1887

MASTER

COO-1689-6

**RADIATION PASTEURIZATION OF
FRESH MEATS AND POULTRY**

Annual Report, February 15, 1969--February 14, 1970

June 1971

Department of Food Science
Michigan State University
East Lansing, Michigan

UNITED STATES ATOMIC ENERGY COMMISSION • DIVISION OF TECHNICAL INFORMATION

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

This report has been reproduced directly from the best available copy.

Available from the National Technical Information Service, U. S. Department of Commerce, Springfield, Virginia 22151.

Price: Paper Copy \$3.00
Microfiche \$0.95.

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

COO-1689-6
RADIOISOTOPE AND RADIATION
APPLICATIONS (TID-4500)

RADIATION PASTEURIZATION OF
FRESH MEATS AND POULTRY

Prepared By:

Walter M. Urbain, George G. Giddings and William W. Ballantyne

DIVISION OF ISOTOPES DEVELOPMENT
UNITED STATES ATOMIC ENERGY COMMISSION

CONTRACT NUMBER

AT(11-1)-1689

ANNUAL REPORT
FOR THE PERIOD

February 15, 1969 through February 14, 1970

June 1971

Department of Food Science
Michigan State University
East Lansing, Michigan 48823

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED
Card

TABLE OF CONTENTS

	<u>Page:</u>
List of Tables and Figures	iii-iv
Abstract	v
Preface	vii
Summary	1
Introduction	3
Experimental Results	4
Eating Quality Evaluations	4
Phosphate Application Methods	23
Color Studies	31
Conclusions	52
Selected References	56

LIST OF TABLES AND FIGURES:

<u>Table No:</u>		<u>Page:</u>
1.	Effect of water dip and phosphate dip on eating quality of irradiated, unstored fresh beefsteaks	6
2.	Effect of phosphate, irradiation and refrigerated storage on eating quality of fresh beefsteaks	9
3.	Comparison of eating quality of irradiated fresh beefsteaks stored in vacuum with that of steaks stored in vacuum followed by aerobic storage	11
4.	Comparison of eating quality and lipid oxidation of irradiated beefsteaks stored in vacuum and stored in vacuum followed by aerobic storage	12
5.	Ditto	14
6.	Effect of ascorbic acid, alone and in combination with added tripolyphosphate, on quality of irradiated-stored fresh beefsteaks	16
7.	Ditto	18
8.	Ditto	21
9.	Comparison of two methods of application of phosphate solution to bulky cuts of beef; the dipping method and the gas entrainment pumping method	26
10.	Comparison of two methods of application of phosphate solution; dipping cuts of beef in phosphate solution after slicing into steaks vs pumping phosphate solution into cuts of beef before slicing into steaks	26
11.	Comparison of the effect on eating quality of TPP-ascorbic acid solution applied by the dipping method and by the pumping method	29
12.	Irradiation of beef slices exposed to air; dose rate vs percentage of each pigment form	32
13.	Irradiation of beef slices under vacuum; dose rate vs percentage of each pigment form	35

14.	Irradiation of beef slices exposed to oxygen above one atmosphere pressure; dose rate vs percentage of each pigment form	37
15.	Comparison of dip solutions with pH values of 9.0-9.2; pigment form and meat pH after reoxygenation	43
16.	Comparison of dip solutions with pH values of 9.0-9.2; pigment form after reoxygenation	43
17.	Effect of pH and Na_2HPO_4 on the oxidation rate of myoglobin; time vs percentage of oxidized pigment	46
18.	Separation of radioactive phosphate from myoglobin on a Sephadex column; fraction location vs the count of $\text{Na}_2\text{H}^{32}\text{PO}_4$	48
19.	Separation of radioactive phosphate from myoglobin by dialysis; time vs count of $\text{Na}_2\text{H}^{32}\text{PO}_4$	48
20.	Association of radioactive phosphate with myoglobin; count of radioisotope in dialysis water vs molar ratios of phosphate to myoglobin	51

FIGURE NO.

1.	Beef slices exposed to oxygen above one atmosphere pressure; dose rate vs the amount of metmyoglobin greater than in the non-irradiated beef	38
2.	The dialysis to equilibrium of radioactive phosphate from a myoglobin solution	49

RADIATION PASTEURIZATION OF
FRESH MEATS AND POULTRY

Walter M. Urbain, George G. Giddings and William W. Ballantyne

Michigan State University
East Lansing, Michigan 48823

CONTRACT AT(11-1)-1689

DIVISION OF ISOTOPES DEVELOPMENT
UNITED STATES ATOMIC ENERGY COMMISSION

ANNUAL REPORT
February 15, 1969 through February 14, 1970

ABSTRACT

The objective of work under the present year's contract was a continuation of the two previous years work, to develop the process of radiation pasteurization of fresh meats and poultry to permit centralized cutting of these products.

An average flavor score of approximately 5 on a 9 point scale was secured with beef phosphate treated, irradiated and stored at 38°F under vacuum for 18 or more days followed by storage in air for 3 or more days. Unstored, untreated beef flavor scores averaged approximately 6. These scores were obtained with a small consumer-type panel.

While the meaning of the flavor difference in terms of acceptance of the stored beef by the public could not be ascertained, efforts to determine its nature and cause were a principal activity of this contract year.

An off-flavor immediately after irradiation was observed in the phosphate-treated beef. This interaction between radiation and the added phosphate solution requires further study.

TBA values, indicative of fat oxidation, were found to increase whenever beef was stored in the presence of oxygen. The addition of ascorbic acid to the phosphate solution led to markedly lower TBA values. Since red meats require exposure to oxygen in order to obtain a normal red color, the use of an antioxidant appears necessary.

Pumping phosphate solution into the meat by a gas entrainment method is more effective than dipping in improving the water holding capacity of the meat. Dipping, however, yields meat of better surface color. A combination of pumping and surface treatment is indicated.

Irradiation of beef in air at high dose rates (above .05 Krad/sec) can cause discoloration. This discoloration can be eliminated by use of lower dose rates or by irradiating in the absence of oxygen.

Efforts were made to understand the action of phosphates in maintaining the normal red color of beef. Studies with meat indicated that the protective action is not simply the result of a pH change. Studies on the isolated meat pigment, however, indicated little effect on oxidation, regardless of pH. These findings suggest that phosphate may be influencing reducing enzymes in the meat, rather than acting directly on the pigment molecule.

Indications of a loose association of phosphate with meat pigment were secured, but there was no evidence of specific chemical bonding. The observed protective action of the phosphates on fresh meat color remains unexplained.

PREFACE

This annual report was prepared in accordance with the terms of Contract No. AT(11-1)-1689 . . Modification No. 3 between the United States Atomic Energy Commission and Michigan State University.

The material included in this third annual report under said contract is based upon experimental work that is continuing. The conclusions stated in this report are therefore tentative, and the interpretation based upon current results may change as investigations continue and new data, observations, information and materials become available.

When the product of a specific manufacturer is stated as having been used in the work described herein, this is not meant to imply an endorsement of said manufacturer, nor is it meant to imply that similar products made by other manufacturers are not equally suitable for the same use.

SUMMARY

The objective of work under the present year's contract was a continuation of the two previous year's work to develop the process of radiation pasteurization of fresh meats and poultry to permit centralized cutting of these products.

An average flavor score of approximately 5 on a 9 point scale was secured with beef phosphate treated, irradiated and stored at 38°F under vacuum for 18 or more days followed by storage in air for 3 or more days. Unstored, untreated beef flavor scores averaged approximately 6. These scores were obtained with a small consumer-type panel.

While the meaning of the flavor difference in terms of acceptance of the stored beef by the public could not be ascertained, efforts to determine its nature and cause were a principal activity of this contract year.

An off-flavor immediately after irradiation was observed in the phosphate-treated beef. This interaction between radiation and the added phosphate solution requires further study.

TBA values, indicative of fat oxidation, were found to increase whenever beef was stored in the presence of oxygen. The addition of ascorbic acid to the phosphate solution led to markedly lower TBA values. Since red meats require exposure to oxygen in order to obtain a normal red color, the use of an antioxidant appears necessary.

Pumping phosphate solution into the meat by a gas entrainment method is more effective than dipping in improving the water holding capacity of the meat. Dipping, however, yields meat of better surface color. A combination

of pumping and surface treatment is indicated.

Irradiation of beef in air at high dose rates (above .05 Krad/sec) can cause discoloration. This discoloration can be eliminated by use of lower dose rates or by irradiating in the absence of oxygen.

Efforts were made to understand the action of phosphates in maintaining the normal red color of beef. Studies with meat indicated that the protective action is not simply the result of a pH change. Studies on the isolated meat pigment, however, indicated little effect on oxidation, regardless of pH. These findings suggest that phosphate may be influencing reducing enzymes in the meat, rather than acting directly on the pigment molecule.

Indications of a loose association of phosphate with meat pigment were secured, but there was no evidence of specific chemical bonding. The observed protective action of the phosphates on fresh meat color remains unexplained.

