1971

Study of Control of Public Health Problems Using Irradiation

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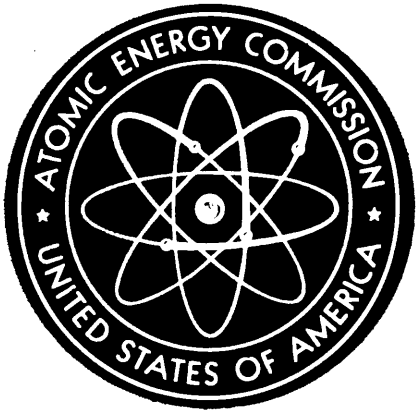

COO-2038-2

STUDY OF CONTROL OF PUBLIC HEALTH PROBLEMS USING IRRADIATION

Annual Report, May 1, 1969—April 30, 1970

May 1971

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UNITED STATES ATOMIC ENERGY COMMISSION · DIVISION OF TECHNICAL INFORMATION

COO-2038-2
RADIOISOTOPE AND RADIATION
APPLICATIONS (TID-4500)

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For the period May 1, 1969 to April 30, 1970

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May 1971

FOR

Division of Isotopes Development

United States Atomic Energy Commission

CONTRACT NUMBER

AT(11-1)-2038

ABSTRACT

Study of Control of Public Health Problems Using Irradiation

Red meat, as exemplified by ground beef and pork, is subject to numerous opportunities for contamination and harborage of pathogenic organisms with the present commercial system of processing and distribution. An exploration was undertaken therefore to determine the usefulness of gamma irradiation for public health protection when applied to red meat. This work indicated that retail products are commonly heavily contaminated. The contamination can be reduced somewhat through central processing as shown by the data obtained from ground beef. Considerable numbers of the contaminants can be destroyed by irradiation at a 68 Krad level.

This work indicates that commercial ground red meat contained a great diversity of contaminants, many of which belong to the family Enterobacteriaceae. Of most interest are salmonellae and other pathogens of lesser notoriety. Many of these organisms are quite sensitive to gamma radiation. While a 68 Krad level of radiation likely will not destroy all these pathogens, a substantial reduction in numbers is possible. Future use of higher dosage should destroy more microorganisms and add further to public health protection.

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SUMMARY

Gamma radiation may be used to destroy pathogenic microorganisms in foods. With low doses the destruction process is limited to the most sensitive species or to a reduction in numbers of the marginally resistant species. An exploration of the usefulness of the gamma radiation process for public health protection when applied to red meat was therefore undertaken.

Red meat, as exemplified by ground beef and pork, is subject to numerous opportunities for contamination and harborage of pathogenic organisms with the present commercial system of processing and distribution. Present retail products are commonly heavily contaminated. This contamination can be reduced somewhat through central processing, and considerably more can be inactivated by irradiation at a 68 Krad level. A somewhat higher dose will be more effective if the problems associated with flavor and color are successfully resolved along with the proof of absence of untoward side effects.

This work indicated that commercial ground red meat contained numerous organisms including a great diversity of contaminants--many of which belong to the family Enterobacteriaceae. Of most interest are salmonellae and other pathogens of lesser notoriety. Many of these organisms are quite sensitive to gamma radiation. While a 68 Krad level of irradiation likely will not destroy all these pathogens, a substantial reduction in numbers is possible. Future use of higher doses should destroy more microorganisms and add further to public health protection.

INTRODUCTION

Gamma radiation may be used to destroy microorganisms in fresh meat and poultry for foods (12). The extent of destruction is dependent on the dose, nature of the microorganisms, and nature of the food. Considerable work has been done to explore the possibility of commercial application of irradiation in food processing. A complete compilation of the literature dealing with irradiation processing of meat and poultry has been made by Urbain et al. (13). Early exploratory work had the primary goal of developing new food processes or economic improvement of presently accepted food processes. The primary mission was shelf-life extension of foods for more convenient and wider distribution of presently accepted products (15).

Some workers have considered the destruction of pathogenic microorganisms in foods, but most of the work has been indirect with pure cultures and laboratory defined media. Work along these lines primarily has been directed toward determining the dose requirements for a complete destruction of a certain specific organism in question; e.g., Salmonella (5). Work on the destruction of sporeforming bacteria has followed a similar pattern, but the work was associated with complete sterilization.

The mission of this project was to explore in a broader sense the usefulness of irradiation processing for public health protection with particular emphasis on application at a pasteurization level. The broad spectrum of pathogenic organisms was to be considered with red meat as the vehicle. Red meat was chosen because it represents a broad exposure

to contamination during processing and distribution. It is an essentially universal product. It is also a product in commercially acceptable fresh forms that is not amenable to present methods of pasteurization. Red meats are not unique since a pasteurization process is not available for many other fresh foods; e.g., vegetables, fruits, fresh formulated foods, etc. Work with irradiation pasteurization of red meats ultimately should be contributive to similar processing of other products.

The approach to irradiation and public health protection from foods was taken because it was realized that an acceptable irradiation process may not destroy all the pathogens, but the concept is to destroy as many as possible, therefore reducing the likelihood of objectionable organisms and hazards to public health therefrom.

Irradiation processing, though not a proven system, offers a potentially ideal system for pasteurizing solid and semi-solid foods. Rapid penetration is a unique property of the system. Food could be processed near the source of raw material, where the initial contaminants and quality loss are minimized. Irradiation pasteurization after proper packaging would destroy contaminants of public health significance and prevent subsequent contamination during transportation and marketing.

Technology and public health protection may well be expected to develop together. Work on the technology of the processes has progressed to indicate a feasibility as exemplified by the work of Urbain (14).

The general plan for this work was to explore the broad spectrum of the problem of pathogens in red meat products and the impact of irradiation thereon. This knowledge could be used as guidelines for more definitive work and the ultimate adoption of the process for commercial uses. Products to be examined were to be chosen to exemplify the greatest challenge within the industry. This involved highly processed products commonly exposed to the greatest number of sources of contamination.

METHODS

Irradiation

A shipboard irradiator with 28,000 Curies of Cobalt 60 (November 1968) was used (11). Temperature control was limited to placing the refrigerated samples in the well of the irradiator for the short exposures and then returning them to refrigerated storage. The absorbed dose by the samples was determined by Fricke dosimetry (7).

The sample size, shape, and location was chosen so that the dose differential was well below the 1.25 limit. Furthermore, the irradiation unit was always operated so that each sample received a two side treatment. The largest sample size for ground beef was 1/4 lb. Irradiation of red meat samples was performed in polyethylene pouches, tightly closed and stored at the appropriate temperature until used. Neither truly aerobic nor anaerobic conditions were sought but an intermediate environment that might prevail with widespread use conditions.

The dose level selection was 68 Krads or less, which was an arbitrary low level to explore the feasibility of irradiation for public

health protection. High doses may be applicable when the maximum permissible dose has been established with consideration of physico-chemical and color changes.

Sample Selection

Early work involved a survey of local market fresh ground beef and fresh ground pork sausage. Ground beef was the subject for the majority of the subsequent work, because it presented the apparently greatest challenge for contamination control, and our supplies seemed to be more ideally suited for pursual of this product. A source of high quality ground beef was readily available and appeared to simulate the ultimate goal of central processing and irradiation of an initially high quality product. We had no indications, however, that the problems with ground beef were unique rather than exemplifying red meat *in toto*.

