Shelf-Life Extension and Sanitation of Fresh Pork Loin by E-Beam Treatment

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ABSTRACT

The usefulness of electron beam (E-beam) irradiation to increase the shelf life of whole fresh pork loin stored at 4°C has been studied. The shelf life was extended from 5 to 11 and 20 days after the application of 1 and 2 kGy, respectively. If a temperature abuse situation were to occur during product distribution (e.g., increase to 8°C), the shelf life would be extended from 3 to 8 and 15 days, respectively, after application of the same doses. When considering Listeria monocytogenes from a public health point of view, the irradiated whole fresh loin may be marketable for periods longer than 2 weeks, thus guaranteeing a practically Listeria-free product. Irradiation produced no important changes in the rheological characteristics of the meat. Although the sensory quality of irradiated meat was scored lower than the control immediately after irradiation, after 5 days in storage, irradiated meat scored higher than or not different from the control.

The loin (longissimus dorsi) is perhaps the highest quality piece of meat from the pig carcass. The whole loin wrapped in laminated film bags of low gas permeability is often distributed under refrigeration to retailers and large supermarkets, where family portions are prepared from it, usually slices, to be cooked at home by consumers. Because of its nutrients, pH (about 5.5), and water activity (0.99), the loin has a very short shelf life and does not last any longer than 5 to 7 days under refrigeration. Spoilage follows the pattern of fresh meat and is commonly due to the growth of the slime-producing psychrotrophic gram-negative aerobic microbiota, mainly Pseudomonas spp. (26). Any action applied to increase the shelf life of this product would be very useful from a commercial point of view. Several approaches may be taken to reach this goal. The most effective one is freezing, because frozen meat may be stored for many months. However, freezing causes a significant loss of quality that primarily affects the texture and the water holding capacity (WHC). Frozen meat is not well accepted by the consumer and undergoes considerable devaluation. Another less aggressive preservation method is packaging the meat in a CO₂-enriched atmosphere (11, 24, 69). The most common spoilage aerobic gram-negative bacteria (mainly Pseudomonas spp.) are effectively inhibited by the CO₂ (21) and are replaced by CO₂-resistant organisms, e.g., lactic acid bacteria (LAB) and Brochothrix thermosphacta (11, 66). In this modified atmosphere, myoglobin becomes the limiting factor in the shelf life, and the use of CO₂- and O₂-enriched atmospheres delays metmyoglobin formation (11). However, concerns have been expressed (22, 54) that the increase in the shelf life of meat packaged in modified atmospheres through inhibition of spoilage bacteria may provide sufficient time for human pathogens to grow to dangerous levels while the food remains attractive to the consumer. In this context, Listeria monocytogenes is the organism of most concern because of its ubiquity, psychrotrophic character, and facultative anaerobic ability. This bacterium is inhibited by CO₂ at very high concentrations, i.e., 75 to 100% (36), but at a concentration of 50% the CO₂ is less inhibitory (32). The risk may be increased by the potential growth of foodborne pathogens under conditions of temperature abuse by retailers and consumers (70), which unfortunately occurs frequently. A third strategy is the combined application of several preservation methods according to the hurdle model (53), but many inhibitory factors must be precisely controlled.

In this work, treatment with electron beam (E-beam) radiation is proposed as a pork preservative. Several authors (18–20, 23) have reported that irradiation is an effective method for killing pathogenic bacteria and decreasing the number of spoilage microorganisms.

Yersinia enterocolitica also is prevalent in pork and pork products. It is a facultative anaerobe that can grow at 4°C on fresh pork chops, and its growth rate is enhanced under CO₂-enriched conditions, where it can reach very high populations within 35 days of storage. Thus, the presence of this pathogen on fresh meat can present a microbiological risk when stored under modified atmospheres (77). However, Y. enterocolitica has not been used in irradiation experiments because its resistance to irradiation is much lower than that of L. monocytogenes, e.g., 0.1 to 0.2 kGy (31, 47) versus 0.35 to 0.5 kGy (18, 20).
Similarly, *Salmonella* Enteritidis is also of concern; its ability to multiply in fresh pork loin and cause illness has been clearly documented (9). This bacterium is not able to grow at refrigeration temperatures nor is the risk as high under conditions of temperature abuse occurs compared with that of *L. monocytogenes*. Several authors (19, 57, 58) have reported higher generation times for *Salmonella* than for *L. monocytogenes*, e.g., 14 h versus 7 h in cooked ham at 10°C, respectively (19).

Therefore, whichever treatment is used to eliminate *L. monocytogenes* to reach a food safety objective will be adequate to reduce the *Yersinia* and *Salmonella* to the lowest levels at the end of the shelf life. To attain this goal, experiments were conducted to optimize E-beam treatments to find a compromise for achieving an acceptable shelf-life extension and adequate microbial safety.

**MATERIALS AND METHODS**

**Organisms.** As a surrogate for *L. monocytogenes*, *L. innocua* NCTC 11288 was used. Fresh cultures were prepared for each experiment by removing a piece of frozen culture (~40°C in Trypticase soy broth with 10% glycerol) from vials, inoculating it into 9 ml of Trypticase soy broth, and incubating it at 32°C for 24 h. The culture was then centrifuged at 4°C, and the pellet was suspended in a sterile test tube with 10 ml of sterile saline, which yielded a bacterial level close to 10^8 CFU/ml. The handling, subculture, and inoculum preparation for *L. innocua* and sample contamination have been previously described (18, 20). In all experiments, a large number of cells were used to precisely calculate the radioresistant parameters.

