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Identification of the gamma irradiation dose applied to ground beef that reduces Shiga toxin producing *Escherichia coli* but has no impact on consumer acceptance

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ABSTRACT

The aims of the present study were: a) to estimate the minimal dose of gamma irradiation required to reduce 5 log CFU/g of native O157 and non-O157 Shiga toxin-producing *Escherichia coli* population in ground beef samples inoculated with high inoculum; b) to assess its effectiveness in samples with low inoculum and 3) to evaluate consumer acceptance. Based on the results, 1 kGy was estimated as the minimal dose of gamma irradiation required to reduce 5 log CFU/g of STEC in ground beef. However, when samples with low inoculum level were subjected to 1 kGy, 3.9% of the samples were positive for *stx* and *eae* genes after an enrichment step. Consumer acceptance analysis was carried out with samples subjected to 2.5 kGy and no significant differences were found between irradiated and control samples. Therefore, 2.5 kGy was identified as the gama irradiation dose that reduces STEC but has no impact on consumer acceptance of ground beef.

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens that can cause bloody diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (HUS). STEC O157:H7 is the most common serotype associated with human diseases worldwide, including Argentina. However, recent increases in the number of outbreaks and sporadic cases were due to non-O157 STEC serogroups O26, O45, O103, O111, O121 and O145 (Gould et al., 2013). The origin of a large number of outbreaks of human illnesses have been traced to the consumption of undercooked ground beef (Kintz, Brainard, Hooper, & Hunter, 2017). The percentage of isolation of *E. coli* O157 in ground beef ranged from 0.1 to 0.8% and for non-O157 STEC ranged from 1 to 16% (Barlow, Gobius, & Desmarchelier, 2006; Mora et al., 2007; Rhoades, Duffy, & Koutsoumanis, 2009; Samadpour et al., 2006)). In Argentina, Leotta et al. (2016) reported that the percentage of isolation in ground beef ranged from 3.5 to 11.6% for *E. coli* O157 and from 7 to 12.8% for non-O157 STEC.

Gamma irradiation has been proposed as an effective technology to control foodborne pathogens (Parnes & Lichtenstein, 2004). Several authors have assessed the effectiveness of gamma irradiation in the inactivation of STEC in ground beef (Clavero, Monk, Beuchat, Doyle, & Brackett, 1994; Sommers et al., 2015; Thayer & Boyd, 1993). This kind of assays usually involves samples that are artificially inoculated with a high concentration of bacteria and subjected to different gamma irradiation doses. Survival microorganism are determined by plate count and bacterial resistance is estimated by D_{10} values, which represents the absorbed radiation dose required to inactivate 90% of a viable bacterial population.

Samples inoculated with a low concentration of bacteria are particularly useful to try to mimic an actual situation where, if STEC is present

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in ground beef, its concentration is very low. Moreover, it provides an opportunity to assess the irradiation effectiveness against STEC by real time PCR after an enrichment step, which is the method described in ISO/TS 13136:2012 for the detection of STEC in products intended for human consumption (ISO/TS, 2012).

To the best of our knowledge, there are no other studies that have assessed the effectiveness of gamma irradiation in samples with low inoculum. Therefore, the aims of the present study were: a) to estimate the minimal dose of gamma irradiation required to reduce 5 log CFU/g of native O157 and non-O157 Shiga toxin-producing *Escherichia coli* population in ground beef samples inoculated with high inoculum; b) to assess its effectiveness in samples with low inoculum and 3) to evaluate consumer acceptance.

2. Materials and methods

2.1. Experimental design

Assay 1. Estimation of the minimal dose of gamma irradiation required to reduce 5 log CFU/g of STEC O157 and non-O157 artificially inoculated in ground beef. The samples consisted of 25 g of ground beef inoculated both, individually and as a pool, with STEC O157, O26, O103, O111 and O145 at a final concentration of 7 log CFU/g. The gamma irradiation doses evaluated were: 0.26; 0.44; 0.67 and 0.86 kGy. Microbial analysis was performed by plate count. Surviving microbial counts were used to build inactivation curves and the results were expressed as D_{10} values (reciprocal of the slope). Each treatment was carried out 3 times with 3 replicates each.

Assay 2. Assessment of the effectiveness of the minimal gamma irradiation dose, estimated in assay 1, in ground beef samples inoculated with a low concentration of STEC. The samples consisted of 25 g of ground beef inoculated individually with the strain that achieved the highest D_{10} value in assay 1 and with a pool of STEC O157, O26, O103, O111 and O145 at a final concentration of 4 CFU/g. The gamma irradiation dose was determined based on the results of assay 1. Microbial analysis was performed by real-time polymerase chain reaction (RT-PCR) after an enrichment step. Results were expressed as a percentage of positive samples to *stx* and *eae* genes. The treatment was carried out 3 times with 30 replicates each.