Microbiological Methods

The general microbiological procedures were those outlined in Standard Methods (1). The fresh red meat products were examined by weighing an 11 g sample into a previously sterilized Waring Blendor jar, then adding 99 ml of phosphate buffer. The total aerobic microbial count was determined with standard plate count agar with incubation at 32°C. The coliform count routinely was determined with violet red bile agar. The coliform count was made as a broad indicator of both contamination of sanitary significance and as an indicator of bacteria of the family *Enterobacteriaceae*. When comparing growth of *E. coli* and/or *S. typhimurium* in

irradiated and unirradiated samples, sufficient inocula were made to the sample to be irradiated so that the population after irradiation was at approximately the same level as the inoculated unirradiated sample. When more specific pathogens (salmonellae) were observed, appropriate references to the methods will be given in the results. Table 1 contains a summary indicating the media and incubation conditions. Other members of the family Enterobacteriaceae were to be observed in a later phase of the work.

In certain experiments the location of the microflora relative to surface and subsurface conditions was observed by obtaining plug samples using the method of Kastner and Hendrickson (3). Sections were then blended and plated.

The general microflora of the ground products was studied by taking isolates through random design. Nature of the isolates was determined by Gram staining, catalase production, spore formation, gas production in brilliant green lactose bile broth for indication of coliform bacteria, characteristic growth in litmus milk, and proteolysis on plate count agar plus skim milk.

RESULTS

Microbial Population in Ground Beef From Supermarkets

The total count and coliform count in ground beef from supermarkets were determined at the time of purchase and after 6 days storage at 5°C. Average results of three trials from each of 6 stores are given in Table 2. The counts showed that there were more than 1,000,000 microorganisms per gram in all samples with the highest showing an average count of 150 million microorganisms per gram.

The coliform counts on the ground beef from these stores ranged from near 8,500 per gram to 410,000 per gram. After incubation to simulate storage in a home refrigerator, the average total count on the sample with the lowest initial count reached 110 million. While other samples were higher in total bacterial population, all samples were judged spoiled to the point of a typical person's rejection. After storage the coliform counts were 100 to 260 million per gram.

These counts were in harmony with magnitude of contamination reported by others (2, 4, 8), indicating the products in this geographical area are not unique and that the problem with ground beef remains.

The highest counts on the fresh product were obtained from a supermarket located at the lowest income area catering to the price oriented consumer. Samples from the supermarket catering to the highest income group had only slightly less count than those from the typical supermarkets of the chains.

Microbial Population in Pork Sausage From Supermarkets

Fresh pork sausage was obtained from the same supermarkets as were the samples of ground beef. Total counts and coliform counts were

made on these fresh products. The results from an average of three trials from each of 6 stores are given in Table 3. Total counts ranged from a low of 6.4×10^4 per gram for Store No. 2 to a high of 1.0×10^8 per gram for Store No. 1. Coliform counts on the fresh product ranged from 48 per gram to 3.1×10^5 per gram.

The samples of pork sausage were stored for 6 days at 5°C after which the same microbial tests were repeated. After 6 days storage at 5°C, total counts and coliform counts increased appreciably in all samples except those obtained from Store No. 1. It is interesting to note that samples from Store No. 1, which had the highest initial count both on ground beef and on pork sausage, showed little increase in counts during storage.

Some additional exploration for microorganisms of public health significance was also made. Table 3 indicates enterococci and coagulase positive staphylococci were commonly found in these products. The pork sausage samples were also screened for salmonellae using the method of Sperber and Deibel (10). Two samples were found to be positive at the time of purchase, but only one was found to be positive after 6 days storage at 5°C.

Studies on Ground Beef to Simulate Central Processing

Microbial observations were made on fresh ground beef from a commissary, which emphasized high quality products for further distribution. Six samples collected at different time periods showed that the initial count was 1.1×10^5/g and the coliform count averaged 1.1×10^2/g (Table 4). It is apparent that this was a much higher quality product than was obtained from the retail stores.

These samples were subjected to irradiation and subsequent storage at 5°C. Microbial observations were made immediately and after 6 days. A compilation of the results is given in Table 4. The data show that gamma radiation was quite destructive to the microflora in that 34 Krads reduced the total flora approximately 84%, while 68 Krads reduced the total flora approximately 97.5%. Apart from lowering the total count, other objectionable bacteria were reduced to an appreciable extent.

The total count and coliform count increased rapidly in unirradiated samples on storage at 5°C similar to that observed earlier for ground beef obtained from retail stores. After 6 days storage the total count in the samples irradiated with 68 Krads of gamma radiation, however, did not attain the level of the average of the fresh samples from the supermarkets.

Observations on the Nature of the Microflora in High Quality Beef Before and After Low Dose Irradiation

Isolates from plates used to make total counts were observed further for identification and classification purposes. Results on 147 isolates from fresh product and 176 from stored product are given in Table 5. In fresh unirradiated samples gram-positive asporogenous rods, micrococci, and pseudomonads constitute a major portion of the total microflora. Most of the asporogenous gram-positive rods were catalase negative implying them to be lactobacilli. On storage the major change was an increase in the number of gram-negative rods and a decrease in the micrococci.

Irradiation eliminated most of the gram-negative rods leaving predominantly micrococci with a few gram-positive rods. On storage, however, the gram-positive asporogenous rods became predominant.

Outgrowth of Microorganisms in Unirradiated and Irradiated High Quality Ground Beef Stored at 2° and 5°C

The above reported studies were done at 5°C to simulate the practical conditions of storage temperature in household refrigerators and in supermarkets. To obtain further data on behavior of microflora at more ideal storage temperatures, the comparative bacterial counts in unirradiated and irradiated high quality ground beef were obtained after storage at 2° and at 5°C. Since it is difficult to determine at what point the sample becomes unacceptable to consumers, a count of 5.0×10^7 to 1.0×10^8/g was taken as an indication of deterioration of a sample to the threshold of unacceptability. Observations, therefore, were continued until the count reached approximately 5.0×10^7/g at 2°C storage.

Figure 1 shows the comparative microbial growth occurring in unirradiated ground beef at 5°C and 2°C through 6 days storage. The data represent averages from three samples. The total count did not increase appreciably until 48 hr, after which multiplication was much faster at 5°C than at 2°C with the result that at 6 days the count was 1.1×10^9/g at 5°C, while it was only 5.0×10^7/g at 2°C.

The corresponding data for irradiated ground beef are also plotted in the same figure. At 2°C it took 14 days to reach a count of 5.0×10^7/g, while at 5°C it took only 8 days. The combination of

irradiation and low temperature storage up to 9 days limited the count to less than that obtained in even the best of the samples from commercial retail stores.

Salmonellae and Indicator Organisms in Ground Red Meat

Salmonallae have been found as contaminants of most red meat products as indicated by the literature (6). The previously described exploratory runs indicated the products in the geographical area of Lincoln, Nebraska, were similar in quality and contamination to those of other geographical areas. Surveys on the occurrence of salmonellae gave similar data as an indication of contamination. Samples contaminated with salmonellae might be expected to be contaminated with other members of the family Enterobacteriaceae. Thus, coliform organisms were taken as indicator organisms of a broad spectrum of contamination. It was felt that observations should be made to substantiate the belief that behavior of coliform organisms, as exemplified by E. coli, was similar to the pathogens of most popular concern at the present time, salmonellae.

Comparative Behavior of S. typhimurium and E. coli when Stored in Ground Beef at 5°C

The fate of S. typhimurium was compared to the fate of the general microflora in unirradiated and irradiated samples. Data are shown in Figure 2. There was a slow decrease in the number of S. typhimurium in the irradiated sample during the test period, but the unirradiated sample showed an insignificant change. In contrast, the total microflora changed little during the first 24 hr but increased rapidly beyond that time. The increase in total microflora during storage was much

greater for the unirradiated than for the irradiated sample.

In the unirradiated samples the coliforms multiplied slowly for 72 hr after which there was rapid multiplication. The count increased from an initial of 1.5×10^3/g to 1.6×10^5/g at the end of the test period (Figure 4). Thus, it is apparent that coliforms in unirradiated samples at 5°C grow, while S. typhimurium does not grow. In the irradiated samples, however, the coliform count, which was primarily from the inoculum of E. coli, decreased steadily. This difference in behavior of species of coliform bacteria was attributed to the various lower limits of growth, as some natural contaminants of coliforms grew at 5°C (Figure 5). Proof of this latter contention of selective outgrowth will be delineated more completely in scientific papers in preparation and will be available for the next report.

