APPLIED MICROBIOLOGY, July 1968, p. 1061-1066

Vol. 16, No. 7
Printed in U.S.A.

Radiation Treatment of Foods

I. Radurization of Fresh Eviscerated Poultry[1]

EDMUND S. IDZIAK AND KALMAN INCZE[2]
Department of Microbiology, Macdonald College, McGill University, Montreal, Canada

Received for publication 2 April 1968

Radurization processing of fresh eviscerated poultry has been microbiologically studied. The recommended dose of 0.5 Mrads extended the shelf life at 5 C by approximately 14 days. This treatment also effected a 10 and 11 log reduction in the number of viable *Salmonella* species and *Staphylococcus aureus* strains, respectively. Recycling these organisms at sublethal doses of irradiation resulted in strains possessing increased irradiation resistance. Shifts in the microbial ecology after irradiation and storage resulted, in some instances, in the isolation of organisms tentatively identified as *Moraxella* sp. and *Herellea vaginicola*.

The potential and practical uses of γ energy from Co^{60} sources as a means of preserving meat and meat products have been studied for many years.

Initially, sterilization doses (radappertization) were used. When the results indicated that in many instances the quality of the food deteriorated (10), lower or pasteurization doses (radurization or radicidation) were used to merely extend the shelf life of the product at refrigeration temperatures.

These studies usually involved an evaluation of the organoleptic spoilage (4, 5, 9) and the enumeration of the total and "most common" organisms found in the product after irradiation and storage (5, 18). The effect of irradiation and storage on the total microflora found in the product was rarely determined and the influence of abusive processing practices, whether by accident or design, was seldom considered.

In this study, the effect of radurization treatment of fresh eviscerated poultry on the extension of shelf life was microbiologically evaluated. The radiation resistance of food poisoning organisms commonly associated with poultry was determined and considered in the selection of a recommended dose.

MATERIALS AND METHODS

Source of poultry. Two- to 3-lb broilers were obtained from a local poultry producer. They were processed in the normal manner with one exception. Whereas the carcasses are normally packed directly into crushed ice for shipment, these birds were placed individually into plastic pouches (Dow polyethylene no. 309-T-1, 2 mil thickness) which were sealed with a wire twist, and then were packed in the crushed ice. Delivery was by insulated refrigerated company van; the carcasses reached the laboratory approximately 5 hr after slaughter.

Laboratory handling of samples. Upon receipt, the entire shipment was stored at refrigeration temperatures (5 C) until the following day. At this time, the carcasses were removed and cut up into eight pieces: two legs, two wings, two back pieces, and two breast pieces. These pieces were then individually packed loosely into new plastic pouches. Initially, only one piece from the entire shipment was used to determine the initial microbial load but in later experiments a piece from each bird was analyzed.

Irradiation. The plastic pouches containing the chicken pieces were packed into cylindrical Lucite containers [outer diameter, 6 inches (15.2 cm); height, 7.5 inches (19 cm)], and the entire assemblage was put into the Gammacell 220 (Atomic Energy of Canada Ltd.) at room temperature ($\pm$ 22 C). Exposure times necessary to obtain the specified doses were calculated, taking into consideration the decay rate of the source, the density of the material to be irradiated, and the size and shape of the container. Nonirradiated samples served as controls. At the time of these experiments, the rate of absorbed energy was approximately 1 Mrad per hr.

Microbiological evaluation. The number of viable cells before and after irradiation and after the various periods of storage was determined by homogenizing 10 g of skin material from the chicken pieces with 90 ml of physiological saline solution containing 0.1% peptone (SP) in a Waring Blendor. Serial dilutions were prepared in SP, and standard pour plate and surface streak techniques were employed with Difco Tryptone Glucose Yeast Extract Agar (TGYEA) as the plating medium. The plates were incubated at the temperatures indicated. When 5 C was used as the incubation temperature, the SP diluent and poured plates were cooled to 5 C prior to use.

