



## Use of mild irradiation doses to control pathogenic bacteria on meat trimmings for production of patties aiming at provoking minimal changes in quality attributes



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### ARTICLE INFO

#### Article history:

Received 15 April 2014

Received in revised form 24 June 2014

Accepted 24 June 2014

Available online 4 July 2014

#### Keywords:

Trimmings

Irradiation

*Listeria monocytogenes*

*Escherichia coli* O157:H7

Beef patties

Food safety

### ABSTRACT

The objectives of the present work were to assess the use of moderate doses of gamma irradiation (2 to 5 kGy) and to reduce the risk of pathogen presence without altering the quality attributes of bovine trimmings and of patties made of irradiated trimmings. Microbiological indicators (coliforms, *Pseudomonas spp* and mesophilic aerobic counts), physicochemical indicators (pH, color and tiobarbituric acid) and sensory changes were evaluated during storage. 5 kGy irradiation doses slightly increased off flavors in patties. Two pathogenic markers (*Listeria monocytogenes* and *Escherichia coli* O157:H7) were inoculated at high or low loads to trimming samples which were subsequently irradiated and lethality curves were obtained. Provided that using irradiation doses  $\leq 2.5$  kGy are used, reductions of 2 log CFU/g of *L. monocytogenes* and 5 log CFU/g of *E. coli* O157:H7 are expected. It seems reasonable to suppose that irradiation can be successfully employed to improve the safety of frozen trimmings when initial pathogenic bacteria burdens are not extremely high.

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### 1. Introduction

Physicochemical composition of meat provides the conditions for the growth of both microorganisms (banal and pathogenic) and the precursor compounds for the development of aromas and flavors, desirable or undesirable. Physicochemical parameters such as pH, color and lipid oxidation and sensory attributes are gross indicators of meat quality (Brewer, 2004; Lorenz et al., 1983; Shahidi, 1994). Bovine trimmings are the main ingredient of patties produced worldwide. Since this meat results from mechanical disruption of several muscles, assessing microbiological markers becomes mandatory and this is used as a trade standard. In particular, mechanically recovered meat, ground meat and meat mixes containing spices, shall all comply with specifications of microbiological markers 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, respectively) have emerged as important food-borne pathogens, especially O157, O26, O103, O111, O145, O45, O91, O113, O121 and O128 serogroups (Momtaz, Farzan, Rahimi, Safarpoor Dehkordi, & Souod, 2012). The pathogenic capacity of STEC resides in a number of virulence factors, including Shiga toxins (*stx1* and *stx2*), protein intimin (*eae*) and enterohemolysin (*ehly*) (Law, 2000). The Shiga toxins produced may cause from diarrhea to hemorrhagic colitis, which can progress into hemolytic uremic syndrome (HUS), (EFSA, 2011). Cattle is a reservoir of zoonotic STEC which are transmitted to humans through meat and meat products (Caprioli, Morabito, Brugere, & Oswald, 2005; Momtaz, Dehkordi, Rahimi, Ezadi, & Arab, 2013). Different incidences of STEC meat contamination have been reported from various sites in the world, ranging from 2 to 50%, with strain O157:H7 the most frequently reported (Ojo et al., 2010).

Outbreaks from *L. monocytogenes* are not common compared with those caused by other pathogens like *Salmonella spp*. However, they receive considerable attention because they usually cause serious symptoms cases and even deaths. In 2010, 1601 confirmed cases of listeriosis were reported in Europe, 17% of which ended fatally (EFSA, 2012). USA authorities reported an incidence of 0.3 listeriosis cases per 100,000

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populations during 2010 with a high mortality rate of 13% (CDC, 2011). *L. monocytogenes* is ubiquitous in the environment. Its ability to proliferate at low temperatures, pH values around 6 and water activities above 0.97, such as those of many meat products and ingredients, allow many strains of *L. monocytogenes* to grow during refrigerated storage, showing high prevalence in processing plants (Talon et al., 2007) and domestic refrigerators (EFSA, 2007; Hereu, Dalgaard, Garriga, Aymerich, & Bover-Cid, 2012; ILSI, 2005; Jofré, Aymerich, Grèbol, & Garriga, 2009; Warriner & Namvar, 2009). Its presence in foods is often caused by mistakes of workers at manufacturing plants that are found to be typically out of compliance with existing regulations; the outbreaks have major economic consequences, especially if the products affect international trade (Todd & Notermans, 2011).

Irradiation may be applied to packaged products extending their shelf-life and improving their microbiological safety with minimal effects on their chemical composition, and on their nutritional and sensory properties. The effects of ionizing radiation on living organisms depend on the total dose absorbed, the rate of absorption, and the environmental conditions (mainly temperature and gas atmosphere) during irradiation (Brewer, 2004). Food spoilage microorganisms are generally very susceptible to irradiation; a 90% reduction of most vegetative cells can be accomplished with 1.0–1.5 kGy (ICGFI, 1996; Olson, 1998a, 1998b; Thayer, Boyd, Fox, Lakritz, & Hampson, 1995). Irradiation followed to storage at refrigerating temperatures was found to be a very effective way to reduce initial microbial loads in ground beef, improve safety and extend shelf life without affecting sensory quality. Irradiation dose was directly related to the elimination of pathogens such as *L. monocytogenes* and *E. coli* O157:H7 (Fu, Sebranek, & Murano, 1995).