INTRODUCTION

This third annual report on the contract covers work completed subsequent to that covered in the first and second annual reports. Considerable background information and literature review was included in these prior annual reports to which reference should be made by the reader.

The experimental results section which constitutes the bulk of the report is divided into roughly three sections: storage studies for which "taste" panels and "taste" panels plus TBA analysis are employed for evaluation; a phosphate application methods section; and a section on color and pigment studies. It was decided to include complete results of qualitative microbiological studies in a subsequent report rather than include a discussion of methods and materials and preliminary type investigations in this report.

EXPERIMENTAL RESULTS

Eating Quality Evaluations

1. Effect of phosphate dip upon eating quality of irradiated-unstored fresh beefsteaks:

A series of six replicate taste panel evaluations was run in order to compare the effect on eating quality attributes, particularly flavor and juiciness, of fresh beefsteaks dipped in tripolyphosphate solution (TPP) vs. steaks dipped in tap-water vs. steaks not dipped. Since TPP or a combination of TPP and hexametaphosphate are considered to be the most likely food-grade phosphate compound(s) for use in improving water holding capacity of red meats, there is need to know if such a treatment affects eating quality attributes. There has been some indication (1) that phosphate treatment has an effect upon flavor and juiciness of the meat after cooking as well as its effects upon color and water-holding-capacity (WHC) of the raw meat. WHC and juiciness are known to be related.

Steaks, each approximately 8 oz, cut from fresh beef round (U.S. Grade Choice) were used in each of the six tests comprising this series. The steaks were bulk vacuum-packed in flexible laminate gas-impermeable plastic bags. Each of the three variables was packed separately. One set of steaks was dipped in 10% TPP at 38°F, another in water at 38°F, and a third set was untreated. The three variables were each given 100 Krad of gamma radiation from ⁶⁰Co, and the steaks were cooked and subjected to taste panel evaluation within 24 hours after completion of irradiation. Taste panels were conducted as described previously (1). The panelists were untrained and were considered to represent general consumers. Results of the six panels are summarized in

Table 1.

While the composite averages of the six panels indicate no differences in overall quality among the three pre-irradiation treatment variables, the data for the five individual quality parameters suggest that phosphate treated samples were rated slightly lower for odor and flavor and slightly higher for tenderness and juiciness than the samples either dipped in plain water or untreated. Verbal comments by panelists tended to support this interpretation. Thus it seems that a slight negative effect on flavor and odor is balanced by a slight positive effect on tenderness and juiciness. The lack of a sharp, clearcut trend in the data cautions against drawing any firm, unequivocal conclusions. It is known, however, that too high a level of added phosphate can cause a recognizable flavor alteration in meat, (for example, a metallic taste in ham at a 0.75% phosphate level). Data presented previously (2) indicated that for our work a one-minute dip in a 10% TPP solution results in a phosphate level of about 0.5% in dipped-drained fresh beefsteaks. The dipping method, however, may result in a concentration gradient, with the phosphate being more concentrated near the surface. This could give rise to a detectable flavor alteration, but this has not been demonstrated experimentally. If this should be the case, however, the gas entrainment pumping method presently being studied should influence such an effect by providing a more even distribution of phosphate solution in the meat.

2. Effect of phosphate, irradiation and refrigerated storage on the eating quality of fresh beefsteaks as compared with frozen controls:

Of particular interest in this panel is how the irradiated samples,

Table 1. Effect of water dip and phosphate dip on eating quality of irradiated, unstored fresh beefsteaks.

Treatment		Cooked color	Odor	Flavor	Texture (tender-ness)	Juici-ness	Overall quality	Sig. diff. @ 95% level*
Panel 1 - (24 panelists)								
P-I	Av	6.4	5.6	5.6	6.0	6.0	5.8	W-I
	Range	3-8	3-7	3-8	4-8	4-8	4-8	O-I
W-I	Av	6.2	6.0	6.2	6.1	6.6	6.3	
	Range	4-8	3-8	3-8	3-9	5-8	5-8	
O-I	Av	6.8	6.2	6.3	6.0	6.8	6.2	
	Range	5-8	4-8	4-8	3-7	2-9	3-8	
Panel 2 - (24 panelists)								
P-I	Av	6.6	6.1	6.3	6.4	6.4	6.3	
	Range	4-8	3-8	3-8	3-9	2-9	4-8	
W-I	Av	6.2	6.2	5.6	4.9	5.1	5.4	P-I
	Range	3-8	3-8	3-7.5	2-7	4-8	3-7	
O-I	Av	6.6	6.4	5.9	5.1	5.2	5.6	P-I
	Range	5-8	4-8	3-8	2-8	3-8	4-8	
Panel 3 - (24 panelists)								
P-I	Av	7.1	5.8	6.0	6.0	7.4	6.3	O-I
	Range	4-9	2-8	2-8	2-9	5-9	3-9	
W-I	Av	7.0	6.5	6.6	5.8	7.0	6.5	O-I
	Range	4-9	4-8	5-8	3-8	3-8	4-8	
O-I	Av	7.2	6.6	6.9	6.6	7.3	6.9	
	Range	5-9	5-9	4-8	4-9	5-9	5-8	
Panel 4 - (24 panelists)								
P-I	Av	6.8	6.0	6.3	6.9	7.2	6.4	
	Range	5-9	3-9	2-9	3-9	5-9	3-9	
W-I	Av	6.8	6.2	6.4	6.7	6.8	6.3	
	Range	4-9	3-8	2-8	3-9	5-8	3-8	
O-I	Av	6.5	6.4	6.3	6.1	7.0	6.4	
	Range	4-9	4-9	4-8	2-9	4-9	5-8	

Table 1 (continued):

Panel 5 - (24 panelists)

P-I	Av	7.0	6.5	6.3	6.6	6.5	6.2	W-I
	Range	4-9	3-8	4-8	4-9	4-8	4-8	
W-I	Av	7.0	6.7	6.6	6.5	6.7	6.5	
	Range	5-8	5-8	4-8	4-9	4-9	5-8	
O-I	Av	7.0	6.8	6.1	6.4	6.0	6.2	W-I
	Range	5-9	4-9	1-9	3-9	1-8	3-8	

Panel 6 - (24 panelists)

P-I	Av	6.6	6.0	6.2	6.1	6.0	6.0
	Range	4-8	3-8	4-8	3-8	4-8	4-7.5
W-I	Av	6.6	6.7	6.2	6.0	6.0	6.0
	Range	5-8	4-9	4-8	2-8	3-9	3-8.5
O-I	Av	6.7	6.8	6.5	5.8	5.7	6.0
	Range	5-8	5-8	4-8	3-8.5	3-8	3-8

Composite averages for the six panels:

P-I	6.8	6.0	6.1	6.3	6.6	6.2
W-I	6.6	6.4	6.3	6.0	6.4	6.2
O-I	6.8	6.5	6.3	6.0	6.3	6.2

CODE:

P-I: Dipped in 10% tripolyphosphate solution, 100 Krad.

W-I: Dipped in water, 100 Krad.

O-I: No pre-irradiation treatment, 100 Krad.

* Refers to "overall quality" scores only. Variables appearing in this column have significantly higher overall quality scores (at 95% confidence level) than the individual attribute variables for the same horizontal row.

handled according to the proposed procedure, compared with non-irradiated controls (held frozen until the day of the taste panel test). The data of Panel No. 2 of Table 2 indicate that irradiated beefsteaks stored for 21 days in vacuum followed by three days in air at 38°F are at least as acceptable as frozen controls (cut from the same carcass and muscle). The relatively high overall quality score received by the phosphated irradiated variable appears due to superior tenderness and juiciness. Surprisingly, the phosphated frozen variable was rated lower in juiciness than the non-phosphated frozen variable. The reverse would be expected. Further testing of this is indicated.

3. Comparison of the eating quality of irradiated fresh beefsteaks stored in vacuum with that of steaks stored in vacuum followed by aerobic storage:

The development of a detectable objectionable off-flavor and off-odor in irradiated and non-irradiated refrigerated fresh meat stored first under vacuum and later in air was believed to be connected with the terminal stage of storage during which time oxygen from the atmosphere is interacting with the meat components. In order to investigate this the following test was carried out. Steaks from a single beef top-round were separated into groups which were treated and stored as indicated in Table 3. Prior to irradiation, the steaks to be stored were bulk vacuum packed in large gas impermeable plastic bags. After 18 days of vacuum storage at 38°F, the steaks were removed from half of the irradiated vacuum bags and placed in clear polystyrene meat trays and overwrapped with polyvinylchloride fresh meat wrapping film. The other half remained in the vacuum bags. All steaks were stored for an additional three days at 38°F, making the total storage period 21 days.

Table 2. Effect of phosphate, irradiation and refrigerated storage on eating quality of fresh beefsteaks.

