

Low-dose Gamma Irradiation and Refrigerated Storage *in vacuo* Affect Microbial Flora of Fresh Pork

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ABSTRACT

Vacuum-packaged ground fresh pork samples absorbed gamma radiation doses of 0, 0.57, 1.91, 3.76, 5.52, or 7.25 kGy at 2°C. Samples were analyzed after 1, 7, 14, 21, 28, or 35 days storage at 2°C for presence and number of aerobic and anaerobic mesophiles and endospore formers, and aerobic psychrotrophs. Conventional plate counts did not detect surviving microflora in any sample that received an absorbed dose of 1.91 kGy or higher, even after refrigerated storage for up to 35 days. The microflora in the control were predominantly Gram-positive for the first 21 days; however, *Serratia* predominated at 28 and 35 days. *Staphylococcus*, *Micrococcus*, and yeast species predominated in samples that received 0.57 kGy.

Key Words: pork, vacuum storage, low dose gamma irradiation

INTRODUCTION

THE U.S. Food & Drug Administration (FDA) approved the treatment of pork meat and products with a minimum dose of 0.3 kGy and a maximum of 1.0 kGy of ionizing radiation to control *Trichinella spiralis* (Anon., 1985). The USDA Food Safety and Inspection Service likewise approved the processing regulation (Anon., 1986). Although doses of ionizing radiation below 1 kGy are adequate to control helminths, they are not generally considered adequate to control many food-borne pathogens or to provide notable extension of shelf life. Government and industry have indicated interest in ionizing radiation doses in excess of 1.0 kGy but less than 10 kGy to increase shelf life and improve safety of products for consumers. Two important concerns remained about the effects of a 1 to 10 kGy treatment of meats: the effect on nutrients and the impact of such processing on microflora especially when the product was vacuum packaged.

Mattison et al. (1986) studied the effects of a 1 kGy gamma radiation dose at $\approx 0^\circ\text{C}$ on microflora, sensory characteristics, and fat stability of vacuum-packaged pork loin. Microbial evaluations were performed at 2, 7, 14, and 21 days on samples stored at 4°C. Radiation treatment reduced the numbers of mesophiles, psychrotrophs, anaerobic bacteria, and staphylococci. Differences between irradiated and nonirradiated populations tended to increase during refrigerated storage. They reported clostridial counts were significantly lower for irradiated pork than for nonirradiated pork.

Ehioba et al., (1987, 1988) characterized bacterial cultures from vacuum-packaged irradiated and nonirradiated ground pork stored up to 12 days at 5°C. Treating the meat with radiation decreased the numbers of mesophiles, psychrotrophs, and anaerobes and extended the shelf life by 2–3 days (from 8 to 11.5 days). Initial microflora gradually shifted from Gram-negative to Gram-positive in nonirradiated samples. In contrast, the majority of microflora in irradiated (1 kGy) samples were Gram-positive shortly after irradiation and increased to 97% after 9 days at 5°C. On the day of preparation *Pseudo-*

monas and *Enterobacter* species predominated in nonirradiated meat. After 12 days refrigerated storage *Lactobacillus* and coryneform bacteria predominated in the irradiated meat.

Lebepe et al., (1990) studied the effects of a 3 kGy radiation dose on microflora and other characteristics of fresh, vacuum-packaged pork loins stored at 2°C up to 98 days. Treating fresh pork with 3 kGy reduced populations of aerobic, psychrotrophic, and mesophilic bacteria. The anaerobic and facultative anaerobic mesophiles were below detection level. Surviving populations increased during the next 40 days of refrigerated storage at 2–4°C. Gram-positive isolates predominated in the irradiated samples. *Hafnia* and *Serratia* were the most prevalent genera among the *Enterobacteriaceae* in nonirradiated samples. *Staphylococcus aureus* was detected consistently in nonirradiated samples only. The microbial shelf life of vacuum-packaged pork was extended from 41 days to 90 days by a 3 kGy radiation dose.