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 (fatty acids and amino acids) are particularly susceptible to free radical attack. This is why even at low doses, irradiation can initiate or promote lipid oxidation resulting in undesirable off-odors and flavors (Lescano, Narvaiz, Kairiyama, & Kaupert, 1991; Thakur & Singh, 1994). Temperature may determine the ratio and kind of radiolytic products generated due to irradiation. Reducing the temperature during irradiation reduces the effects on odor/flavor and color. The effect of irradiation on volatile compounds among muscle types within species, though beef meat has proven to show similar oxidation indexes for both, high or low fat contents.

Irradiating fresh beef at doses sufficient to extend shelf life and reduce pathogen load may result in rapid development of brown, green or, in some cases, bright red oxymyoglobin-like pigments. Irradiation produces a variety of color changes which are related to the myoglobin concentration, the state of myoglobin prior to irradiation, pH, water activity, presence of reducing equivalents, temperature and gas atmosphere during irradiation. Studies reporting color changes in irradiated raw meat differ significantly with animal species and among muscles within animal species (Ahn, Olson, Jo, et al., 1998; Brewer, 2004). The effect on  $L^*$  and  $a^*$  values of different meats varies widely whereas there seems to be an agreement in the decrease of  $b$  values due to irradiation (Montgomery, Parrish, & Olson, 2000; Murano, Murano, & Olson, 1998; Nam & Ahn, 2003). Elevated doses (above those permitted for food irradiation) of 50 kGy can completely destroy myoglobin. It should be mentioned that for pathogen reduction, a maximum of 4.5 kGy is permitted for uncooked, chilled red meat and 7 kGy is permitted for uncooked, frozen meat (FDA, 2012). The quality changes induced by irradiation can increase with storage time.

The objectives of the present work were to assess the use of moderate doses of irradiation as a tool to reduce (or mitigate) pathogen presence without altering the quality attributes of bovine trimmings and patties made of irradiated trimmings, covering: microbiological indicators during 30 days of storage (coliforms, pseudomonas and mesophilic aerobics counts); physicochemical indicators (pH, color and oxidation); sensory changes during a 180 day storage period at freezing temperatures and

pathogenic markers (counts of *L. monocytogenes* and *E. coli* O157:H7) in inoculated samples.

## 2. Materials and methods

### 2.1. Obtaining of 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 in 2.5 kg and 200 g portions for patty manufacture and trimming analysis, respectively. Trimmings for microbiological analysis were placed into sterile bags (Whirl Pak®) while trimmings for other analyses and for patty manufacture were placed into polyethylene bags (oxygen permeability:  $1200 \text{ cm}^3/\text{m}^2 \cdot 24 \text{ h}$  at  $23 \text{ }^\circ\text{C}/\text{HR} = 0$  as informed by supplier). Bags were transferred under refrigerating conditions to the pilot plant where they were stored at  $(-18 \pm 2) \text{ }^\circ\text{C}$  or  $(2 \pm 2) \text{ }^\circ\text{C}$  (see Fig. 1).

### 2.2. Irradiation method

After 24 h of storage, bags were transferred under frozen or chilled conditions to the irradiation unit (Laboratorio Tecnológico del Uruguay, LATU, Montevideo, Uruguay). Samples were irradiated frozen or chilled. Irradiated and non-irradiated trimmings bags were stored at  $(2 \pm 2) \text{ }^\circ\text{C}$  for 24 h before being analyzed or destined to patty manufacture. Samples destined to evaluation after 30 days were stored at  $(-18 \pm 2) \text{ }^\circ\text{C}$  after irradiation. Irradiation was carried out at room temperature under a Cobalt-60 radiation source (Modular Equipment EMI-9, dry shield, Buenos Aires, Argentina). The process was performed in 23 L cylindrical aluminum containers at a mean dose rate of 20 kGy/h. Measurement of the irradiation absorbed dose was performed with alanine dose-meters (ISO, 2004) using an EPR spectrometer (MS400, Miniscope, Magnetech, Berlin, Germany) and PMMA Ambar (ISO, 2002), using a spectrophotometer Shimadzu UV1800 (Kyoto, Japan).

### 2.3. 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. The cultures were kept frozen at  $(-80 \pm 2) \text{ }^\circ\text{C}$  and they were activated by transferring an aliquot of the stock into nutrient broth–NB (Oxoid, Hampshire, United Kingdom) and incubating overnight at  $37 \text{ }^\circ\text{C}$  and 100 rpm. For the preparation of the inoculum suspension (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, Marcy-l'Étoile, France).

