Effect of mild irradiation doses on *Listeria monocytogenes* and *Escherichia coli* o157:h7 inoculated into beef meat trimmings

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Introduction

Trimmings are portions of meat which are remaining after deboning the carcase and preparing the primal cuts, so microbiological markers must be controlled and are used as a trade standards. There are specifications of microbiological markers for mechanically recovered meat and ground meat which require absence of pathogenic strains.
Among pathogens *Listeria monocytogenes* and *E. coli* O157:H7 need to be seriously taken into account.

There are several types of *E. coli* strains that may cause gastrointestinal illness in humans. Verotoxin-producing or Shiga-toxin producing *E. coli* (VTEC or STEC) have emerged as important food-borne pathogens. Cattle is a reservoir of zoonotic STEC which are transmitted to humans through meat and meat products (Caprioli et al. 2005).

Listeria monocytogenes usually cause seriously affected cases and even deaths. In 2010, 1601 confirmed cases of listeriosis were reported in Europe, 17% of which ended fatally (EFSA, 2012).

Its ability to proliferate at low temperatures, pH values around 6 and high water activities of many meat products allow many strains of *L. monocytogenes* to grow during refrigerated storage, having a high prevalence in processing plants (Talon et al. 2007).

Irradiation is used on packaged products to extend shelf-life and improve microbiological safety with minimal effects on chemical composition, nutritional and sensory properties. When biological materials are exposed to irradiation energy, the atoms or molecules eject electrons producing ions and free radicals. The electron-deficient carbon-carbon double bonds of unsaturated fatty acids and carbonyl groups are particularly susceptible to free radical attack. This is why even at low dose, irradiation can initiate or promote lipid oxidation resulting in undesirable off-odors and flavors (Lescano et al. 1991).

The objectives of the present work were to assess the use of moderate doses of irradiation as a tool to reduce (or mitigate) the presence of pathogens using *L. monocytogenes* and *E. coli* O157:H7 as markers inoculated into bovine trimming samples.

Materials and methods

The effectiveness of an irradiation dose of 3kGy was studied on samples independently inoculated at 10^2, 10^3, 10^4, 10^5 and 10^6 cfu/g of *Listeria monocytogenes* and of *E. coli* O157:H7 (Figure 1). 3 kGy was used as target irradiation dose selected as an acceptable commercial dose taking into account results from sensory trials (absence of off-odors/flavors) in a previous study. Lethality curves were obtained using two inoculum levels and four irradiation doses **(Table 1).**

Inoculum concentration	Irradiation dose (kGy)	
	E. coli O157:H7	*L.monocytogenes*
10^3 cfu/g (low)	0 - 0,4 - 0,7 - 1,0	0 - 0,5 - 1,0 - 1,5
10^6 cfu /g (high)	0 - 0,5 - 1,0 - 1,5	0 - 1,0 - 2,0 - 2,5

Table 1. Lethality curves conditions.