[1] Issued as Macdonald College Journal Series No. 578.

[2] Present address: Hungarian Meat Research Institute, Budapest, Hungary.

Resistance of salmonellae and staphylococci to γ energy. The various *Salmonella* and *Staphylococcus* species were grown on the TGYEA surface in a Roux bottle for 24 to 48 hr at 37 C. The entire growth was washed off with 90 ml of SP. This comprised the "stock suspension," which contained from 10^8 to 10^{10} viable cells per ml.

To determine the resistance to γ energy of these different organisms, two screw-cap vials (70 × 21 mm) containing 14 ml of the stock suspension were prepared for each dose level. These vials were closed and placed in a Food and Drug vial (FDV) rack (6).

In addition, the resistances of both the *Salmonella* and *Staphylococcus* species in poultry meat were also determined. The meat from a fresh carcass was removed, comminuted, and incubated at 55 C for ± 18 hr to decrease the microbial load to less than 10 per gram. Ninety ml of the different "stock suspensions" in SP were added to 110 g of the incubated meat and the mixture was homogenized in a Waring Blendor (three 1-min cycles). Two screw-cap vials for each irradiation dose were filled with portions of homogenized sample, sealed, and placed in the FDV rack.

The racks containing the samples were placed in the cylindrical Lucite containers and the entire assemblage was put into the chamber of the Gammacell 220. Exposure times necessary to obtain specified doses were calculated as mentioned previously. All irradiations were at room temperature (± 22 C).

After being subjected to the predetermined dose, two vials were removed from the radiation chamber. These were replaced by two blank vials containing either 14 ml of water or of chicken homogenate. Subsequently, 5 ml or 5 g was removed from each of the irradiated vials containing the SP or poultry-meat suspensions, respectively, and was added to 90 ml of SP to yield a 1:10 dilution. Further dilutions and techniques for enumeration of viable cells were performed according to standard methods with SP as the diluent and TGYEA as the plating medium. The plates were incubated at 37 C for at least 48 hr.

Effect of repeated irradiation treatment on the resistance of Salmonella and of S. aureus. In this series of experiments, chicken meat was homogenized in a Waring Blendor and subsequently sterilized by treatment with 2 Mrad of γ energy. A 90-ml amount of the salmonella "stock suspension" and 90 ml of the staphylococcus "stock suspension" were each added to 110 g of homogenate in a beaker. The mixtures were stirred and the meat was allowed to settle out. The supernatant fluids were discarded. The resulting inoculated homogenates in the beakers were irradiated with 0.25 Mrad of γ energy (Fig. 1) and were incubated for 2 to 3 days at 37 C and 3 weeks at 5 C. After each incubation period and prior to the transfer to fresh sterile chicken homogenate, smears were made on Difco Brilliant Green sulfa agar (BGS) or Difco Mannitol Salt Agar (MSA) to ensure that the salmonellae and staphylococci, respectively, survived the previous irradiation and incubation treatment.

When salmonellae and staphylococci were detected, a sterile peptone solution was added to the chicken homogenate. This mixture was stirred and the meat was allowed to settle out. The supernatant fluid was decanted to fresh sterile chicken homogenate and mixed. After the meat settled out, the supernatant fluid was discarded and the meat was irradiated with 0.25 Mrad of γ energy. This cycle (Fig. 1) was repeated 12 times.

At various time intervals during the recycling, the radiation resistance of the salmonellae and staphylococci isolated on the BGS and MSA, respectively, was determined in both SP and homogenized poultry meat substrate.