### 2.4. Microbiological analysis

Total aerobic counts were performed on Plate Count Agar–PCA (Oxoid, Hampshire, United Kingdom) and incubated at  $(35 \pm 1) \text{ }^\circ\text{C}$  for 2 days. For Coliform enumerations, Most Probable Number methodology with Lauryl Tryptose Broth (Oxoid, Hampshire, United Kingdom) was used. The inoculated broth tubes were incubated at  $(35 \pm 1) \text{ }^\circ\text{C}$  for  $(48 \pm 2) \text{ h}$ . Confirmation of positive tubes was performed using EC broth (Oxoid, Hampshire, United Kingdom) and Tryptone Water (Oxoid, Hampshire, United Kingdom). To enumerate *Pseudomonas* spp., Pseudomonas Agar Base (Oxoid, Hampshire, United Kingdom) supplemented with CFC (10 mg/L Cetrimide, 10 mg/L Fucidin, 50 mg/L Cephaloridine) was used and incubated at  $25 \text{ }^\circ\text{C}$  for  $(48 \pm 2) \text{ h}$ .

*L. monocytogenes*. The inoculated samples were hydrated with 1125 mL of Half Fraser Broth (HFB) (Fraser broth base with Half Fraser selective supplement SR0166, Oxoid, Hampshire, United Kingdom), and incubated at  $(30 \pm 1) \text{ }^\circ\text{C}$  for  $(24 \pm 2) \text{ h}$ . The detection was done

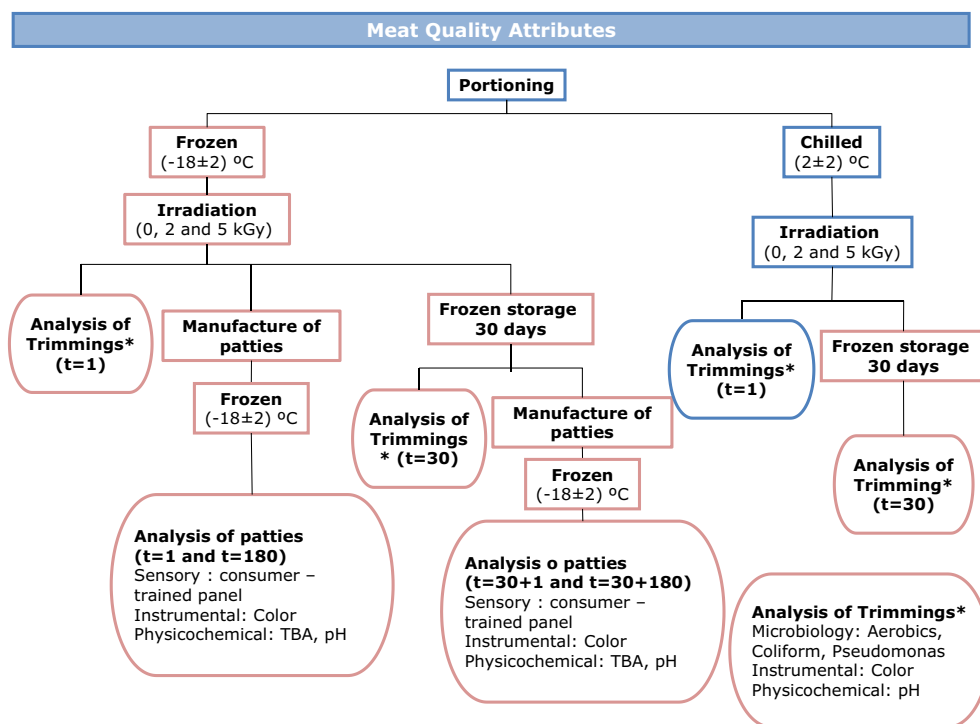


Fig. 1. Schematic procedure of sample treatments for commercial quality study.

by PCR and by the traditional ISO method for *Listeria* (ISO, 1996). When PCR was used, 5 mL of the Half Fraser Broth (HFB) was transferred into 45 mL of MOPS-BLEB Broth (Oxoid, Hampshire, United Kingdom) and incubated at  $(35 \pm 1)^\circ\text{C}$  for  $(24 \pm 2)$  h. Detection of *L. monocytogenes* was done by PCR, using the “BAX® System PCR Assay for *L. monocytogenes*” (Dupont, Wilmington, Delaware, USA). In the cases when the results were “weak positive” the grown MOPS were streaked on Agar *Listeria* Ottavani & Agosti—ALOA (Oxoid, Hampshire, United Kingdom) and incubated at  $(37 \pm 1)^\circ\text{C}$  for  $(48 \pm 2)$  h to confirm the results. To follow the traditional methodology, 5 mL of HFB was transferred to 45 mL Fraser Broth—FB (Fraser broth base with Fraser selective supplement SR0156, Oxoid, Hampshire, United Kingdom) and incubated for  $(48 \pm 2)$  h at  $(35 \pm 1)^\circ\text{C}$ . The HFB was re-incubated at  $(30 \pm 1)^\circ\text{C}$  for 1 more day. Both broths, HFB and FB, were streaked on Palcam Agar (Oxoid, Hampshire, United Kingdom) and ALOA and incubated for  $(48 \pm 2)$  h at  $(37 \pm 1)^\circ\text{C}$ .

The detection of *E. coli* O157:H7 was done using PCR “BAX® System PCR Assay for Screening *E. coli* O157:H7 MP” (Dupont, Wilmington, Delaware, USA). The enrichment was made in Tryptic Soy Broth, modified with 8 mg/L of novobiocine and acid digest of casein—mTSB (Acumedia, Neogen, USA) incubated at  $(41 \pm 1)^\circ\text{C}$  for 16–20 h. To confirm “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 CHROMagar™ O157 (CHROMagar, Paris, France) or Cefixime Tellurite Sorbitol.