**Sample preparation and irradiation treatment.** Twenty-four hours postmortem, whole loin pieces (3.5 to 5 kg in weight) were purchased at a local market, divided into samples (about 500 g), and packaged in laminated film bags of low gas permeability (diffusion coefficient of 35 cm^2/24 h/m^2 bar for O_2 and 150 cm^2/24 h/m^2 bar for CO_2). Samples were then transported to the irradiation plant (Tarancón, Cuenca, Spain, 60 km from the laboratory) in refrigerated boxes. At the plant, samples were treated with an industrial E-beam radiation source, which operates at 10 MeV. The system included a device for turning over the samples to irradiate them on both sides. The radiation doses used were 0.2 and 3 kGy. The dose absorbed by samples was verified with cellulose triacetate dosimeters (7) simultaneously irradiated. To determine the radioresistance of *L. innocua*, a portion of fresh loin was cut into slices of about 0.5 cm thick with an electric machine, whose rotating blade and contact surfaces were previously deeply cleaned. The slices were inoculated as previously reported (20). Experiments were performed at room temperature (18 to 20°C) in triplicate. The temperature increase during treatment was less than 2°C. After treatment, samples were stored at 4 and 8°C; samples stored at the latter temperature represented a temperature abuse scenario during product distribution.

**Microbial analysis.** To enumerate survivors, about 1 g of treated sample was homogenized with 10 ml of a sterile saline solution in a stomacher bag. Superficial indigenous microbiota were enumerated by aerobic plate counts (APCs) using the pour-plate method with plate count agar (Difco, BD, Sparks, MD) plus 2% NaCl (55) as the culture medium. LAB were counted in double-layer de Man Rogosa Sharpe agar (Conda-Pronadisa, Madrid, Spain) at pH 5.6 (55). Samples were incubated at 32°C for 48 h. *Enterobacteriaceae* were enumerated on violet red bile glucose agar (Oxoid, Basingstoke, UK) after incubation for 24 h at 37°C. *Pseudomonas* spp. were determined after incubation for 48 h at 25°C on *Pseudomonas* agar base supplemented with cetrimide, fucidin, and cephalosporin (Oxoid). Palcam medium (Oxoid) was chosen for *Listeria* spp. to enumerate survivors (at 37°C for 48 h) and to avoid the growth of endogenous microbiota. Colonies were enumerated with a Digital S colony counter (J.P. Selecta, Barcelona, Spain). The growth curves were constructed according to the Baranyi and Roberts model (13).

The shelf life of pork loin was determined by periodically counting the surface bacterial flora. Nonirradiated samples were used as controls. From a microbiological point of view, the end of shelf life was established when the APC exceeded 5 × 10^7 CFU/cm². Analyses were performed just after E-beam treatment (0 days) and during storage until the end of the shelf life.

**Physicochemical characteristics.** The dry matter (oven air-drying method) was analyzed following AOAC (10) procedures. The pH of the meat was determined in a homogenate of loin with distilled water (1:10, wt/vol) with a Digit-501 pH meter (Crispin Instruments Ltd., Barcelona, Spain). The a_w was measured at 25°C with a CX1 hygrometer (Decagon Devices Inc., Pullman, WA).

The WHC was measured with the Carver press method (48). A meat sample (0.3 g) was placed on a piece of filter paper (Whatman No. 1, 125 mm; Whatman, Clifton, NJ), set between two Plexiglas plates, and pressed with a mechanical force of 345 kPa for 5 min. The WHC was calculated as the percentage of water retained based on water content in the product before pressing. Four replicate measurements were recorded for each sample.

The exudate loss in raw samples treated at 0, 1, and 2 kGy was determined according to the method of Honikel (42). Pork loin samples (about 25 to 35 g) were packed in bags and treated by E-beam irradiation under the above conditions. For the drip determination, the meat samples were removed from the bags, placed in a perforated support, and then suspended in an inflated bag, ensuring that the sample did not make contact with the bag. After 12 h at 4°C, samples were weighed. Drip loss was expressed as a percentage of the initial weight.

Steaks (2 cm thick) from nontreated and treated pork loin were used to determine the cooking loss according to the method of Aaslyng et al. (1). Samples were heated on a grill pan at a temperature of 160°C. The steaks were turned every 30 s, and the temperature was determined at the center of each steak with a handheld probe (model 735, Testo, S.A., Barcelona, Spain). The steaks were removed from the pan when the temperature reached 70°C. Steaks were weighed before and after cooking to determine cooking losses (expressed as a percentage of the initial sample weight).

WCH, pH, a_w, and drip and cooking loss were determined immediately after E-beam treatment (0 days) and 5 days after treatment during storage at 4°C.

**Texture measurements.** The texture profile analysis (TPA) and tensile test were carried out with a TA.XT2i SMS texture analyzer (Stable Micro Systems Ltd., Goldaming, UK) using a cylindrical probe P/25 for TPA and a tensile grip (A/TGT) for the tensile test. Both tests were performed as previously described (41). The TPA was determined in cylinders (1.5 cm high by 2 cm wide). The tensile test was performed on rectangular pieces (7.5 cm long, 2 cm wide, and 0.3 cm thick) of raw pork loin. Measurements...
TABLE 1. Effect of E-beam treatment on pH, water activity ($a_w$), water holding capacity (WHC), cooking loss, and drip loss of raw pork loin after 0 and 5 days of storage at 4°C.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Storage time (days)</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>0</td>
<td>5.55 ± 0.04</td>
<td>5.57 ± 0.03</td>
<td>5.49 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.61 ± 0.05</td>
<td>5.52 ± 0.06</td>
<td>5.54 ± 0.06</td>
</tr>
<tr>
<td>$a_w$</td>
<td>0</td>
<td>0.989 ± 0.001 A</td>
<td>0.987 ± 0.002 A</td>
<td>0.986 ± 0.002 A</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.984 ± 0.001 B</td>
<td>0.983 ± 0.001 B</td>
<td>0.984 ± 0.001 B</td>
</tr>
<tr>
<td>WHC (%)</td>
<td>0</td>
<td>32.76 ± 4.17 B a</td>
<td>30.99 ± 2.82 B ab</td>
<td>28.53 ± 1.40 B b</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>52.75 ± 2.15 A a</td>
<td>52.11 ± 3.97 A ab</td>
<td>46.94 ± 1.56 A b</td>
</tr>
<tr>
<td>Cooking loss (%)</td>
<td>0</td>
<td>31.23 ± 2.65</td>
<td>31.69 ± 1.37</td>
<td>30.77 ± 3.12</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>32.78 ± 2.17</td>
<td>30.49 ± 3.70</td>
<td>29.59 ± 4.26</td>
</tr>
<tr>
<td>Drip loss (%)</td>
<td>0</td>
<td>2.72 ± 1.47 b</td>
<td>4.73 ± 1.09 b</td>
<td>7.24 ± 1.89 a</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3.04 ± 1.11 b</td>
<td>3.19 ± 1.70 b</td>
<td>9.74 ± 2.10 a</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations. Within a row, means with different lowercase letters are significantly different ($P < 0.05$). Within a column for the same physicochemical characteristics, means with different uppercase letters are significantly different ($P < 0.05$).