Proof of the above contention that psychrotolerant coliforms were involved was obtained by using ground beef from the commercial source known to be highly contaminated with coliform organisms. Microbial counts were made on unirradiated and irradiated samples when fresh and after storage at 5°C. The results are presented in Figure 5. It is apparent that the total count and the coliform count increased during storage.

Behavior of S. typhimurium, Coliform Organisms, and Total Microflora in Ground Beef Stored at 25°C

Unirradiated and irradiated samples of ground beef, which had been inoculated with S. typhimurium prior to irradiation, were incubated at 25°C to simulate the mishandling of room temperature storage. Data representing an average of three trials are presented in Figure 3. In

unirradiated ground beef the total microbial population did not change appreciably until after 6 hr. For the remaining 5 hr, however, the multiplication was rapid attaining a total population density of 1.7×10^7/g. At the 11th hour most unirradiated samples indicated a distinct off odor, which would indicate the rejection of the product as being inedible by most people. The Salmonella in the irradiated samples decreased in numbers for the first 4 hr then increased rapidly thereafter. The decrease in numbers was likely from the failure of injured cells to recover in the meat. Inocula of S. typhimurium in the unirradiated samples showed no apparent lag and increased rapidly throughout the test period.

In the irradiated samples stored at 25°C, the total count was essentially static for the first 4 hr, beyond which there was growth at an increasing rate throughout the test period. In these samples after the lag, the salmonellae multiplied at a faster rate than the total population.

The results with E. coli (Figure 6) were similar to those for S. typhimurium when compared to the behavior of the total microflora.

Microorganisms Surviving Irradiation

While most of the microorganisms surviving irradiation treatment appear to be gram-positive asporogenous rods, a few gram-negative cocci were apparent in most freshly irradiated products as well as those sampled after storage. Since this group of microorganisms has not been generally recognized as a common contaminant of unirradiated products, their presence indicated a shift in the nature of the microflora as a result of irradiation treatment. Thus, observations were made to

determine the nature of these microorganisms. They were found to be quite radiation resistant. An example of the relative resistance is shown by the data in Figure 7, which represent the results of an average of three trials with one of the isolates. Exploratory work indicated there is considerable difference in the radio resistance of the various isolates. It is interesting to note that the plot of dose level against logarithm of numbers does not produce a straight line. Further evaluation of the other isolates will be made later. The physiological and morphological characteristics of this group of resistant microorganisms were observed and they were found to belong to the loosely defined genera Moraxella, Herrellea, and/or Mima. The morphology and physiology appeared similar to the gram-negative cocci observed by Wolin et al. (15). Further observations indicated that the organisms were present in the unirradiated product but had apparently gone without note, because of the far larger number of other bacteria. Furthermore, failure to note their presence in the past may have been attributable to their being relatively inert as compared to other members of the microflora either in unirradiated or irradiated products. While there is no assurance of public health significance of these microorganisms, their likely source is cattle with some levels of infection.

Attenuation as a Factor in the Destructive Effect of Gamma Radiation

While it is generally assumed that the attenuating effect of a few inches of meat would not be a major consideration in altering the destructive effect toward microorganisms, the importance in public health warranted the proof of this point. It is assumed the applicable dose would be evaluated as the position of question in the product being

considered. A comparison was made by treating known cultures with and without the attenuating effect of 10.6 cm of ground beef. The time of exposure was adjusted so that comparable doses were given as determined by Fricke dosimetry. Comparative destructiveness under the two systems of exposure was determined by total plate counts and coliform counts. The results, which are presented in Table 6, indicated that the two exposure systems were equal. Thus, reliance can be given Fricke dosimetry irrespective of the position being considered in red meat.

Position of Contamination

Beef for commercial ground beef represents a variety of cuts generally assembled from a number of sources. If this product represents almost totally surface contamination, then sanitation should be an over-riding force in maintaining a high quality product. Some observations of the location of the contamination were made by taking cuts of meat typical of the production of ground beef and treating to the following sequence: washing, rewashing, and grinding. Various systems were used to treat the surfaces to minimize contamination of the inner tissue. The first two steps represented microorganisms removed and the results are expressed in terms of organisms per gram of the original product. The microbial recovery was determined at each step in the sequence. Average results from four trials with unirradiated samples are given in Table 7. The data indicate surface growth is greater, but growth occurs throughout the sample. The distribution of the microflora indicates no unique position in location of types of microorganisms (Table 8). The results showed that more microorganisms were recovered from the very first washing treatments than from subsequent treatments, but the

general pattern was such as to indicate the contamination was much deeper than a simple surface contamination and subsequent growth phenomenon.

Factors of irradiation at 68 Krads and storage at 5°C were included to determine the effect on location of the outgrowth of microorganisms. Average results from four trials with irradiated samples are given in Table 9, which shows lower counts than on the unirradiated samples, but the same general growth pattern prevailed. There was no clear pattern of change in nature of the microflora (Table 10).

Observations were also made on the depth of contamination by the use of a coring device (3) from which it was possible to take samples of large cuts of meat. These results also indicated that the outer surfaces harbored most of the contamination, but the inner parts contained significant contaminants.

Serratia marcescens as an Indicator of Vectors of Contamination for Ground Red Meat

Exploratory work was carried out on the use of S. marcescens as an indicator organism for determining the vectors and fate of contaminants associated with ground red meat. Preliminary indications are that this organism can be used with surface plating on plate count agar. The primary criterion in the use of surface plating is to emphasize color differences from pigmentation by S. marcescens. Through the application of other work in our laboratory, it was found that the reliability of the test could be improved considerably by adding a small amount of Nacconol to the medium thereby enhancing pigmentation of S. marcescens. It was also shown that the general behavior of S. marcescens on surfaces of wood, glass, and stainless steel was similar to those of coliform organisms such as E. coli.

Thus, the S. marcescens should be useful as a tracer of vectors since its longevity and growth characteristics are fairly similar to a common index of sanitation, coliform organisms.

DISCUSSION

Many of the systems for handling "fresh" foods have evolved from the practice of home production and fresh consumption. With the progressive geographical separation of production and market operations to great distances, problems of protection of fresh products have grown. Beef, for example, is exposed to numerous vectors of contamination, which may originate from equipment or personal contact with carriers of pathogenic microorganisms. An item such as ground beef is a particularly vulnerable product, because it is derived from a diversity of primal cuts. Under certain conditions it is a salvage operation.

The present system of in store processing of red meats makes close control by regulatory sanitarians an almost insurmountable task. Details of the difficulty may be exemplified by the prevention of use of common tools without proper sanitization between pork and beef processing. More obvious contamination may arise from the addition of pork to beef by the unscrupulous operator when it is to his economic advantage. The latter process is most likely to occur with salvage operations.

Ground beef as presently distributed at the supermarket contains a level of contaminating microorganisms that prevents it being stored in a household refrigerator to meet the demands of a weekly shopping schedule. Thus, the housewife must adjust to the pattern of freezing the meat, or to use it at an early part of the weekly shopping schedule. This system

of marketing, storage, and decision making results in the product being used until it is on the threshold of organoleptic rejection. This criterion may even include tasting, which is unwise from a public health standpoint but a common practice among housewives. It should be emphasized that products frozen in the home refrigerator must be thawed prior to use even in cooking, because the product must be formed into patties, etc. Thawing then becomes an opportunity for microbial growth particularly when thawing is at room temperature. There is an inherent lack of precise control of the endpoint of thawing. Tasting raw meat is a fairly common practice. Actual consumption of raw beef is not unique, and consumption of extremely rare ground beef is quite common. Pathogenic organisms in these products are obviously a hazard to public health.