RESULTS

The experiments were performed to determine whether irradiation has any potential in extending the shelf life of commercially produced fresh poultry. Therefore, no attempt was made to regulate or alter the processes in the plant (Fig. 2, Table 1). In general, fresh carcasses shipped from the plant were contaminated with from 10^5 to 10^6 organisms per gram or 10^4 to 10^5 per cm^2 of skin material. This level of contamination is

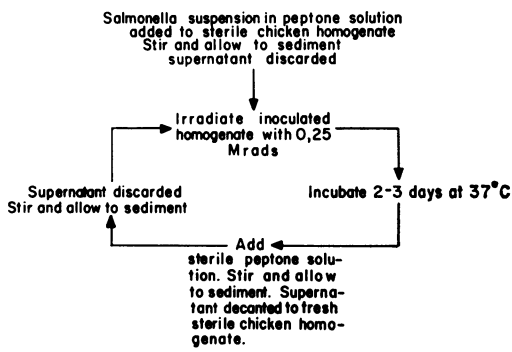

FIG. 1. *Scheme followed to subject the various Salmonella species and Staphylococcus aureus strains, suspended in chicken homogenate, to repeated 0.25-Mrad doses of γ energy.*

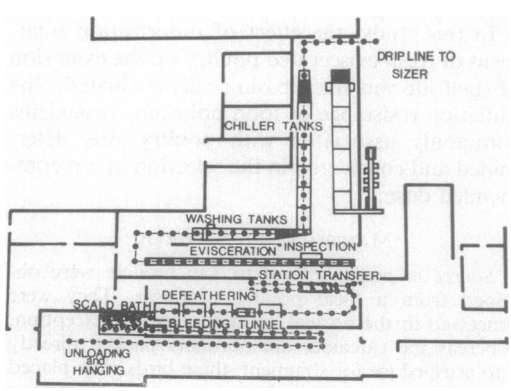

FIG. 2. *Schematic diagram of the processing plant from which samples of eviscerated poultry were received (see Table 1).*

TABLE 1. *Operations of processing plant from which samples of eviscerated poultry were received (see Fig. 2)*

Area	Distance	Travel time	Temp
	ft		*F*
Bleeding tunnel	75	100 sec	
Scald bath	40	90 sec	140–144
Defeathering	123	100 sec	
Evisceration	90	150 sec	
Washing tanks	40	7 min	55
Chiller tanks (rotary drum)	90	30–32 min	55[a] 32[b]
Drip line	200	8 min	

[a] Pre-chiller tank.
[b] Chiller tanks.

TABLE 2. *Percentage of irradiated poultry carcasses organoleptically spoiled at different intervals of storage at 5 and 25 C*

Irradiation level (Mrad)	Storage time (days)	5 C		25 C	
		Total no. of birds analyzed	Percentage spoiled	Total no. of birds analyzed	Percentage spoiled
0.0	1	18	0	18	100
	6	18	33		
	9	18	100		
0.25	1	—[a]	—	18	66
	3	—	—	18	100
0.5	1	—	—	18	66
	3	—	—	18	100
	16	36	0		
	20	36	16		
	30	36	83		
0.7	1	—	—	18	0
	3	—	—	18	66
	5	—	—	18	100
	19	18	0		
	24	54	22		
	29	52	22		
	37	36	66		
	40	18	66		

[a] No tests performed.

similar to those recorded by Clark [3.3×10^4 per cm^2 (3)], Thornley [1.8×10^5 per cm^2 (16)], and Coleby et al. [6×10^5 per cm^2 (4)], but is greater than that reported elsewhere (4×10^3 per cm^2 at Holly Farms Inc., N.C., *personal communication*).

At normal refrigeration temperatures (5 C), fresh carcasses were found to be organoleptically acceptable for periods as long as 6 to 9 days (Table 2). At this time, the bacterial load had, in general, increased by 4 logs above the initial number (Fig. 3). Irradiation at both 0.5 and 0.7 Mrad resulted in an immediate reduction in bacterial numbers. Upon storage, the surviving organisms multiplied, the rate being faster on 0.5 Mrad- than on 0.7 Mrad-irradiated birds. As a result, the 0.5 Mrad treatment extended the shelf life to approximately 16 to 20 days; the 0.7 Mrad treatment to 19 to 24 days (Table 2). In some instances, individual carcasses were not organoleptically spoiled after extended storage even though their microbial load approached 10^8 organisms per gram of skin material (Fig. 3). It should also be noted that in all these experiments the number of viable organisms determined by the surface streak and pour plate techniques was basically the same.