were recorded 0, 5, and 10 days after E-beam treatment during storage at 4 and 8°C.

**Color measurements.** Color measurements were made with a tristimulus colorimeter (Chroma Meter CR300, Minolta Corporation, Wayne, NJ). The L*, a*, and b* (lightness, redness, and yellowness) measured six times on the surface of the samples at three analysis times: 0, 4, and 24 h after opening the package and exposing the contents to air. After the first color measurement, samples were kept at 6 ± 2°C and 64% ± 2% relative humidity without protection (similar to conditions in a refrigerated display case or a domestic refrigerator). Color parameters were determined in nontreated and treated samples at 0, 5, and 10 days of storage at 4°C.

**Sensory analysis.** The sensory analyses were performed by 20 tasters selected from the Department of Nutrition, Food Science and Food Technology (Complutense University of Madrid). Triangular, rank order, and descriptive tests were performed as previously described (14). Analyses were carried out in individual booths built according to the International Organization for Standardization (45) DP 66.58 criteria. For the flavor analysis, pork loin steaks (0.5 cm thick) were cooked for 2 min on each side in a grill pan previously heated to 150°C. The temperature inside the steaks reached approximately 70°C, as measured with a portable digital thermometer (model 735, Testo). This treatment was sufficient to obtain an adequate final degree of doneness. The appearance and odor were evaluated in raw and cooked samples. Only samples stored at 4°C were used for sensory analysis. The range order test was performed until the end of the shelf life of nontreated samples. Triangular and descriptive tests were performed until the end of the shelf life of the nontreated and treated samples.

**Statistical analysis.** Survival curves were obtained by plotting the logarithm of the number of survivors against the radiation dose assayed. Decimal reduction doses ($D$-values) were calculated from the linear regression equation of survival curves. Regression equations, coefficients of determination ($R^2$), and the error bars were calculated with Excel (Microsoft, Redmond, WA). For data from the physicochemical analysis, the differences among means were established by an analysis of variance (ANOVA) and Duncan’s multiple comparison procedure. The effects of the individual variables associated with E-beam treatment and storage (dose and time and temperature of storage) and their interactions were analyzed with a multivariable ANOVA. These statistical analyses were performed using the Statgraphics Plus 5.0 program (Statpoint Technologies, Warrenton, VA).

**RESULTS AND DISCUSSION**

**Physicochemical characteristics.** The fresh pork loin used in this research had a dry matter content of 27.3% ± 1.6%. The effect of E-beam irradiation (1 and 2 kGy) on selected properties (pH, $a_w$, WHC, and cooking and drip loss) of the fresh product is recorded in Table 1. Significant differences were not observed for pH values ($P > 0.05$). This confirmed observations by several authors who reported that the pH of pork loin is not affected by irradiation treatment at 2 to 3 kGy (4, 51). A significant effect ($P < 0.05$) of the E-beam treatment was a decrease in WHC, which in turn could explain the increase in the drip loss in samples irradiated at 2 kGy. Other authors (51, 82) have also reported a decrease in WHC and an increase in soluble protein and exudates loss in irradiated pork longissimus dorsi muscle. These effects may be associated with changes produced by radiation on the muscle tissue structure, e.g., shrinkage of the myofibrils, as observed by Yoon (81) in chicken breast irradiated at 2.9 kGy. Although a significant increase ($P < 0.05$) in drip loss (7 to 8% in irradiated loin versus 2 to 4% in the control) was found in the samples treated at 2 kGy, the $a_w$ value remained unchanged ($P > 0.05$). This finding could be explained by the general sorption isotherm of high-moisture foods (34), which predicts that at $a_w$ values of 0.94 to 0.99 a large water loss is required to detect a minor decrease in the $a_w$ value. The amount of fluid lost by leakage in these loin samples was not enough to cause a detectable reduction of the $a_w$.

**Cooking loss, no significant differences ($P > 0.05$) were observed between control and irradiated samples, probably because the dose was too low. Bowes and Moss (15) reported changes in the collagen fibers in response to radiation at 5 and 50 Mrad (50 and 500 kGy).

**Shelf-life aspects.** To explore the effect of irradiation treatment on loin shelf life, the fate of the indigenous
Changes in aerobic plate counts (APCs) of control fresh pork loin samples (■) and samples irradiated at 1 (▲) and 2 (●) kGy and then stored at 4°C (a) and 8°C (b). The dotted line indicates the end of the shelf life.