Assay 3. Consumer acceptance test. The samples for this assay were beef burgers prepared with both, irradiated and non-irradiated ground beef. The gamma irradiation dose was 2.5 kGy. Overall liking and acceptance related to specific attributes (appearance, texture and flavor) were assessed. The test was carried out with 108 consumers familiar with the product category (see 1.8 section below).

2.2. Raw material

The ground beef was provided by "Cooperativa Obrera", a local supermarket. For assay 1 and 2, after freezing (−18 °C), it was irradiated with 19 kGy to eliminate the interference of local microbiota and divided into samples of 25 g each and placed into stomacher bags (Nasco Whirl-Pak; USA).

2.3. Bacterial strains and inoculum preparation

For this study, we used STEC strains included in IGEVET culture collection. STEC O26 (*stx*$_1$/*eae*) and O157 (*stx*$_2$/*eae*) were isolated from beef products, O145 (*stx*$_2$/*eae*) was isolated from a patient with HUS, and O103 (*stx*$_1$/*eae*) and O111 (*stx*$_2$/*eae*), both were isolated from patients with diarrhea. The strains were kept in frozen culture at −80 °C. Then, subcultures were prepared by inoculating a test tube containing 10 ml of tryptic soy broth (TSB, Biokar, France) with a single colony grown in tryptic soy agar (TSA, Biokar, France). Cultures were individually incubated overnight at 37 °C. Cells were harvested by centrifugation at 4000 ×*g* for 10 min (Unicen 21; Ortoalresa; Spain). The pellets were washed twice with phosphate-buffered saline (PBS, pH 7.2, Oxoid, UK). In order to guarantee that all strains had similar concentrations, the optical density was measured at 600 nm and fixed at the same value using a spectrophotometer (Metrolab 330, Metrolab, Argentina). For the first assay, all strains were inoculated both, independently and as a pool. For the second assay, samples were inoculated with both, a STEC pool and the strain that achieved the highest D_{10} value in assay 1.

2.4. Inoculation procedure

The inoculation procedure was carried out in a biological safety cabinet (BSL-2). For the inoculation procedure, 50 μl of the STEC inoculum were added to the samples previously placed in a stomacher bag and mixed by pressing the bag externally. Finally, the stomacher bags were closed and kept at refrigeration until processing. The final concentration for samples with high inoculum level was 7 log CFU/g. For assay 2, the same procedure was carried out with the caveat that the 50 μl were from a diluted inoculum of STEC. The final concentration for these samples was 4 CFU/g (low inoculum level samples).

2.5. Irradiation treatments

Treatments were carried out in a semi-industrial irradiation facility (cobalt-60 source) at the Centro Atómico Ezeiza, Comisión Nacional de Energía Atómica, Argentina (activity, 820 kCi; temperature, average dose rate, 8.7 kGy/h; average dose uniformity, 1.05 kGy). An electron paramagnetic resonance (*E*-scan Bruker) with BioMax™ alanine dosimeter film (Kodak) was used to measure the absorbed dose. The calibration curve was provided and is traceable to the primary laboratory NIST (National Institute of Standards and Technology, USA). The gamma irradiation doses evaluated in the first assay were as follows: 0.26, 0.44, 0.67 and 0.86 kGy. The gamma irradiation dose evaluated in the second assay (1 kGy) was dependent on the results of the first assay and the gamma irradiation dose evaluated in the third assay was 2.5 kGy.

2.6. Microbiological analysis of samples with high inoculum level (assay 1)

A total of 225 ml of 0.1% peptone water (PW, Biokar, France) was added to the sample in the bag. Immediately after, samples were stomached (easy Mix, AES, France) for 60 s and serial dilutions were prepared. STEC counts were performed in TSA. A duplicate set of plates was incubated overnight at 37 °C. Surviving bacteria counts, expressed as log CFU/g, were used to build the inactivation curves and the D_{10} values were determined by the reciprocal of the slope.

2.7. Microbiological analysis of samples with low inoculum level (assay 2)

A total of 225 ml of modified TSB (mTSB, Biokar, France) was added to the sample in the bag. Immediately after, samples were incubated at 42 °C for 20 h. Following the enrichment step, samples were tested for the presence of *stx*$_1$, *stx*$_2$ and *eae* genes by RT-PCR (Pall Corporation, USA).

