AD

TECHNICAL REPORT

NATICK/TR-81-005

INJURY AND DESTRUCTION OF MORAXELLA - ACINETOBACTER IN THE RADAPPERTIZATION PROCESS

by

R. B. Maxcy

D. B. Rowley

DAAK60-78-C-0039

February 1981

UNITED STATES ARMY
NATICK RESEARCH and DEVELOPMENT LABORATORIES
NATICK, MASSACHUSETTS 01760

Food Sciences Laboratory

FSL

Approved for public release; distribution unlimited.

Citation of trade names in this report does not constitute an official indorsement or approval of the use of such items.

Destroy this report when no longer needed. Do not return it to the originator.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER NATICK/TR-81/005	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* INJURY AND DESTRUCTION OF MORAXELLA-ACINETOBACTER IN THE RADAPPERTIZATION PROCESS		5. TYPE OF REPORT & PERIOD COVERED Final - April 78 through 1 May 80 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) R. B. Maxcy and D. B. Rowley		8. CONTRACT OR GRANT NUMBER(s) DAAK60-78-C-0039
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Nebraska, Dept. of Food Service and Technology Lincoln, Nebraska 68583		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 1L162724AH99 6.2 DB054
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Natick Research and Development Labs. ATTN: DRDNA-YMM Natick, MA 01760		12. REPORT DATE 23 May 80 13. NUMBER OF PAGES 24
14. MONITORING AGENCY NAME & ADDRESS*(if different from Controlling Office)*		15. SECURITY CLASS. *(of this report)* Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Irradiation	Salt sensitivity	Freezing
Radiation resistance	Food preservation	Thawing
Heat resistance	Stability	Meat(s)
Injury	Radappertization	

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Some highly radiation-resistant Moraxella-Acinetobacter (M-A) may survive the radappertization process for meat preservation, because these vegetative bacteria are more resistant than spores to radiation. They are, however, more susceptible than spores to other destructive factors. This work was to determine the effect of some environmental factors that influence the radappertization process. M-A, M. radiodurans, and B. cereus spores varied greatly in their response to changes in temperature of radiation and menstruum in which they were suspended. Available water was critical in response of vegetative cells to

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

radiation. Salts at the level incorporated into meat for the radappertization process suppressed growth of both injured and uninjured M-A. This effect was attributed to reduction in water activity of the menstruum. Freezing and thawing of M-A indicated some destruction and some injury. The injured cells recovered during subsequent incubation. Thus, specific food products and conditions of radappertization must be considered for setting processing parameters.

When all the factors of injury, destruction, and suppression of microbial growth are considered in the radappertization process, it is apparent there is little likelihood any of the low number of naturally occurring M-A cells would survive. If they survive the initial treatments, constraints in the environment would not allow their growth.

PREFACE

The potential for radappertization of meats is well established, and the usefulness of this preservation of fresh-like quality without refrigeration is apparent. The few remaining challenges to the widespread adoption of radappertization include an understanding of the role of highly radiation-resistant vegetative bacteria in the process. Bacteria of this type were isolated for further study (Welch and Maxcy, 1975) and their radiation resistance was determined (Maxcy et al., 1978). The results in this report constitute a continuation of observations on the nature and potential significance of the highly radiation-resistant bacteria in the overall process of radappertization preservation of meats. Environmental factors must be considered to evaluate radiation injury, destruction, and suppression of growth of microorganisms. Results showing the effect of critical environmental factors are given in this report.

TABLE OF CONTENTS

ILLUSTRATIVE DATA

INJURY AND DESTRUCTION OF MORAXELLA-ACINETOBACTER IN THE RADAPPERTIZATION PROCESS

INTRODUCTION

Some species of asporogenous bacteria of the Moraxella-Acinetobacter group, commonly termed M-A, are highly radiation resistant.[1,2] Their resistance to radiation is greater than the resistance of spores. These bacteria, on the other hand, are considerably more sensitive than spores to other destructive factors such as chemicals and heat.[3,4]

There are factors other than radiation that are involved in destruction, injury, and suppression of growth in the process of radappertization described by Anellis et al. (1979).[5] The highly radiation-resistant M-A are susceptible to injury when heated at 72°C for 5 to 10 min. The injury is apparently independent of the death process, and in the injured state they may be intolerant of unfavorable environmental factors (those factors having a direct effect on a cell) such as the presence of glucose[6] and sodium chloride.[7] A high proportion of injured

[1]Welch, A. B., and R. B. Maxcy. 1975. Characterization of radiation-resistant vegetative bacteria in beef. Appl. Microbiol. 30: 242.

[2]Maxcy, R. B., D. B. Rowley, and A. Anellis. 1978. Radiation and heat resistance of Moraxella-Acinetobacter in meats. T.R. 78-010. U.S. Army, Natick Research and Development Command, Natick, Mass.

[3]Gulistani, A. W. 1977. The ecology and resistance of Moraxella-Acinetobacter. Ph.D. Thesis, University of Nebraska-Lincoln.

[4]Firstenberg-Eden, R., D. B. Rowley, and E. Shattuck. 1980. The thermal inactivation and injury of Moraxella-Acinetobacter cells in beef. Appl. Environ. Microbiol. 39: 159.

[5]Anellis, A., D. B. Rowley, and E. W. Ross, Jr. 1979. Microbiological safety of radappertized beef. J. Food Prot. 42: 927.

[6]Bruns, M. A., and R. B. Maxcy, 1978. Effect of selected solutes on growth and recovery of a radiation-resistant Moraxella sp. J. Food Sci. 43: 1386.

[7]Maxcy, R. B., and D. B. Rowley. 1978. Radiation-resistant vegetative bacteria in a proposed system of radappertization of meats, pp. 347-359. In "Food Preservation by Irradiation." Vol. 1. International Atomic Energy Agency, Vienna.

cells in relation to the total population is obtained when a culture is heated to destroy 90-99% of the total population.

The magnitude of effectiveness of factors other than radiation, individually and collectively, in controlling microorganisms in the radappertizing process is not known. For example, the highly radiation-resistant M-A have been shown to be sensitive to solute concentration equivalent to the water activity in fresh meat.[8] Furthermore, sodium chloride and other yet unidentified dialyzable substances in Tryptic Soy Agar were responsible for slow growth and reduced recovery of M-A after irradiation.[9] These may be important environmental factors in preservation of meat by radappertization.

