

Effects of Ionizing Radiation and Anaerobic Refrigerated Storage on Indigenous Microflora, *Salmonella*, and *Clostridium botulinum* Types A and B in Vacuum-Canned, Mechanically Deboned Chicken Meat†

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ABSTRACT

Vacuum-canned, commercial, mechanically deboned chicken meat was challenged with either *Clostridium botulinum* spores (20 strains of types A and B, proteolytic; final spore concentration of ca. 400/g of meat) or *Salmonella enteritidis* (ca. 10⁸ CFU/g of meat) followed by irradiation to 0, 1.5, and 3.0 kGy and storage at 5°C for 0, 2, and 4 weeks. None of the samples stored at 5°C developed botulin toxin; however, when these samples were temperature abused at 28°C they became toxic within 18 h and had obvious signs of spoilage, i.e., swelling of the can and a putrid odor. During 4 weeks of refrigerated storage the log₁₀ of the population of *S. enteritidis* in nonirradiated samples decreased from 3.86 to 2.58. *S. enteritidis* CFU were detectable in samples irradiated to 1.5 kGy at 0 weeks but not in samples irradiated to 3.0 kGy. Log levels of aerobic and facultative mesophiles increased during 4 weeks of refrigerated storage from 6.54 to 8.25, 4.03 to 8.14, and 2.84 to 5.23 in samples irradiated to 0, 1.5, and 3.0 kGy, respectively. Based on taxonomic analyses of 245 isolates, the bacterial populations depended upon radiation dose and storage time. The change was predominantly from gram-negative rods in nonirradiated samples to gram-positive streptococci in samples irradiated to 3.0 kGy and stored for 4 weeks. Spoilage organisms survived even the 3.0 kGy treatment.

Key words: *Salmonella*, clostridium, irradiation, botulism, meat

Regulatory agencies are concerned that if vacuum-packaged meat were to receive ionizing radiation doses less than those required to inactivate *Clostridium botulinum* spores, the meat could become toxic yet lack those signs of spoilage that are obvious to the consumer. This is because the ionizing radiation dose required to inactivate 90% of *C. botulinum* spores (i.e., the D₁₀ value) is much greater (1.29 to 3.34 kGy) (13) than that needed to eliminate ordinary spoilage organisms (4, 5, 6). For example, the D₁₀ value for *Escherichia coli* was reported to

be 0.26 kGy by Patterson (17), while Firstenberg-Eden et al. (5) found that *C. botulinum* type E spores survived on irradiated (3 kGy) chicken skin and produced toxin at 30°C under either aerobic or anaerobic conditions; however, no toxin was found on skins incubated at 10°C. They found that toxin was not detected prior to characteristic spoilage by the natural flora surviving 3 kGy. They also found that at 10°C the natural flora on chicken skins survived a dose of 3 kGy and produced spoilage odors within 8 days. The surviving spores of *C. botulinum* type E did not produce toxin within 14 days (6). Dezfulian and Bartlett (4) found that *C. botulinum* types A and B spores failed to germinate and grow after a gamma radiation dose of 3 kGy and did not produce toxin on chicken skins during aerobic or anaerobic incubation at 10°C. The spoilage odor of meat, poultry, and fish stored at refrigeration temperatures is due mainly to the actions of microorganisms and not to tissue autolysis (7); therefore, it is important to determine the fate of the normal flora under anaerobic conditions following gamma irradiation. Barnes and Impey (3) reported that before refrigerated storage at 4°C *Acinetobacter* and *Flavobacterium* spp. predominated on all chicken carcasses. After storage, *Flavobacterium* spp. predominated on eviscerated chicken carcasses wrapped in polyethylene film. Welch and Maxcy (20) reported that predominantly gram-negative coccobacilli, e.g., *Moraxella*, *Acinetobacter*, and *Achromobacter*, survived nonsterilizing doses of radiation on ground beef. Hughes and Patterson (11) reported that yeast, *Moraxella*, and *Acinetobacter* spp. dominated the microflora of chicken skin following a radiation dose of 2.5 kGy. When these irradiated chicken skins were stored at 4°C for 17 days, the population of gram-positive, nonsporeforming rods increased. Gill et al. (8) observed that vacuum-packaged chicken carcasses stored at 3°C for 2 weeks spoiled with persistent putrid odors. Two of three carcasses that were examined after 1 week of storage had mild odors of H₂S. The total population of aerobic or facultative mesophiles exceeded 10⁸ CFU per carcass after 1 week of storage.

† Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Enterobacter dominated the spoilage flora; lactobacilli replaced the *Enterobacter* as the predominant flora on carcasses stored for 3 weeks (8).

Three parallel studies were conducted with the same lot of vacuum-canned, mechanically deboned, gamma irradiated (0, 1.5, and 3.0 kGy) chicken meat (MDCM) that was stored at 5°C for 0, 2, 4, and 8 weeks before analysis. Odor and appearance were determined with uninoculated MDCM. Survival of the indigenous microflora and of inoculated *Salmonella enteritidis* was determined with one set of samples. The final set of samples was inoculated with proteolytic *C. botulinum* types A and B spores and the germination, growth, and toxin production were determined with and without temperature abuse. Because low doses (>3 kGy) of gamma radiation would have relatively little effect on the highly radiation-resistant *C. botulinum* spores, they were expected to survive irradiation and storage, but not to multiply or to produce toxin at 5°C. It was assumed that all samples of vacuum-canned MDCM inoculated with *C. botulinum* spores would become toxic when temperature abused. The principal concerns were whether signs of spoilage would be present before toxin production occurred and whether there would be an increased potential for toxin production in irradiated MDCM.

MATERIALS AND METHODS

Mechanically deboned chicken

Nonfrozen MDCM was obtained from a local manufacturer of chicken frankfurters on the day it was received at the manufacturing plant. The proximate analysis (means of two commercial analyses) of the MDCM was 12.6% protein, 22.0% fat, 66.7% water, and 1% ash by weight.

Experimental design

Forty-five 60-g samples of uninoculated MDCM, forty-five 60 g samples of MDCM inoculated with *S. enteritidis*, and ninety-six 60-g samples of MDCM inoculated with a spore suspension of 10 proteolytic strains of types A and B *C. botulinum* were vacuum packed in cans, gamma irradiated to absorbed doses of 0, 1.5, and 3.0 kGy, and stored at 5°C for 0, 2, 4, and 8 weeks.

MDCM inoculated with *S. enteritidis* from two cans from each treatment after 0, 2, and 4 weeks of refrigerated storage was analyzed to determine the presence of aerobic and facultative mesophiles, staphylococci, *S. enteritidis*, coliforms, *Pseudomonas*, and aerobic organisms capable of multiplication at 15°C and the populations were estimated, as described below. Representative colonies (total of 245) were selected from each treatment and identified to species where possible. MDCM inoculated with *S. enteritidis* from five cans selected from each treatment was analyzed for the presence of *S. enteritidis* following enrichment.

At day zero the MDCM inoculated with *C. botulinum* from 10 cans from each treatment was analyzed to determine the most probable number (MPN) of spores and to confirm the absence of toxin. The spores were confirmed as toxigenic by analysis of the culture medium used for the MPN determination following incubation. After 2 weeks of refrigerated storage the MPN of the spores in the MDCM from two cans from each treatment was determined. After 8 weeks of refrigerated storage the MDCM from 10 cans from each treatment was tested for toxin with and without temperature abuse at 28°C.

The uninoculated MDCM from five cans from each treatment was examined by two investigators for color and odor following storage for 0, 2, and 4 weeks.