2.8. Acceptance test (assay 3)

For the acceptance test, beef burgers were prepared as described by Szerman, Ferrari, Sancho, and Vaudagna (2019). The burger formulation was: 90% meat and fat; 1.5% NaCl; 0.25% sodium tripolyphosphate and 8.25% water. Samples were cooked according to AMSA guidelines (AMSA, 2015) from a frozen state, for ≈13 min (to achieve an internal temperature of 71 °C) in a preheated electric grill (155 ± 5 °C) (George

Foreman®, Spectrum Brands, USA). The acceptance test was carried out with 108 consumers. Samples were cooked and immediately served. Each consumer received two pieces of each sample (2 cm wide by 1 cm thick) in insulated thermal containers coded with random three-digit numbers. Consumers were asked to assess the acceptability of the samples (in general and for the specific attributes appearance, texture and flavor). A verbal nine-point hedonic scale was used for the evaluation where 9: like extremely and 1: dislike extremely.

2.9. Statistical analysis

Assay 1 was carried out 3 times with 3 replicates each while assay 2 was carried out 3 times with 30 replicates each. ANOVA was used to analyze the effect of the irradiation dose on microbial counts. Statistical analysis functions of MS Excel (Microsoft Corp., Redmond, WA) were used for regression analysis and the D_{10} determination. Consumer acceptance test (assay 3) was carried out with 108 consumers and a two-