This project was to determine the effect of factors associated with radiation injury, destruction, and suppression of microorganisms in radappertization preservation of meats.

MATERIALS AND EXPERIMENTAL PROCEDURES

Cultures

Two isolates of Moraxella-Acinetobacter (M-A Isolate 4 and M-A Isolate 7)[10] as well as Micrococcus radiodurans ATCC 13939 were used. These cultures were propagated in m-Plate Count Broth (PCB; Difco) at 32°C on a rotary shaker with storage at 5°C. Bacillus cereus was obtained from Dr. J. H. Martin of South Dakota State University, and the spores were produced by the method of Martin and Harper (1963).[11]

[8] Snyder, L. D., and R. B. Maxcy. 1979. Effect of a_w of meat products on growth of radiation-resistant Moraxella-Acinetobacter. J. Food Sci. 44: 33.

[9] Bruns, M. A., and R. B. Maxcy. 1978. Effect of selected solutes on growth and recovery of a radiation-resistant Moraxella sp. J. Food Sci. 43: 1386.

[10] Welch, A. B., and R. B. Maxcy. 1975. Characterization of radiation-resistant vegetative bacteria in beef. Appl. Microbiol. 30: 242.

[11] Martin, J. H., and W. J. Harper. 1963. Germination response of Bacillus licheniformis spores to amino acids. J. Dairy Sci. 46: 663.

The general procedures for culture growth, dilution, plating, and counting were those outlined in American Public Health Association (1966)[12] and American Public Health Association (1976)[13] publications. Some alterations in these procedures were made and will be given in the section labeled Results.

Heat treatments for cell injury

Cultures of 5 ml in 15 x 125 mm test tubes were heated in a 72°C water bath for a "come up" time of 3.5 min plus 5 min for M-A Isolate 7 and 10 min for M-A Isolate 4. Approximately 90% of the colony forming units was destroyed in cultures that had grown to near their maximum population density of approximately 10^9/ml.

Evaluation of injury of cells in an M-A culture

Total counts of colony forming units were made on Plate Count Agar (PCA; Difco). Comparative counts were made on PCA with 0.5 or 0.8% sodium chloride added. The difference between counts on PCA plus sodium chloride was considered attributable to injured cells incapable of initiating growth in the presence of the added salt.[14,15]

Irradiation

A Cobalt-60 source similar to the one described by Teeny and Miyauchi (1970)[16] provided a dose rate of approximately 5 krad/min. Subfreezing temperature of irradiation was -30 ± 10°C. Ambient temperature of irradiation was approximately 30°C.

[12]American Public Health Association. 1966. "Recommended Methods for the Microbiological Examination of Foods." Ed. Sharf, J. M. 2nd ed. American Public Health Association, Inc., New York, N.Y.

[13]American Public Health Association. 1976. "Compendium of Methods for the Microbiological Examination of Foods." Ed. Speck, M. L. American Public Health Association, Washington, D.C.

[14]Maxcy, R. B., and D. B. Rowley. 1978. Radiation-resistant vegetative bacteria in a proposed system of radappertization of meats, pp. 347-359. In "Food Preservation by Irradiation." Vol. 1. International Atomic Energy Agency, Vienna.

[15]Firstenberg-Eden, R., D. B. Rowley, and E. Shattuck. 1980. The thermal inactivation and injury of Moraxella-Acinetobacter cells in beef. Appl. Environ. Microbiol. 39: 159.

[16]Teeny, F. M., and D. Miyauchi. 1970. Irradiation of Pacific Coast fish at sea. J. Milk Food Technol. 33: 330.

Evaluation of combinations of pentasodium tripolyphosphate (STPP) and sodium chloride

To determine relative resistance of M-A to sodium chloride and STPP, additions to exceed the tolerance of M-A were made to PCA and heat sterilized. The first medium was poured in a square petri dish to obtain a depth of approximately 1 mm on one side and 4 mm on the other. The second medium was added to obtain the same depth but in an opposite gradient direction. Finally, a gradient of PCA was poured at right angles to the previous gradients. The test culture was then surface inoculated and incubated for observation of visible growth.

Effect of sub-atmospheric pressure on the fate of M-A

A model system was used to maximize culture medium surface area yet allow available water for M-A growth. A culture to be observed therefore was inoculated into tubes of PCB from which 0.1 ml was transferred to a 15 by 125 mm sterile test tube which was then sealed in a flexible pouch in a vacuum of 4, 14, or 68 mm of mercury. A control sample inoculated in a similar manner and sealed in a flexible pouch at atmospheric pressure was included.

General

The results of each experiment were based on exploratory trials plus a minimum of two complete independent trials. Sufficient trials with M-A Isolate 4 and M-A Isolate 7 were made to assure the commonality of their behavior.

RESULTS

Comparative radiation resistance of M-A and M. radiodurans

The extremely high radiation resistance of certain M-A[17,18] indicated them to be far more resistant than the most radiation-resistant strains of Clostridium botulinum. Likewise, the M-A were more resistant than Micrococcus radiodurans

[17]Welch, A. B., and R. B. Maxcy. 1975. Characterization of radiation-resistant vegetative bacteria in beef. Appl. Microbiol. 30: 242.

[18]Maxcy, R. B., and D. B. Rowley. 1978. Radiation-resistant vegetative bacteria in a proposed system of radappertization of meats, pp. 347-359. In "Food Preservation by Irradiation." Vol. 1. International Atomic Energy Agency, Vienna.

according to results in the literature[19]. Direct comparisons of radiation resistance of M. radiodurans and M-A under identical conditions, however, had not been made.