Treatment		Cooked color	Odor	Flavor	Texture (tender-ness)	Juici-ness	Overall quality	Sig. diff. @ 95% level*
Panel 1 - (24 panelists):			One hour post-irradiation					
0-0	Av	6.5	6.3	6.3	6.2	6.4	6.2	
	Range	3-9	4-9	3-9	2-9	4-9	3-8	
0-I	Av	6.4	5.6	5.4	6.6	6.1	5.6	0-0 P-I
	Range	3-9	3-8	1-8	4-9	4-9	4-8	
P-0	Av	5.3	5.7	5.8	6.1	6.5	5.8	0-0 P-I
	Range	3-8	3-9	3-9	2-8	3-9	3-9	
P-I	*Av	6.2	6.2	6.5	6.3	6.0	6.2	
	Range	3-8	3-8	4-8	4-8	3-8	4-8	
Panel 2 - (24 panelists):			21 days in vacuum + 3 days in air at 38°F					
0-0	Av	6.3	6.3	5.5	6.2	5.8	5.8	P-I
	Range	4-8	4-8	2-8	3-8	2-8	3-8	
0-I	Av	6.1	6.4	6.2	5.9	5.4	5.7	P-I
	Range	3-8	5-8	3-8	2-9	3-8	3-7.5	
P-0	Av	5.7	6.6	5.1	6.0	5.2	5.4	0-0 0-I P-I
	Range	3-8	4-8	1-8	3-9	2-7	2-8	
P-I	Av	6.5	6.3	5.8	6.4	6.3	6.2	
	Range	4-8	3-8	3-8	2-9	4-8	4-8	

CODE:

0-0: Untreated, 0 Krad.

0-I: No pre-irradiation treatment, 100 Krad.

P-0: Dipped in 10% TFP solution, 0 Krad.

P-I: " " 100 Krad.

The non-irradiated controls (0-0 and P-0) were held frozen for 24 days before cooking and serving in Panel 2. The two irradiated variables (0-I and P-I) were held for 21 days under vacuum plus 3 days in air at 38°F before cooking and serving to Panel 2.

* See Table 1 note.

According to the data shown in Table 3, the samples stored in air during the final three days before testing were judged to be clearly inferior in odor, flavor and in overall quality as compared with steaks held constantly under vacuum. The data and verbal comments left no doubt as to the adverse effect on odor, flavor and overall eating quality of holding in air during the final few days. As in the previous study, the frozen control apparently suffered somewhat from excessive fluid loss on thawing as evidenced by the low juiciness score. Results of the "zero time" test (Panel 1) indicate inferior odor, flavor, juiciness and overall quality for the phosphated-irradiated variable. As mentioned earlier, this may be due to an uneven distribution of phosphate in the meat resulting from the method of application (dipping).

4. Lipid oxidation studies:

Having established the aerobic terminal stage of storage as the principal period of off-flavor and off-odor development, a series of tests was conducted for the purpose of correlating sensory evaluation data with objective lipid oxidation measurements. Ascorbic acid was tested as a possible means of minimizing lipid and other oxidations during the critical aerobic stage of storage.

- a) Comparison of eating quality and lipid oxidation of irradiated beefsteaks stored in vacuum, and in vacuum followed by aerobic storage:

This test was carried out in the same manner as the previous test except that samples were analyzed for lipid oxidation using the thiobarbituric acid (TBA) test in an attempt to correlate the taste panel evaluation with objective data. The data in Table 4 indicate a sharp

Table 3. Comparison of eating quality of irradiated fresh beefsteaks stored in vacuum with that of steaks stored in vacuum followed by aerobic storage.

Treatment		Cooked color	Odor	Flavor	Texture (tender-ness)	Juici-ness	Overall quality	Sig. diff @ 95% level*
Panel 1 - (24 panelists) "zero time":								
0-0	Av	6.5	6.4	6.4	6.4	6.5	6.3	
	Range	3-8	4-8	2-8	3-8	5-8	2-8	
0-I	Av	6.4	6.0	6.6	6.1	6.4	6.0	0-0
	Range	4-8	4-8	2-8	3-8	4-8	2-8	
P-I	Av	6.5	5.5	5.4	6.1	5.6	5.5	0-0
	Range	3-8	2-8	3-8	3-7	2-8	3-8	0-I
Panel 2 - (25 panelists):								
0-I 18+3	Av	5.8	5.8	4.9	4.9	5.0	4.9	0-0
	Range	3-8	3-8	2-9	1-9	2-9	2-9	0-I: 21 P-I: 21
0-I 21	Av	6.7	6.4	6.1	6.1	5.5	6.1	
	Range	3-9	4-8	3-8	5-9	1-8	4-8	
P-I: 18+3	Av	6.0	5.8	4.5	5.1	5.8	5.0	0-I: 21
	Range	3-9	3-8	1-7	1-8	2-9	3-8	P-I: 21 0-0
P-I: 21	Av	6.7	6.3	5.6	5.8	5.9	5.9	
	Range	4-9	4-8	2-9	4-8	2-8	3-8	
0-0: Frozen	Av	6.0	6.1	6.1	5.2	4.7	5.6	0-I: 21
	Range	2-9	2-8	3-8	2-8	1-7	3-8	P-I: 21

CODE:

0-0: Non-phosphated, non-irradiated.

0-I: " " , 100 Krad.

P-I: 1 min dip in 10% TPP, 100 Krad.

21: Held under vacuum 21 days at 38°F.

18+3: Held under vacuum 18 days, then in air 3 days at 38°F.

Frozen control was held at 0°F until day of test.

* See Table 1 note.

Table 4. Comparison of eating quality and lipid oxidation of irradiated beefsteaks stored in vacuum and stored in vacuum followed by aerobic storage.

Treatment		Cooked color	Odor	Flavor	Texture (tender-ness)	Juici-ness	Overall quality	TBA No.
Panel 1 - (24 panelists) "zero time":								
0-0	Av	6.9	6.5	6.9	6.5	7.2	6.8	0.14
	Range	4-9	4-9	4-9	4-9	2-9	4-8	
0-I	Av	6.6	6.2	6.8	7.0	7.3	6.8	0.16
	Range	4-8	3-8	4-8	5-9	4-9	4-8	
P-I	Av	6.8	6.0	6.0	6.9	7.4	6.5*	0.08
	Range	4-9	4-9	3-9	4-9	5-9	3-8	
Panel 2 - (24 panelists)								
0-I: 18+5	Av	6.2	6.1	5.1	5.8	5.7	5.6	2.70
	Range	3-8	2-8	1-7	3-8	3-9	1-8	
0-I: 23	Av	6.4	6.4	6.0	5.6	5.4	5.7	0.16
	Range	3-9	3-8	3-8	2-8	3-8	3-7	
P-I: 18+5	Av	5.8	5.8	5.1	5.6	5.2	5.3*	4.00
	Range	3-8	2-9	2-8	3-8	3-7	2-8	
P-I: 23	Av	6.2	5.8	5.6	5.7	5.5	5.7	0.12
	Range	3-9	3-8	3-9	2-8	3-8	3-8	

CODE:

0-0: Non-phosphated, non-irradiated.

0-I: " " , 100 Krad.

P-I: 1 min dip in 10% TPP, 100 Krad.

23: Held under vacuum 23 days at 38°F.

18+5: Held under vacuum 18 days, then in air 5 days at 38°F.

* Overall quality score is significantly lower (95% level) than for the other treatments.

increase in TBA values, and therefore in lipid oxidation during the terminal aerobic phase of storage. There was essentially no change in TBA values during 23 days of vacuum storage. The magnitude of the TBA values for the 18 + 5 day variables indicate that an objectionable level of lipid oxidation was reached in meat so held. The TBA analysis results correlate well with the flavor score trend in particular, and, to a lesser extent, with odor and overall quality scores. The latter two indices did not exhibit as clearcut trends as they did in the previous study.

Another study was conducted which was essentially a repeat of the previous one (Table 4) except for the slightly different storage times. The data given in Table 5 indicate a very slight increase in TBA value during constant vacuum storage, possibly due to air entrapped during vacuum packing, and a sharp increase during post-vacuum aerobic storage. Again, flavor scores correlate quite well with the TBA data, as do overall quality and odor scores in the case of the non-phosphated variables. Differences in odor and in overall quality are of a lesser magnitude in the case of the phosphated samples; they follow, however, the same trend as with the non-phosphated samples.

- b) Effect of ascorbic acid alone and in combination with TPP on the eating quality and other attributes of irradiated stored fresh beefsteaks:

The data of Tables 4 and 5 establish that the exposure of the meat to atmospheric oxygen results in lipid oxidation, and this presumably is the explanation of the off-flavor observed in the cooked meat. In order to obtain the normal red color of fresh meat, exposure to atmospheric oxygen is required. For this reason the control of lipid oxidation through

Table 5. Comparison of eating quality and lipid oxidation of irradiated beefsteaks stored in vacuum and stored in vacuum followed by aerobic storage.