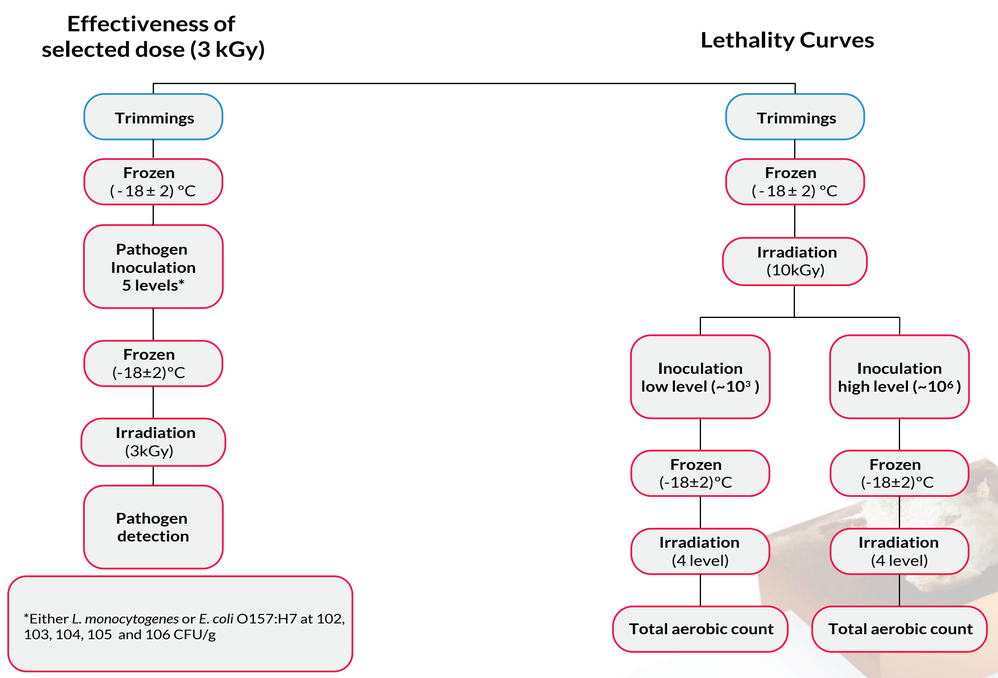

Figure 1. Schematic procedure of sample treatment for determination of irradiation effects on pathogenic cells.

Obtaining beef trimmings

Beef trimmings (20% fat) were obtained from a local slaughter house. Fresh trimmings (0 days age) from grass-fed animals were divided at deboning room into sterile sampling bags.

Bacterial cultures and inoculation of samples

Reference strains of *L. monocytogenes* (ATCC 19111) and non-pathogenic *E. coli* O157:H7 (NCTC 12900) were used to artificially contaminate the samples to be irradiated.

Irradiation

Irradiation process was carried out at room temperature under a Cobalt-60 radiation source (Modular Equipment EMI-9, dry shield, Buenos Aires, Argentina). Non-irradiated (NI, 0 kGy) samples were used as control.

Microbiological analysis

Detection of *L. monocytogenes* and *E. coli* O157:H7 was done by PCR, using the "BAX® System PCR Assay for *L. monocytogenes*" and the "BAX® System Screening *E. coli* O157:H7 MP" (Dupont, Wilmington, Delware, USA), respectively . To confirm *L. monocytogenes* "weak positive" results, the grown MOPS were streaked on Agar Listeria Ottavani & Agosti—ALOA (Oxoid, Hampshire, UK) and to confirm *E. coli* O157:H7 "weak positive" results, m-TSB was immunoconcentrated for *E. coli* O157:H7 using VIDAS® Immuno-Concentration *E. coli* O157—ICE (BioMérieux, Marcy-l'Étoile, France) and streaked on CHROMagarTM O157 (CHROMagar, Paris, France) or Cefixime Tellurite Sorbitol.

Total aerobic counts were performed for lethality curve construction (it was assumed that only inoculated pathogen cells represent the majority of the bacteria population in inoculated samples).

Results and discussion

Effectiveness of an irradiation dose of 3kGy on *Listeria monocytogenes* and of *E. coli* O157:H7

Table 2 shows that irradiation close to 3 kGy reduced below detectable levels a 2,5 log cfu/g of *L. monocytogenes* and 4,3 log cfu/g of *E. coli* O157:H7 that is in agreement with Gumus et al. 2008 and Samelis et al. 2005. Higher resistance of *L. monocytogenes* was expected because gram positive bacteria are often found to be more resistant than gram negative bacteria in foods (Farkas 2001).

Irradiation at 3 kGy			
L.monocytogenes		*E. coli O157:H7*	
Inoculum (log cfu/g)	Presence	Inoculum (log cfu/g)	Presence
Control	–	Control	–
1,52	–	1,32	–
2,52	–	2,32	–
3,52	+	3,32	–
4,52	+	4,32	–
5,52	+	5,32	+

Table 2. Detection of *L. monocytogenes* and *E. coli* / O157:H7 (–) not detectable (+) presence.

Lethality Curves

Initial counts of *E. coli* O157:H7 and *L. monocytogenes* in inoculated trimmings destined to be irradiated at different doses can be seen on **Fig. 2, Fig. 3, Fig. 4 and Fig. 5.**

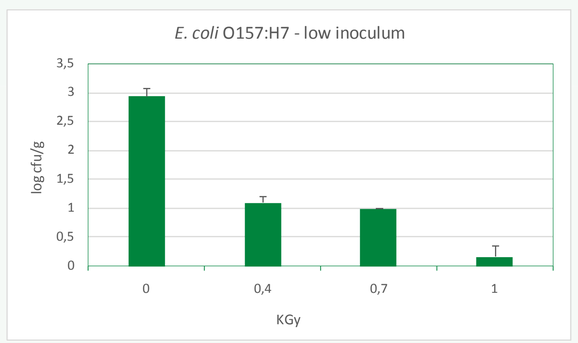

Fig. 2. *E. coli* O157:H7 counts (log cfu/g) of irradiated trimmings previously inoculated at low concentration. (n = 6)