Comparative radiation resistance of M-A Isolate 4, M-A Isolate 7, and M. radiodurans in ground beef at -30°C was determined. The results from an average of three trials are shown in Figure 1, where it is apparent that M. radiodurans is more resistant than the M-A under these conditions. The major differences between these results and those reported by Duggan et al. (1963b)[19] may have been attributable to menstruum and irradiation temperature. The effect of these factors is shown in Figure 2. Freezing provided a much greater protective effect than indicated by Duggan et al. (1963b)[19]. Temperature, menstruum

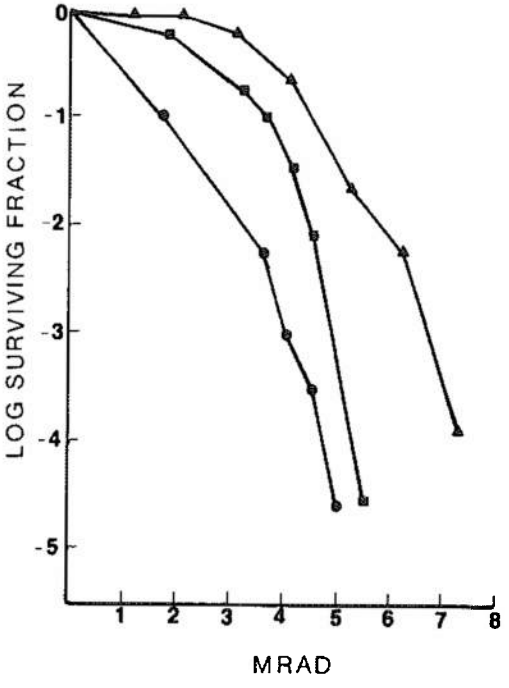

Figure 1. Comparative radiation resistance of M. radiodurans, M-A Isolate 4, and M-A Isolate 7 in beef at -30 ± 10°C. Symbols: ▲, M. radiodurans ■, M-A Isolate 7; ●, M-A Isolate 4.

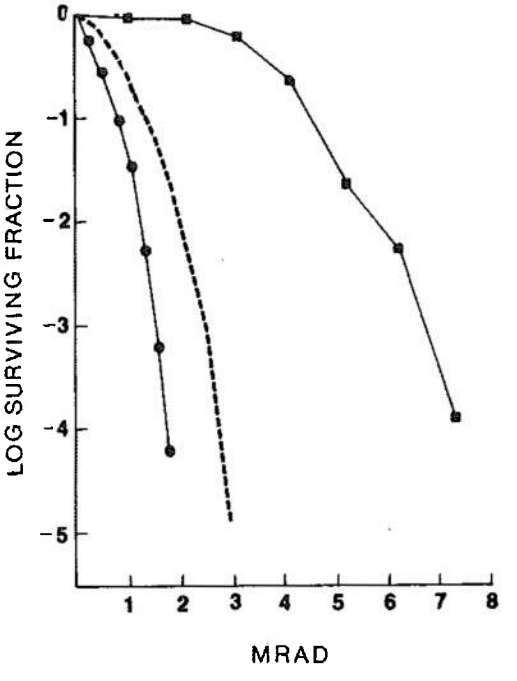

Figure 2. Comparative radiation resistance of M. radiodurans in beef. Symbols: ■, -30 ± 10°C; ●, 30 ± 10°C. Broken line is replotted for M. radiodurans in beef puree at 5°C (Fig. 1, Duggan et al., 1963a).

[19]Duggan, D. E., A. W. Anderson, and P. R. Elliker. 1963b. Inactivation of the radiation-resistant spoilage bacterium Micrococcus radiodurans. II. Radiation inactivation rates as influenced by menstruum temperature, preirradiation heat treatment, and certain reducing agents. Appl. Microbiol. 11: 413.

and its physical state are important factors in determining radiation resistance of bacteria as will be emphasized later.

Available water as a factor in radiation resistance of M-A

The radiation resistance of M-A lyophilized in PCB compared to radiation resistance in liquid and frozen medium is shown in Figure 3. The difference in radiation resistance of cultures in a frozen and an unfrozen state is much less in lyophilized PCB than in unlyophilized PCB.

The effect of lyophilization (Vitris Model No. 10/145 MRBA) with a prior heat treatment to destroy approximately 90% of the vegetative cells is shown in Figure 4. M-A, as well as M. radiodurans, were far more resistant than spores of B. cereus in the lyophilized state. Preheating prior to lyophilization increased the destructive effect of radiation of M-A and M. radiodurans. Heating of B. cereus spores for 15 min at 90°C prior to lyophilization had little, if any, effect on their sensitivity to radiation.

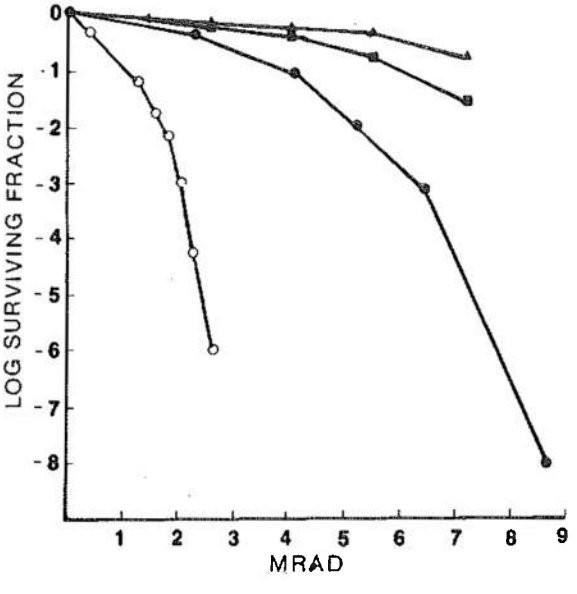

Figure 3. Comparative radiation resistance of M-A Isolate 4 in PCB and lyophilized in PCB before irradiation. Symbols: ○ in PCB at ambient temperature; ● in PCB at -30°C; ■ lyophilized in PCB at ambient temperature; ▲ lyophilized in PCB at -30°C.