FIGURE 1. Changes in aerobic plate counts (APCs) of control fresh pork loin samples (■) and samples irradiated at 1 (▲) and 2 (●) kGy and then stored at 4°C (a) and 8°C (b). The dotted line indicates the end of the shelf life.

microbiota on the fresh pork loin after irradiation was studied. Figure 1 shows the changes in APCs in nontreated (control) and E-beam–treated (1 and 2 kGy) fresh loin samples during storage at 4 and 8°C. The superficial indigenous microbiota of loin stored at 4°C (Fig. 1a) accounted for 4.0 log CFU/cm² in nontreated samples, and after treatment at 1 and 2 kGy decreases of about 2.0 and 3.6 log CFU/cm², respectively, were observed. From these data, a D-value of 0.61 kGy was roughly estimated. This value is in the range usually reported for vegetative bacteria, which is close to the D-value of LAB (67, 80), enterococci (2), and the pathogens Staphylococcus aureus (19, 39), Salmonella (2, 20, 39), and L. monocytogenes (18, 61) but higher than the D-value of other pathogens such as Y. enterocolitica and many gram-negative bacteria, for which D-values of 0.2 to 0.3 kGy have been commonly reported (31, 47).

The initial APC in the fresh product (4.0 log CFU/cm²) can be considered a usual bacterial load, and the meat could be judged as acceptable in terms of microbiological quality (29). At 4°C, the shelf life of control samples (when the APC reached the value of 7.5 log CFU/cm²) was about 5 days. A doubling time (g-value) of 10.3 h was roughly estimated, similar to that reported by other authors (8). Findings were similar when samples were stored at 8°C, but the g-value decreased significantly (6.2 h). The E-beam treatment at 1 kGy resulted in a decrease in the original bacterial load, leading to a significant increase in shelf life at 4°C: 7.5 log CFU/cm² was reached only after 11 days, with an estimated g-value of 14.8 h (0.25-fold higher than that of the nonirradiated microbiota), i.e., the growth rate of the microbiota was decreased by the E-beam treatment. The same effect was previously observed for S. aureus and L. monocytogenes in cooked ham (19) and for LAB in cheese (80), which was attributed to repair of damage caused by radiation. Therefore, the shelf-life extension of the products is due to both the lethal effect of the E-beam radiation and the decrease in the growth rate of the surviving indigenous microbiota. When a dose of 2 kGy was applied, the shelf life at 4°C was extended to around 18 to 20 days, with a rough g-value of 19 h, which is enough for safe domestic and large-scale distribution of fresh pork loin under refrigeration (4°C). Therefore, results obtained in the present study indicate that E-beam irradiation, even at low doses, is a useful strategy for extending by either twofold (1-kGy treatment) or fourfold (2-kGy treatment) the shelf life of fresh pork loin (and presumably other pork cuts).

During meat handling and transportation (mainly for long distances) under refrigerated conditions, temperature abuse may occur (e.g., 8°C) for a variable period of time, which may promote the growth of any organisms present on the meat. Figure 1b shows the changes in the APCs of nontreated and E-beam–treated (1 and 2 kGy) fresh loin stored at 8°C. An acceleration of the growth rate of the indigenous bacteria was observed. Accordingly, the estimated shelf life was 3, 8, and 15 days at doses of 0, 1, and 2 kGy, respectively. From the curves, g-values of 6.2, 9.4, and 15.6 h were calculated, respectively. Similar results have been reported by other authors (19, 38, 60). Although these findings indicate that meat stored at 8°C is acceptable for consumption for a shorter period of time than that stored at 4°C, the E-beam irradiation extended the shelf life of fresh meat even after temperature abuse; shelf lives for treated samples were close to threefold (at 1 kGy) and fivefold (at 2 kGy) longer than those for the nontreated samples.

The initial LAB level in the nontreated loin samples was about 1.5 log CFU/cm² (Fig. 2). Just after E-beam treatment at 1 kGy, the level decreased approximately 1 log CFU/cm² (0.5 log CFU/cm² was recorded), and at 2 kGy the level was below the detection limit (<100 CFU/cm²) of the method, although LAB were detectable later. At 4°C (Fig. 2a), 11, 12, and 15 h were estimated for the g-values in the control samples and those treated with 1 and 2 kGy, respectively. These values were slightly lower when samples were stored at 8°C (Fig. 2b): 10, 11, and 13 h, respectively. After 20 days of storage, LAB had not reached the critical level of 5 × 10⁷ log CFU/cm², and they represented less than 1% of the APC, i.e., LAB were not the main microbiota responsible for the meat spoilage.

For pseudomonads, the original level in control samples was 2.5 log CFU/cm² (approximately 3% of APC).
Enterobacteriaceae and L. monocytogenes (25, 62). L. monocyto-
sl% spp. strain in cooked ham (30, 40, 43, 61). L. Pseudomonas putida (~ is one of the most radioresistant strains in various foods and under various L. monocytogenes ~ (25, 35, 52). Several (12, 68) | surrogates for | at 4 and 8 Enterobacteriaceae to grow because of its psychrotrophic of the microbiota when the meat D levels reached 4. Among the heterogeneous fresh meat microbiota, the organisms with the fastest growth rates and that can utilize glucose are the aerobic gram-negative pseudomonads (35). In the present study, these organisms were responsible for the spoilage observed, although at the beginning they represented a minor percentage of the APC. This finding agrees with those of several authors (25, 62). Morgan et al. (62) reported that Pseudomonas spp. accounted for up to 90% of the microflora when the meat became spoiled during refrigerated storage.