### 2.5. Physicochemical and instrumental analysis

pH measurements were made with a pH Meter (SevenMultiTM, METTLER TOLEDO, Greifensee, Switzerland) equipped with a temperature sensor and a combined penetration electrode previously calibrated with pH 4.00 and 7.00 buffer solutions. Five samples of each condition were measured and averaged.

To determine tiobarbituric acid (TBA), samples were processed in a RETSCH Grindomix GM200 knife mill (Haan, Germany) at range mode with 600 rpm for 10 s for 3 times to prevent overheating. Prior to TBA analysis, fat was removed from the samples according to the Folch

method. TBA analysis was performed according to the AOCS (2009) —TBA Value Direct Method. Values were calculated in mg of malonaldehyde/kg meat.

Color analysis of beef trimmings and patties was performed on a Hunterlab LabScan® XE Colorimeter (Hunter Associates Laboratory Inc., Reston, Virginia, USA) with illuminant A/10 and open cell of 44 mm. Parameters  $L^*$  (lightness),  $a^*$  (redness) and  $b^*$  (yellowness) were obtained and the saturation index was calculated according to AMSA (2012). For beef trimmings, 5 samples of each condition were measured 3 times each, while for patties, 3 samples were measured on both sides 3 times each.

### 2.6. Sensory evaluation.

Sensory properties of cooked patties were evaluated by a trained panel and the acceptability of samples was evaluated by consumers. Both analyses were carried out in a standardized test room (ISO 8589:2007). Patties were grilled for 4–6 min until reaching  $70^\circ\text{C}$  at the center of the piece. Temperature was monitored with a digital thermometer Fluke 54 II (Fluke Corporation, Everett, Washington, USA). The samples were cut into quarters, wrapped in aluminum paper, coded with three digit random numbers and kept warm until they were served. Sample presentation order was randomized to prevent any flavor carryover effects. Mineral water was provided for mouth-rinsing. A nine-member trained descriptive attribute panel was screened, selected, and trained according to AMSA (1995). The assessors evaluated aroma intensity, off-aroma, flavor intensity, off-flavor, tenderness (initial), tenderness (final impression) and initial juiciness. They used a 0–10 unstructured line scale for all attributes. 35 to 50 consumers between 18 and 65 years old, evaluated the overall acceptability of the three samples in one session using a 9-point hedonic scale (1 = “dislike extremely”, 9 = “like extremely”).

### 2.7. Meat quality attributes

Quality attributes of trimmings, irradiated at freezing  $(-18 \pm 2)^\circ\text{C}$  or chilling  $(2 \pm 2)^\circ\text{C}$  temperatures, and patties made of irradiated





**Table 2**

pH mean values ( $n = 5$ ) on beef trimmings irradiated at Ni (0 kGy), D1 (2 kGy) and D2 (5 kGy) under chilling ( $2 \pm 2$  °C) or freezing ( $-18 \pm 2$  °C) temperatures. Column mean values with the same superscript (a) do not differ ( $P > 0.05$ ).

Irradiation dose (kGy)			Storage time (days)		Irradiation temperature	
Ni	D1	D2	1	30	Chilled	Frozen
5.62 <sup>a</sup>	5.61 <sup>a</sup>	5.61 <sup>a</sup>	5.60 <sup>a</sup>	5.62 <sup>a</sup>	5.63 <sup>a</sup>	5.59 <sup>a</sup>

of *L. monocytogenes* and of *E. coli* O157:H7 were used to study selected 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 10 kGy to eliminate interference of microorganisms present in the samples.

### 2.8.2. Irradiation of inoculated samples

As stated before, 3 kGy was used as target for selected dose taking into account results from sensory trials (absence of off-odors/flavors and highest limit of absorbed dose in D1, see Section 2.7.1). The actual doses absorbed were 3.3 kGy for *L. monocytogenes* and 3.1 kGy for *E. coli*. To obtain lethality curves, the following target doses were selected: 0.4; 0.7; 1 and 0.5; 1; 1.5 kGy to 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* concentrations, respectively. Irradiation process was carried out as explained in Section 2.2.

Pathogen detection was used to determine the effectiveness of the selected dose and total aerobic counts (Section 2.4) were performed for lethality curve construction because it was assumed that only inoculated pathogen cells represent the majority of the bacteria population in inoculated samples (see Section 2.8.1).

### 2.8.3. Statistical analysis

Each experiment was performed at least two separate times with three samples analyzed in each replicate. Analyses of variance (ANOVA) was performed over instrumental color and pH data using the statistical software Infostat/L version 2013 (Universidad Nacional de Córdoba, Argentina). Microbiological counts were converted to log cfu/g. Dose, storage time and temperature of irradiation and storage were considered as sources of variation. Data of sensory analysis and microbiology counts were analyzed by ANOVA using XLSTAT Version 2011 (Addinsoft 1995–2010, France). A post-hoc Tukey test was used to obtain paired comparisons among sample means. Level of significance was set to 0.05.