Treatment		Cooked color	Odor	Flavor	Texture (tender- ness)	Juici- ness	Overall quality	TBA No.
Panel 1 - (24 panelists) "zero time"								
0-0	Av	7.0	6.4	6.2	5.0	5.8	5.6	0.00
	Range	5-9	5-8	3-9	2-9	3-8	2-8	
0-1	Av	6.9	6.8	6.9	5.8	6.6	6.4*	0.02
	Range	4-9	5-9	5-9	2-9	3-9	3-8	
P-1	Av	6.4	6.5	6.4	5.3	5.6	5.8	0.05
	Range	4-9	5-8	4-8	2-8	2-9	1-8	
Panel 2 - (24 panelists)								
0-1: 25+3	Av	5.4	5.9	5.1	4.9	4.7	5.0	1.80
	Range	2-8	3-8	1-8	2-8	1-7	2-7	
0-1: 28	Av	6.8	6.6	6.2	6.1	6.2	6.0*	0.12
	Range	5-8	4-8	3-8	3-8	3-9	4-8	
P-1: 25+3	Av	6.1	6.2	5.1	6.2	6.4	5.8**	1.20
	Range	2-8	4-8	1-8	2-8	2-9	2-8	
P-1: 28	Av	6.4	6.6	6.1	5.6	5.8	6.1*	0.12
	Range	4-8	5-8	3-8	2-8	2-9	3-8	

CODE: Refer to Table 4. In this study, samples were held either 28 days in vacuum or 25 days in vacuum followed by 3 days in air at 38°F.

* Overall quality score is significantly higher (95% confidence level) than that of other samples in panel.

** Overall quality score significantly higher than that for "0-1:25+3".

an antioxidant is indicated.

In the following three tests, ascorbic acid was employed as an aqueous dipping solution both alone and in combination with TPP. The effects of the different treatments on appearance and eating quality were evaluated by informal observation and by taste panel evaluation of cooked steaks. TBA analyses on the raw steaks were also made. For the first test, the dipping solutions consisted of 2% w/v ascorbic acid (U.S.P.) and/or 10% w/v TPP. Treated samples were dipped for one minute in either of the solutions, and were then allowed to drain for 15 minutes before being bulk vacuum packed and irradiated. Non-dipped steaks were bulk vacuum packed, and half of these were irradiated, the other half serving as non-dipped, non-irradiated controls. Results of the first test are given in Table 6.

It was apparent at the time of the "zero time" test that the 2% w/v level of ascorbate in the dipping solutions was too high. Steaks treated with the ascorbate developed a greenish surface "scum" after cooking although the steaks were uniformly red with no observable discoloration before cooking. Steaks treated with both phosphate and ascorbate were greener than steaks treated with ascorbate only. This is contrary to the view that green discoloration caused by the interaction of heme pigment and ascorbate (e.g., cholemyoglobin) is associated with a low pH condition (the phosphate treatment raises the pH of the meat). In addition to generating green discoloration on the cooked meat surfaces, the ascorbate appeared to impart an objectionable, somewhat bitter flavor note, which likewise seemed more intense in the presence of added phosphate. The results from Panel 2 also

Table 6. Effect of ascorbic acid, alone and in combination with added tripolyphosphate, on quality of irradiated-stored fresh beefsteaks.

Treatment		Cooked color	Odor	Flavor	Texture (tender-ness)	Juici-ness	Overall quality	TBA No.
Panel 1 - (24 panelists) "zero time"								
O-O	Av	6.2	6.2	6.3	6.2	6.5	6.1*	0.16
	Range	4-9	4-8	4-8	4-8	4-8	4-8	
O-I	Av	6.6	6.1	6.0	6.0	5.9	6.1*	0.39
	Range	4-9	4-8	3-9	4-8	3-9	4-8	
P-I	Av	6.0	5.7	5.4	5.9	5.8	5.5	0.58
	Range	2-9	4-8	2-8	4-8	3-9	3-8	
AA-I	Av	5.5	5.5	5.4	5.8	6.4	5.6	0.21
	Range	1-9	2-8	3-8	2-8	4-9	3-8	
P-AA-I	Av	3.3	5.4	4.7	5.5	5.4	4.6**	0.23
	Range	1-7	3-8	1-8	3-9	3-8	1-8	
Panel 2 - (24 panelists) 18 days in vacuum + 3 days in air at 38°F								
O-O Frozen	Av	6.8	6.2	6.8	6.5	6.1	6.6*	0.55(21)
	Range	5-8	4-8	5-8	3-9	4-8	5-8	
O-I	Av	6.7	6.1	6.1	6.0	5.9	6.1	0(18) 4.5(18+3)
	Range	3-8	4-8	4-8	3-9	2-8	3-8	
P-I	Av	6.1	6.1	5.8	6.5	6.0	5.9	0(18) 2.80(18+3)
	Range	1-8	4-8	3-8	3-8	1-8	3-8	
AA-I	Av	5.4	5.6	5.0	5.1	5.0	4.9**	0.12(18) 0.51(18+3)
	Range	2-8	2-8	2-7	2-8	2-8	1-7	
P-AA-I	Av	5.4	5.7	5.4	5.7	5.6	5.5***	0.14(18) 0.50(18+3)
	Range	3-8	4-8	2-8	3-8	2-8	3-8	

* Significantly (95% level) highest overall quality score in panel.

** " " " lowest " " " "

*** " " " lower " " " than for O-I and P-I.

CODE:

O-O: Fresh control for Panel 1. Frozen control for Panel 2.

O-I: No ascorbate, no phosphate, 100 Krad.

P-I: " " TPP dip, "

AA-I: Ascorbate dip, no phosphate, "

P-AA-I: Combined ascorbate-phosphate dip, 100 Krad.

(18): 18 days in vacuum at 38°F; (21) = days in vacuum at 0°F.

(18+3): 18 days in vacuum + 3 days in air at 38°F.

Ascorbic acid was 2% w/v of dipping solution, and tripolyphosphate was 10% w/v of dipping solution where employed.

observed the same discoloration and off-flavor in the samples held 18 days in vacuum followed by 3 days storage in air. The ascorbate, however, both alone and in combination with phosphate, appears to have reduced lipid oxidation during its storage as indicated by TBA data, as well as by visual observation before cooking. Also, the effectiveness of ascorbate in keeping the meat pigment in the reduced, oxygen binding state was readily observable. Ascorbate treated steaks, (with and without phosphate) were uniformly bright red at the time of cooking, whereas steaks not treated with ascorbate, especially non-phosphorated samples, exhibited slight darkening and browning. All samples, however, appeared salable.

The TBA analytical data suggest that a reduction in compounds responding to the TBA test occurred during refrigerated vacuum storage of all samples. Since this was more pronounced with samples not treated with ascorbic acid, it would seem that the mechanism operating was different from that which accounts for the effectiveness of ascorbate in maintaining the pigment in the oxygen-binding state during post-vacuum holding in air.

For the second in the series of ascorbic acid tests the level of ascorbate in the dipping solutions was lowered to 0.5% w/v in an attempt to avoid the discoloration and off-flavor associated with the 2% w/v level used in the previous test. The results of this second test are summarized in Table 7.

Judging from visual observation, and comments and ratings of the panelists, the lowered level of ascorbate appeared to yield the desired protection against discoloration and high TBA values in the raw meat, as well as not causing any noticeable greenish color and/or bitter off-flavor

Table 7. Effect of ascorbic acid, alone and in combination with added tripolyphosphate, on quality of irradiated-stored fresh beefsteaks.

Treatment		Cooked color	Odor	Flavor	Texture (tender-ness)	Juici-ness	Overall quality	TBA No.
Panel 1 - (25 panelists) "zero time"								
O-0	Av	6.7	6.4	6.6	6.6	7.2	6.6*	0.26
	Range	4-8	4-8	4-9	4-9	4-9	4-9	
O-I	Av	6.5	6.1	6.0	6.1	6.0	6.1	0.22
	Range	3-9	3-8	3-8	4-8	3-9	4-8	
P-I	Av	6.7	5.9	6.2	6.5	6.7	6.3	0.43
	Range	4-9	2-8	4-8	4-9	4-9	4-8	
AA-I	Av	6.0	6.4	6.1	6.4	6.6	6.1	0.27
	Range	4-8	5-8	3-8	4-9	5-8	4-8	
P-AA-I	Av	6.3	6.4	6.3	6.5	6.7	6.3	0.16
	Range	4-8	4-8	4-8	4-9	5-8	4-8	
Panel 2 - (25 panelists) 21 days in vacuum + 5 days in air at 38°F								
O-0 Frozen	Av	7.0	6.4	6.0	5.7	6.4	6.0*	0.10(26)
	Range	5-9	4-8	3-8	3-9	4-9	3-8	
O-I	Av	6.2	5.6	3.8	4.8	5.2	4.5**	0.30(21) 4.30(21+5)
	Range	4-9	2-8	1-7	2-7	2-8	1-7	
P-I	Av	5.2	5.8	4.6	5.1	4.9	4.5**	0.20(21) 2.80(21+5)
	Range	1-8	3-8	2-8	2-8	1-9	2-7	
AA-I	Av	5.6	5.6	4.4	4.3	4.7	4.5**	0.18(21) 0.39(21+5)
	Range	2-8	2-8	1-8	1-7	1-8	1-8	
P-AA-I	Av	6.3	5.7	4.2	5.9	6.3	5.0***	0.15(21) 0.17(21+5)
	Range	4-8	3-8	1-8	3-8	3-9	1-8	

* Significantly (95% level) highest overall quality score.