Pseudomonads were not detected in samples that had been treated with E-beam radiation at 1 and 2 kGy until these samples had been stored for several days at either 4 or 8°C, i.e., the levels were lower than 100 CFU/cm². The pattern for APC counts for nontreated samples was similar to that observed for treated samples (Fig. 1). However, the pseudomonads in treated samples at both doses were unable to grow at the same rate. At 4°C, they were able to reach only about 6 log CFU/cm². This low growth may be related to the selective culture medium used to inhibit the growth of other organisms present in the samples or to the culture medium used for enumeration of survivors, which was supplemented with cetrimide, fucidin, and cephalosporin as inhibitors of native microflora. The E-beam irradiation may have sensitized the pseudomonads, which then may have been inhibited by the supplemental antimicrobials. At 4°C, the temperature may act as an additional disgenesic agent. Therefore, the actual count is not reflected by that obtained on the pseudomonad-selective medium but rather by the APC reported in Figure 1.

Pseudomonad generation times of 6.6, 10.8, and 17.6 h were calculated for samples treated at 0, 1, and 2 kGy, respectively, and stored at 4°C. When the pork loin was stored under temperature abuse conditions (8°C), the generation times calculated were 4.56, 10.6, and 16.0 h at 0, 1, and 2 kGy, respectively. Pseudomonads are very sensitive to irradiation, e.g., D-values of 0.08 and 0.13 kGy have been reported for Pseudomonas putida (71) and Pseudomonas spp. (59). These values suggest that in a mixed microbiota, such as that of fresh meat, the most radiosensitive bacteria will be effectively killed, which explains the response of the pseudomonads. The most radioresistant organisms will survive, but they may not necessarily cause spoilage; these survivors may either grow more slowly than the radiosensitive organisms or they may not be able to compete at refrigeration temperatures.

B. thermosphacta and cold-tolerant Enterobacteriaceae also can be found in aerobically stored meat, but because of their slower growth rate they are poor competitors for the pseudomonads (25, 35, 52). In the present study, at the end of the loin shelf life Enterobacteriaceae levels reached 4 and 5 log CFU/cm² at 4 and 8°C, respectively, in nontreated fresh loin samples (controls). In irradiated samples, counts were lower than the detection limit during the entire storage time at both temperatures.

Food safety aspects. As expected, the response of L. innocua (surrogate for L. monocytogenes) to the irradiation treatment fitted first-order inactivation kinetics, according to the following regression equation: log CFU/cm² = 7.67 − 2.31 × dose ($R^2 = 0.9955$). From this equation, a D-value of 0.43 kGy was calculated, slightly lower than the 0.49 kGy determined for the same L. innocua strain in cooked ham (18). This difference is be considered normal because evidence suggests that bacterial radioresistance may be influenced by the food matrix (12, 68). The 0.43 kGy value also falls within the range previously reported for different Listeria strains in various foods and under various conditions (39, 74).

Among non–spore-forming pathogens, L. monocytogenes is one of the most radioresistant (30, 40, 43, 61). The extended shelf life of the pork loin resulting from the E-beam treatment may provide an opportunity for L. monocytogenes to grow because of its psychrotrophic nature, thereby increasing the product risk when consumed. Thus, the potential for an increase in L. monocytogenes levels during the storage period must be considered. In the U.S. Food and Drug Administration report (79), several

![Graph](https://example.com/graph.png)
TABLE 2. Effect of several variables on breaking strength and texture profile of fresh pork loin

<table>
<thead>
<tr>
<th>Main effect (F ratio)(^a)</th>
<th>Breaking strength (N/cm(^2))</th>
<th>Hardness (N)</th>
<th>Adhesiveness (N/s)</th>
<th>Cohesiveness (10(^{-2}) m)</th>
<th>Springiness (J)</th>
<th>Chewiness (I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-beam treatment (A)</td>
<td>0.72 NS</td>
<td>1.36 NS</td>
<td>2.42 NS</td>
<td>3.85*</td>
<td>0.84 NS</td>
<td>4.17*</td>
</tr>
<tr>
<td>Storage time (B)</td>
<td>3.29 NS</td>
<td>0.63 NS</td>
<td>13.21**</td>
<td>0.98 NS</td>
<td>17.7***</td>
<td>9.47***</td>
</tr>
<tr>
<td>Storage temp (C)</td>
<td>0.24 NS</td>
<td>9.64**</td>
<td>1.38 NS</td>
<td>1.53 NS</td>
<td>6.07*</td>
<td>0.69 NS</td>
</tr>
</tbody>
</table>

Interaction (F ratio)\(^b\)

| A vs B                      | 0.11 NS                          | 0.52 NS      | 2.66 NS            | 0.32 NS                     | 1.55 NS        | 3.04 NS      |
| A vs C                      | 0.59 NS                          | 0.36*        | 0.04 NS            | 0.31 NS                     | 1.00 NS        | 2.18 NS      |
| B vs C                      | 0.01 NS                          | 11.94**      | 1.38 NS            | 1.34 NS                     | 7.57*          | 0.89 NS      |
| A vs B vs C                 | 0.76 NS                          | 0.36 NS      | 0.04 NS            | 0.31 NS                     | 1.00 NS        | 2.18 NS      |

Irradiation dose effects\(^b\)

| 0 kGy                      | 3.63 ± 0.35 A                    | 31.44 ± 5.57 A | 0.57 ± 0.13 A      | 0.50 ± 0.30 AB             | 0.20 ± 0.50 A  | 0.029 ± 0.50 A |
| 1 kGy                      | 3.92 ± 0.42 A                    | 25.47 ± 6.11 A | 0.46 ± 0.80 A      | 0.45 ± 0.40 B             | 0.21 ± 0.70 A  | 0.021 ± 0.50 B |
| 2 kGy                      | 3.74 ± 0.40 A                    | 28.31 ± 7.20 A | 0.47 ± 0.70 A      | 0.51 ± 0.40 A             | 0.17 ± 0.40 A  | 0.023 ± 0.50 A |

\(^a\) Significance: NS, not significant (P > 0.05); * P < 0.05; ** P < 0.005; *** P < 0.0005.