Jenkins et al. (1989) investigated the effects of radiation dose, cooking, and post-irradiation storage on thiamin content of ground pork loin and microbial flora of raw meat. Results of treatments on thiamin content were reported (Jenkins et al., 1989). The results of microbiological analyses of the irradiated samples are presented here. The objectives of these studies were to determine the effects of gamma radiation dose (0, 0.57, 1.91, 3.76, 5.52, and 7.25 kGy at $2 \pm 2^\circ\text{C}$) and storage time of treated samples *in vacuo* at 2°C on the microbial populations and to determine if the treatments caused shifts in composition of those populations. The 0.57 kGy dose was selected to represent pork irradiated to control *Trichinella spiralis* since the minimum dose would be 0.30 kGy and the maximum dose 1.0 kGy. The higher doses would be more appropriate to control food-borne pathogens and to extend shelf life of irradiated pork.

MATERIALS AND METHODS

Experimental design

The experimental design included 6 gamma radiation doses and 6 storage periods of vacuum-packaged ground fresh pork for a total of 36 treatments. Each treatment was performed in triplicate for a total of 108 independent samples.

Sample preparation and packaging

Samples were prepared and packaged as described previously (Jenkins et al., 1989). Briefly, samples (15 g each) of lean ground pork from two matched pairs of pork loins, obtained 2 days post-mortem, were placed in 13 cm \times 18 cm plastic barrier pouches (All-Vak #13; International Kenfield Dist. Co., Rosemont, IL). The pouches had a food contact layer of polyethylene and an oxygen permeability of 15.5 $\text{cm}^3/\text{m}^2/24 \text{ hr}$ at 25°C. The pouches were vacuum sealed to a dial reading of -690 mm Hg . All samples were refrigerated at $2 \pm 2^\circ\text{C}$.

Irradiation and storage

Samples were irradiated at 0.129 kGy/min using a self-contained cesium-137 gamma source (Shieh et al., 1985). The temperature was maintained at $2 \pm 2^\circ\text{C}$ during irradiation. Absorbed dose measurements were made using ferrous-cupric sulfate dosimetry (Jarrett and Halliday, 1979). Variation between maximum and minimum calculated doses was $\approx 4.6\%$. The measured treatment doses were 0, 0.57,

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IRRADIATION AND REFRIGERATION OF FRESH PORK . . .

Table 1—Storage at 2°C and/or gamma radiation effects on the microbial flora of ground pork

Colony type	Dose kGy	Log number of colony forming units ± SD ^a					
		0	7	14	21	28	35 days
Aerobic	0	2.76 ± 0.06	2.35 ± 0.08	2.81 ± 0.83	2.30 ± 0.44	4.11 ± 0.04	6.50 ± 0.35
Mesophile	0.57	2.50 ± 0.15	2.30 ± 0.19	1.95 ± 0.62	2.97 ± 0.80	5.15 ± 0.41	3.88 ± 0.50 ^d
Anaerobic	0	0.91 ± 0.55	2.09 ± 0.15	2.79 ± 0.83	2.85 ± 0.75	4.07 ± 0.28	5.38 ± 1.12
Mesophile ^b	0.57	2.22 ± 0 ^d	1.76 ± 0.49	1.73 ± 0.77	3.36 ± 0.18	5.00 ± 0.28	2.61 ± 2.24
Growth at 10°C	0	2.05 ± 0.24	2.27 ± 0.05	3.04 ± 0.52	3.56 ± 0.38	3.84 ± 0.07	6.79 ± 0.10
	0.57	ND ^c	ND ^c	3.26 ± 0.08	4.28 ± 0.84	5.32 ± 0.35 ^d	7.44 ± 0.10 ^d

^a SD represents standard deviation of means.

^b Anaerobic mesophile include facultative organisms.

^c ND represents not detected (< 10 CFU/g).

^d Indicates that the value for the irradiated sample differs significantly (P < 0.05) from that for the nonirradiated sample in the same data set.