Historically, the argument has been given that ground beef is cooked before it is used thus excusing a relatively high level of contamination including certain pathogens. At the present level of socio-economic development, this concept of safety should no longer be acceptable without challenge. Raw products carried into the home are handled in the raw state by the housewife without subsequent precautions to prevent the contamination of other noncooked foods. The latter foods may be handled and stored at room temperature. These exposures are in addition to the previously mentioned practices of consuming raw meat.

Central processing has tremendous potential for the improvement of public health protection in the processing and distribution of ground beef. Many of the previously mentioned hazards could be overcome through the organization of a central control system, specialization of labor, sophisticated equipment, and more elaborate control systems. Irradiation

processing could be included to add a far greater improvement to public health protection than the previously mentioned sanitary control systems.

Irradiation processing could be performed near the prime source of raw material after processing and packaging. The data given in this report indicate that a major reduction in the pathogens of public health significance could be attained through irradiation treatments. Irradiation can be applied after the product is processed and packaged to prevent further contamination. Furthermore, irradiation is most effectively applied to a high quality product which is attainable near the source of raw material. The data presented in this report were obtained on samples irradiated at a lower level than will probably be attainable. Other factors as flavor, color, safety from irradiation hazards, etc., must be considered in setting the ultimate standard.

The extreme effectiveness of gamma radiation in destroying salmonellae and E. coli indicates the process has still great unexplored potential in destroying other members of the family Enterobacteriaceae. These are of public health significance though are not presently receiving the emphasis of public health concern that salmonellae are.

REFERENCES

1. American Public Health Association. 1966. Recommended methods for the microbiological examination of foods. 2nd ed. APHA, Inc. New York, N. Y.
2. Jay, J. M. 1964. Beef microbial quality determined by extract-release volume (ERV). Food Technol. 18: 1637-1641.
3. Kastner, C. L. and R. L. Hendrickson. 1969. Providing uniform meat cores for mechanical shear force measurement. J. Food Sci. 34: 603-605.
4. Kirsch, R. H., F. E. Berry, C. L. Baldwin, and E. M. Foster. 1952. The bacteriology of refrigerated ground beef. Food Res. 17: 495-503.
5. Licciardello, J. J., J. T. R. Nickerson, and S. A. Goldblith. 1968. Effect of repeated treatment with gamma rays on radio-resistance, virulence, and culture characteristics of certain pathogenic bacteria. Report to U.S. Atomic Energy Commission, Washington, D.C.
6. National Academy of Sciences. 1969. An evaluation of the Salmonella problem. Publication No. 1683. National Academy of Sciences, Washington, D.C.
7. Rizzo, F. X. 1968. Atomic Energy Commission Food Irradiation Manual. Brookhaven National Laboratory, N. Y.
8. Rogers, R. E. and C. S. McCleskey. 1957. Bacteriological quality of ground beef in retail markets. Food Technol. 11: 318-320.
9. Saraswat, D. S., W. S. Clark, Jr., and G. W. Reinbold. 1963. Selection of a medium for the isolation and enumeration of enterococci in dairy products. J. Milk Food Technol. 26: 114-118.

10. Sperber, W. H. and R. H. Deibel. 1969. Accelerated procedure for Salmonella detection in dried foods and feeds involving only broth cultures and serological reactions. App. Microbiol. 17: 533-539.

11. Teeny, F. M. and D. Miyauchi. 1970. Irradiation of Pacific coast fish at sea. J. Milk Food Technol. 33: 330-334.

12. Urbain, W. M. 1965. Radiation preservation of fresh meat and poultry. In: Radiation Preservation of Foods. pp. 87-98. National Academy of Sciences, Washington, D. C.

13. Urbain, W. M., P. S. Belo, and G. G. Giddings. 1969. Centralized processing of fresh meat and poultry including radiation pasteurization - a bibliography. U.S. Atomic Energy Commission. Washington, D.C.

14. Urbain, W. M., G. G. Giddings, P. S. Belo, and W. W. Ballantyne. Radiation pasteurization of fresh meats and poultry. 1969. Report to U.S. Atomic Energy Commission, Washington, D.C.

15. Wolin, Eileen F., J. B. Evans, and C. F. Niven, Jr. 1957. The microbiology of fresh and irradiated beef. Food Research (J. Food Sci.) 22: 682-686.

Table 1. Methods used for microbial counts

Count	Medium employed	Plating method	Incubation
Total	Plate Count Agar (Difco)	Pour plate	32°C/48 hrs
Coliform	Violet Red Bile Agar (Difco)	Pour plate with overlay	37°C/24 hrs
Enterococcal	Citrate azide agar of Saraswat et al. (6)	Pour plate	37°C/48 hrs
Staphylococcal	Staphylococcus medium #110 (Difco)	Surface plating	37°C/48 hrs

Table 2. Total count and coliform count per gram of ground beef from six stores at the time of purchase and after 6 days storage at 5°C

Store	Count at the time of purchase		Count after 6 days at 5°C	
	Total	Coliform	Total	Coliform
1	1.5×10^8	4.1×10^5	6.9×10^9	6.0×10^7
2	4.7×10^6	2.6×10^4	8.1×10^9	2.6×10^8
3	4.1×10^7	1.6×10^5	1.1×10^8	9.2×10^5
4	8.7×10^6	8.5×10^3	1.9×10^9	6.6×10^6
5	3.7×10^6	9.0×10^3	5.8×10^9	2.2×10^7
6	4.5×10^6	1.9×10^5	1.5×10^9	1.2×10^8

Table 3. Microbial counts per gram of pork sausage obtained from six stores, at the time of purchase and at 6 days storage at 5°C

Store	Count at the time of purchase				Count after 6 days at 5°C			
	Total	Coliform	Entero-cocci	Staphy-lococci	Total	Coliform	Entero-cocci	Staphy-lococci
1	1.0×10^8	1.3×10^5	23	<10	1.4×10^8	1.0×10^5	250	1.0×10^4
2	6.4×10^4	1.8×10^2	43	<10	1.2×10^8	2.1×10^5	460	3.6×10^4
3	5.6×10^7	3.1×10^5	33	1.3×10^3	2.2×10^9	1.8×10^8	94	3.5×10^4
4	1.5×10^7	1.1×10^5	140	150	6.5×10^8	8.3×10^6	95	4.0×10^3
5	1.8×10^7	2.3×10^4	130	1.0×10^3	3.6×10^8	1.6×10^7	70	2.4×10^3
6	2.9×10^5	4.8×10^1	43	<10	1.3×10^8	1.2×10^5	68	1.0×10^4

Table 4. Microbial counts per gram of unirradiated and irradiated freshly ground beef and after storage at 5°C

Radiation dose	Total Count			Coliform			Enterococci			Staphylococci		
	Storage time (days)			Storage time (days)			Storage time (days)			Storage time (days)		
	0	2	6	0	2	6	0	2	6	0	2	6
0	1.1×10^5	3.7×10^6	1.3×10^9	1.1×10^2	4.5×10^2	2.2×10^2	44	67	51	20	30	<10
34 Krads	1.8×10^4	3.0×10^4	5.7×10^7	<10	<10	13	<10	<10	<10	<10	<10	<10
68 Krads	2.8×10^3	5.5×10^3	2.6×10^6	<10	<10	<10	<10	32	14	<10	<10	<10

Table 5. Predominant groups of bacteria in unirradiated and irradiated high quality ground beef and the changes occurring on storage at 5°C

Group	Fresh			After storage for 6 days at 5°C		
	Radiation dose			Radiation dose		
	0	34 Krads	68 Krads	0	34 Krads	68 Krads
Gram-positive non-sporeforming rods	38[1]	19	6	44	86	98
Bacilli	2	0	2	2	2	0
Micrococci	44	79	90	2	2	0
Streptococci	2	2	2	0	10	0
Coliform	0	0	0	9	0	0
Pseudomonads	14	0	0	44	0	2

[1]The figures represent per cent of isolates based on 147 picked from fresh product and 176 picked from the samples stored for 6 days.