\(^b\) Values are means ± standard deviations for breaking strength and texture attributes. Within a column, means with different letters are significantly different (P < 0.05).

Authors provided evidence of increased L. monocytogenes levels in various products, with an average increase of 0.2 log units per day in fresh meat stored at 4 to 5°C and 0.35 log units per day in meat stored at 8°C (19, 79). Assuming contamination of the fresh loin at 10 cells per cm\(^2\) (1 log CFU/cm\(^2\)), as suggested by the International Commission on Microbiological Specifications for Foods (44), the L. monocytogenes population in nonirradiated loin would be about 10\(^2\) CFU/cm\(^2\) at both 4°C (shelf life of 5 days) and 8°C (shelf life of 3 days). These values do not meet either the microbiological criterion for L. monocytogenes of 100 CFU/cm\(^2\) stipulated by the European Union or the zero-tolerance policy of the U.S. Department of Agriculture. Nevertheless, E-beam treatment provokes 2.33 \(D\) and 4.65 \(D\) reductions with the application of 1 and 2 kGy, respectively. Therefore, the E-beam treatment would reduce the level of Listeria to 4.7 \(\times\) 10\(^{-2}\) and 2.2 \(\times\) 10\(^{-4}\) CFU/cm\(^2\), respectively. Because this bacterium is able to grow under refrigeration conditions, levels would increase during storage in a way that, assuming the same growth rate, they would be around 8 and 2 CFU/cm\(^2\), respectively, at the end of the shelf life at 4°C (18 to 20 days), lower than the 100 CFU/cm\(^2\) criterion but very far from the zero-tolerance criterion (absence in 25 g). In cooked ham, the growth of Listeria that survived the E-beam treatment was significantly reduced because of increases in both the lag phase and the g-value (19). For example, when 2 kGy was applied, the lag phase of the surviving population at 7°C was about 5 days and the g-value increased from 25 h (nontreated cells) to 125 h. Therefore, the level of Listeria at the end of the product shelf life, even under temperature abuse conditions, probably will be lower than the microbiological limit stipulated by the regulatory agencies, and in general consumer health should be safeguarded and cross-contamination minimized. Temperature abuse usually does not occur during the entire shelf life; usually the temperature rises only occasionally. However, under the worst-case scenario, in which temperature abuse (8°C) occurs during the entire storage period, a calculation similar to that for storage at 4°C would yield about 30 CFU/cm\(^2\) at the end of the storage period (shelf life of 8 days) when a 1-kGy radiation treatment is applied at 40 CFU/cm\(^2\) at the end of the storage period (shelf life 15 days) when a 2-kGy treatment is used. Therefore, the results obtained for products stored at 4°C in relation to the microbiological limit criteria also are valid for products stored at 8°C.

Texture and breaking strength analyses. Table 2 shows the effect of E-beam treatment and storage (time and temperature) on selected rheological characteristics of fresh pork loin. The multifactor ANOVA results indicated that only cohesiveness and chewiness were significantly affected (P < 0.05) by E-beam treatment. The lowest values for these rheological parameters were obtained at 1 kGy (about 0.45 and 0.021 J, respectively). However, no differences (P > 0.05) were found in the mean breaking strength (3.76 N/cm\(^2\)), hardness (28 N), adhesiveness (0.5 N/s), and springiness (0.19 \(\times\) 10\(^{-2}\) m). These results indicate that doses higher than 2 kGy would be needed to produce considerable changes in the rheological features of meat. In other studies (50, 75), doses higher than 10 kGy resulted in myofibril fragmentation and a decrease in tensile strength of muscles. Changes in secondary and tertiary structures of protein and induced cross-binding also have been found at higher doses (78). However, no marked difference was reported in the texture of fresh pork loins (raw and cooked) treated at 6 kGy (49).

Unlike E-beam treatment, a significant effect of sample storage parameters (time and temperature) on the TPA parameters (except cohesiveness) was observed (Table 2). The storage time significantly affected the adhesiveness (P < 0.005) and the chewiness (P < 0.005). In general, adhesiveness and chewiness increased with an increase in storage time (P < 0.005), which is probably related to the
formation of slime by gram-negative bacteria, e.g., pseudomonads, after the count exceeded 10^8 CFU/cm² (26, 35). The springiness also was significantly affected (P < 0.0005) by storage time, although it depended on the storage temperature; a significant interaction (P < 0.05) between both storage factors was found. Springiness increased (P < 0.05) only as the storage time increased when the temperature was 8°C. For hardness, a significant effect (P < 0.0005) of storage temperature was found. Significant interactions between storage variables (P < 0.0005) and between storage temperature and E-beam treatment (P < 0.05) also were found. This latter interaction indicated that the effect of the E-beam treatment on the hardness is dependent on storage temperature. This interaction explains the lower hardness values (P < 0.05) found for the treated samples stored at 8°C, whereas no significant differences (P > 0.05) were observed between nontreated and prepared samples stored at 4°C. The minimum hardness (13.74 N) was found at the end of the shelf life of the products treated at 2 kGy and stored at 8°C. This result is an accordance with previous reports of the acceleration of a number of enzymatic and chemical reactions (76, 82) during temperature abuse and fluctuation during meat storage. No additional interactions were found between storage variables and E-beam treatment (Table 2). Therefore, E-beam treatment had less of an effect on the rheological parameters than did the storage conditions (temperature and time).