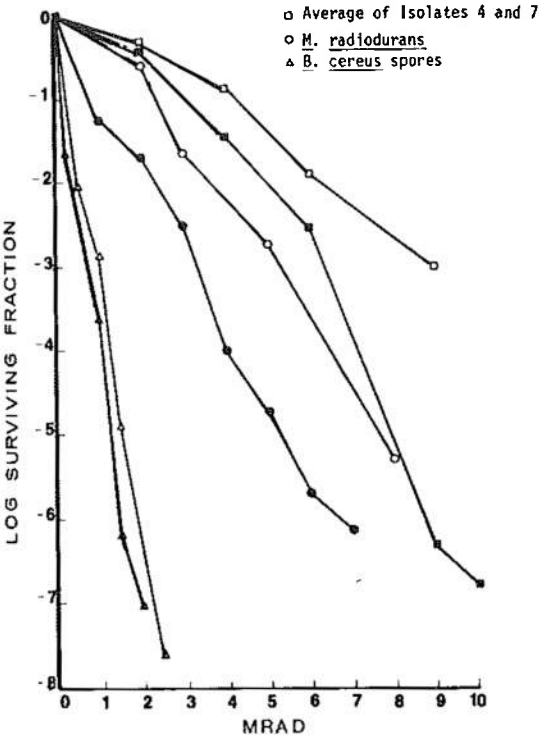

Figure 4. Comparative effect of radiation on lyophilized cultures that had been heat treated. Closed symbols represent cultures that were preheated prior to lyophilization. The heat treatments were: M-A Isolate 4, 10 min at 72°C; M-A Isolate 7, 5 min at 72°C; M. radiodurans, 3 min at 60°C; and B. cereus spores, 15 min at 90°C.

The data in Figure 5 show that water absorbed by the menstruum in 1 hr of exposure after lyophilization markedly reduced the resistance of M-A Isolate 4 to radiation.

Sensitivity of M-A to salts

The sensitivity of highly radiation resistant M-A to sodium chloride[20,21] and lowered water activity[22] is a factor of potential importance in radappertization preservation of meats. Phosphates may also be a factor as the data in Figure 6 indicate that pentasodium tripolyphosphate (STPP) of 0.3% or more auto-

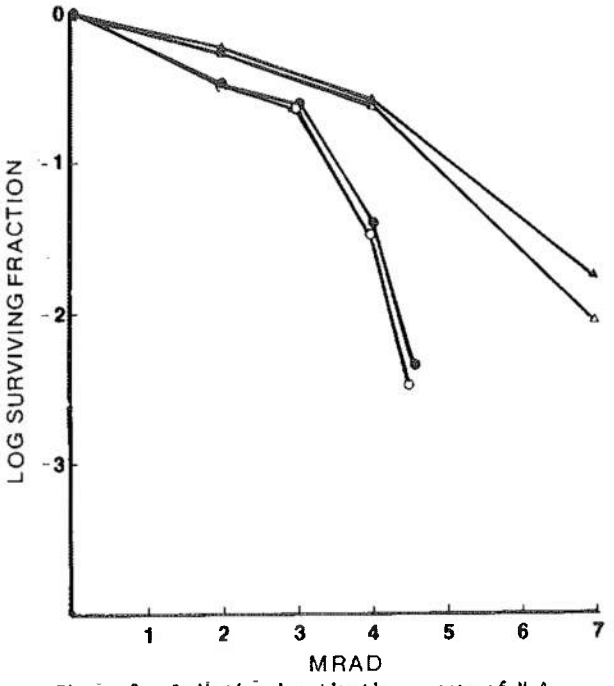

Figure 5. Radiation inactivation curves of M-A Isolate 4 lyophilized in PCB and subsequently exposed to a moist atmosphere for 1 hr at 25°C (○,●) and M-A Isolate 4 which was lyophilized in PCB but kept dry (△,▲). Closed symbols indicate recovery on PCA. Open symbols indicate recovery on PCA with 0.5% NaCl. Irradiation was at ambient temperature.

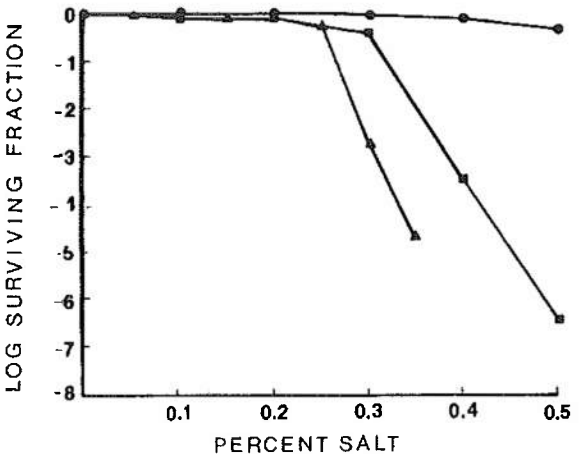

Figure 6. The effect of various concentrations of salts in PCA for enumeration of M-A Isolate 7 after incubation in PCB at 32°C for 24 hr on a shaker incubator. Symbols: ▲ , NaCl and STPP in equal concentrations; ■ , STPP; ● , NaCl.

[20]Maxcy, R. B., and D. B. Rowley. 1978. Radiation-resistant vegetative bacteria in a proposed system of radappertization of meats, pp. 347-359. In "Food Preservation by Irradiation." Vol. 1. International Atomic Energy Agency, Vienna.

[21]Firstenberg-Eden, R., D. B. Rowley, and E. Shattuck. 1980. The thermal inactivation and injury of Moraxella-Acinetobacter cells in beef. Appl. Environ. Microbiol. 39: 159.

[22]Snyder, L. D., and R. B. Maxcy. 1979. Effect of a_w of meat products on growth of radiation-resistant Moraxella-Acinetobacter. J. Food Sci. 44: 33.

claved in PCA suppresses growth of M-A Isolate 7. Figure 6 also shows that 0.3% STPP plus 0.3% NaCl was more inhibitory than either alone at the 0.3% level.

With STPP in PCB the fate of M-A Isolate 7 over a seven-day period at 32°C is shown in Figure 7. A concentration of approximately 0.3% STPP autoclaved in PCB appeared to be the approximate growth-limiting level for M-A Isolate 7. The effect of combinations of sodium chloride and STPP is shown in Figure 8. Inclusion of sodium chloride reduced the level of STPP that M-A Isolate 7 could tolerate (compare Figure 7 and Figure 8). The approximate maximum concentration of STPP that M-A Isolate 7 could tolerate appeared to be 0.3% in liquid media but somewhat more on solid media. The extreme sensitivity of the cultures could not be attributed to changes in pH, because the addition of phosphates altered the pH of the medium less than 0.2 unit from neutrality and the highly radiation resistant M-A grew over a pH range of 5.5 to 8.5. The reason for the greater sensitivity of M-A to STPP in PCB than in PCA is not apparent.

