

Low-Dose Gamma Irradiation and Refrigeration to Extend Shelf Life of Aerobically Packed Fresh Beef Round

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ABSTRACT

A 2-kGy gamma (low-dose) irradiation was applied to fresh top round from beef animals slaughtered and fabricated at commercial facilities. Cuts were packed in polyethylene film and stored at 1°C. Temperature abuse (9°C/24h) was simulated during storage. Psychrotroph counts on nonirradiated samples reached 10⁷ CFU/cm² between 8 and 11 d of storage, while similar counts were found after 28 d of storage on low-dose irradiated samples. Pseudomonads, *Enterobacteriaceae*, and *Brochotrix thermosphacta* were strongly inhibited on irradiated samples. No changes in organoleptic attributes were observed by a trained panel on treated samples. Low-dose irradiated samples had an average of 17 more shelf life days than the nonirradiated counterparts based on psychrotroph counts status and under the experimental condition being tested in this study.

Meat progressively undergoes spoilage from the time of slaughtering to consumption even if it is kept under refrigeration. Several interrelated factors such as storage temperature, gaseous environment, pH, water activity, light, indigenous enzymes, and the presence of microorganisms affect meat stability (shelf life) and keeping quality (3,7). Microbial growth is by far the most important factor in the shelf life and keeping quality of fresh meat and is influenced by many of the factors noted. Losses up to 20 million pounds of meat due to microbial spoilage have been reported in the United States (2), and 90% of these losses would be accounted for slaughter, fabrication, and in the retail area. Losses due to spoilage of unprocessed meat might account for higher figures than those aforementioned, particularly in markets with a lack of an appropriate cold food chain. Current practices of production and distribution of fresh meat are inadequate to protect consumers (19).

Since some bacterial carcass contamination is encountered under current slaughtering technology, preservation or decontamination methods of meat cuts needs to be pursued. Merchandising patterns are strongly affected by meat perishability, particularly in markets such as Argentina where meat is usually marketed fresh. Control of microbial spoilage in fresh meat has been reviewed in depth by Gill (7). Ionizing radiation has been applied on several meat and poultry items to improve food safety and to extend shelf life (4,19). It appears obvious that under current meat industry practices, there is a need for further improvements.

The impact that irradiation might have on major beef producer countries involving various scenarios has been discussed (8). Hayes et al. (8) claim that irradiation would greatly affect patterns of world meat trade. Also, survey results by Moss et al. (18) have shown that most consumers, when informed about the risks of poultry related foodborne diseases, were willing to accept and pay for a safer chicken treated with irradiation or chemical agents. The Food Safety and Inspection Service of the U.S. Department of Agriculture have endorsed the use of ionizing radiation on meat and poultry to provide consumers with a safe, wholesome, and nutritious food supply (1). Low-dose irradiation (LDI) has been employed to increase the shelf life of beefburgers and minced meat in combination with other preservation methods (5,21). It has also been reported in lamb (22), in whole chicken carcasses (9), in vacuum-packaged pork loins (13), and in vacuum-packed beef cuts (20). Lakritz and Maerker (10) reported that irradiation of meat between 1-10 kGy may reduce proteolysis caused by some indigenous enzymes. Yet, the use of this technology is somewhat limited in the meat industry. Reports on LDI in retail fresh beef packed under aerobic environments are scarce.

The aims of this research were to evaluate the effect of low-dose gamma irradiation on spoilage flora of "naturally" contaminated fresh beef top round and to characterize its shelf life through spoilage indicator counts under refrigeration storage and temperature abuse.

MATERIALS AND METHODS

A design was constructed to simulate the production of a fresh beef item under commercial production, deboning, and centralized shipping practices. Slaughtering, fabrication, and packaging were carried out at two beef operations (trials 1 and 2, respectively) that follow good manufacturing practices (11).

Beef cuts

Top round from Aberdeen Angus crossbreed steer (average 30 months old) carcasses were used. The carcasses were aged between 30 and 36 h at 2-3°C and were deboned under commercial practices. Samples were randomly assigned to the treated (low-dose irradiated) or to the control (nonirradiated) group. Seventy-two top round samples were used in the first trial, and eighty-four cuts were used in the second trial.

Packaging

An 80- μ m thick polyethylene wrap was used. Each top round cut was individually packaged by commercial meat handlers at the beef plants.

