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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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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 ≤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 cm^3/m^2.24 h at 23 °C/HR = 0 as informed by supplier). Bags were transferred under refrigerating conditions to the pilot plant where they were stored at (−18 ± 2) °C or (2 ± 2) °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 ± 2) °C for 24 h before being analyzed or destined to patty manufacture. Samples destined to evaluation after 30 days were stored at (−18 ± 2) °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, Magnettech, 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 ± 2) °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 °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 ± 1) °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 ± 1) °C for (48 ± 2) 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 ° C for (48 ± 2) 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 ± 1) °C for (24 ± 2) h. The detection was done

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