Table 6. The comparative effect of gamma radiation with and without attenuation of 10.6 cm of ground beef on the destruction of microorganisms

Trial	Initial count		Counts per gram after irradiation			
			Coliforms		Total Count	
	Coliform	Total	Unattenuated	Attenuated	Unattenuated	Attenuated
3/17	2,300,000	77,000,000	3,300	4,500	1,300,000	1,600,000
3/20	16,000,000	50,000,000	6,500	12,000	1,800,000	1,200,000
3/26	450,000	7,600,000	360	470	100,000	150,000
3/31	32,000,000	170,000,000	130,000	120,000	2,500,000	1,900,000
4/9	2,800	20,000,000	15	5	780,000	710,000

Table 8. The nature and location of microbial contamination in unirradiated beef

	0 days				2 days			
	Per cent of isolates				Per cent of isolates			
	Control	1st wash	2nd wash	Ground	Control	1st wash	2nd wash	Ground
Gram positive non-sporeforming rods	38	27	33	27	17	30	25	27
Bacilli	0	0	5	2	3	3	0	3
Micrococci	22	15	23	22	12	0	0	3
Streptococci	0	5	0	0	0	0	0	5
Coliforms	2	7	5	10	3	20	25	5
Pseudomonads	28	31	27	33	62	45	50	57
Gram-negative cocci	10	15	7	7	3	3	0	0
No. of isolates studied	60	59	60	60	40	40	40	40

	4 days				6 days			
	Per cent of isolates				Per cent of isolates			
	Control	1st wash	2nd wash	Ground	Control	1st wash	2nd wash	Ground
Gram-positive non-sporeforming rods	20	10	33	43	15	15	28	38
Bacilli	3	0	0	0	5	3	0	0
Micrococci	0	0	0	0	3	0	0	0
Streptococci	0	0	0	0	0	0	0	5
Coliforms	3	3	0	3	5	8	3	3
Pseudomonads	70	87	67	53	73	75	70	55
Gram-negative cocci	3	0	0	0	0	0	0	0
No. of isolates studied	30	30	30	30	40	40	40	40

Table 7. Recovery of microorganisms from fresh beef and from samples stored at 5°C.

Storage days	Microorganisms per gram			
	Control sample	Washed samples		After grinding
		1st	2nd	
0	690,000	320,000	98,000	180,000
2	8,700,000	2,300,000	1,600,000	3,300,000
4	590,000,000	220,000,000	65,000,000	70,000,000
6	590,000,000	660,000,000	140,000,000	110,000,000

Table 9. The effect of storage at 5°C on the recovery of microorganisms from beef irradiated at 68 Krads.

Storage days	Microorganisms per gram			
	Control sample	Washed samples		
		1st	2nd	After grinding
0	100,000	45,000	14,000	7,700
2	170,000	44,000	13,000	32,000
4	380,000	61,000	35,000	67,000
6	2,400,000	750,000	460,000	1,000,000

Table 10. The nature and location of microbial contamination in beef irradiated at 68 Krads

	0 days				2 days			
	Per cent of isolates				Per cent of isolates			
	Control	1st wash	2nd wash	Ground	Control	1st wash	2nd wash	Ground
Gram-positive non-sporeforming rods	0	3	3	0	7	5	5	8
Bacilli	0	0	0	0	0	0	0	0
Micrococci	28	15	13	15	7	10	8	0
Streptococci	0	0	0	0	0	3	0	0
Coliforms	3	0	3	8	3	8	13	13
Pseudomonads	5	8	16	8	7	13	23	30
Gram-negative cocci	64	75	66	70	77	63	53	50
No. of isolates studied	39	40	38	40	30	40	40	40

	4 days				6 days			
	Per cent of isolates				Per cent of isolates			
	Control	1st wash	2nd wash	Ground	Control	1st wash	2nd wash	Ground
Gram-positive non-sporeforming rods	63	43	59	70	77	77	84	83
Bacilli	0	0	0	3	0	0	0	0
Micrococci	0	10	14	3	0	0	0	0
Streptococci	0	0	0	0	0	0	3	0
Coliforms	0	3	7	7	5	8	5	10
Pseudomonads	19	10	14	3	18	15	8	8
Gram-negative cocci	19	33	7	13	0	0	0	0
No. of isolates studied	27	30	29	30	39	39	38	40

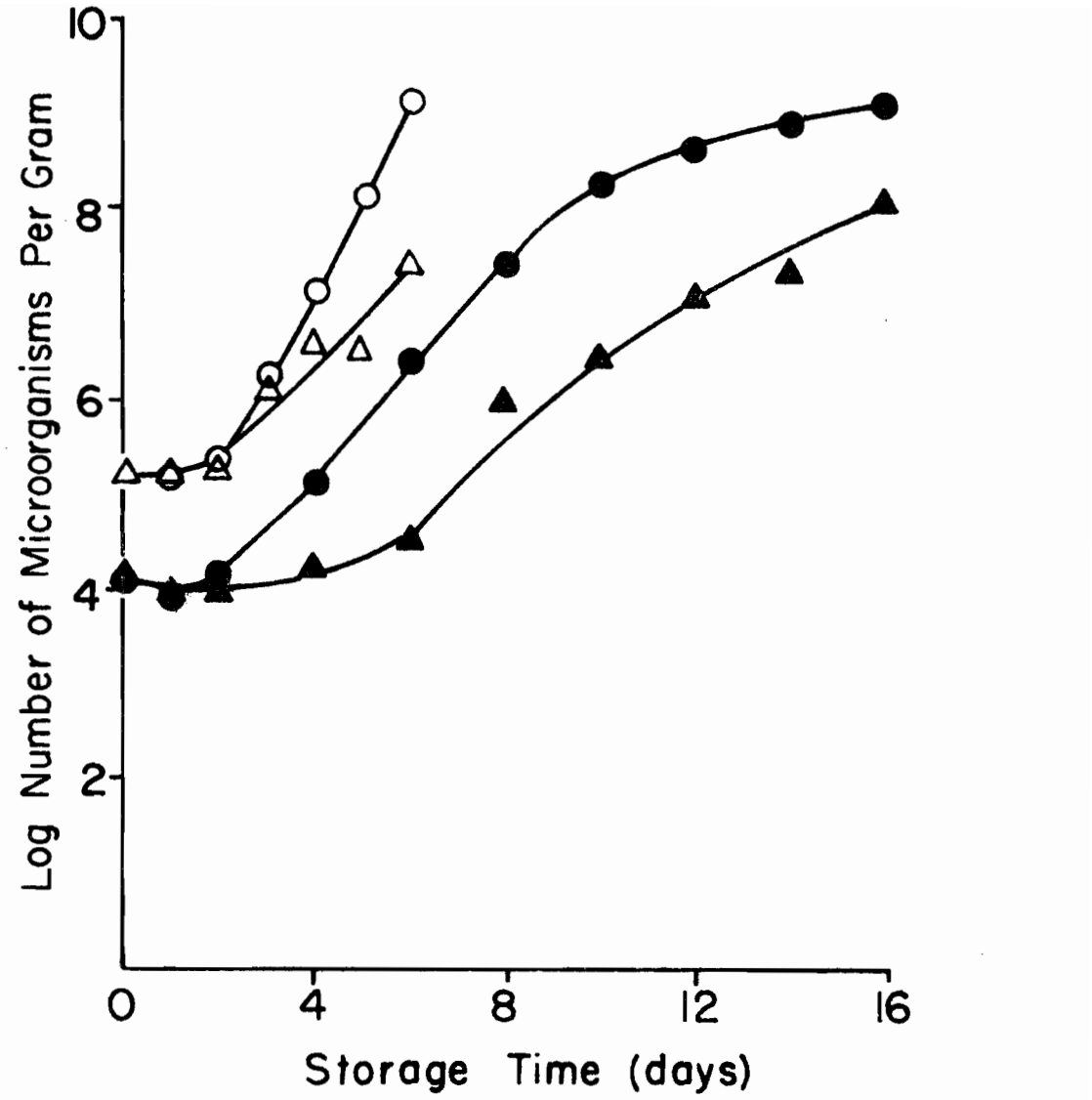

Figure 1. Total bacterial count of unirradiated and irradiated (68 Krads) ground beef stored at 2°C and 5°C.