Irradiation

Treated samples were irradiated with 2.0 kGy using a ^{60}Co power source from the Atomic Energy Commission (CNEA, Ezeiza, Argentina) 3 h after being fabricated. Samples were kept in expanded 60 x 30 x 30-cm polystyrene boxes through the entire process at room temperature (22°C). To determine the true radiation dose received by the beef round, ampoules containing nitrate-nitrite were distributed at equal distances among and within the boxes. Dosimeters had 0% deviation when tested against the Frick dosimeter by the International Atomic Energy Agency.

Storage

Temperature of samples after irradiation was 2.5°C. Irradiated and nonirradiated samples were held at 1°C in a cooler facility at the Instituto de Tecnología de Carnes, INTA. Samples were individually allocated to the cooler shelves and periodically analyzed.

Shipping and retailing

Actual practices that might correspond to temperature abuse during meat handling were simulated. All abused samples were held at 9°C during the last 24 h of storage after an appropriate storage period at 1°C (See Tables 1 and 2 for storage time and temperature abuse schedule).

Sampling

Six treated and control beef top round samples were analyzed at each appropriate day (See Tables 1 and 2). An area of 22 cm² and 4 mm depth was sampled in an aseptic manner with a sterile scalpel and tweezers under a laminar flow from each one of the samples.

Microbiological assays

Samples described above were placed in a sterile bag containing 50 ml of sterile 0.01% peptone water and homogenized for 2

min in a Stomacher 400 (Lab Blender 400, Seward Med, England). From this homogenate, suitable decimal dilutions in 0.01% peptone water were prepared. Total viable mesophile (mesophiles), total viable *Enterobacteriaceae* (enterobacteria), and most probable number of total coliforms were performed as described in Lasta et al. (11). Total viable psychrotroph (psychrotrophs) values were determined by spread plating 0.02 ml of an appropriate dilution on a quarter of a plate containing plate count agar (Difco Lab, Detroit, MI). Plates were incubated for 10 d at 5°C. Pseudomonads counts were determined by the spread plate technique utilizing 0.02 ml of appropriate dilution on a quarter of a plate containing CFC medium (17) and incubated 2 d at 25°C. *Brochothrix thermosphacta* were determined by the spread plate method utilizing 0.04 ml in a half plate containing STTA medium (6) and were incubated 5 d at 25°C.

Organoleptic characteristics

Color (appearance), odor (aroma), and overall acceptability were evaluated by an eight-member trained (average 8 years in evaluating meat products) panel immediately after sampling for the microbiological analyses. Attributes of color and aroma were evaluated by using a 10-point numerical scale, where 1 corresponded to "extreme change" (undesirable product) and 10 to "characteristic of a product of high quality". Acceptability was evaluated with a 10-point hedonic scale, where 1 corresponded to "dislike extremely" and 10 "like extremely".

Shelf life

To evaluate shelf life objectively, data related to the storage time in days required for each sample replicate to reach a count value higher than 10⁵ CFU/cm² (colony forming units per square centimeters) on two consecutive samplings were used. This value was chosen considering that temperature abuse or mishandling, which can increase the count number, might happen before the cuts reach the consumer. Confidence intervals (95%), as an expression of shelf life in days, were calculated based on counts of the main spoilage organisms tested. A cutoff value of 10⁷ CFU/cm² was used to indicate the end of shelf life.

TABLE 1. Microbial counts on low-dose (2 kGy) irradiated and nonirradiated beef top round during aerobic refrigeration storage (1°C) and with temperature abuse (9°C/24 h) (Trial 1). Count values are expressed as log CFU/cm².^a

Microorganism		Storage time (d)						
		0	7	8	14	15	27	28
		NA ^b	NA	A ^c	NA	A	NA	A
Psychrotrophs	I ^d	<1.58	<1.58	1.89	2.92	3.45	7.20	6.65
	NI ^e	4.11	6.33	6.93	7.76	8.22	-- ^f	--
Mesophiles	I	<1.58	<1.58	2.22	1.84	2.16	5.55	3.58
	NI	3.69	6.44	6.77	7.73	7.77	--	--
Pseudomonads	I	<1.58	<1.58	1.83	2.09	2.10	4.45	4.49
	NI	3.11	5.99	6.96	7.35	7.49	--	--
Enterobacteria	I	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30	<0.30
	NI	1.80	2.27	1.25	3.45	3.12	--	--
Coliforms	I	0.48	0.48	0.48	0.48	0.48	0.48	0.48
	NI	1.80	2.27	1.25	3.45	3.12	--	--

^a Count values are average of six replicates;

^b Nonabused samples (temperature was kept at 1°C);

^c Abused samples (the last 24 h temperature was elevated to 9°C);

^d Irradiated samples;

^e Nonirradiated samples;

^f Nontested.