Examination of the organisms isolated from irradiated birds stored for long periods revealed that the microflora usually consisted of relatively few species and, in some instances, of one or two different organisms (Table 3). During these preliminary studies, we isolated in relatively pure culture a new species of *Moraxella* and *Herellea vaginicola*. The significance of these results is the subject of another paper.

Under exaggerated abnormal conditions of storage (25 C), fresh birds spoiled within 1 day. During this time interval, the numbers of organisms increased by 3 logs (Fig. 4). Treatment with increasing doses of irradiation resulted in progressively lower numbers of viable organisms

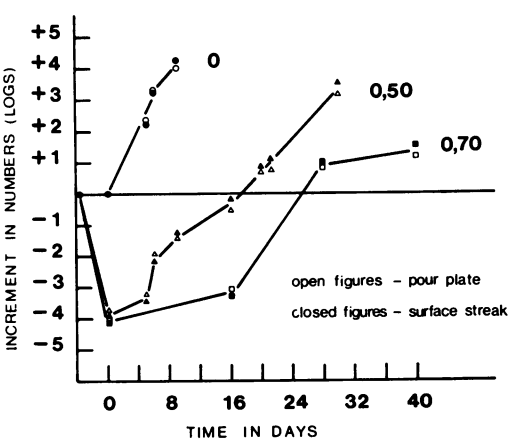

FIG. 3. *Logarithmic increments in the total numbers of microorganisms of untreated and irradiated carcasses (0.25, 0.5, and 0.7 Mrad) after different periods of storage at 5 C. The number of viable organisms was determined at both 25 (pour plate and surface streak) and 5 C (surface streak) on Tryptone Glucose Yeast Extract Agar.*

TABLE 3. *Identification of organisms selected at random from the surviving microflora of eviscerated poultry carcasses after irradiation (0.5 Mrad) and storage at 5 C*

Organism	Characteristic colony	Morphology	Gram reaction	Genus[a]
a	Smooth, cream colored	Pleomorphic coccoid	−	*Moraxella*
b	Smooth, brown-orange	Rod	+	*Serratia*
c	Rough, spreading colony, white	Yeast	+	*Pullularia*
d	Smooth, ochre colored	Pleomorphic rods	−	*Herellea*
e	Rough, white	Unicellular mycelium	+	*Actinomyces*

[a] Genus determined from results of biochemical and physiological tests. Designations are tentative.

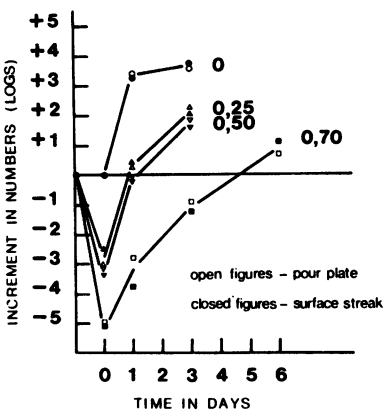

FIG. 4. *Logarithmic increments in the total numbers of microorganisms of untreated and irradiated carcasses (0.25, 0.5, and 0.7 Mrad) after different periods of storage at 25 C. The number of viable organisms was determined at 25 C on Tryptone Glucose Yeast Extract Agar.*