**Color.** Significant effects (P < 0.0001) of E-beam treatment, air exposure time (0, 4, and 24 h), and storage time were detected for a*, b*, and L* values (Table 3). The loin E-beam treated samples had lower a* and higher b* and L* values than did the nontreated samples (Table 3). These results are in agreement with the color changes observed by other authors (63, 64) in meat and meat products after irradiation. Irradiation reduced the redness (a*) of ground beef significantly (63). The color changes induced by irradiation differ depending on animal species, muscle type, meat product, dose, and packaging type (16). An important role has been attributed to the meat pH and the concentration and state of myoglobin (56, 72). Some authors have speculated (37) that free myoglobin binding sites could react with free radicals such as hydroxyl or sulfuryl produced by irradiation of metmyoglobin and sulfmyoglobin. When the myoglobin is primarily in the MbFe^3+ form, irradiation produces a pigment with a spectral curve similar to that of oxymyoglobin (72), which results in an increase in the a* value. However, when the pigment is primarily in the MbO₂ form, the radiation converts the pigment into MbFe^3+, thus decreasing the a* value.

The packaging atmosphere (aerobic or anaerobic) has a greater effect on meat color than does irradiation alone (6). Tappel (75) reported that the color of pork irradiated in an oxygen atmosphere changed from pink to brown. The irradiation of aerobically packaged ground pork decreased the a* values (less red) and increased the visual gray and brown colors (73). These statements are in total agreement with the results obtained in the present study (Table 3). Previous literature also indicates that irradiation increases the yellowness (b*) in all species of meat (16).

In general, the a* value decreased with increased storage time (P < 0.05) but increased with increasing air exposure time (P < 0.05). The b* value also increased with increases of both storage and air exposure times (P < 0.05). An inverse reaction was observed for the L* value, which could be related to the loss of surface water (33).

The E-beam treatment effect on the three instrumental color parameters was independent of the time that samples were exposed to air; no interactions (P > 0.05) between these variables were found (Table 3). However, the contribution of E-beam treatment to meat color was affected by

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**Table 3. Effect of several variables on redness (a*), yellowness (b*), and reflectance (L*) of fresh pork loin**

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>a*</th>
<th>F ratio</th>
<th>P</th>
<th>b*</th>
<th>F ratio</th>
<th>P</th>
<th>L*</th>
<th>F ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-beam treatment (A)</td>
<td>31.46</td>
<td>0.0000</td>
<td>15.57</td>
<td>0.0000</td>
<td>48.53</td>
<td>0.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Air exposure time (B)</td>
<td>10.09</td>
<td>0.0001</td>
<td>51.33</td>
<td>0.0000</td>
<td>64.29</td>
<td>0.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Storage time (C)</td>
<td>16.90</td>
<td>0.0000</td>
<td>52.23</td>
<td>0.0000</td>
<td>15.82</td>
<td>0.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A vs B</td>
<td>4.45</td>
<td>0.018</td>
<td>21.42</td>
<td>0.0000</td>
<td>8.74</td>
<td>0.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A vs C</td>
<td>4.45</td>
<td>0.018</td>
<td>21.42</td>
<td>0.0000</td>
<td>8.74</td>
<td>0.0000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B vs C</td>
<td>1.35</td>
<td>0.2500</td>
<td>1.78</td>
<td>0.1000</td>
<td>3.13</td>
<td>0.0200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A vs B vs C</td>
<td>2.30</td>
<td>0.0200</td>
<td>1.78</td>
<td>0.1000</td>
<td>3.37</td>
<td>0.0010</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irradiation dose effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 kGy</td>
<td>7.90 ± 0.28 A</td>
<td>6.39 ± 0.35 B</td>
<td>53.87 ± 0.83 C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 kGy</td>
<td>6.49 ± 0.29 B</td>
<td>8.29 ± 0.65 A</td>
<td>55.48 ± 0.87 B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 kGy</td>
<td>6.47 ± 0.34 B</td>
<td>8.05 ± 0.67 A</td>
<td>57.74 ± 0.77 A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Values are F ratios and associated P values.
b Values are means ± standard deviations for a*, b*, and L*. Within a column, means with different letters are significantly different (P < 0.0001).
the storage time ($P < 0.05$). The minimum average values of $a^*$ and the maximum $b^*$ were obtained ($P < 0.05$) at the end of the shelf life (20 days at 4°C) for samples treated with 2 kGy. For the $a^*$, significant differences between nontreated and treated samples were always detected. For $b^*$ and $L^*$, significant differences ($P < 0.05$) between samples treated at 1 and 2 kGy were only found at the end of the shelf life, whereas immediately after E-beam treatment (0 days), nontreated and treated samples had similar values ($P > 0.05$). A significant interaction ($P < 0.001$) between the three variables (dose, storage time, and air exposure time) was found (Table 3) for the $L^*$ value. At all storage times, freshly opened samples that had been treated at 2 kGy had the highest $L^*$ values ($P < 0.05$). This result is probably associated with the lower WHC and drip loss (Table 1).

**Sensory aspects.** The effects of the E-beam treatments on the sensory attributes of fresh pork loin stored at 4°C are shown in Tables 4 and 5. Significant differences in appearance ($P < 0.05$) were noted between nontreated and treated (1 and 2 kGy) samples immediately after treatment (0 days). In the descriptive subjective analyses, samples treated at 1 kGy were judged to be pale pink and those treated at 2 kGy were rated as slightly grayish pink, although they were still considered acceptable for sale. These subjective sensory results coincided perfectly with the results obtained with the color analysis instrument (Table 3). A similar trend was reported for aerobically packaged pork loin samples irradiated at 1 kGy, which were more discolored than nontreated samples (51). With the triangular test, the only significant differences ($P < 0.05$) were found after 5 days of storage for samples irradiated at 2 kGy compared with those irradiated at 0 and 1 kGy (Table 4). According to the results obtained in the descriptive subjective analyses, control samples were dark, dull, and aged and had a grayish pink color, whereas samples treated at 2 kGy were bright pink and samples treated at 1 kGy were pale pink. These appearance differences would explain the increased levels of acceptance revealed by the rank order test for treated samples (mainly those treated at 2 kGy) after 5 days of storage at 4°C (Table 5). However, no significant differences were found between nontreated and irradiated samples, probably because the control samples had less drip loss, which undoubtedly contributed to a better sample appearance (Table 5). This result is also related to the lower WHC detected in the treated samples (Table 1). In the descriptive subjective analyses conducted at the end of the shelf life of the E-beam–treated samples (11 and 20 days at 1 and 2 kGy, respectively), color features similar to those mentioned above were described. There were no significant differences ($P > 0.05$) in appearance between nontreated and treated samples after they were cooked.