** " " lowest " "

*** Overall quality score of 5.0 significantly higher than 4.5.

CODE: Refer to Table 6 for letter code.

(21) = 21 days in vacuum at 38°F.

(21+5) 21 days in vacuum + 5 days in air at 38°F.

(26) 26 days in vacuum at 0°F.

Ascorbic acid was 0.5% w/v of the dipping solution and TPP 10% w/v of the dipping solution where employed.

in the cooked meat. The storage times in this test were more severe than in the previous test, and this time there appeared to be an ascorbate-phosphate synergism in reducing TBA values as well as in protecting against discoloration of the raw meat.

Refrigerated samples without ascorbate, and especially those without phosphate, exhibited an objectionable level of discoloration at the end of 21 ± 5 days. In contrast, ascorbate treated steaks were uniformly bright red, the non-phosphated being noticeably paler than the phosphated. The apparent reduction in TBA active compounds during refrigerated vacuum holding indicated by the data in Table 6 is not evident from the data in Table 7. This may possibly reflect differences in raw material such as have been observed with pigment reducing capacity and water holding capacity. Incipient organoleptic spoilage due to microbial growth was observed and the low flavor and overall quality scores received by the irradiated-refrigerated samples apparently were largely a result of this. The samples had been stored post-vacuum in air for five days instead of three. A higher irradiation dose (i.e., 150 Krad) and/or lower storage temperature would likely have provided the extra margin needed from the microbiological viewpoint to satisfactorily achieve the lengthy storage time. The TBA data indicated that lipid oxidation did not reach an objectionable level (TBA No. ca. 0.5) in the ascorbate treated samples even after five days of post-vacuum storage in air. TBA values for the samples not treated with ascorbate showed severe lipid oxidation.

For the third test in the series the level of ascorbate in the dipping solution was further reduced to 0.25% w/v to determine if this level of ascorbate provides the same effect vis-a-vis TBA values and color, while

further reducing the possibility of an adverse effect upon flavor, and color of the cooked meat. The data from this test are given in Table 8. The "zero time" test results suggest that at this level of ascorbate in the dipping solution there is no adverse effect upon cooked color and odor. However, judging from the TBA values, this level of ascorbate may not be sufficiently high enough to hold lipid oxidation below objectionable levels (i.e., below a TBA value of about 0.5). The optimal level of ascorbic acid under the conditions used in this work appears to be between 0.5 and 0.25% w/v.

The main point to be emphasized from the ascorbate work thus far is that the compound is effective in lowering lipid oxidation, as measured by TBA values. The level of ascorbate needed to significantly lower lipid oxidation seems to be below the level at which adverse effects on flavor and color of the cooked meat become detectable. Further, this relatively low level of ascorbic acid has, not unexpectedly, a pronounced positive effect upon the color of the raw meat (over and above the positive effect of phosphate) with respect to keeping the pigment in the reduced, oxygen binding state during aerobic holding.

The tests reported above provide only chemical evidence that ascorbic acid does reduce lipid oxidation. Eating tests did not determine that the off-flavor noticed without ascorbic acid was controlled. In one test too much ascorbic acid was used (Table 6) and this resulted in an off-flavor which prevented evaluation of flavor associated with lipid oxidation. In the other test (Table 7) incipient microbiological spoilage resulting possibly from a longer than normal aerobic storage marked the oxidation flavor.

Table 8. Effect of ascorbic acid, alone and in combination with added tripolyphosphate, on quality of irradiated-stored fresh beefsteaks.

Treatment		Cooked color	Odor	Flavor	Texture (tender-ness)	Juici-ness	Overall quality	TBA No.
Panel 1 - (25 panelists) "zero time"								
O-O	Av	6.6	6.2	6.3	6.4	6.2	6.2	0.164
	Range	3-9	4-9	4-8	4-8	3-8	4-8	
O-I	Av	6.9	6.6	6.7	6.8	6.8	6.7*	0.04
	Range	5-8	4-8	5-8	5-9	4-9	5-8	
P-I	Av	6.4	6.4	5.9	6.7	6.9	6.3	0.01
	Range	4-8	4-9	3-8	2-9	3-9	4-9	
AA-I	Av	6.7	6.4	5.9	5.7	6.6	6.0	0.014
	Range	5-8	4-9	4-8	3-8	4-9	4-8	
P-AA-I	Av	6.2	6.4	6.2	6.0	6.8	6.1	0.01
	Range	4-8	5-8	3-8	2-8	5-9	5-8	
Panel 2 - (25 panelists) 21 days in vacuum at 38°F								
O-O	Av	6.4	5.9	6.4	5.4	5.6	5.9*	0.15
	Range	4-8	2-8	2-9	2-9	2-9	2-8.5	
O-I	Av	5.8	5.2	5.1	4.7	4.4	4.8**	0.03
	Range	2-8	2-8	1-8	2-8	1-7	2-7	
P-I	Av	6.3	5.3	5.4	5.6	5.2	5.4	0.00
	Range	3-8	2-8	2-8	2-8	1-8	2-8	
AA-I	Av	6.0	5.6	5.5	5.8	5.2	5.5	0.09
	Range	4-8	3-8	2-9	3-9	2-9	2-8	
P-AA-I	Av	5.8	5.3	5.3	5.5	4.6	4.9**	0.16
	Range	3-8	3-7	2-9	1-8	1-8	1-7	

Table 8 - Continued:

Panel 3 -- (25 panelists) 21 days in vacuum + 4 days in air at 38°F

0-0	Av	6.6	6.2	6.2	5.6	5.4	6.0*	
	Range	5-8	4-8	3-8	3-7	2-8	3-8	0.16
0-1	Av	6.3	5.9	5.4	5.4	5.5	5.5	
	Range	1-9	4-9	3-8	2-8	3-8	3-8	3.40
P-1	Av	6.7	6.2	6.0	6.1	6.4	6.1*	
	Range	3-9	3-9	3-9	2-9	2-8	4-8	4.70
AA-1	Av	6.0	6.1	5.2	5.2	5.2	5.4	
	Range	2-9	3-8	2-9	3-8	2-8	3-8	1.91
P-AA-1	Av	6.0	6.0	5.9	6.2	5.8	5.7	
	Range	2-9	4-9	2-8	3-9	1-8	2-8	1.17

CODE: Refer to Table 6.

Control (0-0) samples were held at 0°F until day of tests 2 and 3.

Ascorbic acid was 0.25% w/v of dipping solution and tripolyphosphate was 10% of dipping solution where used.

* Significantly highest overall quality scores.

** " lowest " " "

While the TBA values suggest that ascorbic acid can provide a solution to the lipid oxidation problem, additional work is needed to establish this.

Phosphate Application Methods:

With a gas entrainment meat pumping apparatus just received, a series of tests was conducted in order to compare the dipping method of phosphate application with that of gas entrainment pumping of phosphate solution, and to generally study the merits and problems associated with this particular pumping method. The results, data and discussion of this work follow.

The gas entrainment pumping equipment used was a development model of a commercial unit (3)(4). The development model is manual in its operation, whereas the commercial unit is fully automatic.

Briefly, on the downstroke, nitrogen gas is forced under 20 psi through the needle holes (two rows of needles, seven needles per row) into the meat, thus "opening up" the meat structure somewhat. On the up-stroke phosphate solution is forced through the needle holes as a fine spray under 25-30 psi pressure. Phosphate solution is thereby rather evenly distributed throughout the bulk of the meat. The gas and the fluid pressures are adjustable up to about 100 psi; however, the pressures cited above appear perfectly adequate. The manufacturer recommends that the pressure driving the fluid into the needles be about 5-10 psi higher than the pressure of the gas which combines with the fluid at the needle tips to form a spray. The principal factor controlling the amount of fluid being delivered to the meat is the rate at which the needles are withdrawn from the meat at the end of each downstroke (when the needle tips are at the "bottom" of the meat).

This is because fluid is being delivered into the meat only on the up-stroke as the needles are being withdrawn through the bulk of the meat.

The commercial model of the pumping apparatus is automated to the degree that the pumping rate, and thus the weight gain or amount of fluid added to the meat, is automatically and reproducibly controlled. Thus the desired amount of fluid is added to the meat and over- or under-pumping is avoided. The development model of the apparatus used in this work is completely manual in operation and in control. Therefore, the amount of fluid added to the meat is subject to some variability. Pumping was of interest because of two possibilities: 1) thick pieces of meat could not absorb sufficient phosphate for drip control by treating only the surface; and 2) all cuts, regardless of size, could be made from previously pumped larger pieces (e.g., primals) and, in this way, a more efficient phosphate application method be utilized.