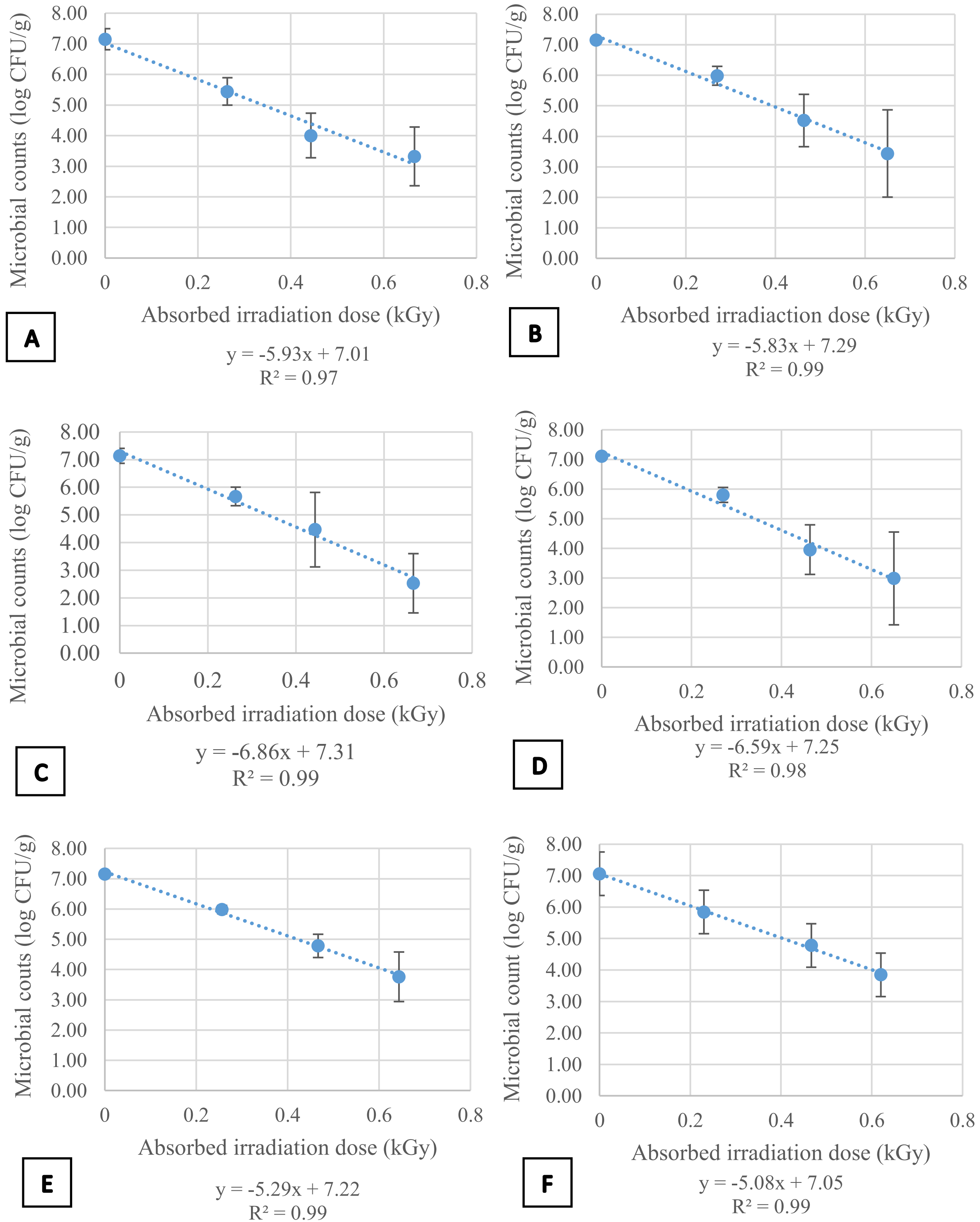

Fig. 1. Gamma irradiation inactivation curves for STEC O26 (A), O103 (B), O111 (C), O145 (D) O157 (E) and for the pool of STEC (F) cells inoculated in ground beef. Results are expressed as mean; n = 9 per dose. Error bars represent the standard deviation.

way ANOVA was used to evaluate data from sensory analysis, considering as factors both, consumer and sample (Kilcast, 2010). The software used was Infostat v 2018.

3. Results

3.1. Assay 1

The inactivation curves are shown in Fig. 1 (A-F). The STEC D_{10} values were 0.15 kGy for O103 and O145, 0.17 kGy for O26 and O111, and 0.19 kGy for O157. As to the STEC pool, the D_{10} value was 0.20 kGy. The R^2 values were as follows: 0.97, 0.99, 0.99, 0.98, 0.99 and 0.99 for O26, O103, O111, O145, O157 and for the STEC pool, respectively. Based on the results, the strain that achieved the highest D_{10} value was STEC O157 and the minimal dose of gamma irradiation required to reduce 5 log CFU/g of STEC in ground beef was 1 kGy (5*0.20 kGy).

3.2. Assay 2

Samples with low inoculum level were irradiated with 1 kGy, enriched overnight and tested by RT-PCR. The results were as follows: in repetition number 1, none of the irradiated samples were positive for *stx* and *eae* genes. In repetition number 2, four-out of thirty samples inoculated with STEC O157 were positive for *stx* and *eae* genes, while none of the samples inoculated with the STEC pool were positive. In repetition number 3, three-out of thirty samples inoculated with the STEC pool were positive for *stx* and *eae* genes while none of samples inoculated with STEC O157 were positive. Taking into consideration all three repetitions, 4 out of 90 (4.4%) samples inoculated with STEC O157 were positive for *stx* and *eae* genes and 3 out of 90 (3.3%) samples inoculated with a STEC pool were positive. Therefore, 1 kGy was not enough to guarantee total STEC inactivation. As to control samples, all of them (100%) were positive for *stx* and *eae* genes.

3.3. Assay 3

The irradiation dose selected for this assay was 2.5 kGy. The selection of this value was based not only on the dose range expected during commercial operation (the dose uniformity ratio, D_{max}/D_{min} should be lower than 3) (Eichholz, 2003; International Atomic Energy Agency, 2006), but also by the fact that 1 kGy was not enough to guarantee total STEC inactivation. With higher irradiation doses, STEC cells should not be able to recover. Our previous research demonstrated that after a treatment with 2 kGy the same STEC strains as those used in the assay inoculated on beef trimmings instead of ground beef, were all negative for *stx* nor *eae* genes by RT-PCR after an enrichment step (Cap et al., 2020). The irradiated and non-irradiated ground beef was used to prepare the beef burgers that were consumed during the acceptability test. The ANOVA results showed no significant differences ($P > 0.01$) between non-irradiated and irradiated samples, both in terms of overall liking as well as in particular attributes acceptability (Fig. 2). Mean values obtained were in all cases between “like slightly” and “like moderately”.

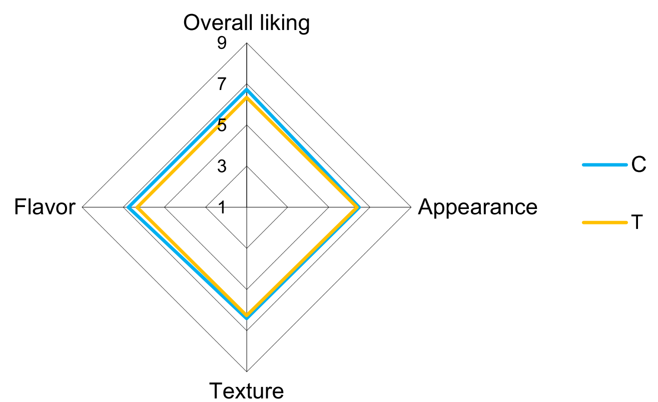

Fig. 2. Star diagram of acceptance test results.
C: control samples (non-irradiated); T: Treated samples (irradiated).

