Using gamma irradiation and low temperature on microbial decontamination of red meat in Iran

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Abstract Gamma irradiation can be used as one of the most efficient methods to reduce microorganisms in food. The irradiation of food is used for a number of purposes, including microbiological control, insects control and inhibition of sprouting and delay of senescence of living food. The aim of this study was to study effects of gamma irradiation, refrigeration and frozen storage as the combination process for improvement of red meat shelf-life. The bovine meat samples were treated with 0, 0.5, 1, 2 and 3 kGy of gamma irradiation and kept in refrigerator for 3 weeks and in freezer for 8 months. The control and irradiated samples were stored at 4–7°C and at –18°C for refrigeration and frozen storage, respectively; and microbial and chemical analyze was done at 1 week and 2 months intervals. In this study the optimum dose of gamma radiation in order to decrease the total count of *Mesophilic bacteria*, *Coliforms*, *Staphylococcus aureus* and especially for elimination of *Salmonella* was obtained at 3 kGy. Microbial analysis indicated that irradiation and storage at low temperature had a significant effect on the reduction of microbial loads. There was no significant difference in chemical characteristics during freezing storage in bovine meat. Also, irradiated meat samples (3 kGy) were stored in 4–7°C for 14 days, compared to 3 days for non irradiated samples.

Key words: Gamma irradiation · Red meat · Microbiology · Contamination · Low temperature

Introduction

Food irradiation is used for a number of purposes, including microbiological control, insects control and inhibition of sprouting and delay of senescence of some food such as: potatoes, onions, fruits, vegetables and etc¹. The aims of irradiation of meat and poultry are: 1. Elimination inactive, pathogenic microorganisms present in fresh or frozen meats, poultry and thereby make these foods safer for human consumption. These pathogenic microorganisms may be either certain bacteria such as *Salmonella* or *Campylobacter* or parasites such as *Trichinella spiralis*. 2. Extending the shelf life of the fresh meats by reducing their microbial population, primarily vegetative forms of bacteria which are the cause of the spoilage².

Many researchers recognized and reported that gamma irradiation in low doses, below 10 kGy; kills most organisms without deterioration of food quality^{3,4,5}. In 1997, the FDA approved the irradiation of meat products (fresh and frozen beef, lamb and pork) for controlling disease causing microorganisms such as *Escherichia Coli*, *Salmonella*, *Listeria* and other food borne pathogens⁶. Freezing is known to reduce viable cell counts by 1–2 log units, with extended storage causing additional, time-dependent reductions⁷. In this study, effects of gamma irradiation on bovine meat and its effect on extending shelf life in refrigeration and freezing condition (–18°C) as combination methods were examined. Also this study has been set out to evaluate the microbiological and chemical characteristics of bovine meat and to apply gamma irradiation for preservation of red meat against bacterial contamination and to increase storage life of bovine meat at commercial scale.

Materials and Methods

Sample preparation: Thirty freshly packed bovine meat samples weighting 2000 ± 100 g were purchased at a

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slaughterhouse (Qazvin, Iran) on the day of slaughtering. The samples were divided into 15 equal parts and then were packed in commercial freezing bags. Each sample divided into 5 groups (each groups contain 3 equal parts weighting 100 ± 10 g). One group of each sample wasn't exposed to gamma ray and kept as control and 4 groups were exposed 0.5, 1, 2 and 3 kilo-gray (kGy). The meat samples were stored at refrigerator ($4-7^{\circ}\text{C}$). Also other 30 packed bovine meats weighted 2000 ± 100 g purchased at the above slaughterhouse and divided as in the above procedure and irradiated 0, 0.5, 1, 2 and 3 kGy but stored at freezing condition (-18°C).

Irradiation: The samples were irradiated by a Co-60 source (Gamma cell Px-30, dose rate = 0.749 Gy/sec and activity = 4600Ci) in Nuclear Agriculture Department of Agricultural, Medical and Industrial Research Institute, Atomic Energy Organization of Iran.