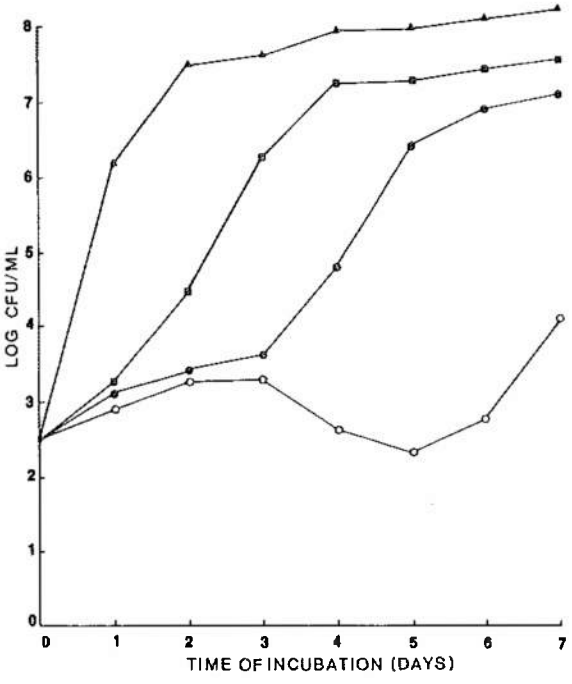

Figure 7. Effect of various concentrations of STPP in PCB on the growth rate of Isolate 7 at 32°C. Symbols: ▲, 0% STPP; ■, 0.2% STPP; ●, 0.25% STPP; ○, 0.3% STPP.

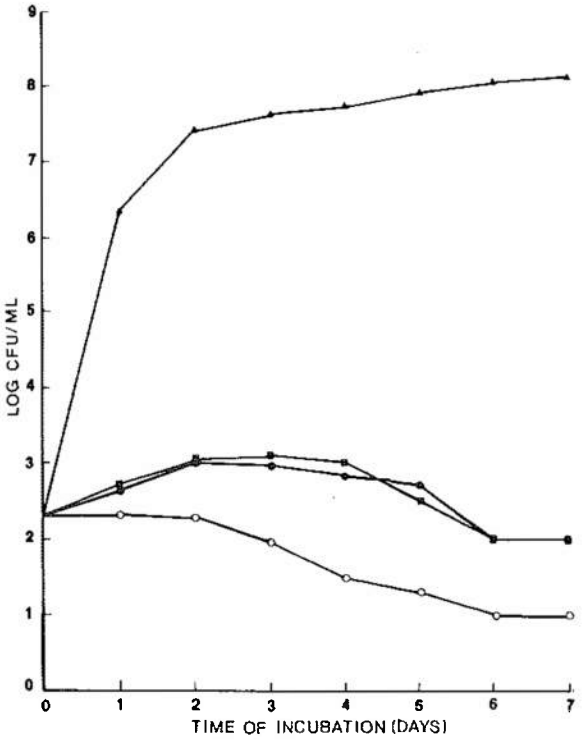

Figure 8. The combined effect of NaCl and STPP in PCB on the rate of growth of Isolate 7 at 32°C. Symbols: ▲, PCB; ■, 0.2% NaCl + 0.2% STPP; ●, 0.2% NaCl + 0.25% STPP; ○, 0.2% NaCl + 0.3% STPP.

By the use of a gradient plate technique it was possible to check for synergistic or antagonistic effects of combinations of STPP and sodium chloride. The limits of growth as judged by the slow appearance of small colonies was parallel to the gradient of solutes, therefore indicating the effect of the salts to be simply additive.

The above experiments utilized media with STPP added prior to heat sterilization. Heating may alter the form and thereby the effectiveness of some forms of phosphates. Comparisons therefore were made with filter sterilized and heat treated phosphates added to PCB. Tolerance was determined by visual observation for turbidity within 72 hr at 32°C on a shaker incubator. Positive tubes were streaked to check for contamination. The heat treatment of autoclaving or the lower heat treatment, which was comparable to the heat treatment in radappertization preservation, reduced the inhibitory effectiveness of STPP and disodium pyrophosphate but only autoclaving reduced the effectiveness of tetrasodium pyrophosphate (Table 1).

Table 1. Tolerance levels of M-A Isolate 7 in the presence of three different sodium phosphates in PCB prepared in various ways.

Treatment	Phosphates		
	Pentasodium tripolyphosphate (STPP)	Tetrasodium pyrophosphate	Disodium pyrophosphate
Filter sterilization	0.1%	0.1%	0.1%
Autoclaving	0.2-0.3%	0.2-0.3%	above 0.5%
Heating at 70°C for 9 hours after filtering*	0.2-0.3%	0.1%	0.2%

*Treatment to simulate heating in radappertization preservation of meat.

It should be noted that the previously reported results involved heat sterilization of STPP in the media. Therefore, if less severe heat treatments with STPP in the media were used, the results would be expected to show M-A to be less tolerant of STPP.

Effect of sub-atmospheric pressure on the fate of M-A

Cultures of M-A were grown to near maximum population density, heat shocked to destroy approximately 90% of the cells, and divided into aliquots of 5 ml per tube. The tubes were centrifuged to pellet the cells, which were then washed with 5 ml of fresh PCB before recentrifuging and decanting. Control tubes with pellet were maintained at atmospheric pressure. Other tubes were evacuated to 68, 14, and 4 mm of mercury, sealed in air impervious flexible pouches, and incubated for 6 or 24 hr at 32°C. None of the samples showed a change in population density as measured by counts with PCA, and there was only slight recovery from injury as measured by counts with PCA plus sodium chloride.

Effect of freezing and thawing on M-A

Heat shocked cultures were divided into two groups. One group was frozen immediately after heat shock and the other was incubated 4 hr at 32°C before freezing. The results showed that more than one log cycle of colony forming units as determined on PCA was destroyed by freezing and thawing (Figure 9).