Cultures

S. enteritidis ATCC 9186 was obtained from the American Type Culture Collection, Rockville, MD, and maintained on tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI). *S. enteritidis* was cultivated aerobically, agitated at 150 rpm for 18 h at 35°C in tryptic soy broth (TSB) (Difco), and used to inoculate MDCM to a final level of approximately 10⁴ colony-forming units (CFU) per gram of meat. Spore suspensions of 10 proteolytic strains of *C. botulinum* type A (2 OPLALC, 3, 33, B1218, 25763, 62, 69, 78, 426, and 429) and 10 of type B (4, 169, 383, 642, 999, 8688R, 770B, 53, 17409-1A, and 7949) were used. The origin and preparation of the spore suspensions were described previously (12). MDCM was mixed, aseptically, by hand with the spore suspension for an inoculum level of ca. 400 spores per gram. To ensure thorough mixing, the spore suspension was added to the MDCM and mixed into the meat. The inoculated meat was subdivided, and each portion was remixed separately. These portions were then recombined and remixed.

Canning

Samples of 60 g of MDCM were placed in 208 by 107 aluminum tab cans and sealed with a nitrogen flush in a Rooney vacuum canner to 35 to 40 torr.

Radiation source and irradiation technique

A self-contained ¹³⁷Cs gamma-radiation source with 134,000 Ci (4.95 PBq) and a dose rate of 0.114 kGy min⁻¹ was used for the study. The dose rate was established using National Physical Laboratory (Middlesex, United Kingdom) dosimeters. Variations in absorbed dose were minimized by placing samples within a uniform portion of the field. Samples were maintained at 5 ± 0.5°C by injecting the gas phase from liquid nitrogen into the irradiation chamber. Sample temperature was monitored continuously during irradiation.

Microbiological analysis

For microbiological analysis 5.0-g aliquots of MDCM were removed aseptically from each can and macerated for 90 s with a Stomacher 400 (Tekmar Co., Cincinnati, OH) in enough buffered 0.1% peptone water (Difco) to represent a dilution of 1/10. Appropriate serial dilutions were prepared in 0.1% peptone water. Colony-forming units of aerobic mesophiles incubated at 35°C for 48 h and CFU of aerobic bacteria capable of colony formation at 15°C in 120 h were counted after growth in TSA pour plates. An incubation temperature of 15°C was selected on the basis of our past experience in analyzing microbial flora of meats: we frequently found much higher counts using an incubation temperature below 35°C. This temperature was also expected to favor the growth of psychrophiles with optimum growth at 10 to 15°C and of psychrotrophs. Microbial populations were estimated as follows: *Pseudomonas* spp. CFU in 72 h at 35°C on Pseudosel agar (Baltimore Biological Laboratories [BBL], Baltimore, MD); *S. enteritidis* CFU on brilliant green sulfa agar (Difco) in 24 h; *Staphylococcus* CFU on Baird-Parker agar with tellurite (Difco) in 48 h; and coliforms CFU producing gas on Petrifilm coliform count plates (3M, St. Paul, MN) (16). Colony counts were made using a New Brunswick Scientific Biotran II automated colony counter (New Brunswick Scientific Co., Inc., Edison, NJ); results were the average counts of three petri dishes for each test. Tests were performed for the presence of *Salmonella* on MDCM from five cans from each treatment using the 1-2 test (BioControl Systems, Inc., Bothell, WA)

according to the manufacturer's instructions following a 24 h enrichment at 43°C in buffered peptone and tetrathionate broth with brilliant green (2).

Colonies were selected for identification from each of three TSA plates of aerobic and facultative mesophiles; the number of colonies selected from each plate was the square root of the mean of the average number of CFU. Selected colonies were cloned on TSA at 35°C and stored on TSA slants at 5°C. When the slant was inoculated and a gram stain was made to select appropriate identification methods. Isolates were tested for the presence of catalase, hemolytic activity, coagulase, and cytochrome oxidase and identified with the aid of Vitek AMS Automicrobic System GNI or GPI cards, as appropriate (bioMérieux Vitek, Inc., Hazelwood, MO) (1, 14).

Most probable number counts of *C. botulinum* spores

C. botulinum spore counts of inoculated MDCM were made by a three-tube MPN procedure starting with three 22-g samples. Each 22-g sample was added to 55 ml of fluid thioglycollate medium (FTB) (Difco) and mixed with a sterile thioglycollate depressor for a total of four dilutions of the original MDCM. The suspensions were heated at 80°C for 10 min and were serially diluted 1:9 in FTB. The diluted samples were incubated for 3 days in an anaerobic incubator at 33°C under N₂ before the presence of growth and toxin were determined.