Microbial analysis: Microbial analysis of *Aerobic bacteria*, *Coliforms*, *St aureus* and *Salmonella spp* was carried out a day after the arrival day and on 1, 2 and 3 weeks for storage in refrigeration condition ($4-7^{\circ}\text{C}$) and on months 2, 4, 6 and 8 months for freezing condition (-18°C)⁸. For total count of aerobic Mesophilic bacteria, 25 g from each bovine meat sample was isolated aseptically and diluted with 225 ml sterile peptone water (%0.1) and blended for 3 min to prepare 1:10 dilution. Subsequent dilutions were prepared 1:100 and 1:1000. For bacterial counts 1 ml of each dilution was added into the sterile plates in duplicate, plate count agar media (Merck) was added and incubated for 48 h at 35°C . The number of viable bacterial colonies expressed as microbial counts (log CFU/g). For *Coliform* counts, 1 ml of each dilution was spread in duplicate on plates containing Violet Red Bile Agar (Merck) and incubated for 48 h at 37°C , also the Maximum Possibility Number (MPN) of *Coliform* was obtained by the 3 tubes method in Lactose broth media for 24 h at 37°C and 1 ml of each tube, which was positive for gas production, was added to another tube containing Brilliant Green Broth (Merck)⁹. For *St aureus* counts, 1 ml of each dilution was spread, in duplicated on plates containing of Bird Parker Agar (Merck), coagulase test using rabbit plasma was conducted to confirm *St aureus*¹⁰. For isolation of *Salmonella spp*, 25 g of each sample was incubated at 37°C in 225 ml of Lactose Broth for the first enrichment. Selective enrichment was done in Selenite Enrichment Broth at 43°C followed plating on Shiglla-Salmonella Agar (Merck) to detect of characteristics colonies of *Salmonella* for 48 h at 37°C incubation¹¹.

Chemical analysis: Some of the volatile Nitrogen substances were made by the effects of active microorganisms on food proteins. These substances are such as: Amoniac, Indol, Scatol, Cadaverin and etc. Total Volatile Nitrogen

(TVN) was determined as described by Mwansyemela¹². There is a direct correlation between the amount of TVN and the spoilage of meat. If the TVN was in the range of $17-20$ mg/g it shows fresh meat, if TVN was $20-30$ mg/g the meat is acceptable and if TVN was $30-50$ mg/g, it shows meat spoilage.

Peroxide Value (PV) determined lipid oxidation. Unsaturated fatty acids absorb oxygen molecules and make peroxide; amount of peroxide can be measured by Iodometry method. Briefly 1 g of bovine fat from each sample was cut and dissolved in the acetic acid: chloroform mixture, 0.5 ml of saturated Potassium Iodine (KI) (83.2 g solid KI/40 ml H_2O) was added and mixed vigorously, then 30 ml deionized water were added, the solution was mixed again. The mixture was allowed to stand for 5–10 min at room temperature, and then titrated by 0.01 M $\text{Na}_2\text{S}_2\text{O}_3$ gradually. During the titration, 0.5 ml of starch indicator 1% was added. Color of the upper aqueous layer ranged from light purple to dark purple and the lower layer remained white to gray. The end-point of titration was established when the color of the upper aqueous layer disappeared. The peroxide value was measured as miliequivalent/kg of sample fat (mPV). The mPV was calculated employing the following formula^{1, 12}:

$$\text{mPV} = \frac{(S) (N) 1000}{W} \quad S = \text{Na}_2\text{S}_2\text{O}_3 \text{ for sample titration}$$

$$N = \text{Normality of Na}_2\text{S}_2\text{O}_3 \quad W = \text{sample weight (g)}$$