Symbols: o——o Unirradiated stored at 5°C

Δ——Δ Unirradiated stored at 2°C

o——o Irradiated stored at 5°C

Δ——Δ Irradiated stored at 2°C

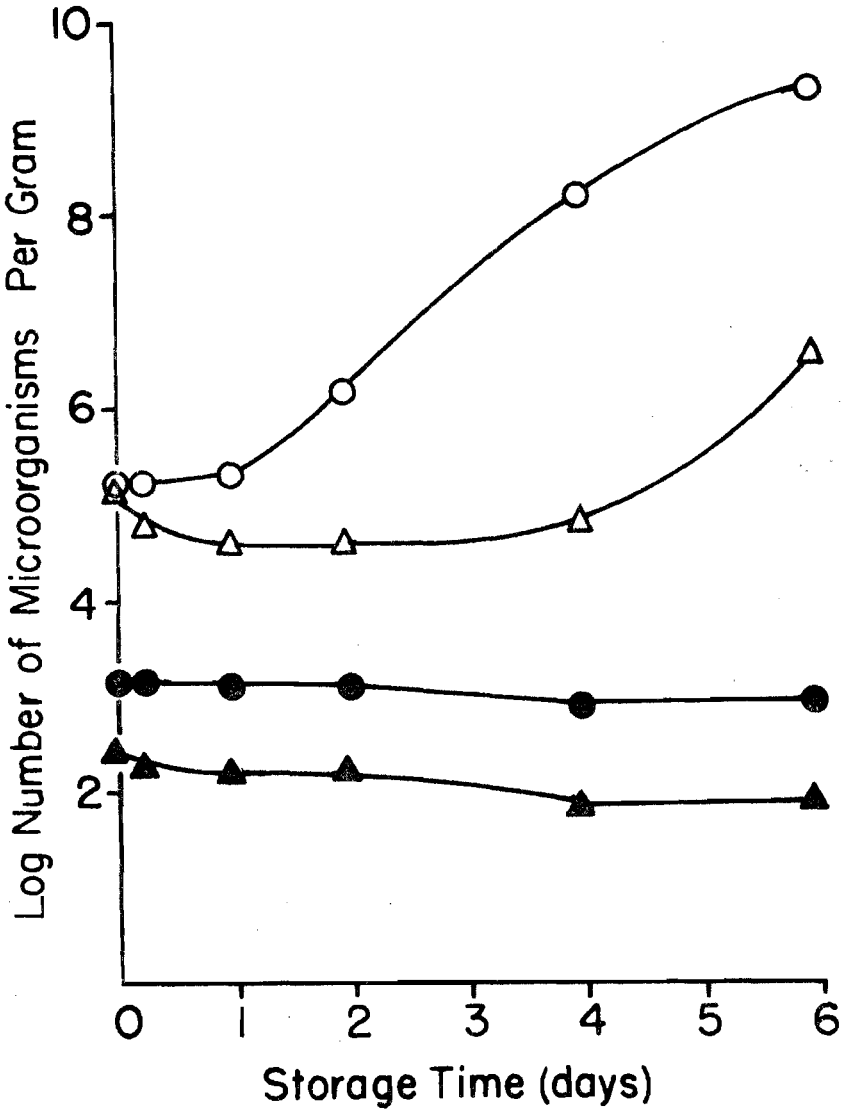

Figure 2. Fate of S. typhimurium and the total microflora at 5°C in unirradiated and irradiated (68 Krads) ground beef.

Symbols: o——o Total count of unirradiated sample

Δ——Δ Total count of irradiated sample

o——o S. typhimurium count of unirradiated sample

Δ——Δ S. typhimurium count or irradiated sample

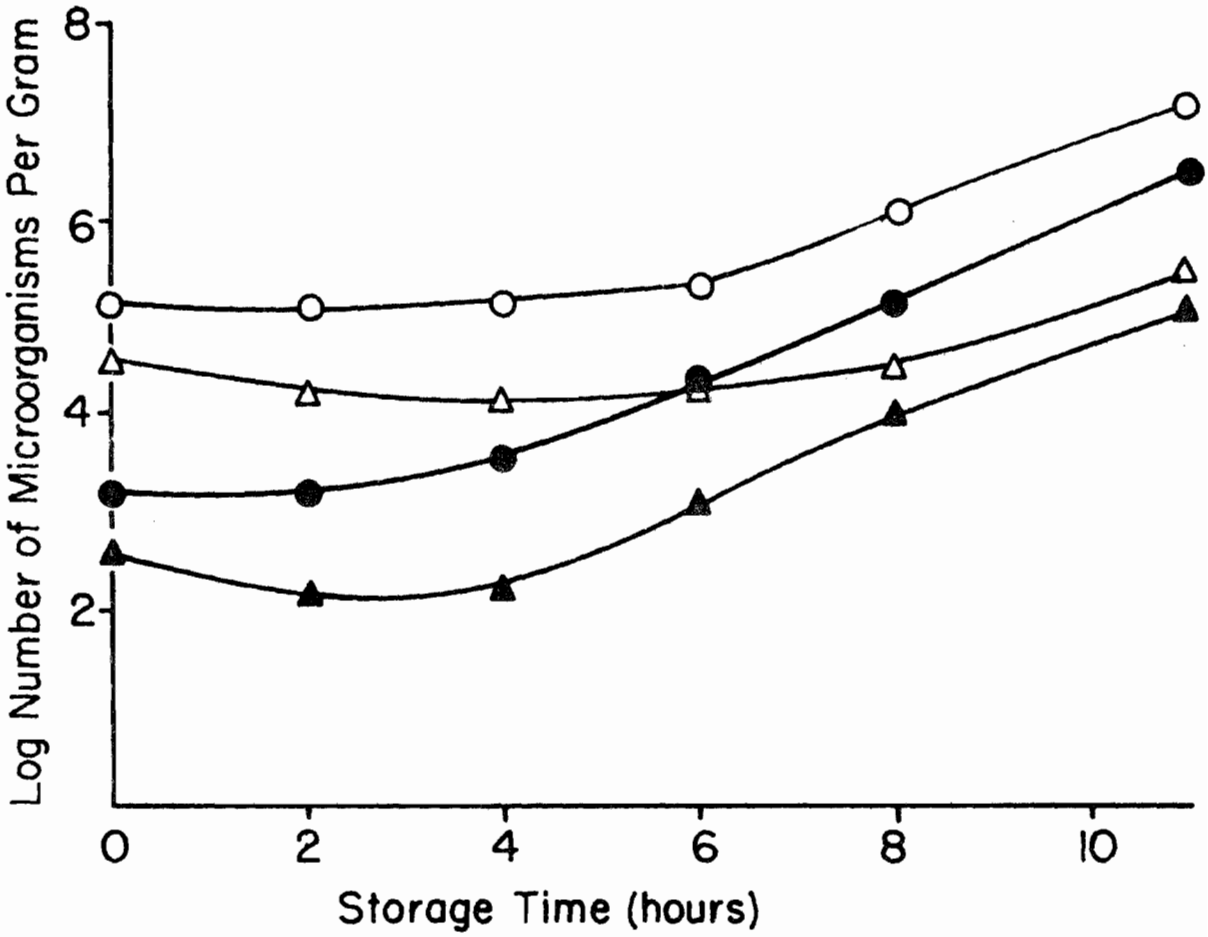

Figure 3. Growth of S. typhimurium and the total microflora at 25°C in unirradiated and irradiated (68 Krads) ground beef. Symbols:

o———o Total count of unirradiated sample

Δ———Δ Total count of irradiated sample

o———o S. typhimurium count in unirradiated sample

Δ———Δ S. typhimurium count in irradiated sample

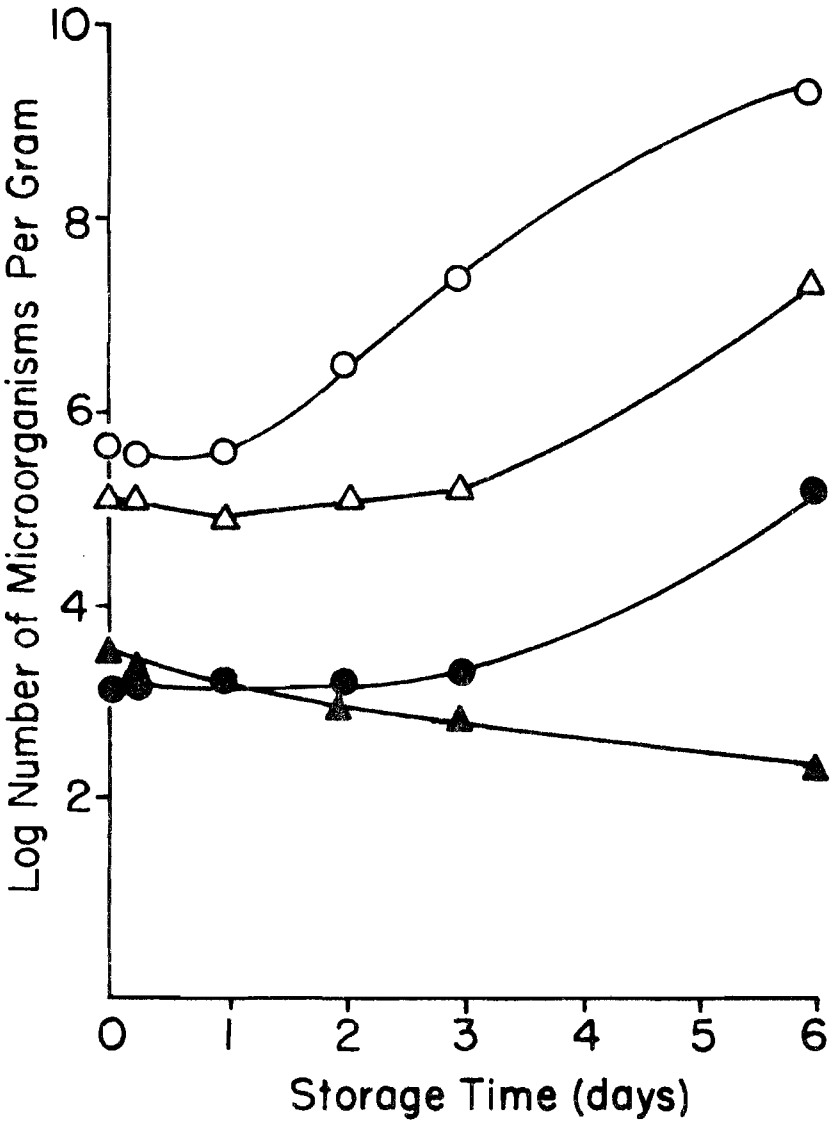

Figure 4. Fate of E. coli and the total microflora at 5°C in unirradiated and irradiated (68 Krads) ground beef.

Symbols:

o———o Total count of unirradiated sample

Δ———Δ Total count of irradiated sample

o———o E. coli count of unirradiated sample

Δ———Δ E. coli count of irradiated sample

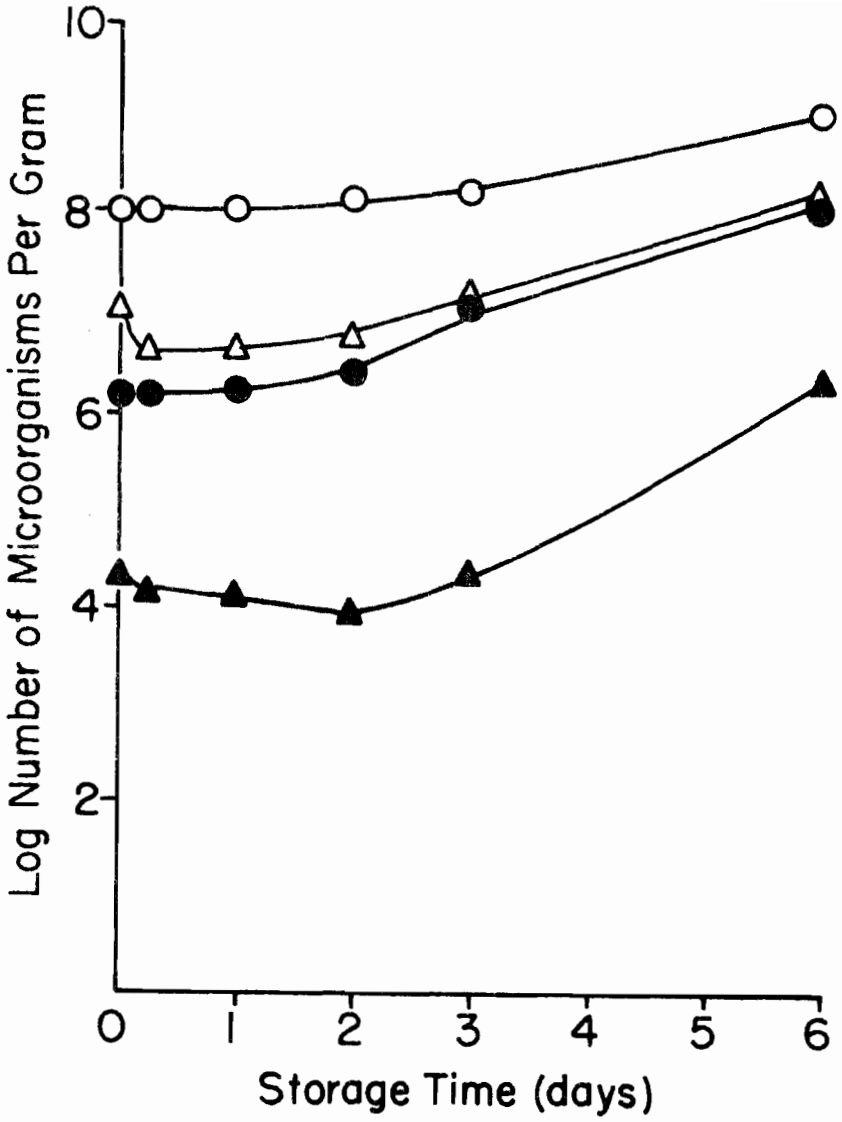

Figure 5. Growth of coliforms and total microflora at 5°C in unirradiated and irradiated (68 Krads) ground beef.

