EFFECT OF MILD IRRADIATION DOSES ON LISTERIA MONOCYTOGENES AND ESCHERICHIA COLI O157:H7 INOCULATED INTO BEEF MEAT TRIMMINGS

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Abstract - The objectives of the present work were to assess the use of moderate doses of irradiation (less than 3kGy) as a tool to reduce the risk of pathogen presence in bovine trimmings destinated to elaborate patties. Two pathogenic markers (*Listeria monocytogenes* and *Escherichia coli* O157:H7) were inoculated at high or low loads (10^6 or 10^3 CFU/g, respectively) to trimmings samples which were subsequently irradiated and lethality curves were obtained. For *E. coli* O157:H7 irradiation doses of 0.5, 0.7, 1.0 and 1.5 kGy provoked reductions of: 2.6±0.5; 3.3±0.3; 3.53±0.0 and 5.75±0.03 log CFU/g, respectively. For *L. monocytogenes* irradiation doses of 1, 2 and 2.5 kGy produced reductions of 1.2±0.4; 2.32±0.04 and 2.8±0.1 log CFU/g, respectively. Decimal reduction value coefficient (D_{10} value) was estimated from the lethality curves as 0.28 (R^2 0.98) for *E. coli* O157:H7 and 0.71 (R^2 0.99) for *L. monocytogenes*. Provided that using moderate gamma irradiation doses of less than 3 kGy, reductions of 2log CFU/g of *L. monocytogenes* and 5log CFU/g of *E. coli* O157:H7 were achieved, it seems reasonable to suppose that irradiation can be successfully employed to improve the safety of frozen trimmings when initial burdens of pathogenic bacteria are not extremely elevated.

I. INTRODUCTION

Meat has an appropriate physicochemical composition for pathogenic microorganisms growth. Trimmings are portions of meat which are remaining after deboning the carcase and preparing the primal cuts, so microbiological markers must be controlled and it is used as a trade standard. There are specifications of microbiological markers for mechanically recovered meat and ground meat such as: total mesophilic counts, *Escherichia coli* counts and 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. Vero-toxin-producing or Shiga-toxin producing *E. coli* (VTEC or STEC) have emerged as important food-borne pathogens, especially O157, O26, O103, O111, O145, O45, O91, O113, O121 and O128 serogroups. The Shiga toxins produced may cause from diarrhea to hemorrhagic colitis, which can progress into hemolytic uremic syndrome [1]. Cattle is a reservoir of zoonotic STEC which are transmitted to humans through meat and meat products [2].

Listeria monocytogenes receive considerable attention because they usually cause seriously affected cases and even deaths. In 2010, 1601 confirmed cases of listeriosis were reported in Europe, 17% of which ended fatally [3]. *L. monocytogenes* is ubiquitous in the environment. 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 [4].

Irradiation is used on packaged product 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 [5].

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 trimmings samples.

II. MATERIALS AND METHODS

The effectiveness of an irradiation dose of 3kGy was studied on samples independently inoculated with both pathogenic strains at different levels. Lethality curves were obtained using two inoculum levels and four irradiation

doses which were chosen using the data collected in the previous experiments. (See Figure 1)

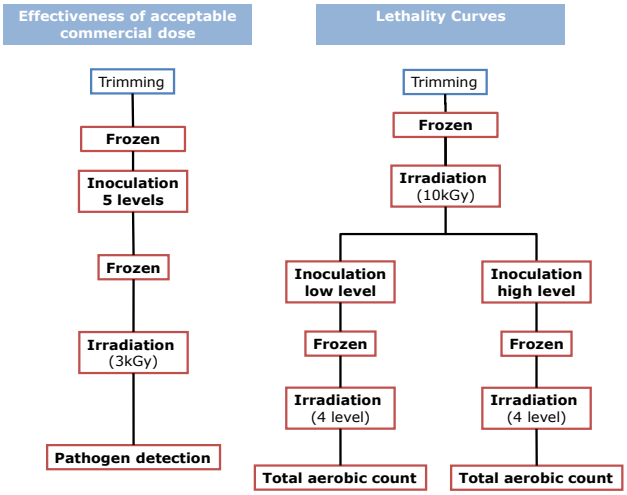

Figure 1. Schematic procedure of sample treatments.

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 containing 125g (qualitative stages) or 11g (quantitative stages) and frozen 18-24hs. 100µL of the *L. monocytogenes* or *E. coli* O157:H7 inoculum suspension (IS) was inoculated into the meat sample and thoroughly mixed.