Results

Total count of aerobic bacteria decreased with increase of irradiation dose (Table 1). Mean of bacterial total count, *Coliform* count and *St aureus* count were 2.81×10^6 , 3.57×10^4 and 9.68×10^3 colony forming units (CFU) per gram at control sample (0.0 kGy) respectively (Table 1). According ANOVA analysis, the number of *Aerobic bacteria*, *Coliform* and *St aureus* decrease with increase of irradiation, therefore irradiation significantly ($P < 0.05$) reduced them (Table 1 and 2). In this study irradiation and frozen storage was more effective than either treatment alone at decreasing total of *bacteria*, *Coliform* and *St aureus* counts (Table 2). The optimum dose of gamma radiation to reduce the microbial contamination and eliminate *Salmonella* was obtained at 3 kGy. Also, irradiation meat samples (3 kGy) can be stored in refrigerator without any spoilage for 2 weeks, compared to 3 days for non-irradiation meat (Table 1). According to this study the optimum dose of gamma radiation to decrease bacterial total count, *Coliform* count, *St aureus* count and especially for elimination *Salmonella* was calculated as 3 kGy. Effects of various levels of gamma irradiation on PV are shown in Table 3. There was no significant difference ($P > 0.05$) between PV

Table 1 Microbial means for control (0.0 kGy) and irradiated (0.5, 1, 2 and 3 kGy) bovine meat during refrigeration storage (4–7 °C).