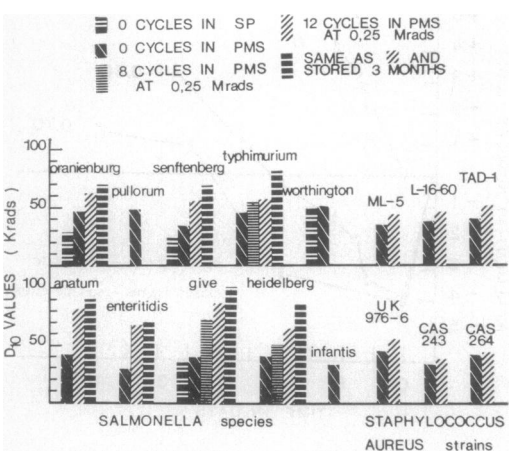

FIG. 5. *Effect of repeated treatment with 0.25 Mrad of γ energy and subsequent storage on the radiation resistance of various Salmonella species and Staphylococcus aureus strains. Irradiations were performed at room temperature with interim incubation at 37 C for 24 to 48 hr. SP, physiological saline solution containing 0.1% peptone; PMS, poultry meat substrate.*

which multiplied upon subsequent storage. Treatment with either 0.25 or 0.5 Mrad of γ energy increased only slightly the shelf life of fresh poultry, whereas 0.7 Mrad extended the shelf life by 2 to 5 days as determined by appearance, texture, and odor.

The resistance to γ energy of the various *Salmonella* and *S. aureus* organisms in different menstrua and after storage is presented in Fig. 5. With the salmonellae, a change in the suspending medium from SP to poultry meat resulted in increased resistance in all instances. Recycling and subsequent storage at room temperature further increased substantially the resistance of all of the *Salmonella* species studied. A doubling in resistance by recycling alone was effected with *S. anatum*, *S. enteritidis*, and *S. give*, whereas *S. typhimurium* was relatively unaffected. The subsequent storage, after recycling, had the greatest influence on *S. typhimurium* in increasing resistance. Recycling at sublethal doses of γ energy had little effect on the resistance of the different *S. aureus* strains (Fig. 5). It was not possible to recycle the *Salmonella* species at 5 C, as there were no survivors after the first cycle.

DISCUSSION

The results of simple organoleptic evaluations indicated that γ energy can be successfully used as a means of extending the shelf life of fresh eviscerated poultry (5, 9–11, 15). Results of this nature are, however, of minor significance in determining the safety of the process from a public health standpoint. The most common spoilage organisms indigenous to poultry carcasses, i.e., *Pseudomonas*, *Achromobacter*, *Flavobacterium*, etc. (1, 8, 18), are normally not detected immediately after an irradiation treatment of 0.5 Mrad or more (5, 18). Depending on the storage conditions after irradiation, however, these organisms may gain ascendancy. Thornley et al. (18) found that when spoilage of irradiated poultry (0.5 Mrad) occurred at 1 C, *Pseudomonas* species were predominant, whereas when spoilage occurred at 3 C *Achromobacter* species [*Acenitobacter* phenons

3 and 4, Thornley (19)] were greater in number. In addition, it is possible that in some localities various radiation-resistant organisms may be indigenous. As a result, depending on the radiation dose and the time and temperature of storage, different types of organisms may be enumerated in high numbers with or without appreciable organoleptic spoilage. Some of these organisms may also be pathogenic.

In our preliminary experiments, we isolated a species of *Moraxella* and *H. vaginicola*-like organisms in relatively large numbers (10^7 per gram of skin tissue) in poultry carcasses which did not show objectionable organoleptic spoilage. When injected intraperitoneally into mice, both organisms caused conjuctivitis. These organisms have not been associated to a great extent with food products. In 1967, Snodgrass and Koburger (17), however, showed that they are frequent contaminants of a variety of untreated foods. Thornley (19) has shown that, although her poultry isolates were similar to different *M. lwoffi* strains at the 86% level, the latter organisms formed a separate grouping at the 92.5% level. The question still remains whether the moraxella organisms constitute a group distinct from Thornley's poultry isolates.