TABLE 2. Microbial counts on low-dose (2 kGy) irradiated and nonirradiated beef top round during aerobic refrigeration storage (1°C) and with temperature abuse (9°C/24 h) (Trial 2). Count values are expressed as log CFU/cm².^a

Microorganism		Storage time (d)											
		0	3	4	7	8	10	11	15	16	21	22	28
		NA ^b	NA	A ^c	NA	A	NA	A	NA	A	NA	A	NA
Psychrotrophs	I ^d	1.66	-- ^e	--	--	--	3.11	--	5.10	5.03	6.16	6.64	7.54
	NI ^f	3.45	4.40	5.26	6.06	6.25	6.55	7.35	--	--	--	--	--
Mesophiles	I	<1.58	--	--	--	--	2.65	--	2.58	2.81	3.13	3.92	2.40
	NI	2.84	3.67	3.10	6.11	4.11	4.98	5.54	--	--	--	--	--
Pseudomonads	I	<1.58	--	--	--	--	<1.58	--	<1.58	<1.58	<1.58	2.83	5.43
	NI	2.54	4.19	4.77	5.49	5.62	6.38	7.52	--	--	--	--	--
Enterobacteria	I	<0.30	--	--	--	--	<0.30	--	<0.30	<0.30	<0.30	<0.30	<0.30
	NI	0.30	0.44	0.90	0.77	1.98	1.98	3.36	--	--	--	--	--
<i>Brochothrix</i> ^g	I	<1.58	--	--	--	--	2.30	--	4.90	4.92	<3.58	<3.58	<3.58
	NI	2.92	4.00	5.10	5.30	5.43	6.01	6.37	--	--	--	--	--

^a Count values are average of six replicates;
^b Nonabused samples (temperature was kept at 1°C);
^c Abused samples (the last 24 h temperature was elevated to 9°C);
^d Irradiated samples;
^e Nontested;
^f Nonirradiated samples;
^g *Brochothrix thermosphacta*.

Statistical analysis

Actual microbial counts expressed as CFU/cm² were transformed to log. Differences for each group of microorganisms between trials and between appropriate storage time, for irradiated and control samples, were tested by using the "t" test (23).

RESULTS

Microbial counts on LDI and nonirradiated samples are shown in Tables 1 and 2. Naturally occurring microflora from low-dose gamma irradiated samples significantly decreased (P < 0.05) when compared with nonirradiated top round samples. A delay of psychrotrophs growth on treated samples also occurred (Fig. 1 and 2). Samples exposed to a 24 h/9°C temperature abuse period showed an increase of approximately 1 log in the microbial counts (Fig. 2). There were,

however, some exceptions such as enterobacteria on trial 2 (Table 2) at 11 d of storage which increased 1.38 log. Furthermore, an increase higher than 1 log was only observed on nonirradiated beef rounds. On treated samples, an increase of 0.79 log in mesophile count at 22 d of storage was noted in trial 2 (Table 2), and this value was the higher one found in LDI samples. Results of dosimetry showed an 1.069 maximum to minimum dose ratio.

Nonirradiated samples showed marked evidences of spoilage (undesirable odor and color) between 8 and 10 d of storage, which was in agreement with the high microbial counts (Tables 1 and 2). Undesirable organoleptic changes were observed as psychrotrophs reached a 10⁷ CFU/cm²

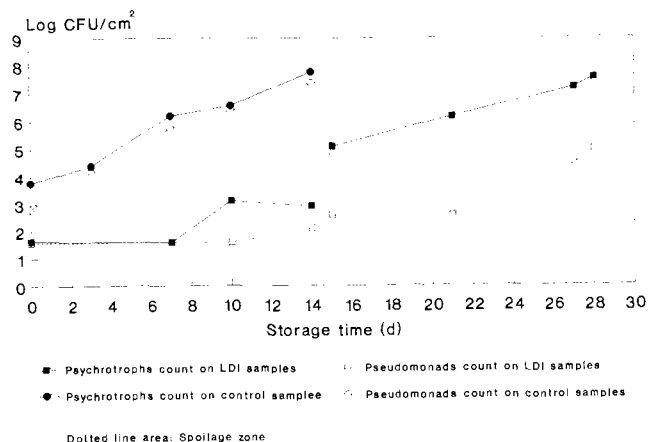


Figure 1. Growth patterns of psychrotrophs and pseudomonads on control and low-dose (2 kGy) irradiated (LDI) beef round samples stored under refrigeration (1°C).