ID (kGy)	TC ^a (Mean ± SE)					CC ^b (Mean ± SE)					SC ^c (Mean ± SE)				
	0	0.5	1	2	3	0	0.5	1	2	3	0	0.5	1	2	3
T															
1 day	2.8×10 ⁶ ± 2.8×10 ⁶	2.3×10 ⁵ ± 2.3×10 ⁵	2.7×10 ³ ± 2.7×10 ³	1.2×10 ² ± 1.2×10 ²	3.7×10 ¹ ± 3.7×10 ¹	3.6×10 ⁴ ± 3.6×10 ⁴	2.4×10 ² ± 2.4×10 ²	1.8×10 ² ± 1.8×10 ²	9.7×10 ¹ ± 9.7×10 ¹	2±1	9.7×10 ³ ± 9.7×10 ³	1×10 ³ ± 1×10 ³	1.5×10 ² ± 1.5×10 ²	2.7×10 ¹ ± 2.7×10 ¹	0
7 days	1.2×10 ⁶ ± 1.2×10 ⁶	5.5×10 ⁴ ± 5.5×10 ⁴	1.4×10 ³ ± 1.4×10 ³	1.1×10 ² ± 1.1×10 ²	1.2×10 ¹ ± 1.2×10 ¹	2.2×10 ⁴ ± 2.2×10 ⁴	1.1×10 ² ± 1.1×10 ²	1.4×10 ² ± 1.4×10 ²	3.2×10 ¹ ± 3.2×10 ¹		3.2×10 ⁶ ± 3.2×10 ⁶	3.2×10 ² ± 3.2×10 ²	3.2×10 ¹ ± 3.2×10 ¹	1.2×10 ¹ ± 1.2×10 ¹	0
14 days	2.7×10 ⁷ ± 2.7×10 ⁷	2.7×10 ⁶ ± 2.7×10 ⁶	1.5×10 ⁴ ± 1.5×10 ⁴	2.8×10 ³ ± 2.8×10 ³	2.0×10 ² ± 2.0×10 ²	7.5×10 ⁵ ± 7.5×10 ⁵	5.1×10 ³ ± 5.1×10 ³	3.7×10 ³ ± 3.7×10 ³	1.4×10 ² ± 1.4×10 ²	1×10 ³ ±3	4.6×10 ⁵ ± 4.6×10 ⁵	7×10 ⁴ ± 7×10 ⁴	3.6×10 ³ ± 3.6×10 ³	8.2×10 ¹ ± 8.2×10 ¹	0
21 days	3.2×10 ⁶ ± 3.2×10 ⁶	3.4×10 ⁵ ± 3.4×10 ⁵	1.2×10 ⁴ ± 1.2×10 ⁴	1.3×10 ³ ± 1.3×10 ³	1.2×10 ² ± 1.2×10 ²	3.2×10 ⁵ ± 3.2×10 ⁵	3.2×10 ³ ± 3.2×10 ³	1.2×10 ³ ± 1.2×10 ³	3.2×10 ¹ ± 3.2×10 ¹	4×10 ¹	3.2×10 ⁵ ± 3.2×10 ⁵	3.2×10 ⁴ ± 3.2×10 ⁴	2.3×10 ³ ± 2.3×10 ³	3.2×10 ¹ ± 3.2×10 ¹	0
	4×10 ⁷ ± 4×10 ⁷	3.3×10 ⁷ ± 3.3×10 ⁷	2.8×10 ⁵ ± 2.8×10 ⁵	2.1×10 ⁴ ± 2.1×10 ⁴	3×10 ³ ± 3×10 ³	3.5×10 ⁷ ± 3.5×10 ⁷	4.2×10 ⁵ ± 4.2×10 ⁵	2.4×10 ⁵ ± 2.4×10 ⁵	4×10 ³ ± 4×10 ³		NC	NC	5.1×10 ⁵ ± 5.1×10 ⁵	5.6×10 ² ± 5.6×10 ²	0
	2.2×10 ⁷ ± 2.2×10 ⁷	4.2×10 ⁶ ± 4.2×10 ⁶	1.6×10 ⁵ ± 1.6×10 ⁵	3.2×10 ⁴ ± 3.2×10 ⁴	2.2×10 ³ ± 2.2×10 ³	1.3×10 ⁷ ± 1.3×10 ⁷	3.2×10 ⁵ ± 3.2×10 ⁵	3.2×10 ⁵ ± 3.2×10 ⁵	3.2×10 ³ ± 3.2×10 ³	3×10 ¹	NC	NC	4.2×10 ⁵ ± 4.2×10 ⁵	2.5×10 ² ± 2.5×10 ²	0
	1.5×10 ⁸ ± 1.5×10 ⁸	7.4×10 ⁷ ± 7.4×10 ⁷	4.6×10 ⁵ ± 4.6×10 ⁵	8.2×10 ⁴ ± 8.2×10 ⁴	1.8×10 ⁴ ± 1.8×10 ⁴	NC	7×10 ⁶ ± 7×10 ⁶	6.7×10 ⁶ ± 6.7×10 ⁶	1.5×10 ⁴ ± 1.5×10 ⁴	1×10 ²	NC	NC	NC	9.8×10 ² ± 9.8×10 ²	0
	1.2×10 ⁷ ± 1.2×10 ⁷	6.2×10 ⁶ ± 6.2×10 ⁶	4.1×10 ⁵ ± 4.1×10 ⁵	3.6×10 ⁴ ± 3.6×10 ⁴	3.2×10 ³ ± 3.2×10 ³		3.4×10 ⁶ ± 3.4×10 ⁶	3.2×10 ⁶ ± 3.2×10 ⁶	2.2×10 ⁴ ± 2.2×10 ⁴	1.2×10 ²				5.2×10 ² ± 5.2×10 ²	

a = Total count of *Aerobic bacteria*, b = *Coliform* count, c = *Staphylococcus aureus* count, ID= Irradiation Dose (kGy), T= Time (days after irradiation)
 NC = Non Countable, and SE= Standard Error.

Table 2 Microbial means for control (0.0 kGy) and irradiated (0.5, 1, 2 and 3 kGy) bovine meat during frozen storage (-18 °C).