**Table 3**

CIELAB color parameters of beef trimmings for different irradiation treatments and storage time. Within columns mean values with common letters (a, b) do not significantly differ ( $P > 0.05$ ). ns, not statistically significant, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

	L*	a*	b*	Saturation index
<i>Effect of irradiation dose</i>				
(Overall irradiation temperature and storage time)				
Ni (0 kGy)	34.89 <sup>a</sup>	26.03 <sup>a</sup>	19.47 <sup>a</sup>	32.54 <sup>a</sup>
D1 (2 kGy)	34.07 <sup>a</sup>	23.20 <sup>b</sup>	17.54 <sup>b</sup>	29.12 <sup>b</sup>
D2 (5 kGy)	34.66 <sup>a</sup>	23.42 <sup>b</sup>	17.66 <sup>b</sup>	29.38 <sup>b</sup>
Statistical significance	ns	***	***	***
<i>Effect of storage time</i>				
(Overall irradiation dose and irradiation temperature)				
1 day storage	36.05 <sup>a</sup>	24.66 <sup>a</sup>	18.60 <sup>a</sup>	30.92 <sup>a</sup>
30 day storage	33.04 <sup>b</sup>	23.77 <sup>a</sup>	17.85 <sup>a</sup>	29.77 <sup>b</sup>
Statistical significance	***	*	ns	*
<i>Effect of irradiation temperature</i>				
(Overall irradiation dose and storage time)				
2 °C	34.56 <sup>a</sup>	22.93 <sup>a</sup>	17.56 <sup>a</sup>	28.93 <sup>a</sup>
-18 °C	34.53 <sup>a</sup>	25.50 <sup>b</sup>	18.89 <sup>b</sup>	31.76 <sup>b</sup>
Statistical significance	ns	***	**	***

## 3. Results and discussion

### 3.1. Meat quality attributes

Table 1 shows that for D1 irradiation dose (2 kGy), total aerobic counts of 3 log (cfu/g) in trimmings were reduced by at least 1.5 log orders. This result agrees with previous findings of Chouliara et al. (2006) who reported that frozen trimmings irradiated at 2 kGy under Co-60 source reduced total aerobic counts of 6.1 log (cfu/g) in 1.6 log orders. Karadag and Günes (2007) found that for *Pseudomonas* spp, the same dose (2.2 kGy) caused a reduction of at least 1 log order for an initial load of nearly 2 log (cfu/g). The temperature of the different irradiation treatments (chilling vs freezing) did not significantly affect the results. Overall, irradiation at D1 dose had a significant improvement on hygienic quality which was practically similar to that caused by an irradiation dose of 5 kGy. For both irradiation doses, reductions obtained on day 1 after treatment were preserved during a 30 day storage period at freezing temperatures. This implies that irradiation may provide an alternative capable of decreasing the microbial load of meat products. Most spoilage microorganisms in meat are gram negative with *Pseudomonas* spp. predominant for aerobic storage at chilling temperatures.

#### 3.1.1. pH

pH results of all samples tested varied between 5.56 and 5.68 (Table 2). pH values did not show significant differences ( $P > 0.05$ ) for: irradiation dose, temperature and storage time. These results agree with those reported by Fu et al. (1995), who did not observe changes in the pH of fillets irradiated with 0.6 and 1.5 kGy, on 0 and 7 days of storage. According to Brewer (2004), irradiation can only produce very small changes in the pH of meat.

#### 3.1.2. Lipid oxidation

TBA values of patties made of trimmings irradiated at both D1 and D2, and stored 1 and 30 days did not significantly differ ( $P > 0.05$ ; data not shown). Whereas Ni samples of trial 1 showed significant

**Table 4**  
CIELAB color parameters of patties made of fresh or aged trimmings (30 d) for different irradiation treatments, and storage time. Within columns mean values with common letters (a, b) do not significantly differ ( $P > 0.05$ ). ns, not statistically significant, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

	L*	a*	b*	Saturation index
<i>Effect of irradiation dose</i> (Overall trimmings age and storage time)				
Ni (0 kGy)	46.35 <sup>a</sup>	17.00 <sup>a</sup>	18.33 <sup>a</sup>	25.05 <sup>a</sup>
D1 (2 kGy)	45.49 <sup>a</sup>	17.15 <sup>a</sup>	18.14 <sup>a</sup>	24.99 <sup>a</sup>
D2 (5 kGy)	46.19 <sup>a</sup>	18.36 <sup>a</sup>	18.98 <sup>a</sup>	26.44 <sup>a</sup>
Statistical significance	ns	ns	ns	ns
<i>Effect of trimmings age before patty production</i> (Overall irradiation dose and storage time)				
1 day storage	44.86 <sup>a</sup>	16.70 <sup>a</sup>	18.07 <sup>a</sup>	24.63 <sup>a</sup>
30 day storage	47.15 <sup>b</sup>	18.31 <sup>b</sup>	18.90 <sup>b</sup>	26.35 <sup>b</sup>
Statistical significance	***	**	**	**
<i>Effect of storage time</i> (Overall irradiation dose and trimmings age)				
1 day	46.64 <sup>a</sup>	18.28 <sup>a</sup>	18.65 <sup>a</sup>	26.16 <sup>a</sup>
180 days	45.37 <sup>b</sup>	16.73 <sup>b</sup>	18.31 <sup>a</sup>	24.82 <sup>b</sup>
Statistical significance	**	**	ns	*