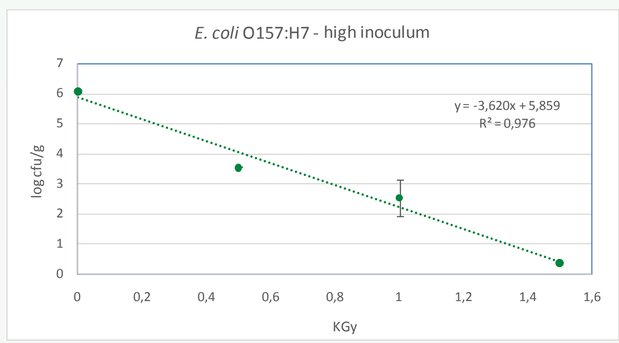

Fig. 3. *E. coli* O157:H7 counts (log cfu/g) of irradiated trimmings previously inoculated at high concentration. (n = 6).

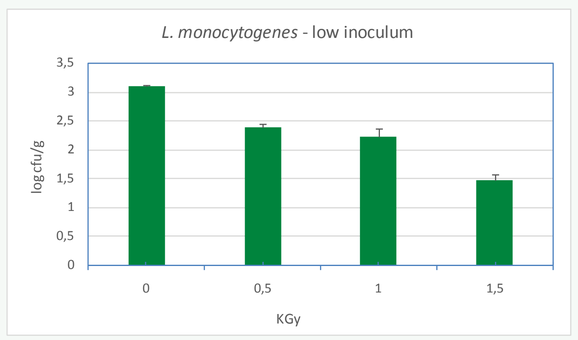

Fig. 4. *L. monocytogenes* counts (log cfu/g) of irradiated trimmings previously inoculated at low concentration. (n = 6)

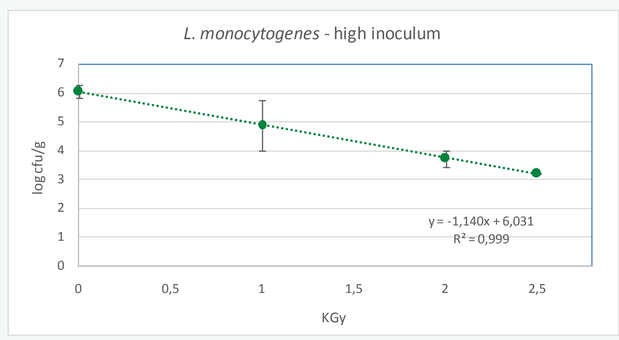

Fig. 5. *L. monocytogenes* counts (log cfu/g) of irradiated trimmings previously inoculated at high concentration. (n = 6).

The reductions as a consequence of the irradiation process were estimated from experiments with high inoculums (Table 3 and 4).

The D10 value estimated from lethality curves was 0,28 (R^2 0,98) for *E. coli* O157:H7 and 0,71 (R^2 0,99) for *L. monocytogenes.*

Reductions of *L. monocytogenes* obtained in trimmings samples are lower than those obtained for *E. coli* O157:H7 for both high and low inoculums assayed.

For the series of experiments carried out at low inoculums, additional reduction values could only be estimated for *L. monocytogenes*: 0,72 ± 0,05 and 1,62 ± 0,07 log cfu/g, respectively for 0,5 and 1,5 kGy. Reduction values obtained for 1,0 kGy were similar to those of high inoculum experiments. The values obtained in this work are similar to those found by Molins 2001.

Irradiation Dose (kGy)	Reductions *E.coli* O157:H7 (cfu/g)
0,5	2,6 ± 0,5
1,0	3,5 ± 0,0
1,5	5,75 ± 0,03

Irradiation Dose (kGy)	Reductions *L.monocytogenes* (cfu/g)
1,0	1,2 ± 0,4
2,0	2,32 ± 0,04
2,5	2,8 ± 0,1

Table. 3 and Table. 4. Reductions of target bacteria inoculated at high concentration.

CONCLUSIONS

This study has been carried out using beef trimmings, representing a huge share of the world trade market of meat destined to elaborate burguers or patties. The pathogenic reductions obtained in this work support the role of irradiation as a useful processing tool for increasing food safety of trimmings. Provided that moderate gamma irradiation doses up to 2,5 kGy were used, at least reductions of 2 log cfu/g of *L.monocytogenes* and 5 log cfu/g of *E. coli* O157:H7 are achieved as deducted from lethality curves. It seems reasonable to suppose that irradiation can be successfully employed to achieve the safety of frozen trimmings when the initial load of pathogenic bacteria is not extremely high.

ACKNOWLEDGMENTS

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