4. Discussion

The STEC D_{10} values were 0.15 kGy for O103 and O145, 0.17 kGy for O26 and O111, and 0.19 kGy for O157. As to the STEC pool, the D_{10} value was 0.20 kGy. These results are in agreement with Sommers et al. (2015) who determined the gamma radiation D_{10} value of a large set of genetically diverse STEC strains, inoculated in ground beef and reported that the D_{10} value ranged from 0.16 to 0.48 kGy. Likewise, authors informed that STEC strains that lacked *eae* gene had a mean D_{10} significantly higher (0.37 kGy) than those with *eae* gene (0.27 kGy). Clavero et al. (1994) evaluated *E. coli* O157:H7 resistance inoculated in beef burgers with two levels of fat content and reported that the D_{10} values were between 0.24 and 0.31 kGy, regardless of the fat content. Thayer and Boyd (1993) applied a surface model to evaluate the combined effect of irradiation dose, temperature and vaccum atmosphere on the inactivation of *E. coli* O157:H7 inoculated on poultry and beef meat. Authors described a significant effect of temperature on the strain resistance as the mean D_{10} value at 4 °C was of 0.27 kGy and the mean D_{10} value at −5 °C was of 0.44 kGy. In addition, they also reported significant differences, dependent upon the phase of the growth curve. If the strains were in the exponential growing phase, the D_{10} value was 0.16 kGy while if strains were in the stationary growing phase, the D_{10} value was 0.27 kGy. The vaccum atmosphere had no effect on bacterial resistance. The strains evaluated in the present study were all positive for the *eae* gene, the irradiation treatment was applied in refrigeration conditions but the strains were in the stationary growing phase. When comparing the D_{10} values informed by the other authors with the D_{10} values reported in the present study, we can conclude that our strains had low resistance to gamma irradiation. Based on our results, the minimal dose of gamma irradiation required to reduce a 5 log CFU/g of STEC in ground beef was 1 kGy and it was estimated by multiplying by 5 the highest D_{10} obtained in assay 1 (0.20 kGy).

The aim of assay 2 was to try to mimic a real situation where if the ground beef presents STEC, its concentration is very low and, where the methodology to detect it, according to international standards, is through a RT-PCR after an enrichment step. Results showed that 4.4% of the samples inoculated with STEC O157 were positive and 3.3% of the samples inoculated with a STEC pool were positive for *stx* and *eae* genes. These results were unexpected as 1 kGy should had been more than enough to guarantee the inactivation of 2 log CFU per sample. A possible explanation is that in some STEC cells the irradiation treatment did not cause a lethal damage, hence, STEC cells were able to repair themselves during the enrichment step and become detectable by RT-PCR. In a recent study, it has been demonstrated that STEC O157:H7 can resist to a non-lethal irradiation dose through important modifications in genes expression and proteins profiles which includes nucleotides excision repair (Gaougaou et al., 2020). With higher irradiation doses, STEC cells should not be able to recover (Cap et al., 2020).

The results of the present study highlight the importance of assessing the efficacy of an antimicrobial treatment with more than one level of inoculum and including an enrichment step in which injured cells are capable of recovering. The existence of injured microorganisms in food and their recovery during culturing procedures is critical, since pathogens in this injured state may constitute a public health hazard (Wu, 2008).

As to consumer’s acceptance, no significant differences ($P > 0.05$) were found between acceptability of the samples irradiated with 2.5 kGy and the non-irradiated samples ones (control samples). Mean values obtained were similar to those reported by Schilling et al. (2009) and by Vickers and Wang (2002) in beef burgers irradiated with 2 kGy and 1.5

kGy respectively. In addition to this, Vickers and Wang (2002) demonstrated a higher acceptance of the burgers when people were provided an USDA leaflet "Ten most commonly asked questions about food irradiation" prior to tasting the samples.

5. Conclusions

Based on the results obtained for samples with a high inoculum level, the minimal dose of gamma irradiation required to reduce 5 log CFU/g of STEC in ground beef was 1 kGy. However, when samples with a low inoculum level were subjected to 1 kGy, 4.4% of the samples inoculated with STEC O157 and 3.3% of the samples inoculated with the STEC pool were positive for *stx* and *eae* genes after an enrichment step. As to consumer acceptance no significant differences were found between samples irradiated with 2.5 kGy and the non-irradiated samples. Therefore, 2.5 kGy was identified as the gamma irradiation dose that reduces STEC but has no impact on consumer acceptance of ground beef.

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Declaration of Competing Interest

None.

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