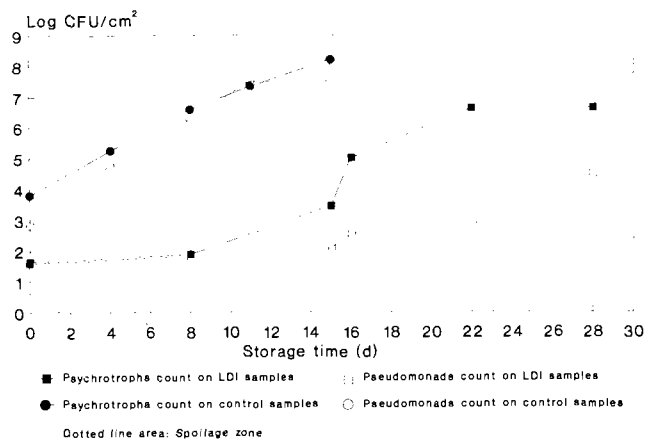


Figure 2. Growth patterns of psychrotrophs and pseudomonads on control and low-dose (2 kGy) irradiated (LDI) beef round samples stored under refrigeration (1°C) and subjected to temperature abuse (9°C/24 h) (See Tables 1 and 2 for temperature abuse schedule).

count (Fig. 1 and 2). Mean scores for appearance and odor on irradiated samples were 7.5 and 6.5, respectively, after 4 weeks of storage and overall acceptability of irradiated samples at the end of storage time was 7.5.

Shelf life, based on microbial counts, of LDI samples varied according to the microbial spoilage group that was analyzed. Shelf life (in days) expressed as 95% confidence intervals (CI) if psychrotrophs were considered was between 15 and 18.7 d. If mesophiles were taken into account shelf life CI was between 18 and 20 d and finally, shelf life CI was between 19.5 and 20.5 d if the pseudomonads group was considered (Table 3). Differences between those counts from LDI and control samples were highly significant ($P < 0.01$).

TABLE 3. Shelf life comparison of low-dose irradiated (2 kGy) and nonirradiated beef top round.^a

Trial	Microbial count					
	Mesophiles		Psychrotrophs		Pseudomonads	
	NI ^b	I ^c	NI	I	NI	I
I	7	27	7	27	7	27
II	10	28	7	21	7	28
Average	8.5	27.5	7	24	7	27.5
Difference	19		17		20.5	

^a Values are expressed as days required for spoilage to become evident.

^b Nonirradiated samples.

^c Irradiated samples.

DISCUSSION

Shelf life extension of treated samples might be explained in terms of a drastic reduction of initial microbial contamination (Tables 1 and 2) and by a delay on microbial growth of remaining viable cells (Fig. 1). It is noted that a \log_{10} 1.58 was the lowest detection limit used for counts. This might mask some bacterial growth in case it had occurred during the first 6 d of storage. Bacterial growth curve for psychrotroph organisms on LDI samples seemed to exhibit a sporadic growth with the lag phases ending around days 8 and 15 (Fig. 1). It has been reported by Lebepe et al. (13) that different DNA repair mechanisms for different bacteria after irradiation injury might explain this behavior. Nonetheless, temperature abuse could also have affected this growth pattern (Fig. 2). Initial counts, particularly regarding psychrotrophs, on naturally contaminated fresh beef top round cuts usually showed less than 10^4 CFU/cm². This value might be obtained through application of good manufacturing practices at the meat plant level (Tables 1 and 2).