ID (kGy)	TC ^a (Mean ± SE)					CC ^b (Mean ± SE)					SC ^c (Mean ± SE)				
	0	0.5	1	2	3	0	0.5	1	2	3	0	0.5	1	2	3
T															
0	2.8×10 ⁶ ± 1.2×10 ⁶	2.3×10 ⁵ ± 5.5×10 ⁴	2.7×10 ³ ± 1.4×10 ³	1.2×10 ² ± 1.1×10 ²	3.7×10 ¹ ± 1.2×10 ¹	3.6×10 ⁴ ± 2.2×10 ⁴	2.4×10 ² ± 1.1×10 ²	1.8×10 ² ± 1.4×10 ²	9.7×10 ¹ ± 3.2×10 ¹	2±1	9.7×10 ³ ± 3.2×10 ⁶	1×10 ³ ± 3.2×10 ²	1.5×10 ² ± 3.2×10 ¹	2.7×10 ¹ ± 1.2×10 ¹	0
2	1.7×10 ⁶ ± 1.2×10 ⁶	1.8×10 ⁵ ± 1.2×10 ⁵	7.6×10 ² ± 4.4×10 ²	9.5×10 ¹ ± 5.2×10 ¹	3.2×10 ¹ ± 1.2×10 ¹	2.4×10 ⁴ ± 3.2×10 ⁴	1.7×10 ² ± 1.2×10 ²	8.1×10 ¹ ± 6.2×10 ¹	2.1×10 ¹ ± 1.3×10 ¹	0	6.8×10 ³ ± 3.5×10 ³	8.5×10 ² ± 6.2×10 ²	7.5×10 ¹ ± 5.1×10 ¹	1.5×10 ¹ ± 1.1×10 ¹	0
4	8.4×10 ⁵ ± 7.2×10 ⁴	1.3×10 ⁵ ± 1.2×10 ⁴	4.2×10 ² ± 3.3×10 ²	8.6×10 ¹ ± 3.2×10 ¹	2.4×10 ¹ ± 1.3×10 ¹	2.1×10 ⁴ ± 1.6×10 ⁴	1.2×10 ¹ ± 1.2×10 ¹	0 ± 1.0 ¹	0 ± 1.0 ¹	0	1.5×10 ³ ± 1.2×10 ³	3.3×10 ² ± 1.4×10 ²	5.2×10 ¹ ± 3.2×10 ¹	5.2×10 ¹ ± 3.2×10 ¹	0
6	5.6×10 ⁵ ± 4.9×10 ⁵	8.7×10 ⁴ ± 2.2×10 ⁴	2.1×10 ² ± 1.5×10 ²	8.7×10 ¹ ± 3.1×10 ¹	2.3×10 ¹ ± 1.2×10 ¹	2.4×10 ³ ± 2.2×10 ²	0 ± 2.2×10 ²	0 ± 2.2×10 ²	0 ± 2.2×10 ²	0	7.2×10 ² ± 5.2×10 ²	1.1×10 ² ± 6.8×10 ¹	1.5×10 ¹ ± 1.1×10 ¹	0 ± 1.1×10 ¹	0
8	1.3×10 ⁵ ± 1.2×10 ⁴	6.5×10 ⁴ ± 4.4×10 ³	9.2×10 ¹ ± 5.2×10 ¹	7.2×10 ¹ ± 4.1×10 ¹	2.2×10 ¹ ± 1.2×10 ¹	1.2×10 ² ± 1.1×10 ²	0 ± 1.1×10 ²	0 ± 1.1×10 ²	0 ± 1.1×10 ²	0	2.4×10 ² ± 1.2×10 ²	9.5×10 ¹ ± 5.6×10 ¹	0 ± 5.6×10 ¹	0 ± 5.6×10 ¹	0

a= Total count of *Aerobic bacteria*, b = *Coliform* count, c = *Staphylococcus aureus* count, ID= Irradiation dose,
 T= Time (months after irradiation), and SE= Standard Error.

Table 3 Chemical attributes means for control (0.0kGy) and irradiated 90.05, 1, 2 and 3kGy) bovine meat during storage.