Symbols:

o——o Total count of unirradiated sample

Δ——Δ Total count of irradiated sample

o——o Coliform count of unirradiated sample

Δ——Δ Coliform count of irradiated sample

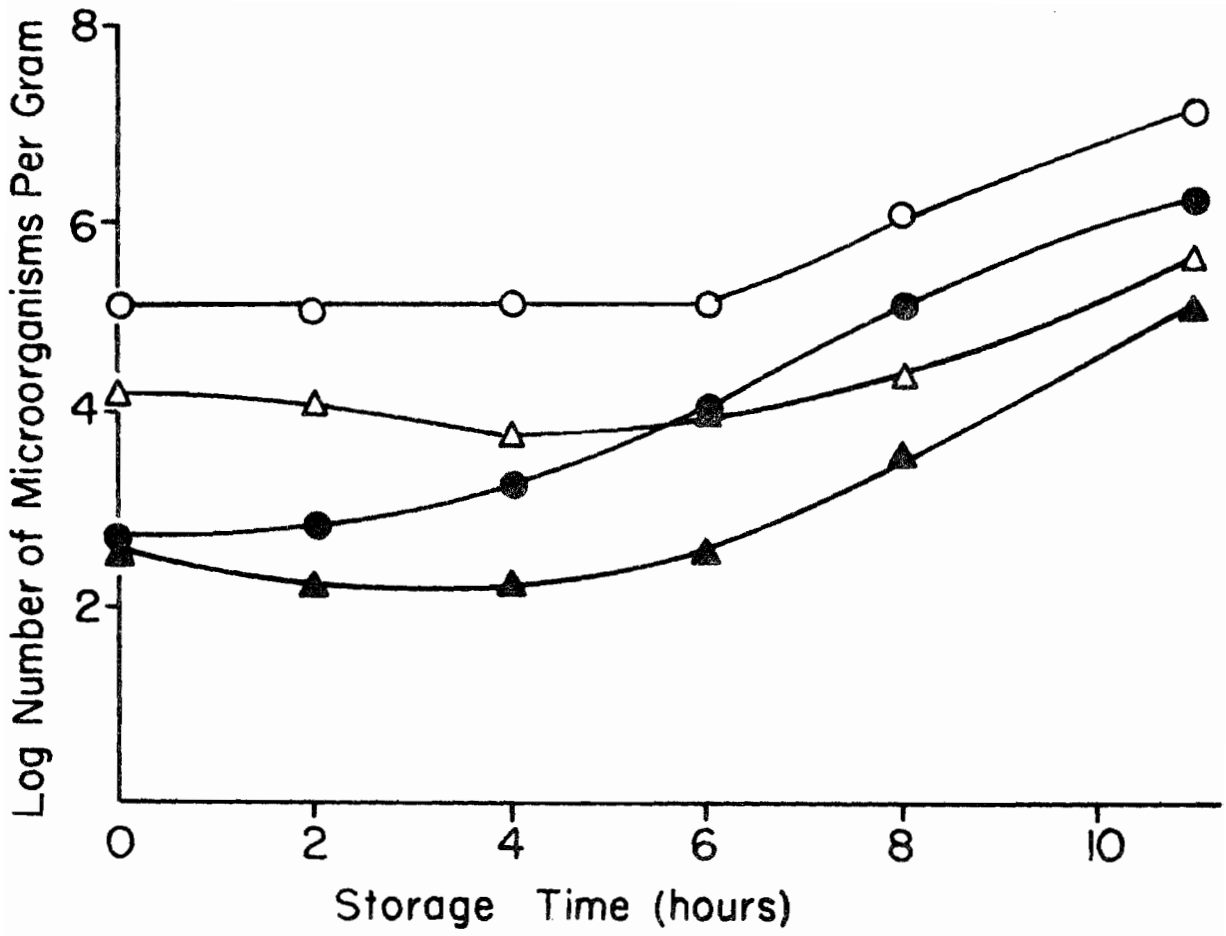

Figure 6. Growth of E. coli and total microflora at 25°C in unirradiated and irradiated (68 Krads) ground beef.

Symbols:

o——o Total count of unirradiated sample

Δ——Δ Total count of irradiated sample

o——o E. coli count of unirradiated sample

Δ——Δ E. coli count of irradiated sample

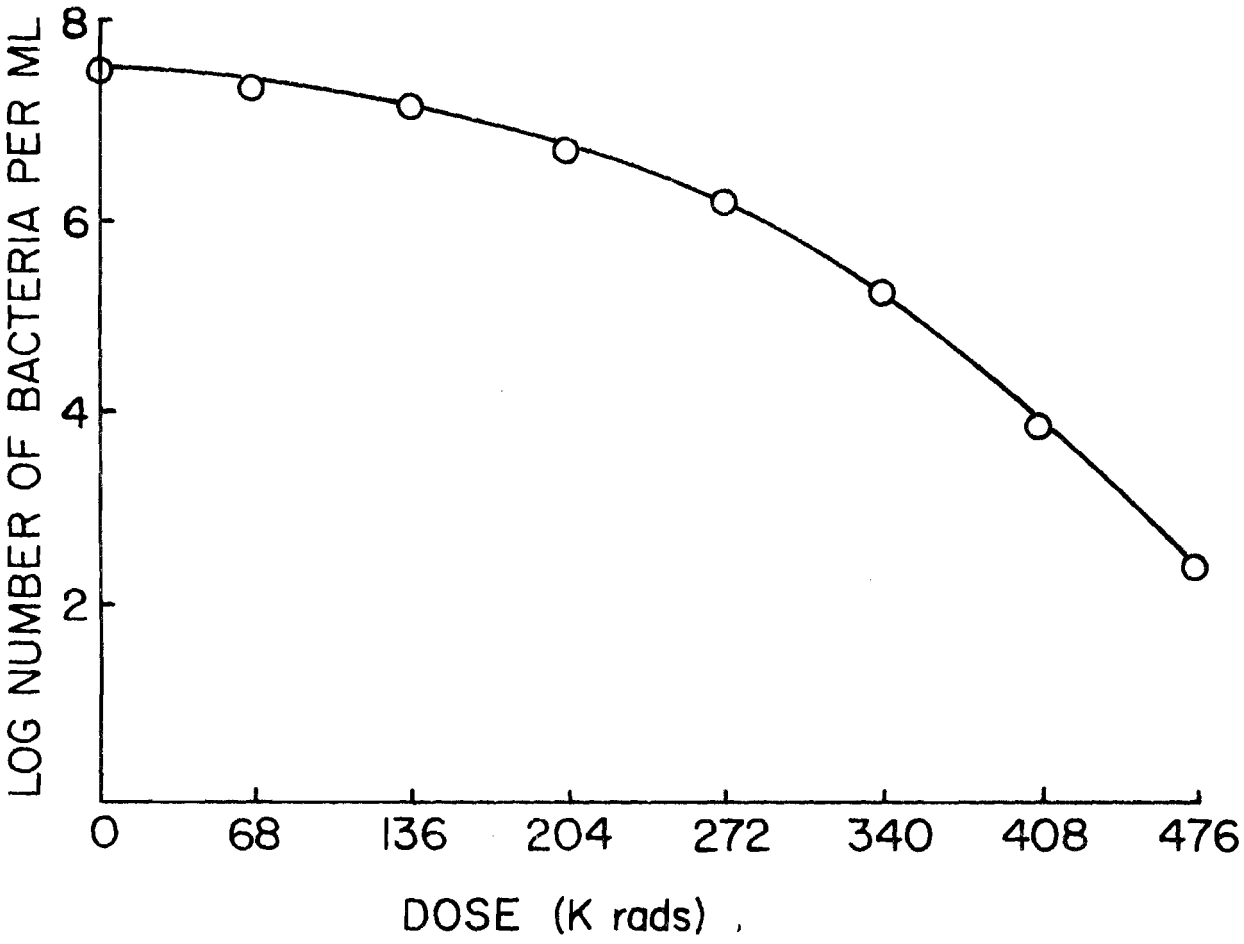

Figure 7. The effect of dose level on the destruction of a pure culture of gamma radiation resistant bacterium isolated from ground beef.