Table 2—Distribution of microbial types on nonirradiated and irradiated (0.57 kGy) vacuum-packaged pork stored at 2°C for 1 to 35 days

Storage (Days)	kGy	Percentage distribution of microflora ^a								
		Bacillus	Enterobacter	Micrococcus	Pseudomonas	Serratia	Staphylococcus	Streptococcus	Yeast	Unknown
1	0	8					64	14		14
1	0.57			18			82		17	7
7	0			7			69		4	4
7	0.57			14			78		11	15
14	0			15			59		17	
14	0.57			4			79			4
21	0		11			29	57		61	
21	0.57			9			30		30	33
28	0			3			7		86	3
28	0.57				8	83				8
35	0						94			6
35	0.57									

^a Pooled analysis of isolates from three replicate samples per treatment.

1.91, 3.76, 5.52, and 7.25 kGy. The samples were stored in the dark at 2 ± 2°C for the required storage periods of 1 day, 1, 2, 3, 4, and 5 wk post irradiation.

Microbiological analysis

At time of withdrawal from refrigerated storage, sub-samples of 5.0 g of the ground pork were removed aseptically from each pouch for microbiological analysis. The remaining portion was retained for vitamin and sensory analyses, as reported (Jenkins et al., 1989). Each sample was vacuum sealed within an All-Vac #13 barrier pouch, frozen rapidly at -50°C and stored frozen until analysis. Preliminary experiments with commercial ground pork revealed less than one log reduction in microbial population of small amounts of meat frozen rapidly in this manner. (The means of five determinations of logarithms of the number of colony forming units (cfu) of aerobic mesophilic bacteria of a commercial ground pork loin before and after freezing were 5.91 ± 0.06 and 5.93 ± 0.02, respectively.) Each sample pouch was opened aseptically and the sample macerated for 90 sec with a Stomacher 400 (Tekmar Co., Cincinnati, OH) in enough 0.1% peptone water (Difco) for a dilution of 1/10. Appropriate serial dilutions were prepared in 0.1% peptone water. Aerobic mesophilic cfu (35°C/96 hr), anaerobic (or facultative) mesophilic CFU (35°C/96 hr) and aerobic microorganisms capable of growth at 10°C (120 hr) were counted after growth in Tryptic soy agar (TSA, Difco, Detroit, MI) pour plates. Aerobic or anaerobic (or facultative) endospore forming cfu were enumerated using TSA spread plates prepared with heat-shocked 1 to 10 dilutions of stomachates (97°C/15 sec); these were incubated for 96 hr at 35°C. Anaerobic conditions were established where necessary with a BBL Microbiology Systems Gas Pack Plus (Cockeysville, MD). The CFU on three petri plates giving 30 to 300 colonies were counted using a New Brunswick Scientific Biotran II automated colony counter (New Brunswick Scientific Co., Inc., Edison, NJ 08818) and averaged.

Aerobic mesophilic colonies (≈35) were selected from the same areas on replicate TSA plates from each treatment. All were recloned on TSA at 35°C and stored on TSA slants at 5°C. A gram stain was made when the slant was streaked, and results were used in selection of appropriate identification methods. About 200 isolates were analyzed taxonomically using the Vitek AMS Automicrobic System (bioMérieux Vitek, Inc., USA, Hazelwood, MO), (Knight et al., 1990); API 20E System (API Analtab Products, Plainview, NY), (Lennette et al., 1985); API Staph Trac System (API Analtab Products, Plainview, NY), (Gemmell and Dawson, 1982); and API Rapid Strep System (API Analtab Products, Plainview, NY), (Tillotson, 1982). Yeasts

were identified from morphology. No attempt was made to identify yeast to genus. Many colonies were pink. Plate-count data were transformed into logarithms, and all data were analyzed by using the Statistical Analysis System (SAS Institute, Inc., 1985).