Reference strains of *Listeria monocytogenes* (ATCC 19111) and non-pathogenic *Escherichia coli* O157:H7 (NCTC 12900) were used to artificially contaminate the. For the preparation of the IS successive dilutions were made in phosphate water to obtain the expected concentration for each stage of the study. The actual load of the IS was confirmed by making counts of the suspension with the automatic enumeration methodology TEMPO TVC (BioMérieux, France).

The inoculated sample was then sealed and kept chilled for 1 hour before being frozen again. Concentrations of 10^2, 10^3, 10^4, 10^5 and 10^6 CFU/g of Listeria and of *E. coli* O157:H7 were used for study at 3kGy irradiation dose effectiveness.
Lethality curves were studied for two levels of pathogens concentrations: 10^3 CFU/g (low concentration) and 10^6 CFU/g (high concentration). Trimmings were previously irradiated at 10kGy to eliminate interference of microorganisms present in the samples.

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 (data not shown). Samples were irradiated after 24h of storage, under frozen or chilled conditions in LATU irradiation unit. Irradiated and non-irradiated trimmings bags were stored at (2±2) °C for 24 h before being analyzed. Irradiation was carried out at room temperature under a Cobalt-60 radiation source.
To obtain lethality curves, the following target doses were selected: 0.4; 0.7; 1 and 0.5; 1; 1.5 kGy for low and high *E. coli* O157:H7 concentrations, respectively, and 0.5; 1; 1.5 kGy and 1; 2; 2.5 kGy to low and high *L. monocytogenes* concentration, respectively.

Analysis. Pathogen detection was used to determine the effectiveness of commercially acceptable dose and total aerobic counts were performed for lethality curves construction because it is assumed that only inoculated pathogen could grow.
L. monocytogenes. The inoculated samples were hydrated with Half Fraser Broth (HFB) and incubated at (30±1)ºC for (24±2)h. The detection was done by PCR and by the traditional ISO method for *Listeria* (ISO 11290-1:1996). Detection of *L. monocytogenes* was done by PCR, using the "BAX® System PCR Assay for *L. monocytogenes*" (Dupont, USA). In the cases when the results were "weak positive" the grown MOPS were streaked on Agar Listeria Ottavani & Agosti - ALOA (Oxoid) and incubated at (37±1)ºC for (48±2)h to confirm the results.
The detection of *E. coli* O157:H7 was done using PCR "BAX® System PCR Assay for Screening *E. coli* O157:H7 MP" (Dupont). The enrichment was made in modified Tryptic Soy Broth, incubated at (41±1) ºC for 16-20hrs. To confirm "weak positive" results, m-TSB was inmunoconcentrated for *E. coli* O157:H7 using VIDAS® Immuno-Concentration *E. coli* O157 – ICE (BioMérieux) and streaked on CHROMagar™ O157 or Cefixime Tellurite Sorbitol.
Statistical analysis. Experiments were replicated twice by analyzing triplicate samples per replicate. Analyses of Variance (ANOVA) were performed using the statistical software Infostat/L version 2013 and XLSTAT Version

2011. A post-hoc Tukey test was used to obtain paired comparisons among sample means. Level of significance was set to $P < 0.05$.

III. RESULTS AND DISCUSSION

Irradiation close to 3 kGy reduced below detectable levels a 2.5 log CFU/g of *L. monocytogenes* and inhibited 4.3 log CFU/g of *E. coli* O157:H7 (Table 1).

Table 1. Detection of *L. monocytogenes* and *E. coli* O157:H7 by PCR.

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	+

Detection of *L. monocytogenes* and *E. coli* O157:H7 by PCR "BAX® System method after irradiation treatment (3 kGy): (-) no detectable (+) presence

Higher resistance of *L. monocytogenes* was expected because gram positive bacteria are found often more resistant than gram negative bacteria in foods [6]. In agreement with our findings, Gumus [7] investigated the irradiation effect on inoculated meatballs and demonstrated that a dose of 3.2 kGy inhibit counts of 4.3 log CFU/g of *E. coli* O157:H7. Samelis et al. [8] studied the use of ionizing radiation to control *Listeria* spp. and *E. coli* O157:H7 on frozen meat trimmings and showed that a dose of 2 kGy caused a 1.6 log CFU/g reduction for an initial count of 6 log *Listeria* spp. whereas the reduction at a 4 kGy dose was 2.5 log CFU/g. For *E. coli* O157:H7 the reduction at 2 and 4 kGy were 2 and >4.5 log CFU/g respectively for an initial count of 5.5 log CFU/g.