Botulinal toxin assay

Extracts of the contents of the cans were prepared by adding 20 ml of gelatin phosphate buffer (0.2% gelatin, 0.36% KH₂PO₄, 0.15% Na₂HPO₄ · 7H₂O, pH 6.2) to 10 g of the MDCM in 50-ml centrifuge tubes. After mixing, the tubes were centrifuged at 3,000 × g for 15 min at 4°C. The supernatants (0.1 ml) were used for the toxin tests by an ELISA procedure previously demonstrated to give excellent results with these proteolytic strains (13). The mouse assay for toxin was used only for nonstored samples. Two Swiss-Webster mice (15 to 20 g) were injected i.p. with 0.5 ml of extract. Surviving mice were observed for typical respiratory symptoms of botulism; if these occurred or if the mice died, the extracts were boiled 10 min and additional mice were injected. Extracts were considered positive if the boiled preparations failed to kill the mice or elicit symptoms.

RESULTS

Effects of gamma irradiation and refrigerated storage on survival of *Salmonella*

The initial inoculum of *S. enteritidis* of 3.86 log₁₀ CFU/g of MDCM decreased during sample storage at 5°C and was further reduced by gamma irradiation (Table 1). The number of positive 1-2 tests for *Salmonella* of the five cans tested for each treatment is as follows: 0 weeks, 0 kGy, 5 of 5; 0 weeks, 1.5 kGy, 5 of 5; 0 weeks, 3.0 kGy, 0 of 5; 2 weeks, 0 kGy, 5 of 5; 2 weeks, 1.5 kGy, 5 of 5; 2 weeks, 3.0 kGy, 1 of 5; 4 weeks, 0 kGy, 5 of 5; 4 weeks, 1.5 kGy, 2 of 5; and 4 weeks, 3.0 kGy, 1 of 5. Although there were fewer than 10 CFU/g of *S. enteritidis* in samples irradiated to 3.0 kGy, a few cells survived the irradiation in isolated cans.

Effect of gamma irradiation and refrigerated storage on indigenous microflora of mechanically deboned chicken meat

Mesophiles. The population of aerobic and facultative mesophilic (growth at 35°C) bacteria in the unirradiated

MDCM samples exceeded 10⁷ CFU/g, and the MDCM had a putrid, spoiled odor after 2 weeks of refrigerated storage *in vacuo*. The population exceeded 10⁸ CFU/g at 4 weeks (Table 1). Because the MDCM was vacuum canned it is assumed that the increased-number of CFU represented multiplication of the facultative bacteria. A level of 10⁷ CFU/g of meat was arbitrarily considered to be spoiled. Populations of mesophilic bacteria in samples that received 1.5 kGy required in excess of 2 weeks refrigerated storage to reach 10⁷ CFU/g, and samples that received doses of 3.0 kGy never reached a population level of 10⁷ CFU/g (Table 1). None of the cans swelled during refrigerated storage, as expected, and when opened only the nonirradiated samples after 2 and 4 weeks had odors indicative of spoilage. Irradiated MDCM had a distinctly pink color compared to that of the nonirradiated MDCM, as was described previously by Hanson et al. (10). All irradiated meat had a pungent odor when the cans were first opened. This odor quickly dissipated so that panelists could not distinguish the irradiated meat from nonirradiated fresh meat by odor after a few minutes. Gram-negative species predominated in the nonirradiated samples and increased to the exclusion of gram-positive species during refrigerated storage. Eight of twenty isolates from nonirradiated samples stored for 4 weeks were *Serratia*. Gram-positive bacteria predominated in the isolates from irradiated samples, particularly after extended refrigerated storage. After 4 weeks of refrigerated storage the majority of the isolates from irradiated samples were streptococci: 23 of 24 isolates at 1.5 kGy and 13 of 17 isolates at 3.0 kGy.