A procedure to establish an improvement in shelf life from LDI samples should be set up based on psychrotroph, pseudomonads, or mesophile counts. These microbial counts may be used to test the keeping quality of a fresh low-irradiated beef commodity. Psychrotrophs are important since they are the main organisms responsible for meat deterioration under an aerobic environment and at chill temperatures (3,7). This group of organisms might also be used to monitor the efficacy of the LDI process. According to these results,

psychrotroph count was the most rigorous indicator of spoilage evidence (samples with lower shelf life). But, a 10-d incubation period is required to obtain this count. The pseudomonads group might be useful in terms of monitoring the sample status since only a 36- to 48-h incubation period is required to obtain this count. The CFC medium allows the growth of primarily *Pseudomonas*, rather than the growth of *Moraxella* and *Acinetobacter* (17). Hence, organisms growing on this medium were denominated pseudomonads in the current assay. This group might also be a better spoilage indicator than mesophiles in refrigerated food items.

Shelf life was analyzed in terms of the time (in days) required for irradiated or control samples to reach a 10^5 CFU/cm² count. Any further count increase due to mishandling or temperature abuse during meat shipping or retailing should lead to spoilage evidence (Fig. 2). In the current study, the end point was considered to be when all sample counts were higher than 10^5 CFU/cm² for each particular microbial count in two consecutive sampling periods (Tables 1 and 2). In case the samples had not reached such values by the last day of sampling, a 10^5 CFU/cm² count value was assigned. This was the case for mesophiles and pseudomonads on LDI samples (Tables 1 and 2). It was deemed that this procedure was very effective in evaluating sample shelf life, and it provided a more rigorous assay condition.

A pronounced effect on reduction of *Pseudomonaceae* and *Enterobacteriaceae* were attained by using 2-kGy irradiation on beef samples and these results agree with those reported by Niemand et al. (20) on flexible pouch vacuum-packed beef sirloin. Reduction of *Pseudomonaceae* might be explained in terms of the D value (decimal reduction dose) for *P. fluorescens* of 0.13 kGy that was reported (16). *B. thermosphacta*, a relevant spoilage organism, was also greatly reduced (Table 2) by the treatment. Reduction of spoilage and other indicator organisms also will be important because it would lower the chances of cross-contamination involving this commodity. Our result on beef shelf life enhancement through radurization also agrees with that reported by Paul et al. (22) for lamb meat. The 2-kGy dose applied in the current research might also have a detrimental effect on other spoilage flora and on certain pathogens and parasites as has been reported (4,25). Tarkowski et al. (25) reported that a dose of 1 kGy caused a reduction of more than 95% in *Salmonella* and 99.9% in *Yersinia enterocolitica* and *Campylobacter jejuni* isolated from beef. Carcasses lightly infested by *Cisticercus bovis* might be rendered fit for human consumption when exposed to 0.20-0.60 kGy (4).

In the current study, trained panelists did not detect development of any "irradiation odor" on the treated samples. This might be explained by a threshold dose of 2.5 kGy that has been reported as responsible for producing such odor in beef (24). In addition, the panelists found a good overall appearance when considering irradiated top round samples after 4 weeks of storage. These results are in agreement with those published on lamb (22) and beef (20). Moreover, thiobarbituric acid values have been reported not to be affected in low-irradiated pork loins as stated by Lebepe et al. (13) and by Mattison et al. (15). Lea et al. (12) reported, however, that a radurization dose level produced a change in color, flavor, and odor of fat and also changed the appearance

and odor of raw and cooked beef. In the current study only raw beef round was evaluated.

The use of irradiation as a food preservation method has been supported by the World Health Organization (26); however, its application on an industrial basis has been somewhat controversial despite its numerous advantages (4,8). There are no scientific or technological reasons for the lack of massive use of irradiation in the food industry; however, public awareness of these advantages, emotional factors, and consumer misinformation appear to be main causes of this status (19). It is noteworthy, however, that the worldwide trend in unconditional approval of food irradiated items has steadily increased over the last years 10 years (14). In the current research, an increase in shelf life of fresh beef due to the use of low-dose gamma irradiation and refrigeration is shown.

Increasing of shelf life of irradiated beef round samples varied according to the microbial group considered. Yet, it is possible to establish an objective criterion to evaluate LDI fresh beef shelf life (Table 3 and Fig. 1). Besides, average shelf life value of irradiated samples has a meaningful merchandising condition; they double the shelf life of beef stored and handled under current commercial practices

CONCLUSION

LDI (2 kGy) might be a reliable preservation tool to obtain an organoleptically stable, retail fresh beef product, while enhancing its shelf life under refrigeration and aerobic environment by reducing naturally occurring spoilage microflora. Psychrotroph counts might be used to monitor the LDI process, while the pseudomonads group might serve to control LDI fresh beef shelf life.

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