Using larger cuts of beef (i.e., roast size) a series of three replicate tests was conducted to compare percentage weight gain immediately after pumping or dipping, as well as the percentage weight loss after a week of refrigerated (38°F) vacuum storage.

Beef bottom round was divided into two portions, roughly on the basis of muscle groupings. Each portion was itself divided into two approximately equal cuts. After weighing, one cut from each portion was dipped for one minute in 10% TPP and drained for 10 minutes. The other cuts were pumped with 10% TPP solution using the gas entrainment apparatus, and drained for 10 minutes. After weighing, each cut was then sliced into approximately one-half inch slices, each slice being identified as to the

cut from which it was derived.

All the slices from a given cut and receiving the same treatment were placed in a gas impermeable plastic bag which was evacuated and sealed. The four groups of slices so prepared were stored at 38°F for one week. On opening the bags at the end of the storage period the combined drained weight of each group of slices was obtained. The volume of released liquid in the bag was also measured.

The data from the three replicate tests comparing the dipping and pumping methods are given in Table 9.

The data clearly demonstrate that the pumping method is capable of putting considerably more fluid into the meat than is the dipping method. In spite of the much higher fluid uptake and weight gain by the pumped meat, the dipped meat released as much or more fluid during the week of storage. This is most noteworthy and probably reflects the fact that pumping distributed phosphate solution throughout the cuts, thus improving the WHC of each steak after slicing, whereas phosphate solution from dipping was essentially confined to a small surface thickness and did not penetrate sufficiently to improve the WHC of steaks cut therefrom. Accepting this line of reasoning, it follows that, while the pumping method appears ideally suited to large cuts, the dipping method is limited in effectiveness to cuts with much greater surface-area-to-volume, such as steaks. This point is explored experimentally in the following tests.

Two replicate tests were conducted in which a comparison was made between 1) the dipping of beef cuts in phosphate solution after slicing into steaks, and 2) the pumping of phosphate solution into larger cuts before

Table 9. Comparison of two methods of application of phosphate solution to bulky cuts of beef; the dipping method and the gas entrainment pumping method.*

	Muscle group No. 1		Muscle group No. 2		
	pumped	dipped	pumped	dipped	
% gain in weight	12.8	1	15.3	1	
ml. fluid loss after 1 week**	36	32	20	24	
% loss in wt (drip loss) after 1 week**	2.0	2.3	3.5	4.0	

* Each figure is an average of three replicates.

** Each cut was sliced into steaks after dipping or pumping. The steaks were bulk vacuum packed in impermeable plastic bags and were stored for one week at 38°F.

Table 10. Comparison of two methods of application of phosphate solution; dipping cuts of beef in phosphate solution after slicing into steaks vs pumping phosphate solution into cuts of beef before slicing into steaks.*

	Muscle group No. 1 and 2	
	Sliced then dipped	Pumped then sliced
% gain in weight	0.8	11.6
ml fluid loss after one week**	90	45
% loss in weight (drip loss) after one week**	4	1.8

* Each figure is the average of two replicates.

** Steaks from the beef cuts were bulk vacuum packed in impermeable plastic bags and stored at 38°F for one week after treating.

slicing into steaks. Again, muscles were taken from beef bottom-rounds and one-half of a given muscle grouping was cut into steaks and then dipped while the other half was pumped before cutting into steaks. Ten percent w/v TPP solution was used with both applications. Phosphate treatments, packaging and storage were as described for the previous test, except that here all the slices from each of the two treatments were combined in one bulk package. Data from the two replicate tests are given in Table 10.

As in the previous test, the total weight of meat comprising the variables being compared was approximately equal. As indicated by the above data, although the pumped meat took up several times more fluid than did the dipped slices, the amount of fluid released and percentage weight loss was approximately half that for the dipped meat. It was noted, however, that the dipped steaks exhibited slightly better bright red surface color retention than did steaks cut from pumped halves of the muscles during post vacuum holding in air at 38°F. The pumped meat began to discolor about a day or two sooner than the dipped meat. One reason might be that the dipped meat had a higher concentration of phosphate at the cut surfaces. It was also observed that the pumped meat assumed a spongy, open appearance and feel. The pumped meat was, however, overpumped at 11.6% weight gain and this no doubt contributed to the sponginess.

To test the effect of the pumping method on eating quality, steaks from a U.S.D.A. Choice grade top and bottom from a beef round were divided into five treatment variables such that steaks from each muscle were evenly distributed among the five variables. This procedure is followed with all taste panel tests and makes it possible for each panelist to receive a set of

variables all from a single muscle, thus eliminating muscle-to-muscle variability. One lot of steaks was set aside as untreated controls and the remaining two lots were treated as follows:

Lot 1: Portions of each muscle were sliced into steaks which were dipped in a chilled solution containing 10% tripolyphosphate (w/v) and 0.5% ascorbic acid (w/v). After draining, the steaks were divided into two equal sub-lots which were separately bulk vacuum packed in flexible, gas impermeable plastic bags. One of the bags of steaks was irradiated at 100 Krad, the other bag was not irradiated.

Lot 2: Portions of each muscle were pumped with a chilled solution identical in composition to that used with Lot 1. Total weight gain after pumping and draining was approximately 6% of which about 0.6% was phosphate and about 0.03% was ascorbic acid.

After slicing the pumped cuts into steaks, Lot 2 was handled and packaged in the same manner as Lot 1, including irradiation. The taste panel results reported in Table 11 were obtained three hours after irradiation was completed. The low flavor score of the untreated control appeared to be due to lack of flavor, possibly due to insufficient aging, rather than to any "off flavor". Also, the control was less tender and juicy than the treated variables which could also contribute to the relatively low flavor score. Three of the four treated variables received impressively high and statistically equal odor, flavor, juiciness and overall quality scores. Tenderness scores were also quite good. The relatively low odor and flavor scores received by the "PAII-pump" variable are likely due to the interaction of two important factors; high moisture content due to the 6% wt/wt solution pickup

Table 11. Comparison of the effect on eating quality of TPP-ascorbic acid solution applied by the dipping method and by the pumping method.

(n = 25)							
Treatment		Cooked color	Odor	Flavor	Texture (tender-ness)	Juiciness	Overall quality
0-0	Av	6.3	6.6	5.9	5.3	5.9	5.6*
	Range	4-8	4-8	4-8	2-8	1-9	3-8
P-AA-0 dip	Av	6.1	6.4	6.3	6.2	6.9	6.4**
	Range	3-8	4-9	3-8	4-8	4-9	4-8
P-AA-I dip	Av	7.0	6.3	6.4	6.2	7.1	6.5**
	Range	5-9	4-8	4-8	3-8	4-9	5-8
P-AA-0 pump	Av	6.2	6.3	6.5	6.4	7.1	6.4**
	Range	3-8	3-8	1-9	2-9	5-9	4-8
P-AA-I	Av	6.2	5.7	5.8	5.8	7.0	5.9***
	Range	2-9	3-8	2-8	1-9	4-9	3-8.5

This test was conducted 3 hours after completion of irradiation.

* Significantly lowest (95% level) overall quality score.

** " highest " " "

*** " higher " " " than for 0-0.

CODE:

0-0 = Untreated control.

PAA0 = Treated with solution consisting of 10% w/v tripolyphosphate plus 0.5% ascorbic acid in chilled water. No irradiation.

PAAI = Same as PAA0 except irradiated to 100 Krad after bulk vacuum packing.

"dip" = Phosphate-ascorbate solution applied by the dipping method.

"pump" = Phosphate-ascorbate solution applied by the gas entrainment pumping method.

and ionizing radiation. The development of off-flavor in irradiated meats with higher than normal moisture levels has been observed previously. The reason for the relatively low tenderness score was not apparent. All variables received satisfactory color scores.

Before cooking the steaks it was noted that those cut from pumped muscle exhibited a slightly spongy, somewhat open texture characteristic of meat pumped with the gas entrainment apparatus, although to a smaller extent than that of the first test, probably because it was pumped to a lesser degree (6% uptake as contrasted with over 10%). If the U.S.D.A. approves the phosphate treatment, the weight gain will probably be limited to 3%, an amount which should not seriously change the meat texture when the gas entrainment pumping method is used.

As a general conclusion from the limited amount of data and observations accumulated thus far, it appears that the pumping method is far superior to the dipping method with respect to improvement of WHC, but that dipping of cuts such as beefsteaks results in slightly better surface color stability than does pumping of larger cuts prior to slicing into steaks, etc. Additional work in which the pumped weight gain is limited, probably to about 3%, and in which the phosphate uptake does not exceed 0.5%, is indicated.