Initial counts of both *E. coli* O157:H7 and *L. monocytogenes* in inoculated trimmings at high concentration destined to be irradiated at different doses can be seen on Figures 2 and 3. Their reductions as a consequence of the irradiation process can be easily estimated from experiments with high inoculums.

For *E. coli* O157:H7 irradiation doses of 0.5, 0.7, 1.0 and 1.5 kGy provoked reductions of: 2.6±0.5; 3.3±0.3; 3.5±0.0 and 5.8±0.0 log CFU/g, respectively. Decimal reduction value coefficient (D_{10} value) was estimated from the lethality curves adjusted linear regression (least square means) as 0.28 (R^2 0.98).

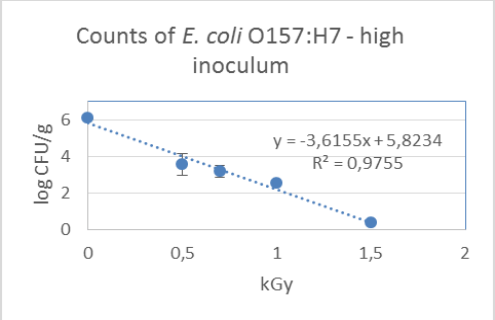

Figure 2. *E. coli* O157:H7 counts* of irradiated trimmings previously inoculated with the target bacteria at high concentration. (n=6). Different letter in bars means that count are significantly different (P<0.05). *counted as mesophilic aerobic.

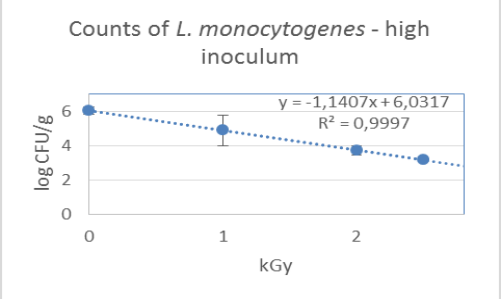

Figure 3. *L. Monocytogenes* counts* of irradiated trimmings previously inoculated with the target bacteria at high concentration. (n=6). Different letter in bars means that count are significantly different (P<0.05). *counted as mesophilic aerobic.

For *L. monocytogenes* irradiation doses of 1, 2 and 2.5 kGy produced reductions of 1.2±0.4; 2.3±0.0 and 2.8±0.1 log CFU/g, respectively. Estimated D_{10} value was 0.7 (R^2 0.99). Both D_{10} values are related to the range assessed for high inoculums experiments where fit to linear approach seemed reasonable in the conditions of this experiment.

For the series of experiments carried out at low inoculums, additional reduction values could only be estimated for *L. monocytogenes*: 0.7±0.1 and 1.6±0.1 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. For *E. coli* O157:H7, count values were often below or proximate to the detection limit of the technique (10^1CFU/g).

The values obtained in this work are similar to those found by Molins [9], although they were higher than reduction values of Sommers et al. [10]). This fact could be explained by differences between food matrices in composition (fat to protein ration, presence of antioxidants, pH, water activity) and irradiation equipment designs.

Technological enhancement of food quality, safety and security are of paramount important in this age. The global food system makes a significant contribution to climate changing greenhouse gas emissions with all the stages in the supply chain, from agricultural production through processing, distribution, retailing, home food preparation and waste, playing a part [11]. The use of thermal or non-thermal preservation technologies contributes to improve the unit efficiency of food production, enhancing extending terms for food distribution (and accessibility) while reducing waste at the same time. Technology may play a key role to address the problems faced reducing environmental impacts and increasing supply. Waste should be minimized through better inventory management, and through approaches that extends the shelf life of foods maintaining its quality.

IV. CONCLUSIONS

Previous and current studies show that irradiation treatments are useful to diminish regular microflora normally present in raw meat or trimmings. This study has been carried out using beef trimmings, representing a huge share of the world trade market of meat destined to elaborate burgers 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 using moderate gamma irradiation doses of less than 3 kGy, at least reductions of 2log CFU/g of *L. monocytogenes* and 5log CFU/g of *E. coli* O157:H7 can be achieved as deducted from lethality curves. It seems reasonable to suppose that irradiation can be successfully employed to improve the safety of frozen trimmings when initial loads of pathogenic bacteria are not extremely elevated [17]

V. REFERENCES

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