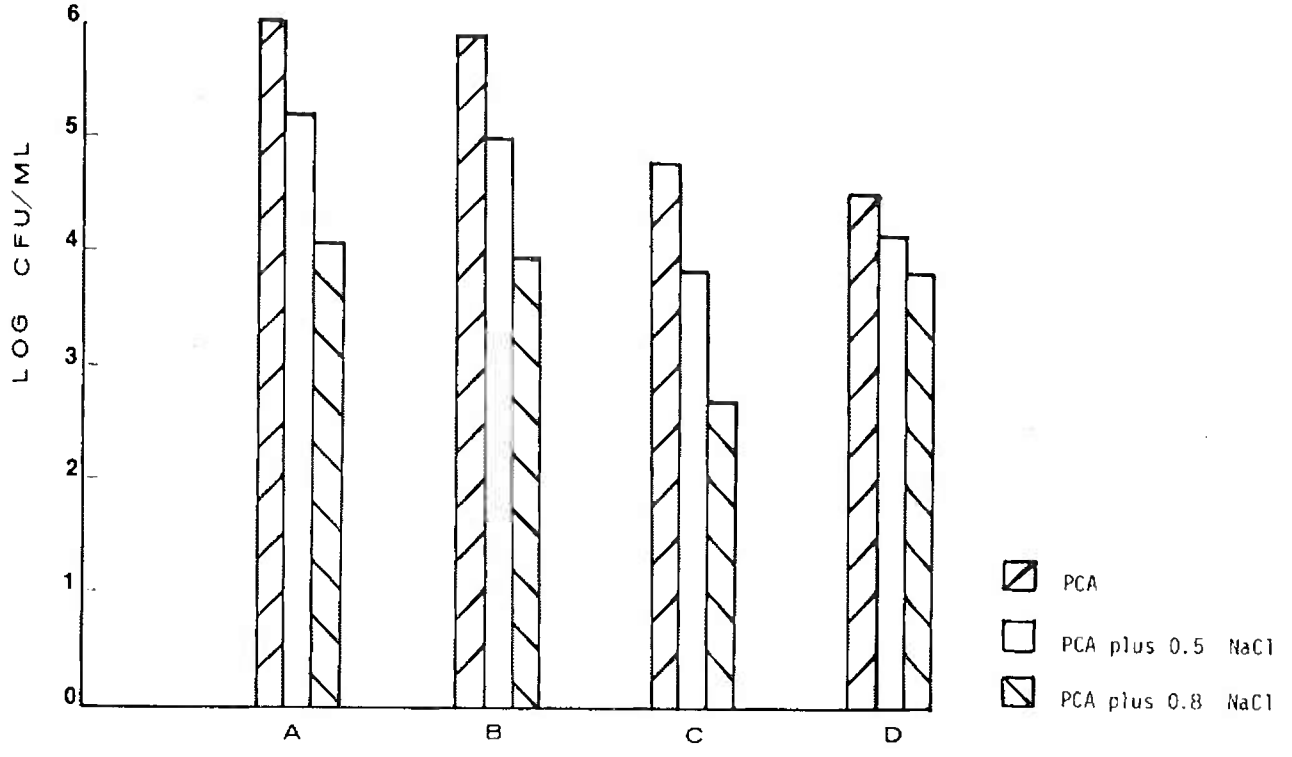

Figure 9. The effect of freezing and thawing on heat shocked M-A immediatel, and after 4 hr at 32°C. A, immediately after heat shock; B, after 4 hr at 32°C; C, frozen immediately after heat shock; D, frozen after 4 hr at 32°C.

A slightly greater proportion of the cells surviving freezing and thawing were injured as determined on PCA plus sodium chloride. Furthermore, cells given 4 hr to recover from heat shock were less susceptible to injury from freezing and thawing. Thus, freezing and thawing are of considerable significance in radappertization preservation of meats.

Destruction of heat shocked M-A during freezing and thawing and during irradiation

M-A that had grown to near the maximum population density in PCB were heated to destroy approximately 90% of the cells and diluted into fresh, sterile PCB. The cultures were incubated for 3 hr at 32°C and then observed for the effects of freezing and thawing as well as irradiation. Results shown in Figure 10 indicated