Color Studies

1. Irradiation of beef slices exposed to the atmosphere:

The semitendinosus muscle of a commercial grade bottom round beef was cut into one-half inch thick slices. All slices were drained for 30 to 60 minutes following a 60 second dip in a 10% solution of TPP. The concentration of the three pigment forms (oxy, reduced, and met-myoglobin) was determined at the meat surface by reflectance measurements using a Bausch and Lomb 505 recording spectrophotometer. The samples were individually wrapped in highly gas-permeable plasticized polyvinyl chloride fresh meat film and irradiated to a total dose of 200 Krad. Samples were electron irradiated at three dose rates (5, 25 and 50 Krad/sec) using a General Electric one MeV Resonant Transformer Generator. Two gamma dose rates of 0.05 and 0.5 Krad/sec were obtained with the Cobalt 60 research gamma irradiator.

Reflectance measurements were taken on irradiated and non-irradiated slices immediately following irradiation and after removal of the air-permeable film. The polyvinyl chloride wrapped samples which were to be stored were vacuum packaged in flexible gas-impermeable laminated plastic bags and held for 21 days at 38°F. Reflectance measurements were taken of samples immediately upon their removal from vacuum and again following one day of exposure to the atmosphere. The air-permeable film was removed for the reflectance measurement.

Table 12 shows both the immediate results and long term effects on myoglobin pigment of different irradiation dose rates. As shown, there was an immediate increase in the amount of metmyoglobin formed at higher

Table 12. Irradiation of beef slices exposed to air; dose rate vs percentage of each pigment form.

Dose rate Krad/sec - Radiation source	Pigment form	Before irradiation %	After irradiation %	Following 21 days storage %	After one day of oxygenation %
50 Electron	Mb	22	33.5	51	21
	MbO ₂	55	22	6	58.5
	MetMb	23	44.5	43	20.5
25 Electron	Mb	22	17	48.5	23
	MbO ₂	56	42	10.5	58
	MetMb	22	41	41	19
5 Electron	Mb	19.5	26.5	41	19.5
	MbO ₂	60	37	15	67
	MetMb	20.5	36.5	44	13.5
0.5 Gamma	Mb	25	41.5	46	12
	MbO ₂	50.5	32	14.5	71.5
	MetMb	24.5	26.5	39.5	17.5
0.05 Gamma	Mb	21	16.5	41.5	19.5
	MbO ₂	59.5	56.5	20.5	61.5
	MetMb	19.5	27	38	19
0 None	Mb	12	2	76.5	22
	MbO ₂	65	74	4.5	67.5
	MetMb	23	24	19	10.5

dose rates. The observed darkening was due to conversion from oxymyoglobin to both reduced myoglobin and metmyoglobin. Visual observations generally indicated significant differences among samples irradiated at the three electron rates and the control, with the highest dose rate producing the darkest sample. The higher gamma rate also caused some darkening, but the lower rate generally resulted in a color that could not be distinguished from the control.

Following the three week storage period, reflectance measurements showed that all the irradiated samples had a similar amount of metmyoglobin, about twice as much as the control. After one day of reoxygenation, spectrophotometric results indicated minor differences among the samples. These differences did not seem dependent on dose rate. Although there was some drop in the metmyoglobin concentration with a corresponding decrease in electron dose rate, both gamma rates resulted in higher ultimate metmyoglobin concentrations than did the lowest electron rate. All the samples had more metmyoglobin than the controls, but spectrophotometrically there was little difference in the values.

However, visual observations after reoxygenation indicated that the highest dose rates, 25 and 50 Krad/sec, may result in metmyoglobin levels too high for acceptability. Samples irradiated with the lowest electron rate and the higher gamma rate were generally acceptable. Those irradiated at the lower gamma rate were almost always acceptable. Thus, following reoxygenation after three weeks vacuum storage, the reflectance spectrophotometric results may not parallel visual observations.

2. Irradiation of vacuum packaged beef slices:

Preparation of vacuum packaged beef slices was similar to that described in the previous section. However, following the phosphate dip, free moisture was immediately removed from each sample with a paper towel. The samples were then wrapped individually or in pairs (one on top of the other) in air permeable film, and vacuum sealed in flexible gas impermeable laminate plastic bags. These samples were irradiated in vacuum bags, in the absence of oxygen.

Results obtained from irradiation of vacuum samples are given in Table 13. Reflectance spectrophotometry indicates that there was a slightly greater conversion to metmyoglobin with the highest dose rate. Although all the samples had about 50% oxymyoglobin before irradiation, there was almost a complete conversion from this form to reduced myoglobin following irradiation at any dose rate. Visual changes were slight, and darkening was generally noted only with the highest electron dose rate. This would indicate that meat in which about half the pigment was already in the reduced form would not appear to become more purple when the reduced myoglobin concentration increased. The darkening at the highest dose rate was observed to be a brown hue on the purple background.

After three weeks of vacuum storage, the reflectance measurements indicated that the higher electron rates may result in slightly greater amounts of oxidized pigment. Following one day of reoxygenation, both the reflectance and the visual observations showed the level of metmyoglobin still to be greatest in the samples treated with the highest dose rate of electron irradiation. Many of these samples were unacceptable in appearance.

Table 13. Irradiation of beef slices under vacuum; dose rate vs percentage of each pigment form.

Dose rate Krad/sec - Radiation source	Pigment form	Before irradiation %	After irradiation %	Following 21 days storage %	After one day of oxygenation %
50 Electron	Mb	44.5	81	73	18
	MbO ₂	50.5	1.5	8	55
	MetMb	5	17.5	19	27

25 Electron	Mb	47	83	71.5	10
	MbO ₂	46	9.5	15.5	86
	MetMb	7	7.5	13	4

5 Electron	Mb	49	93	65	10
	MbO ₂	51	6	24.5	79.5
	MetMb	0	1	10.5	10.5

0.5 Gamma	Mb	53	85	78	15.5
	MbO ₂	42.5	4	12.5	78.5
	MetMb	4.5	11	9.5	6

0.05 Gamma	Mb	43.5	94	85	10
	MbO ₂	53	0	12	80
	MetMb	3.5	6	3	10

0 None	Mb	59	59	83.5	17
	MbO ₂	38	38	6	75
	MetMb	3	3	10.5	8

Samples irradiated at the lower dose rates were usually an acceptable appearing bright red.

3. Irradiation of beef slices exposed to oxygen above one atmosphere pressure:

Samples to be treated under oxygen pressure greater than 760 mm were first phosphate dipped, drained, wrapped in air permeable film, and then held for 10 hrs at 38°F to promote maximum formation of oxymyoglobin. Following this, they were packaged in flexible gas impermeable laminate bags equipped with tygon tubing inserts, vacuum sealed, and backfilled through the tubing with oxygen in excess of 760 mm before irradiation.

Electron dose rates of 0.55, 5, 10, 15, 25 and 50 Krad/sec were used as well as the two gamma rates, 0.05 and 0.5 Krad/sec. These samples were not held in storage, and reflectance measurements taken before and immediately following irradiation were obtained.

Table 14 contains data from samples irradiated under oxygen pressure of slightly greater than one atmosphere. The results exaggerate the effects of the presence of oxygen during irradiation. For this study a larger number of electron dose rates was used than in previous studies. The lowest was almost as low as the higher of the two gamma rates. For the irradiated samples, Figure 1 shows the amount of oxidized pigment greater than that in the control. This differential in oxidized pigment increased as the dose rate increased. The higher gamma rate and the lowest electron rate, which were approximately the same, yielded very similar metmyoglobin concentrations. This would be some verification of the assumption that the effect from gamma rays and electrons is part of a continuum. In contrast to

Table 14. Irradiation of beef slices exposed to oxygen above one atmospheric pressure; dose rate vs percentage of each pigment form.

Dose rate Krad/sec - Radiation source	Before irradiation			After irradiation		
	% Mb	% MbO ₂	% MetMb	% Mb	% MbO ₂	% MetMb
50	22	72	6	31	18	51
Electron						
25	22	73	5	33	18	49
Electron						
15	10	85	5	18	45	37
Electron						
10	17	78	5	25	45	30
Electron						
5	19	75	6	33	43	24
Electron						
0.55	21	75	4	28	52	20
Electron						
0.5	12	86	2	33	50	17
Gamma						
0.05	11	85	4	16	69	15
Gamma						
0	14	82	4	17	83	0
None

Figure 1. Beef slices exposed to oxygen above one atmosphere pressure; dose rate vs the amount of metmyoglobin greater than in the non-irradiated beef.

irradiation under atmospheric or vacuum conditions, irradiation under oxygen pressure resulted in definite oxidation at all dose rates. However, there was little difference in the amount of metmyoglobin produced at the gamma rates of 0.05 and 0.5 Krad/sec and the electron rates of 0.55 and 5.0 Krad/sec. At dose rates greater than 5.0, increases in the dose rate resulted in increases in metmyoglobin production.

Visible darkening of the sample surface was observed for all dose rates used, with greater darkening at higher rates. It was impossible to distinguish between the samples irradiated with the two lowest electron rates and the two gamma rates. The irradiation appeared to cause the conversion of oxymyoglobin to both reduced myoglobin and metmyoglobin, but the conversion to reduced myoglobin was not as great as with the two highest electron rates. The amount of oxymyoglobin which remained increased almost directly with a decrease in dose rate.