differences ( $P < 0.05$ ) between samples stored 1 and 30 days, Ni samples of trial 2 did not show those differences. Lipid stability could be due to the fact that trimmings used were from grass-fed animals. Brito (2005) reported that trimmings from grass-fed animals usually contains high levels of vitamin E (a well-known antioxidant compound) that might help to prevent increases in fat oxidation due to irradiation processing. Furthermore, since ascorbic acid was used as an ingredient for patty formulation at 0.7%, the compound could be responsible of preventing oxidation increases. Ahn and Nam (2004) did not report significant differences on TBA values of ground beef with ascorbic acid at 0.1% irradiated at 2.5 kGy along 7 days, while control samples (without ascorbic acid) did show an increase.

### 3.1.3. Color

Non irradiated (Ni) samples presented higher  $a^*$  and  $b^*$  values than the irradiated ones (Table 3), but there was no significant difference between the two doses (D1 and D2). Visual evaluation also suggested that irradiated samples were less red. Nanke, Sebranek, and Olson (1999) reported, as well, lower  $a^*$  and  $b^*$  values in beef irradiated with doses ranging from 1.5 to 10.5 kGy compared to control samples non-irradiated. According to Lycometros and Brown (1973), who studied pigment behavior on model systems, changing from oxymyoglobin to metmyoglobin at the surface could explain the lower  $a^*$  values on the exterior surface of irradiated beef. Obtained values of  $a^*$  and  $b^*$  were affected by the temperature of the samples at the moment of the irradiation process, and they were significantly lower in chilled samples than in the frozen ones. For non-irradiated samples, this difference was not significant. In agreement with Brewer (2004), this suggests that chilled irradiated samples experience more changes than frozen irradiated samples. Reducing the temperature during the irradiation process reduces the effects on odor, flavor and color. Temperature may determine which

radiolytic products are generated and in what ratios, affecting also food matrix viscosity and water mobility. Ion and free radical dispersion are lower when free water is in the frozen state. Also, free radicals tend to recombine when water in foods is frozen because they are less likely to diffuse and react with other food components (Taub et al., 1975). Storage time did not affect  $a^*$  and  $b^*$  values. Lightness ( $L^*$ ) was not affected by irradiation dose or temperature, but it did decrease with storage time, being lower on samples stored for 30 days after irradiation. The same behavior was observed for the saturation index, decreasing with storage. Nevertheless, Millar, Moss, and Stevenson (2000) suggested that CIELAB parameters may not be sufficiently sensitive indicators of pigment changes following irradiation. Several studies have shown that packaging atmosphere (aerobic or anaerobic) has a greater effect on meat color than does irradiation alone (Ahn, Olson, Lee, et al., 1998; Luchsinger et al., 1996; Murano et al., 1998).

It is important to remark that instrumental color on beef trimmings and patties are not comparable in this study, since color measures on beef trimmings were made exclusively in the muscle (excluding the fat) and patties were made by grinding and mixing these two components (muscle and fat); thus, differences explained by the contribution of fat to final color of patties were expected.

Irradiation dose did not significantly affect  $L^*$ ,  $a^*$  and  $b^*$  values on beef patties ( $p > 0.05$ ) (Table 4). These results agree with those reported by Murano et al. (1998), who found that the only differences between non irradiated and irradiated (2 kGy) beef patties were due to packaging atmosphere. On the other hand, Montgomery et al. (2000) reported lower  $a^*$  and  $b^*$  values and higher  $L^*$  values on beef patties irradiated with 2 kGy compared with non-irradiated samples. All color scores were significantly higher in patties made of trimmings aged for 30 days related to patties made with fresh (1 d) trimmings. Values of  $L^*$ ,  $a^*$  and saturation indexes decreased significantly during the 180 days of storage while  $b^*$  values remained unchanged.

**Table 5**  
Odd flavor intensity mean rates for patties made of fresh (1 d) and aged (30 d) trimmings. Within columns, treatment means with common letters (a–b) are not significantly different ( $P > 0.05$ ). Within rows, treatment means with common letters (x–y) are not significantly different ( $P > 0.05$ ).

	Off flavor intensity (0 to 10)			
	Patties made of 1 d trimmings		Patties made of 30 d trimmings	
Dose	1 d	180 d	1 d	180 d
Ni	0.3 <sup>a,x</sup>	1.5 <sup>a,y</sup>	0.1 <sup>a,x</sup>	0.2 <sup>a,x</sup>
D1	0.7 <sup>a,b,x</sup>	1.3 <sup>a,x</sup>	0.7 <sup>a,b,x</sup>	0.7 <sup>a,b,x</sup>
D2	1.4 <sup>a,b,x</sup>	1.8 <sup>a,x</sup>	1.4 <sup>b,x</sup>	1.7 <sup>a,b,x</sup>

**Table 6**  
Consumer acceptability mean values for patties made of fresh (1 d) and aged (30 d) trimmings. Within columns, treatment means with common letters (a,b) are not significantly different ( $P > 0.05$ ).ns; within rows, treatment means with common letters (x,y) are not significantly different ( $P > 0.05$ ).ns.