For odor, significant differences ($P < 0.05$) were detected with the triangular test when nontreated and treated (1 and 2 kGy) samples were compared (Table 4). In the descriptive analyses, immediately after treatment (0 days) the samples treated at 2 kGy released a slight off-odor defined as “scalded feather,” “poultry,” and “sulfur notes.” For the samples treated at 1 kGy tasters described “less fresh” meat odor and negligible off-odor to “scalded feather” odor. More than 7% of the volatiles found in irradiated foods are hydrocarbons commonly found in heat-treated foods (65). Most chemical changes in irradiated meat are associated with free radical reactions (3). The off-odors probably were responsible for the lower scores assigned to the treated samples (Table 5) in the rank order test. Despite this effect, the irradiated samples, even those treated at 2 kGy, were considered as acceptable for sale. After 5 days of storage, samples treated at 1 kGy were given the highest ratings (Table 5). This result is consistent with those obtained in the descriptive analyses because off-odors associated with the growth of spoilage organisms and the aging of the meat (e.g., pungent, sour, and unpleasant) were detected in the nontreated samples, whereas off-odors formerly perceived in treated samples diminished with increased storage time. Several authors reported similar findings concerning the dissipation of irradiation off-odors during storage (17, 28, 63). Irradiation can increase levels of dimethyl disulfide, dimethyl trisulfide, S-methyl ester, and ethanoic acid. These sulfur compounds are highly volatile and can be eliminated by storing the irradiated meat under aerobic conditions (28). The results obtained by various researchers suggest that aerobic packaging facilitates the reduction of some of the volatile compounds responsible for irradiation odor (17, 63). Other authors reported that cooking can reduce or eliminate irradiation-induced odors (49).

**TABLE 4. Significant differences of appearance (A) and odor (O) of nontreated and irradiated (1 and 2 kGy) fresh pork loin in the triangular test after 0 and 5 days of storage at 4°C**

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>Appearance</th>
<th>Odor</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$A^0, O^0$</td>
<td>$A^0, O^0$</td>
</tr>
<tr>
<td>1</td>
<td>$A^3, O^3$</td>
<td>$A^0, O^0$</td>
</tr>
</tbody>
</table>

**TABLE 5. Sensory evaluation by rank order test of nontreated and irradiated (1 and 2 kGy) fresh pork loin**

<table>
<thead>
<tr>
<th>Dose (kGy)</th>
<th>Appearance</th>
<th>Odor</th>
<th>Flavor</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>60 $A$</td>
<td>60 $A$</td>
<td>40 $A$</td>
</tr>
<tr>
<td>1</td>
<td>30 $B$</td>
<td>34 $B$</td>
<td>42 $A$</td>
</tr>
<tr>
<td>2</td>
<td>30 $B$</td>
<td>26 $B$</td>
<td>38 $B$</td>
</tr>
</tbody>
</table>

$a$ Sum of rank = $[(N_1 \times 1) + (N_2 \times 2) + (N_3 \times 3)]$, where $N_1$, $N_2$, and $N_3$ are the number of panelists that ranked the sample in the position 1 (minimum preference), 2 (intermediate preference), or 3 (maximum preference) in the ranked order test. Within a column, values with different letters are significantly different ($P < 0.05$).
In the flavor analysis of the cooked samples, no significant differences (both immediately after E-beam treatment and after storage at 4 °C) were detected among samples (Table 5). In the descriptive analyses, samples treated at 2 kGy were judged less juicy and had a very slight taint of “burnt” or “hot culture medium” and a very slight astringent aftertaste. Others have reported (27) that postirradiation storage could allow flavor to return to close to that of nontreated products as the volatile compounds are lost. Much of the work on irradiated meat odor and flavor has targeted selected constituents, particularly lipids (5, 17). The reactions of sulfur-containing amino acids with water radiolytic products appear to be the source of hydrogen sulfide and other volatile sulfur-containing compounds, which contribute to off-flavors (46). Irradiation increases the concentration of 3-methylbutanal and 2-methylbutanal, mainly in vacuum-packaged samples (28). However, dimethyl disulfide levels did not differ between irradiated and nontreated samples in aerobic packages (28).

A slightly higher concentration of volatile compounds has been found in irradiated cooked meat than in irradiated raw meat that was subsequently cooked (28).

The results obtained in this study indicate that the shelf life of whole fresh pork loin at 4 °C may be extended from 5 to 11 or 18 to 20 days by the application of 1 or 2 kGy of E-beam radiation, respectively. Likewise, under conditions of moderate temperature abuse (8 °C), the shelf life would be extended from 3 to 8 and 15 days, respectively, by the application of 1 or 2 kGy doses, without compromising the sensory qualities. From a hygienic point of view, E-beam irradiation of fresh pork loin practically guarantees a Listeria-free product during its shelf life, even when temperature abuse occurs, with no significant changes in the main sensory characteristics.

ACKNOWLEDGMENTS

The present work received financial support from projects AGL2010-19158 and CSD 2007-00016 (CONSOLIDER INGENIO 2010) funded by the Spanish Ministry of Science and Innovation.

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