Growth at 15°C. The numbers of CFU on TSA plates incubated at 15°C were similar to those found on plates incubated at 35°C, except that the nonirradiated MDCM initially contained a greater population of bacteria preferring 35°C for multiplication on TSA than of those capable of or preferring multiplication at 15°C (Table 1). This difference tended to disappear during refrigerated storage, which we interpret to mean that mesophilic populations predominated in both nonirradiated and irradiated samples of the MDCM.

Coliforms. The nonirradiated samples had moderate populations of coliforms, based on their ability to form gas in Petrifilm coliform count plates (Table 1). Coliforms were not detected in many of the irradiated samples. Members of the genera *Citrobacter*, *Enterobacter*, *Escherichia*, and *Serratia* were identified among the isolates from nonirradiated samples. The percentage of *Serratia* in the isolates increased during storage of the nonirradiated samples (Table 1). Only *Enterococcus faecium* was isolated from irradiated samples and then only from the nonstored samples.

Pseudomonas. Organisms capable of growth on Pseudose agar, resistant to cetrimide, and presumably *Pseudomonas* did not increase in population during refrigerated storage. Though this medium is very selective for *Pseudomonas*, both oxidase-positive and -negative gram-negative bacilli will grow on the medium. Since members of the genus *Pseudomonas* are strictly aerobic they were not expected to multiply during refrigerated storage *in*

TABLE 1. Survival and multiplication of bacteria at 5°C on mechanically deboned chicken in vacuo

Bacterial Type	Dose (kGy)	Log CFU/gram ± SD		
		0 weeks	2 weeks	4 weeks
Aerobic CFU at 35°C	0	6.54 ± 0.01	7.63 ± 0.04	8.25 ± 0.01
	1.5	4.03 ± 0.38	5.66 ± 0.07	8.14 ± 0.03
	3.0	2.84 ± 0.46	2.44 ± 0.00	5.23 ± 0.73
Aerobic CFU at 15°C	0	6.69 ± 0.22	6.10 ± 2.09	8.30 ± 0.02
	1.5	4.42 ± 0.18	5.68 ± 0.11	8.24 ± 0.10
	3.0	3.01 ± 0.26	3.01 ± 0.26	4.96 ± 0.06
Coliforms	0	3.72 ± 0.14	4.74 ± 0.06	6.59 ± 0.11
	1.5	ND ^a	0.22 ± 0.37	ND
	3.0	ND	0.22 ± 0.37	ND
<i>Pseudomonas</i>	0	3.50 ± 0.50	2.98 ± 0.09	3.39 ± 0.36
	1.5	2.21 ± 0.11	ND	ND
	3.0	1.92 ± 2.07	ND	ND
<i>Salmonella</i> ^b	0	3.88 ± 0.12	4.28 ± 0.20	2.58 ± 0.51
	1.5	1.22 ± 1.37	ND	ND
	3.0	ND	ND	ND
<i>Staphylococcus</i>	0	5.01 ± 0.46	5.28 ± 0.48	6.37 ± 0.17
	1.5	3.19 ± 0.25	3.47 ± 0.01	5.17 ± 0.37
	3.0	1.87 ± 0.49	1.22 ± 1.37	2.00 ± 2.15

^a ND: not detected (detection limit 10 CFU/g).

^b Refers to counts in samples artificially inoculated with *S. enteritidis*. Salmonellae were not detected in noninoculated samples of MDCM.

vacuo. Their population was lower in irradiated samples, and such bacteria were not detected by plate counts or among the isolates from the TSA plates after irradiation and refrigerated storage of MDCM for 2 weeks at 5°C (Table 1).

Staphylococcus. Staphylococci represented approximately 1 out of 40 of the total aerobic or facultative mesophiles in the nonirradiated samples (Table 1). The population of staphylococci increased approximately one log cycle during 4 weeks of refrigerated storage of MDCM. The population of *Staphylococcus* in the samples was decreased, but not eliminated, by radiation doses of either 1.5 or 3.0 kGy. No staphylococci were among the colonies on TSA selected for identification from the nonirradiated samples before storage; however, *Staphylococcus lentus* was isolated from nonirradiated samples after 2 weeks of refrigerated storage. *Staphylococcus hominis*, *Staphylococcus sciuri*, and *Staphylococcus hyicus* were isolated from irradiated samples.