	Consumer acceptability			
	1 day aged trimming		30 day aged trimming	
Dose	1 d	180 d	1 d	180 d
Ni	6.6 <sup>a,x,y</sup>	6.0 <sup>a,y</sup>	7.0 <sup>a,x</sup>	6.7 <sup>a,x,y</sup>
D1	6.6 <sup>a,x</sup>	5.8 <sup>a,y</sup>	6.1 <sup>b,x,y</sup>	6.2 <sup>a,x,y</sup>
D2	6.2 <sup>a,x,y</sup>	5.5 <sup>a,y</sup>	6.0 <sup>b,x,y</sup>	6.5 <sup>a,x</sup>

**Table 7**

Detection of *L. monocytogenes* and *E. coli* O157:H7 by PCR “BAX® System method after irradiation treatment (3 kGy): (–) not detectable (+) presence.

<i>L. monocytogenes</i>		<i>E. coli</i> 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	+

**3.1.4. Sensory evaluation**

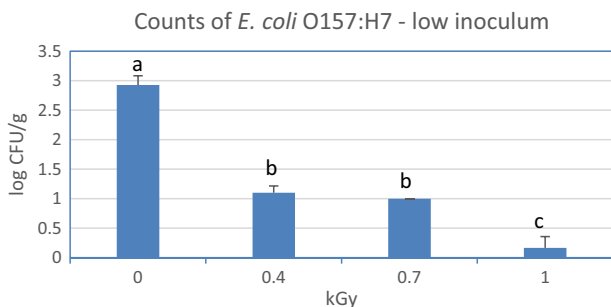
Off-flavor intensity of patties was the only parameter where judges detected differences with increasing irradiation dose. Table 5 shows off-flavor intensity of patties made of irradiated and non-irradiated fresh (1 d) and 30 d stored trimmings. The other sensory attributes assayed showed no significant changes due to irradiation treatment or storage time of trimmings and patties. Their values, in a 0 to 10 continuous scale ranged as follows: odor intensity [5 to 6]; off odor intensity [0.3 to 1.4]; initial tenderness [4.2 to 5.9]; final tenderness [4.7 to 5.8]; initial juiciness [4.2 to 5.7]; final juiciness [3.9 to 5.7] and flavor intensity [5.0 to 6.2].

Table 5 shows that off flavor scores of patties made of aged irradiated trimmings did not differ ( $P > 0.05$ ) from patties made of fresh (1 d) irradiated trimmings. Patties made of trimmings irradiated at D2 significantly differed from patties made of non-irradiated trimmings for both fresh and aged ones. Nevertheless, values obtained for off flavor were all below 2 in a 0 to 10 scale and they experienced no changes during the 180 d storage period at freezing temperature. Giroux et al. (2001) with the use of a trained panel, concluded that there was no significant difference in odor and taste between irradiated (4 kGy) and non-irradiated ground beef patties (23% fat) during 7 days of storage at 4 °C. Sommers et al. (2004) performed a study evaluating frozen ground beef patties (15% fat) irradiated and non-irradiated at doses of 1.35 and 3 kGy. Irradiation had no significant impact on the ratings of any of the sensory attributes either at 1 d or after 6 months of storage. Similar results were found by Zienkewicz and Penner (2004) with 25% fat ground beef.

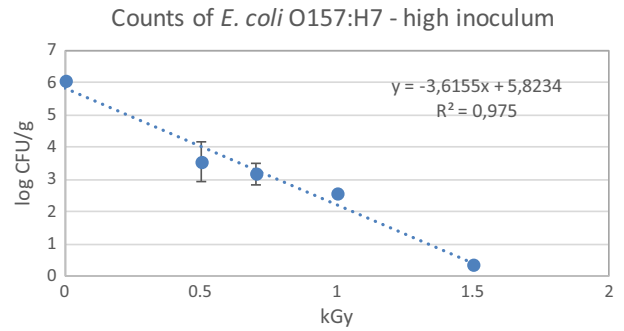
Consumer acceptability is one of the tools used to predict market product success and the likelihood of rejection of consumers if this is the case. Table 6 shows acceptability results for patties made of fresh irradiated and non-irradiated trimmings at 1 and 180 d of frozen storage.

It was observed that irradiated patties did not differ from non-irradiated ones ( $P > 0.05$ ) for both 1 and 180 d storage times. Irradiated patties 180 d old showed less acceptability ( $P < 0.05$ ) than 1 d old patties though these values were proximate to the original ones.

Results from judges (trained panelists) and consumers indicate that there are no sensory differences between patties produced from fresh (1 d) or 30 d stored irradiated trimmings at 2 or 5 kGy, suggesting



**Fig. 3.** *E. coli* O157:H7 counts\* (log cfu/g) of irradiated trimmings previously inoculated with the target bacteria at low concentration. (n = 6) \*counted as mesophilic aerobic.