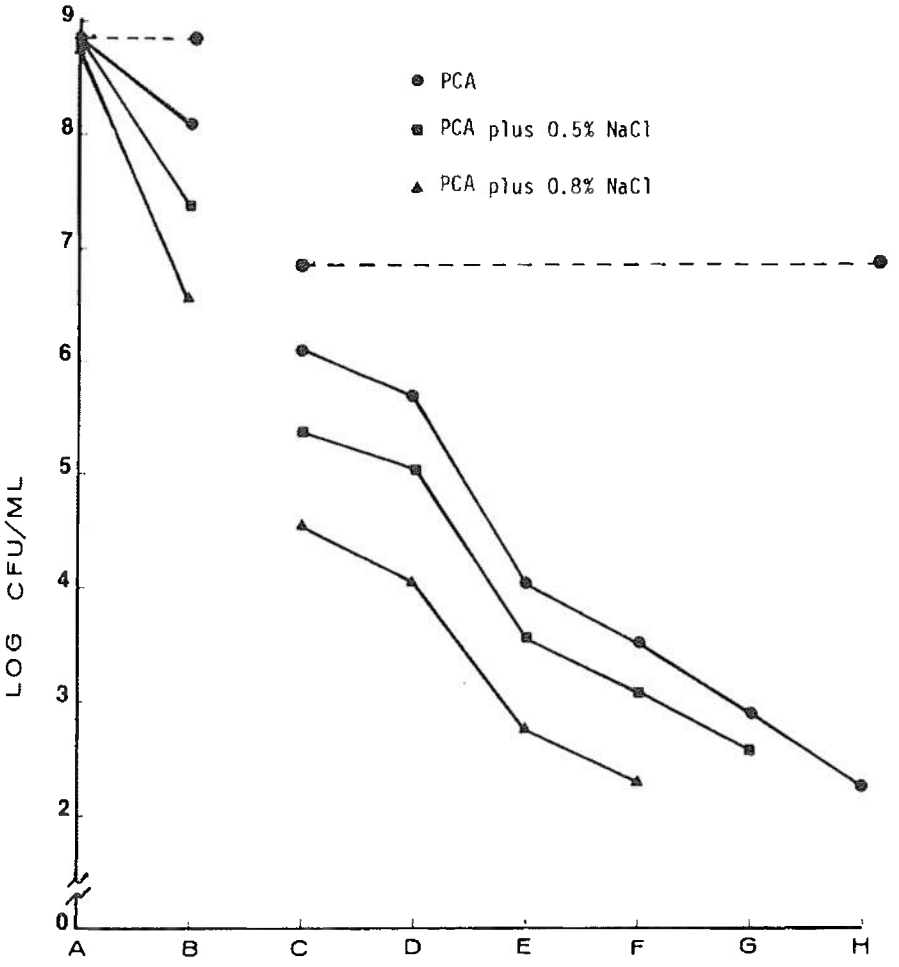

Figure 10. Response of M-A to heat shock, incubation for 3 hr at 32°C, freezing and thawing, and irradiation at approximately -30°C. A is pre-shock, B is post-shock, C is after dilution into fresh medium, D is after 3 hr incubation, E is after freezing and thawing, F is after 1 Mrad, G is after 2 Mrad, and H is after 3 Mrad. The broken line is a hypothetical control based on the expected results from other observations.

that the destructive effects of heat and transfer to a fresh medium persisted through incubation for 3 hr. These cells were sensitive to subsequent freezing and thawing and to radiation. It should be pointed out that a control of unheated cells would have shown insignificant destruction during the above treatments, as unheated cells are insensitive to these treatments.

The quantity of injured cells was greater when expressed as inability to initiate growth on PCA with 0.8% NaCl than on PCA with 0.5% NaCl. This difference persisted throughout subsequent treatments to simulate elements of the radappertization process such as quiescent incubation after heating, freezing and thawing, and irradiation at three dose levels.

When similar experiments were performed only extending the recovery period to 24 hr quiescent incubation at 32°C, injury and radiation resistance were at the level expected for unheated M-A cells (Figure 11). Most of the injured

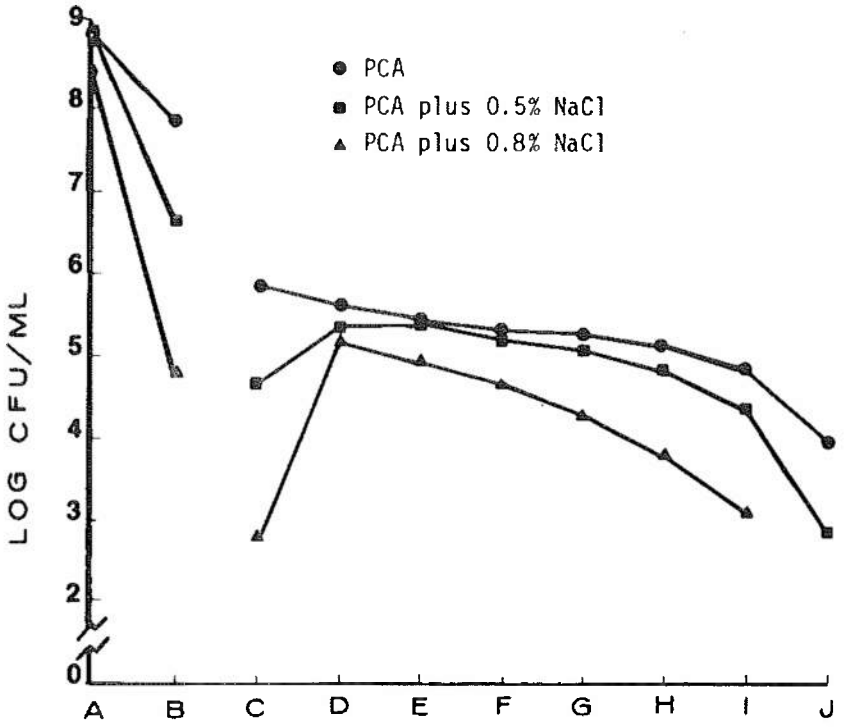

Figure 11. Response of M-A cells to heat shock, incubation for 24 hr at 32°C, freezing and thawing, and irradiation at approximately -30°C. A is pre-shock, B is post-shock, C is after dilution, D is 24 hr incubation, E is after freezing and thawing, F is 1 Mrad, G is 2 Mrad, H is 3 Mrad, I is 4 Mrad, and J is 5 Mrad.

cells recovered during the 24 hr incubation and regained ability to grow on PCA with added salt. There was also recovered resistance to radiation, and the culture regained a shoulder for the death curve.

DISCUSSION AND CONCLUSIONS

The highly radiation-resistant M-A may be a problem in radappertization of meat, if radiation resistance alone under certain conditions is considered. However, there are many bactericidal factors involved in the overall process of radappertization preservation. M-A are quite susceptible to injury, destruction, and suppression by factors other than radiation per se. Thus, the overall process must be considered for proper evaluation.

In the radappertization process, prior to freezing and irradiation, meat is heated to an internal temperature of not less than 73-77°C.[23] The process of heating and holding hot may involve up to 9 hr. Even a heat treatment of 1 hr at 70°C destroys about 8 log cycles of the radiation-resistant M-A.[24] Many survivors are injured and far more susceptible to radiation destruction.[25] The repair process is slow as there was no apparent repair in 3 hr at 32°C after heat injury (Figure 10). The injured cells also would be less tolerant of subsequent adverse conditions such as solutes in the menstruum.[26]

[23]Anellis, A., D. B. Rowley, and E. W. Ross, Jr. 1979. Microbiological safety of radappertized beef. J. Food Prot. 42: 927.

[24]Firstenberg-Eden, R., D. B. Rowley, and E. Shattuck. 1980. The thermal inactivation and injury of Moraxella-Acinetobacter cells in beef. Appl. Environ. Microbiol. 39: 159.

[25]Maxcy, R. B., and D. B. Rowley. 1978. Radiation-resistant vegetative bacteria in a proposed system of radappertization of meats, pp. 347-359. In "Food Preservation by Irradiation." Vol. I. International Atomic Energy Agency, Vienna.