TABLE 4. *Logarithmic reduction in the number of viable cells of different Salmonella species and of Staphylococcus aureus strains effected by 0.5 Mrad of γ energy*

Organisms	Logarithmic reduction per gram of poultry meat		
	Untreated cells	Cells recycled 12 times at a sublethal dose of 0.25 Mrad	Cells recycled 12 times and stored for several months on laboratory media
Salmonella anatum	12	6	6
S. enteritidis	17	7	7
S. give	13	6	5
S. heidelberg	13	8	6
S. infantis	15	—	—
S. oranienburg	10	8	7
S. pullorum	10	—	—
S. senftenberg	15	9	8
S. typhimurium	10	9	6
S. worthington	10	—	—
S. aureus			
UK-976-6	11	9	—
CAS-243	15	13	—
CAS-264	12	12	—
ML-5	15	12	—
L-16-60	14	11	—
TAD-1	13	10	—

We are quite certain that these organisms do not contribute an additional hazard to public health (E. S. Idziak and R. Whitaker, *in preparation*). Nevertheless, such ecological shifts in the microbial population of irradiated and stored poultry must be studied in greater detail before ruling out completely any public health hazards from the potentially pathogenic organisms which are from time to time found in poultry carcasses. In this respect, we are determining the radiation resistances of organisms belonging to the genera *Streptococcus, Corynebacterium, Neisseria, Gaffkya, Pasteurella* (2, 14, 16).

A distinct advantage of the irradiation process is the elimination of *Salmonella* species and *S. aureus* strains without making the product objectionable to the consumer. In 1966, salmonellae were responsible for 12.7% of all food-borne poisoning outbreaks in the United States. Salmonellae isolated from poultry contributed ±10% of the total number isolated from nonhuman sources in the U.S. in the first 11 months of 1967 (13). Woodburn (20), over a 1-year period, found that 27% of the poultry in retail outlets was contaminated with *Salmonella*. Morris and Ayres (12) found that from 7 to 14% of birds received by a processing plant were positive for *Salmonella*. We have estimated the number of salmonellae in our carcasses to be approximately 2 per 100 g of meat. With the proposed dose of 0.5 Mrad, salmonellae and staphylococci can be reduced in the numbers shown in Table 4. This treatment, therefore, eliminates a minimum of 10D of the wild type and 6D of the recycled resistant salmonella organisms. Idziak and Whitaker (*in preparation*) have shown that the recycled *Salmonella* species and *S. aureus* strains can still be identified as such by the standard Food and Drug Directorate methods (7). This appears to be somewhat in conflict with the results of Erdman et al. (6), who showed that recycling of salmonellae produced mutants which were H_2S and citrate negative. The difference may be attributed to the experimental techniques used. Erdman et al. (6) used repeated and increasing doses of irradiation to obtain their ultimate mutants, whereas our recycling was done at one irradiation level. It is quite probable that individual cells within the surviving populations may be altered, but the total population of survivors, in our experiments, retained the characteristics of salmonellae (N. Epps and E. S. Idziak, *unpublished data*). This was also true with the *S. aureus* strains examined.

From the experimental results, the probability of recycling either salmonellae or staphylococci, or both, on poultry can be evaluated. For this phenomenon to occur at an irradiation dose equal

to one-half the recommended dose of 0.5 Mrad, the carcasses must contain at least 10^5 salmonellae and staphylococci per gram of meat. In addition it is necessary that the irradiated carcasses be stored at elevated temperatures (±15 C) to allow proliferation of any surviving salmonellae and staphylococci to levels approaching 10^5 per gram of meat. If this level is not attained, there will be no survivors after the second irradiation treatment. Further, if these conditions are met, the carcasses would in all likelihood be grossly spoiled and would be rejected for further treatment and thus discarded. Considering all these factors, it is quite evident that the probability of recycling of these pathogenic organisms occurring commercially within a poultry processing plant would be minimal.

ACKNOWLEDGMENTS

This investigation was supported by Atomic Energy of Canada Ltd. research grant no. 8033-513.

We thank R. E. Weaver, Communicable Disease Center, Atlanta, Ga., and R. Elder, Ottawa Civic Hospital, for assistance in the identification of the *Moraxella* sp. and *Herellea vaginicola* sp. The technical assistance of Susan Cowan is also greatly appreciated.