Clostridium botulinum. Prior to storage of the MDCM, but after inoculation and irradiation (0, 1.5, and 3.0 kGy), all samples of the MDCM were found to be negative for the presence of *C. botulinum* toxin by both the ELISA and the more sensitive mouse test. Spores in nine out of ten of the nonirradiated samples formed toxin in incubated FTB media and gave a positive ELISA test. Five of ten samples that received 3 kGy were positive for spores by the FTB test and produced toxin in that medium.

After 2 weeks at 5°C, combined samples from two cans were tested for spores by the FTB test. The 22-g samples of nonirradiated meat were positive for spores (3 of 3), but none was positive when diluted (1.0, 0.1, 0.01, and 0.001); all dilutions were negative in samples irradi-

ated at 1.5 kGy while those irradiated at 3.0 kGy tested positive for spores in 3 of 3 of the 1.0 and 0.1 dilutions; 2 of 3 of the 0.01 and 0 of 3 of the 0.001 dilutions were positive. No tests for the presence of toxin in the meat were performed at 2 weeks.

After 8 weeks at 5°C, the MDCM from 10 cans without and 10 cans with 18-h temperature abuse at 28°C from each treatment (0, 1.5, and 3.0 kGy) was tested for *C. botulinum* toxin by the ELISA test. None of the cans had any evidence of swelling before temperature abuse and the MDCM was negative for toxin. This was not surprising since it is well known that these strains do not multiply at 5°C. The cans swelled rapidly (within 18 h) when temperature abused at 28°C, and the MDCM became toxic and had a putrid odor. It is assumed that swelling is due to amino acid decarboxylation. These samples were not tested for surviving spores, but obviously contained such.

DISCUSSION

The results of this study can be interpreted as indicating that the useful shelf life of irradiated, vacuum-canned MDCM would be extended by treatment with gamma radiation, based on the length of time required for the population of aerobic mesophilic bacteria to exceed 10⁷ CFU/g. If coliforms, *Pseudomonas*, and *Salmonella* were present before irradiation their populations would be significantly reduced following irradiation and probably would decline even further during refrigerated storage.

The population of gram-positive bacteria on irradiated vacuum-packed MDCM increased during refrigerated storage. These bacteria were mainly *Streptococcus* spp.,

whereas Hughes and Patterson (11) found the microflora of irradiated chicken skin to be dominated by *Moraxella* and *Acinetobacter*. Thayer and Boyd (19) found that a dose of 0.36 kGy at 0°C would inactivate 90% of stationary-phase CFU of *Staphylococcus aureus* in MDCM. Thus, the results obtained with Baird-Parker agar indicating survival of Staphylococci in MDCM that had been irradiated to doses of 3.0 kGy were not expected. Two isolates, *S. hominis* and *S. sciuri*, were identified in irradiated (1.5 kGy) MDCM and one isolate, *S. hyicus*, in irradiated (3.0 kGy) MDCM. Unfortunately the CFU on the Baird-Parker agar were not identified, so it is unknown if these three species represented most of the presumed Staphylococci surviving treatment with radiation. The ratio of *Staphylococcus* CFU on Baird-Parker agar to aerobic CFU on TSA was 1 to 1700 or approximately 0.06% of the total population in MDCM irradiated to 3.0 kGy and stored for 4 weeks at 5°C (Table 1). None of the three isolated and identified species has been linked to food poisoning.

Acinetobacter, which has been suggested as a possible indicator organism for irradiated meats, was found in only one irradiated sample, whereas *Acinetobacter* represented 16% of the isolates from nonirradiated samples after 2 weeks of storage. These results indicate that with vacuum-canned meats investigators cannot count upon the presence of increased populations of *Acinetobacter* to indicate that the meat has been treated with ionizing radiation.