[26]Bruns, M. A., and R. B. Maxcy. 1978. Effect of selected solutes on growth and recovery of a radiation-resistant Moraxella sp. J. Food Sci. 43: 1386.

Freezing and thawing destroy some M-A and injure others (Figure 9), therefore these phenomena also must be considered in evaluating radappertization preservation of meats.

When considering the resistance to radiation of M-A as well as M. radiodurans, the environment can be extremely important. For example, taking the data of Duggan et al. (1963a; 1963b)[27,28] on the effect of freezing on radiation resistance of M. radiodurans, the data of Welch and Maxcy (1975),[29] and the data of Maxcy and Rowley (1978),[30] one would conclude M-A to be more radiation resistant than M. radiodurans. When these bacteria are observed under the same conditions, however, M. radiodurans may be more radiation resistant (Figure 1) or less radiation resistant (Figure 4) than M-A. Such an environmental change as drying doesn't increase the radiation resistance of B. cereus spores to the level of radiation resistance expected for M-A at ambient temperature (Figures 2, 3 and 4). Thus, radiation resistance is a complex phenomenon and cannot be predicted from one condition to another.

The highly radiation-resistant M-A require a very high a_w as shown by their inability to grow in fresh meat or a laboratory medium with the equivalent a_w of

[27]Duggan, D. E., A. W. Anderson, and P. R. Elliker. 1963a. Inactivation of the radiation-resistant spoilage bacterium Micrococcus radiodurans. I. Radiation inactivation rates in three meat substrates and in buffer. Appl. Microbiol. 11: 398.

[28]Duggan, D. E., A. W. Anderson, and P. R. Elliker. 1963b. Inactivation of the radiation-resistant spoilage bacterium Micrococcus radiodurans. II. Radiation inactivation rates as influenced by menstruum temperature, preirradiation heat treatment, and certain reducing agents. Appl. Microbiol. 11: 413.

[29]Welch, A. B., and R. B. Maxcy. 1975. Characterization of radiation-resistant vegetative bacteria in beef. Appl. Microbiol. 30: 242.

[30]Maxcy, R. B., and D. B. Rowley. 1978. Radiation-resistant vegetative bacteria in a proposed system of radappertization of meats, pp. 347-359. In "Food Preservation by Irradiation." Vol. I. International Atomic Energy Agency, Vienna.

meat.[31] Meat in the radappertization preservation process would be more unfavorable than fresh meat for M-A, because approximately 0.75% NaCl and 0.38% STPP are added to meat for radappertization.[32] These solutes, as shown in this work, are additive in suppressing growth of M-A, though on a weight basis STPP is more than twice as effective as sodium chloride. Furthermore, the results of this work were primarily based on autoclave sterilization of STPP in the medium being used, while less severe heat treatments would increase the inhibitory effectiveness of STPP. It is highly improbable that a localized water activity high enough for these bacteria to grow would occur in radappertized meat, since the phosphate is added to prevent surface accumulation of moisture.

The numerous recognized factors of microbial injury, destruction, and suppression in radappertization preservation of meat combine to assure the absence of detectable M-A in meat so preserved.

[31] Snyder, L. D., and R. B. Maxcy. 1979. Effect of a_w of meat products on growth of radiation-resistant Moraxella-Acinetobacter. J. Food Sci. 44: 33.

[32] Anellis, A., D. B. Rowley, and E. W. Ross, Jr. 1979. Microbiological safety of radappertized beef. J. Food Prot. 42: 927.

REFERENCES

American Public Health Association. 1976. "Compendium of Methods for the Microbiological Examination of Foods." Ed. Speck, M. L. American Public Health Association, Inc., Washington, D.C.

American Public Health Association. 1966. "Recommended Methods for the Microbiological Examination of Foods." Ed. Sharf, J. M. 2nd ed. American Public Health Association, Inc., New York, N.Y.

Anellis, A., D. B. Rowley, and E. W. Ross, Jr. 1979. Microbiological safety of radappertized beef. J. Food Prot. 42: 927.

Bruns, M. A., and R. B. Maxcy. 1978. Effect of selected solutes on growth and recovery of a radiation-resistant Moraxella sp. J. Food Sci. 43: 1386.

Duggan, D. E., A. W. Anderson, and P. R. Elliker. 1963a. Inactivation of the radiation-resistant spoilage bacterium Micrococcus radiodurans. I. Radiation inactivation rates in three meat substrates and in buffer. Appl. Microbiol. 11: 398.

Duggan, D. E., A. W. Anderson, and P. R. Elliker. 1963b. Inactivation of the radiation-resistant spoilage bacterium Micrococcus radiodurans. II. Radiation inactivation rates as influenced by menstruum temperature, preirradiation heat treatment, and certain reducing agents. Appl. Microbiol. 11: 413.

Firstenberg-Eden, R., D. B. Rowley, and E. Shattuck. 1980. The thermal inactivation and injury of Moraxella-Acinetobacter cells in beef. Appl. Environ. Microbiol. 39: 159.

Gulistani, A. W. 1977. The ecology and resistance of Moraxella-Acinetobacter Ph.D. Thesis, University of Nebraska-Lincoln.

Martin, J. H., and W. J. Harper. 1963. Germination response of Bacillus licheniformis spores to amino acids. J. Dairy Sci. 46: 663.

Maxcy, R. B., D. B. Rowley, and A. Anellis. 1978. Radiation and heat resistance of Moraxella-Acinetobacter in meats. T.R. 78-010. U.S. Army, Natick Research and Development Command, Natick, Mass.

Maxcy, R. B., and D. B. Rowley. 1978. Radiation-resistant vegetative bacteria in a proposed system of radappertization of meats, pp. 347-359. In "Food Preservation by Irradiation." Vol. I. International Atomic Energy Agency, Vienna.

Snyder, L. D., and R. B. Maxcy. 1979. Effect of a_w of meat products on growth of radiation resistant Moraxella-Acinetobacter. J. Food Sci. 44: 33.

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

Welch, A. B., and R. B. Maxcy. 1975. Characterization of radiation-resistant vegetative bacteria in beef. Appl. Microbiol. 30:242.