LITERATURE CITED

1. Ayres, J. C. 1959. Effect of sanitation, packaging, and antibiotics on the microbial spoilage of commercially processed poultry. Iowa State J. Sci. **34**:27–46.
2. Ayres, J. C., W. S. Ogilvy, and G. F. Stewart. 1950. Post mortem changes in stored meats. I. Microorganisms associated with development of slime on eviscerated cut-up poultry. Food Technol. **4**:199–205.
3. Clark, D. S. 1967. Comparison of pour and surface plate methods for determination of bacterial counts. Can. J. Microbiol. **13**:1409–1412.
4. Coleby, B., M. Ingram, and H. J. Shepherd. 1960. Treatments of meat with ionizing radiations. III. Radiation pasteurization of whole eviscerated carcasses. J. Sci. Food Agr. **11**:61–71.
5. Coleby, B., M. Ingram, H. J. Shepherd, and M. J. Thornley. 1960. The treatment of meats with ionizing radiations. IV. A comparison of the deterioration in quality during storage of eviscerated carcasses treated with chlortetracycline and radiation. J. Sci. Food Agr. **11**: 678–684.
6. Erdman, I. E., F. S. Thatcher, and K. F. MacQueen. 1961. Studies on the irradiation of microorganisms in relation to food preservation. II. Irradiation resistant mutants. Can. J. Microbiol. **7**:207–215.
7. Food and Drug Directorate Official Methods. 1965. The determination of *Salmonella* in foods. FO-51. Food and Drug Directorate, Ottawa.
8. Gunderson, M. F., K. D. Rose, and M. J. Henn. 1947. Poultry boning plants need bacteriological control. Food Ind. **19**:1516–1517.
9. Hannan, R. S., and H. J. Shepherd. 1959. The treatment of meats with ionizing radiations. I. Change in odour, flavour, and appearance of chicken meat. J. Sci. Food Agr. **10**:286–295.
10. Hanson, H. L., M. J. Brushway, and H. Lineweaver. 1964. Flavour studies of irradiation-sterilized chicken. Food Technol. **18**:141–146.
11. McGill, J. N., A. I. Nelson, M. P. Steinberg, and L. L. Kempe. 1959. Gamma ray pasteurization of whole eviscerated chickens. Food Technol. **13**:75–80.
12. Morris, T. G., and J. C. Ayres. 1960. Incidence of *Salmonellae* in commercially processed poultry. Poultry Sci. **39**:1131–1135.
13. National Communicable Disease Center *Salmonella* Surveillance. 1967. Annual summary for 1966, October 9, and Reports No. 58–68.
14. Pablo, I. S., G. J. Silverman, and S. A. Goldblith. 1966. Microbial growth patterns of rehydrated freeze dried foods. II. Chickens. J. Food Sci. **31**:245–252.
15. Rhodes, D. N. 1965. The radiation pasteurization of broiler chicken carcasses. Brit. Poultry Sci. **6**:265–271.
16. Sadler, W. W., R. E. Corstvet, H. E. Adler, and R. Yamamoto. 1966. Relative prevalence of bacterial agents and histopathology in passed and condemned poultry carcasses and livers. J. Food Sci. **31**:773–780.
17. Snodgrass, C. J., and J. A. Koburger. 1967. The isolation and characterization of the tribe *Mimea* in foodstuffs. J. Food Sci. **32**:589–591.
18. Thornley, M. J., M. Ingram, and E. M. Barnes. 1960. The effects of antibiotics and irradiation in the *Pseudomonas-Achromobacter* flora of chilled poultry. J. Appl. Bacteriol. **23**:487–498.
19. Thornley, M. J. 1967. A taxonomic study of *Acenitobacter* and related genera. J. Gen. Microbiol. **49**:211–257.
20. Woodburn, M. 1964. Incidence of salmonellae in dressed broiler-fryer chickens. Appl. Microbiol. **12**:492–495.