**Fig. 4.** *E. coli* O157:H7 counts\* (log cfu/g) of irradiated trimmings previously inoculated with the target bacteria at high concentration. (n = 6). Different letters in bars mean that counts are significantly different ( $P < 0.05$ ). \*counted as mesophilic aerobic.

that it is possible to commercialize irradiated trimmings as such to markets that require 30 days for transport and beef patties for up to a 180 d period ( $p > 0.05$ ) taking into account only sensory results.

**3.2. Effect on pathogenic cells**

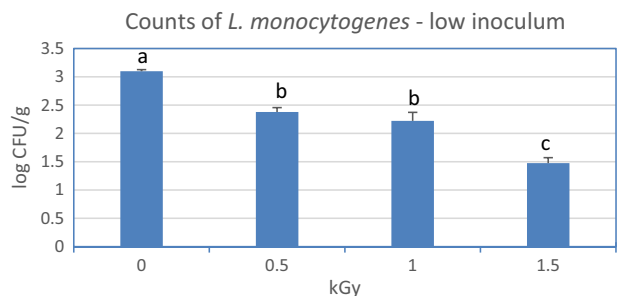
**3.2.1. Effectiveness of selected dose**

In order to determine the effectiveness of the selected dose in terms of pathogenic markers, 3 kGy was chosen as target dose because it was the upper end of the range of absorbed doses assayed for D1 with the trimmings destined for patty manufacture. In addition in sensory evaluation trials, the 5 kGy dose (D2) caused barely higher scores of off flavor intensity.

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 (Table 7). 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). In agreement with our findings, Gumus et al. (2008) investigated the irradiation effect on inoculated meatballs and demonstrated that a dose of 3.2 kGy reduced counts of 4.3 log cfu/g of *E. coli* O157:H7. Samelis, Kakouri, Savvaidis, Rigankos, and Kontominas (2005) 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 6.0 log *Listeria* spp. whereas the reduction at a 4 kGy dose was 2.5 log cfu/g. For *E. coli* O157:H7 the reductions at 2 and 4 kGy were 2 and >4.5 log cfu/g respectively for an initial count of 5.5 log cfu/g.

**3.2.2. Lethality curves**

Initial counts of both *E. coli* O157:H7 and *L. monocytogenes* in inoculated trimmings destined to be irradiated at different doses can be seen on Figs. 3 to 6. 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



**Fig. 5.** *L. monocytogenes* counts\* (log cfu/g) of irradiated trimmings previously inoculated with the target bacteria at low concentration. (n = 6) \*counted as mesophilic aerobic.

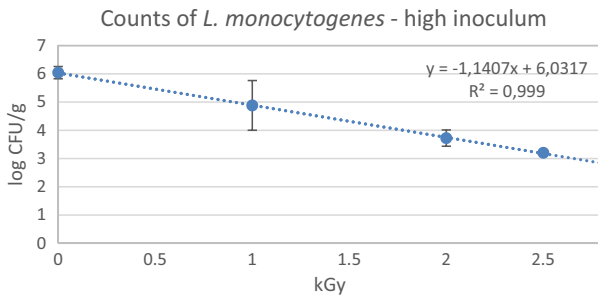


Fig. 6. *L. monocytogenes* counts\* (log cfu/g) of irradiated trimmings previously inoculated with the target bacteria at high concentration. ( $n = 6$ ). Different letters in bars mean that counts are significantly different ( $P < 0.05$ ). \*counted as mesophilic aerobic.

reductions of:  $2.6 \pm 0.5$ ;  $3.3 \pm 0.3$ ;  $3.5 \pm 0.0$  and  $5.75 \pm 0.03$  log cfu/g, respectively. Decimal reduction value coefficient ( $D_{10}$  value) was estimated from lethality curves adjusted by linear regression (least square means) as 0.28 ( $R^2$  0.98). For *L. monocytogenes* irradiation doses of 1, 2 and 2.5 kGy produced reductions of  $1.2 \pm 0.4$ ;  $2.32 \pm 0.04$  and  $2.8 \pm 0.1$  log cfu/g, respectively. Estimated  $D_{10}$  value was 0.71 ( $R^2$  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.

As it was told, 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. This is in agreement with findings regarding not only irradiation technology (Farkas, 2001) but also other non-thermal processes such as high hydrostatic pressure processing (Hereu et al., 2012). For the series of experiments carried out at low inoculums, additional reduction values could only be estimated for *L. monocytogenes*:  $0.72 \pm 0.05$  and  $1.62 \pm 0.07$  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. For *E. coli* O157:H7, count values were often below or proximate to the detection limit of the technique ( $10^1$  cfu/g).

The values obtained in this work are similar to those found by Molins (2001), although they were higher than reduction values of Sommers et al. (2004). 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 importance 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. This has been estimated in up to 28% for developed countries (Garnett, 2011). 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 either extend the shelf life of foods maintaining its quality (Garnett, 2013; WRAP, 2012).

#### 4. 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 for formulation of burgers or patties. The joint indicators studied both from the quality attributes (pH, color, TBA, sensory analysis, coliforms

and mesophilic counts) and from the safety points of view (pathogenic cells), support the role of irradiation as a useful processing tool to guarantee food safety of trimmings and patties slightly altering its physico-chemical and sensory properties. 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 deduced 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.

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