Various species of *Pseudomonas* were isolated from both irradiated and nonirradiated meat in this study, but none of these was isolated from irradiated meat stored at 5°C after it had been stored *in vacuo* for 2 or more weeks. This is not interpreted as meaning that none was present, merely that it was a relatively small percentage of the population. The strictly aerobic *Pseudomonas* cells were not expected to increase in population in vacuum-canned MDCM, but would they survive storage under these conditions and would gamma radiation doses of 1.5 or 3.0 kGy eliminate them from the meat? They did survive in the unirradiated MDCM but the populations were below 10 per g of meat in irradiated MDCM, at both 1.5 and 3.0 kGy, after 2 weeks of storage.

The *Streptococcus* spp. isolated from the irradiated meat would be expected to sour the meat if present in sufficient numbers and probably would have produced a sour taste in any sample in which the aerobic or facultative mesophilic population exceeded 10^7 CFU/g (Table 1). They did not, however, produce a sufficiently objectionable odor for the product to be considered spoiled.

Shewanella putrefaciens (formerly classified as *Aleromonas putrefaciens* or *Pseudomonas putrefaciens*) is a known spoilage organism associated with spoilage of poultry and other meats (7, 9) by anaerobic production of H_2S and methyl mercaptan. Therefore we were expecting to find it; however, it was identified among isolates from only one nonirradiated sample. It apparently was present only as a minor component of the indigenous population

of this meat and may not have survived the treatment with gamma radiation.

The pink color of the irradiated, vacuum-canned MDCM was observed by Lynch et al. (15) in irradiated turkey meat stored in oxygen-impermeable film. Their sensory panel found the pink color preferable in the raw product but objectionable in the cooked meat. Other investigators had noted that irradiated chicken stored in nitrogen was pinker than that stored in air (10, 18).

As expected, the highly radiation-resistant endospores of *C. botulinum* survived radiation doses of 3.0 kGy. So long as these samples were refrigerated the cans did not swell, and demonstrable toxin was not present in the MDCM. Neither the odor nor the appearance of uninoculated MDCM after irradiation to either 1.5 or 3.0 kGy and storage at 5°C for up to 4 weeks indicated obvious spoilage. Samples irradiated to a dose of 1.5 kGy developed mesophilic populations exceeding a spoilage level of 10^7 CFU/g within 4 weeks but did not have an odor indicative of spoilage. Nonirradiated samples, though not swollen, developed off-odors within 2 weeks indicative of spoilage, as did mesophilic populations exceeding 10^7 CFU/g. It is probable, though not established by actual sensory tests, that the high populations of streptococci in the MDCM would have produced a sour taste in the meat after 4 weeks of refrigerated storage. This study demonstrated that indigenous bacterial microflora will survive and multiply in irradiated (3 kGy) vacuum-packaged MDCM stored at 5°C. The indigenous population may still not indicate by means of odor or appearance that the meat has been temperature abused; proof would have required extension of the study until spoilage of refrigerated, vacuum-canned, irradiated MDCM occurred and temperature abuse of noninoculated, vacuum-canned, irradiated (0, 1.5, and 3.0 kGy) MDCM. The indigenous population surviving radiation treatments of 1.5 or 3.0 kGy did not prevent the multiplication and production of toxin by *C. botulinum* in the vacuum-canned MDCM. However, there was no evidence that reduction of the indigenous population by gamma irradiation increased the potential for the formation of botulinum toxin, and refrigerated MDCM did not become toxic before there were obvious signs of spoilage, i.e., swelling of the can and a putrid odor. These results, therefore, support the conclusions of Dezfulian and Bartlett (4) and Firstenberg-Eden et al. (5). Our study used a very large challenge of raw MDCM with *C. botulinum* spores and thus should not be interpreted as indicating that less obvious signs of spoilage with botulinum toxin formation might occur with a much smaller challenge. If a ready-to-eat product were to be treated in the same manner as used here for raw MDCM, there could be some risk to the consumer. Raw products, however, would typically be cooked before eating, inactivating small amounts of toxin. Viable *S. enteritidis* were reduced to less than \log_{10} 1.22 CFU/g from an initial inoculum of \log_{10} 3.88 CFU/g by a gamma radiation dose of 1.5 kGy, and these were below detection limits after 2 weeks of refrigerated storage.

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