 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 D10 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 D10 value (14).

One log of viable E. coli O157:H7 in chicken meat was eliminated by doses of 0.27 kGy at 5°C (15). It was found that the D10 values of E. coli O157:H7 ranged from 0.21 to 0.307 kGy (6). In a similar study the D10 value of E. coli O157:H7 at 4°C 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°C (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 log10 cfu/ml. A similar plot of the log10 of cfu/ml vs. time can also be

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

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>Total Coliforms</th>
<th>E. coli O157:H7</th>
<th>E. coli type 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 0</td>
<td>day 15</td>
<td>day 30</td>
</tr>
<tr>
<td>0.0</td>
<td>2.86 ± 0.16(a)</td>
<td>2.52 ± 0.15(a)</td>
<td>2.47 ± 0.19(a)</td>
</tr>
<tr>
<td>0.5</td>
<td>1.20 ± 0.09(b)</td>
<td>1.11 ± 0.04(b)</td>
<td>1.06 ± 0.02(b)</td>
</tr>
<tr>
<td>1.0</td>
<td>&lt;0.48</td>
<td>&lt;0.48</td>
<td>&lt;0.48</td>
</tr>
<tr>
<td>1.5</td>
<td>&lt;0.48</td>
<td>&lt;0.48</td>
<td>&lt;0.48</td>
</tr>
</tbody>
</table>

(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.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Equation</th>
<th>Regression Coefficient</th>
<th>D_{10}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Coliforms</td>
<td>y = -3.4220x + 2.8560</td>
<td>$R^2 = 1.0000$</td>
<td>0.293</td>
</tr>
<tr>
<td>E. coli type 1</td>
<td>y = -1.8114x + 5.8893</td>
<td>$R^2 = 0.9817$</td>
<td>0.552</td>
</tr>
<tr>
<td>E. coli O157:H7</td>
<td>y = -4.0720x + 5.5713</td>
<td>$R^2 = 0.9968$</td>
<td>0.245</td>
</tr>
</tbody>
</table>

x: Irradiation doses
y: Numbers of bacteria
used, giving a slightly different formula as \( t_g = \frac{\log(2)}{\text{slope}} = 0.301/\mu \). 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 \( t_d = \frac{0.301}{\mu} \) \((16,17)\).

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 \( \mu' \) (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 \( \mu'_\text{ir} = -3.4220 \), \( \mu'_\text{ir} = -1.8114 \) and \( \mu'_\text{ir} = -4.0723 \), respectively. It is important to note that \( \mu'_\text{ir} \) 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 \(-18 \, ^\circ\text{C}\) 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 \(-18 \, ^\circ\text{C}\) 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.

References