RESULTS

No CFU were observed in plate counts of any sample that received a radiation dose of ≥ 1.91 kGy. Populations of aerobic mesophilic bacteria in control nonirradiated samples were not significantly (p > 0.05) different from those in samples that received a radiation dose of 0.57 kGy, and population increases were not apparent during refrigerated storage until after 21 days (Table 1). Populations of anaerobic or facultative mesophilic bacteria in control samples did not differ (p > 0.05) from those in samples that received a radiation dose of 0.57 kGy (Table 1). Populations of the anaerobic or facultative mesophilic bacteria of the samples increased during the first 28 days refrigerated storage (Table 1). Aerobic microorganisms capable of growth at 10°C were not detected in irradiated samples at 1 and 7 days refrigerated storage (Table 1). Populations of aerobic microorganisms that multiplied at 10°C increased in both irradiated and nonirradiated samples from 14 days through 35 days refrigerated storage. The population of the microorganisms that multiplied at 10°C was higher (p < 0.05) at 28 days and 35 days of refrigerated storage in irradiated than in nonirradiated samples. In nonirradiated samples, 25 aerobic and 10 anaerobic or facultative cfu of endospore former gram were detected after 1 day refrigerated storage. None was detected in samples stored 7, 14, 21, and 28 days. After 35 days refrigerated storage, aerobic and anaerobic or facultative endospore forming bacterial counts of nonirradiated meat samples were 1 CFU/g each. In the irradiated (0.57 kGy) meat samples aerobic endospore formers (3/g) were detected only in samples stored 7 days.

Bacillus cereus, *B. coagulans*, and *B. macerans* were identified from colonies picked from the nonirradiated samples at 1 day (Table 2). No *Bacillus* isolates were found in any other sample. *Enterobacter agglomerans* was identified among selected isolates only in the nonirradiated samples after storage 21 days (Table 2). All eleven isolates had identical taxonomic

characteristics. *Micrococcus* isolates from several samples were identified to the genus level (Table 2). No clear pattern to the isolations was identified. *M. roseus* and *M. varians* were identified. The remainder of the isolates were not identified to species. *Pseudomonas fluorescens* and *P. stutzeri* were identified among the isolates (Table 2) from the nonirradiated samples that had been stored for 35 days. *Serratia liquefaciens* became an important component of the total population in nonirradiated samples stored 21 and 28 days and the predominant isolate from samples stored 35 days (Table 2). *Serratia* was not identified among the isolates from irradiated samples.

Staphylococcus species were frequently identified among the isolates from both irradiated and nonirradiated pork meat during the first 21 days of refrigerated storage (Table 2). Twelve *Staphylococcus* species were identified. The ratios of *S. aureus* to total *Staphylococcus* isolates from nonirradiated refrigerated samples were as follows: 0/23, 4/20, 1/16, 0/28, 0/3, and 0/0 after 1, 7, 14, 21, 28, and 35 days refrigerated storage, respectively. The ratios of *S. aureus* to total *Staphylococcus* isolates from the irradiated refrigerated samples were as follows: 3/12, 1/21, 1/13, 0/7, 0/2, and 0/17 after 1, 7, 14, 21, 28, and 35 days refrigerated storage, respectively. The following species were identified: *S. aureus*, *S. auricularis*, *S. epidermidis*, *S. capitis*, *S. haemolyticus*, *S. hominis*, *S. warneri*, *S. sciuri*, *S. lentus*, *S. saprophyticus*, and *S. xylosus*. Several isolates were not identified to species. Four *Streptococcus agalactiae* isolates and one *Enterococcus faecalis* isolate were obtained from the nonirradiated pork at 1 day (Table 2).