Chemical attributes		TVNa (Mean ± SE) mg/ 100 g					PVb (Mean ± SE) Miliequivalent/ kg of sample fat				
ID (kGy)		0	0.5	1	2	3	0	0.5	1	2	3
T	Te										
0	4-7 °C	20±0.66	20±0.62	19.5±0.64	19.5±0.65	19.6±0.67	3.5±0.28	3.6±0.30	3.8±0.28	4.2±0.22	4.6±0.28
7 d	4-7 °C	21.5±0.35	21±0.34	19.6±0.32	19±0.36	19.5±0.29	3.8±0.27	3.9±0.35	3.8±0.29	4.4±0.24	4.5±0.25
14 d	4-7 °C	21.9±0.56	21.5±0.57	19.8±0.45	19.5±0.54	19.4±0.57	4±0.23	4±0.34	4.2±0.22	4.5±0.25	4.8±0.28
21 d	4-7 °C	29.5±0.30	24±0.32	20±0.25	19.3±0.31	19.5±0.28	4.2±0.22	4.4±0.30	4.4±0.31	4.9±0.29	4.9±0.29
2 months	-18 °C	19.5±0.68	19.6±0.58	19.2±0.48	19±0.51	18.6±0.47	3.6±0.24	3.7±0.29	3.9±0.33	4.6±0.26	4.8±0.28
4 months	-18 °C	19±0.40	19.2±0.45	18.5±0.47	18±0.53	17.5±0.52	3.9±0.30	4±0.33	4.3±0.23	4.8±0.28	5±0.25
6 months	-18 °C	18.5±0.55	18.53	17.8±0.45	17.5±0.43	17±0.50	4.0±0.27	4.4±0.34	4.3±0.24	5±0.32	5.5±0.30
8 months	-18 °C	17±0.68	17.5±0.50	17±0.46	16.5±0.46	16±0.42	4.2±0.31	4.5±0.35	4.5±0.25	5.1±0.31	5.8±0.28

a= Total Volatile Nitrogen, b= Peroxide Value, ID= Irradiation Dose, T= Time, and Te = Temperature (°C).

of irradiation and control groups. In the irradiated groups no significant difference ($P>0.05$) was found for TVN and PV as irradiation dose level increased (Table 3). Increased applied dose decreased the rate of TVN formation during frozen storage by reducing the initial levels of the common spoilage bacteria. We can use TVN as a practical and fast method for chemical analysis in irradiated meat.

Discussion

The national advisory committee on microbiological criteria for food (1993) in the USA cited two basic approaches to decontamination of meat. The first approach usually consists of spraying carcass during slaughtering and / or chilling. These procedures can reduce but will not kill all the intrinsic pathogens. The second approach consists of irradiation packed meat¹³. The 3 kGy dose reduced the counts of *St aureus*, *L monocytogenes*, *E faecalis* by more than 3, 1.4 and 4 log units in rabbit meat. On the other hand, irradiation at 1.5 and 3 kGy significantly reduced the count of aerobic *Mesophilic bacteria*, *Psychophylics bacteria* and yeasts and prolonged the refrigerated shelf-life of samples to 12 and 21 days, respectively, compared to 6 days for non-irradiated¹⁴. Also in this study irradiation at 3 kGy reduced the count of *Aerobic bacteria*, *Coliforms*, *St aureus* and eliminated *Salmonella*, also increased the refrigerated shelf-life of meat samples to 14 days, compared to 3 days for control. Thayer et al.⁴ showed that peroxide and iodine value at low irradiation dose (<10 kGy). There was no significant change in any of the meat lipids. Quattara et al. showed that gamma irradiation increased lipid oxidation in ground beef samples¹⁵. It is consistent with the report of Lambert, Smith and Doods who found a rapid fat oxidation in beef samples irradiated at 0.25-1 kGy under O₂ permeable condition¹⁶. In this study,

also PV was greater in irradiated samples, but there was no significant difference between irradiated and control group (Table 3). Badr showed rabbit meat samples that irradiated with gamma ray at doses 0, 1.5 and 3 kGy had no significant effects on their total volatile nitrogen (TVN) contents, while storage in refrigerator significantly increased TVN for irradiated and non-irradiated samples¹⁴. Our study showed the same results for the amount of TVN in non-irradiated and irradiated (0.5, 1, 2 and 3 kGy) samples.

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