DISCUSSION

OUR RESULTS were unexpected in that no surviving microflora were detected in samples exposed to gamma radiation doses >0.57 kGy even after storage at 2°C up to 35 days. Perhaps by chance, the composition and size of the population of microflora of these pork samples were such that none could survive a dose of 1.91 kGy or higher. The initial contamination level of this meat was very low (<10³/g) compared with a commercial ground pork loin. The number of viable vegetative cells would be substantially reduced by a dose of 1.91 kGy. The very small number of bacterial endospores detected in the nonirradiated samples, though substantially more resistant than vegetative cells (D-value for *Clostridium botulinum* in meat at 25°C is ≈ 3.6 kGy), would also be reduced in number. Thayer et al., (1992) found no difference in the response of *Salmonella typhimurium* to gamma radiation when present at 10 or 100 CFU/chicken wing and only small differences when present at 1,000 or 10,000 cfu/wing. Such results might be expected for meat obtained 2 days postmortem from a local abattoir and then carefully prepared in a research facility with due precautions to prevent contamination. Two factors nevertheless make this study relevant when evaluating effects of gamma radiation treatments for the control of trichina. First, the applied radiation dose of 0.57 kGy more closely approximated that which would be used by industry than would studies with an average dose of 1.0 kGy. Second, both the irradiation temperature and absorbed dose were controlled within very narrow limits in our study. Regulations state that maximum dose must not exceed 1.0 kGy, and minimum dose must exceed 0.3 kGy. Since the processor would be working with commercial-sized samples, some parts of the product would approach 1.0 kGy; and some may receive only slightly more than 0.3 kGy. The variation between the minimum and maximum calculated doses in our study was 4.6%. Rather small variations in irradiation temperature (i.e., 10°C) may have significant effects on survival of some food-borne pathogens, such as salmonellae (Thayer and Boyd, 1991).

The predominant genera of the microflora in both the nonirradiated and irradiated (0.57 kGy) meat were Gram-positive at the beginning of the study, as were samples analyzed at 7 and 14 days. The nonirradiated samples opened on the 21st

day of refrigerated storage had significant populations of *Serratia*, as did those opened on the 28th day. Gram-positive genera, however, still predominated. *Serratia* represented 83% of all aerobic mesophilic isolates from nonirradiated samples stored *in vacuo* at 2°C for 35 days. Ehioba et al. (1988) reported mainly Gram-positive organisms in irradiated vacuum-packaged ground pork immediately following a dose of 1 kGy and during 12 days of refrigerated storage, as did we. However, they did not store samples beyond 14 days. Our results differed from those of Ehioba et al. (1988) in that *Staphylococcus* represented a major portion of the microflora in the irradiated (0.57 kGy) samples at each sampling period, whereas they found a predominance of *Lactobacillus* spp. and coryneform bacteria. These results could be simply the results of chance. However, Thayer and Boyd (1992) reported that 90% of *S. aureus* CFU in mechanically deboned chicken meat were killed by a dose of 0.36 kGy. Since the total population of aerobic mesophiles did not exceed 10³ CFU/g at the beginning of our study, possibly the small population of *Staphylococcus* could have been eliminated by a radiation dose of 1.9 kGy and not by 0.57 kGy. The absence of competitive species may have allowed its growth. *S. aureus* was isolated only during the first 2 wk refrigerated storage of the pork. Its presence could be a food safety concern. However, neither *S. aureus* nor any other microorganism was found in the pork that had received a gamma radiation dose of 1.91 kGy or higher.

We did not observe a marked reduction in total population of aerobic mesophiles with a dose of 0.57 kGy, but did in the populations of anaerobic or facultative mesophiles and bacteria capable of growth at 10°C. With 10⁷ CFU/g of aerobic mesophiles as an indicator of bacterial spoilage, then none of the samples including the nonirradiated control spoiled during the 35 days storage at 2°C. Conventional plate counts did not detect surviving cfu in the ground pork following treatments of 1.91 kGy and higher. Consequently, those samples would be expected to have greater shelf life. Because neither enrichment procedures nor abuse tests were performed, we cannot state that there were no microbial survivors at doses of 1.91 kGy or higher. It is also possible that addition of pyruvate or other resuscitative agents to the assay media might have altered results. However, preliminary experiments did not indicate any advantage from addition of such agents. Niemand et al. (1981) reported that a dose of 2 kGy almost doubled the shelf life of fresh meat. Our results do not contradict their conclusion but afford no clear support since the nonirradiated control did not reach a level (10⁷ cfu/g) of aerobic mesophilic microflora indicative of spoilage within the 35 day period.

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