In summary, a meat surface darkening could be observed under certain conditions with pasteurizing doses of irradiation. The darkening, as determined by reflectance spectrophotometry, was due to the conversion of bright red oxymyoglobin pigment to either purple myoglobin or brown metmyoglobin. The conversion, especially to the oxidized pigment form, was dependent on the conditions at the time of irradiation. The effect was most noticeable with irradiation under oxygen pressure, and least noticeable when conditions of vacuum existed during irradiation. Oxidized pigment formation also appeared to be dependent on the dose rate used. Based on visual observations, this effect was minimal when irradiation was carried out with vacuum packaged beef, was generally noticeable at higher dose rates

with meat having access to air, and was pronounced when a pure oxygen atmosphere was present.

The range of dose rates studied was 0.05 Krad/sec with gamma rays to 50 Krad/sec with electrons. The two extremes could probably be considered as normal operational dose rates for the respective sources. Only at the highest dose rate, 50 Krad/sec, was there any significant oxidation of pigment with vacuum conditions. Electron radiation at the rates studied resulted in oxidation under atmospheric conditions, but gamma radiation at lower dose rates did not cause any changes. At all the dose rates studied, both types of radiation caused pigment oxidation when oxygen conditions existed. The amount of reduced pigment formed by the irradiation did not seem to follow any pattern. There was a large increase in reduced, purple myoglobin at all dose rates when vacuum conditions were used. At some dose rates with atmospheric conditions there was no net increase in myoglobin. With oxygen conditions, increases always occurred.

In the storage studies, only vacuum and atmospheric irradiation conditions were compared. After 21 days of vacuum storage, the amount of reduced pigment was a significant determinant of the oxygenation ability of the meat. The amount of reduced pigment present after storage was generally much greater when vacuum irradiation conditions were used. The amount of metmyoglobin after reoxygenation was similar for all dose rates under atmospheric irradiation conditions, but under vacuum irradiation conditions, the 50 Krad/sec rate resulted in slightly greater amounts than did the other rates. Although the reflectance measurements after reoxygenation of all atmospheric irradiation samples were similar, visual observations indicated

that the samples treated with higher dose rates were often unacceptable in appearance. Only the highest dose rate with vacuum irradiated samples had enough metmyoglobin to be often judged unacceptable in appearance.

There appeared to be little difference in surface effects between gamma and electron irradiation at the dose rates used except for dose rate differences. During storage, the initial visible differences disappeared and a fairly uniform color resulted. This generally occurred within a week after irradiation.

4. Effect of TPP on the pigment and color of fresh beef slices:

The extension of shelf-life and distribution time of centrally cut fresh beef poses the problem of pigment oxidation to brown metmyoglobin. This conversion was delayed to some extent by the effectiveness of the metmyoglobin reducing enzymes and by a system that included vacuum packaging. A noted decrease in oxidation rate occurred when various inorganic phosphates were used as treatments to slow fluid exudation or drip.

Experiments were conducted with fresh beef slices in an attempt to quantify some of the effects of inorganic phosphates on fresh meat. Cross-sectional beef slices were trimmed from U.S.D.A. Good grade eye of the round. The slices were dipped for 60 sec into the experimental solutions immediately after cutting, permitted to drain for 30 to 60 min, vacuum packaged, gamma irradiated with 100 Krad, and vacuum stored for a 21 day period at 38°F. Reflectance measurements were taken before irradiation, at 21 days, and following a single day of reoxygenation in air. Surface pH of the fresh meat was determined with a Beckman Expandomatic pH meter. After 22 days of storage, pH measurements were taken both of the surface

of the slices and of ground meat samples. Visual observations of color and appearance were made following the reoxygenation.

The effect of dipping in a 10% w/v solution of TPP was compared with dipping in some other inorganic compounds of similar pH values. A 10% w/v TPP solution has a pH of approximately 9.1. Solutions of reagent grade ammonium and sodium hydroxide and Tris-HCl and carbonate buffers were prepared with pH values in the range 9.0 to 9.2. All solutions were chilled before use. Color differences were noted visually throughout the refrigerated holding period, and reflectance measurements were taken upon termination of storage.

Results of experiments designed to compare the usefulness of some chemicals and buffer systems of high pH are listed in Table 15. Only the dip solutions containing phosphate resulted in favorable reoxygenation color. Although by spectrophotometric determination the other solutions resulted in less metmyoglobin formation than the control, they were not visibly superior. Initially the carbonate and Tris-HCl treated samples were as reduced in appearance as the phosphate treated samples, but they began to show increased oxidation after about two weeks of vacuum storage. The dibasic sodium phosphate treated samples often had a "dark cutter" appearance after a day of reoxygenation. The control was the most oxidized sample.

Experiments were also conducted to compare the dibasic form of orthophosphate, Na_2HPO_4 , with carbonate and borate buffers, NaTPP, and a commercial blend of phosphates called "Kena", supplied by Calgon Corporation. Again, visual observations were made throughout the 21 day vacuum holding

Table 15. Comparison of dip solutions with pH values of 9.0-9.2; pigment form and meat pH after oxygenation.

Treatment	Pigment form			Final pH of meat	
	% Mb	% MbO ₂	% MetMb	Ground	Surface
Control	20	30	50	5.6	5.6
TPP	19	70	11	5.9	5.85
NaOH	20	47	33	5.65	5.65
NH ₄ OH	23	33	44	5.65	5.65
Carbonate	22	45	33	5.7	5.75
Tris-HCl	24.5	44	31.5	5.75	5.75
Na ₂ HPO ₄	26.5	73.5	0	6.15	6.1

Table 16. Comparison of dip solutions with pH values of 9.0-9.2; pigment form after oxygenation.

Treatment	Pigment form		
	% Mb	% MbO ₂	% MetMb
Control	29	49	22
TPP	5	87	8
Carbonate	23	49	28
Borate	5	72	23
Na ₂ HPO ₄	16	84	0
"Kena"	19	81	0

period, and both visual observations and reflectance recordings taken after removal from vacuum and reoxygenation.

Table 16 shows that samples containing added phosphate were again superior in color after reoxygenation. The borate buffer dip was better than the carbonate buffer and the control, but did not give as bright red a color on reoxygenation as phosphate dip. The results obtained with dibasic sodium phosphate were inconsistent. Visually the samples dipped in dibasic sodium phosphate often appeared too purple after reoxygenation, similar to the "dark cutter" appearance of high pH beef. In a few cases, the beneficial effect of the dibasic sodium phosphate seemed to disappear before 21 days and the vacuum-packaged samples would begin to oxidize. The dibasic sodium phosphate seemed to be faster acting than the TPP, which probably had to be hydrolysed by meat phosphatases before it could affect the color.

5. Effect of dibasic phosphate on oxidation rates of Sephadex-purified myoglobin:

The influence of Na_2HPO_4 on oxidation rate was studied. Sephadex-purified oxymyoglobin was dialysed against deionized distilled water to remove the buffer. Quantities of Na_2HPO_4 were added to one ml fractions of the oxymyoglobin in molar ratios of 1:10, 1:1, and 10:1 phosphate ion to oxymyoglobin. The phosphate-myoglobin solutions were added to 3 ml portions of 0.2 M Tris-HCl buffer to give one mixture of each at pH 5.6, 6.1, 6.5 and 7.0. The samples were incubated in 16 X 125 mm test tubes in a water bath at $86^\circ\text{F} \pm 1^\circ\text{F}$.

An immediate spectrophotometric measurement was taken of each for zero time, and again at 15, 30, 60, 90, 120 and 150 minutes. No

provision was made to keep the temperature at 86°F for the brief time of measurement. The pH value of each sample was determined at the end of a test.

The method of Snyder and Ayres (5) and Brown and Dolev (6) was used to determine autoxidation rate. Changes in the peak at 582 nm, characteristic of oxymyoglobin, could be followed with a Bausch and Lomb recording spectrophotometer. The initial absorbance reading from a particular sample was assumed to be representative of 100% oxymyoglobin. With time, oxidation of the pigment resulted in a drop in the height of the peak at 582 nm. After the 150 minute test, a few milligrams of potassium ferricyanide were added, and the resulting absorbance value taken as representative of 100% oxidized pigment. The amount of oxidized pigment as a ratio of the total possible could then be determined for each test.

The effects of Na_2HPO_4 and pH on oxidation rates of purified oxymyoglobin solutions are shown in Table 17. These solutions had not been treated with the reducing agent, sodium hydrosulfite. The results are similar to previous findings, and indicate that as the pH was lowered, oxidation rates increased, Brown and Mebine (7); Yamazaki et al. (8); George and Stratmann (9). The comparison of oxidation rates between the control and phosphate treated samples indicated that a definite decrease occurred at pH 5.6 and a minor, but noticeable decrease at pH 6.1. However, at pH 6.4 and 7.0, the oxidation rates of the control and the treated samples were similar. These effects were independent of the molar ratio of phosphate to myoglobin.

Table 17. Effect of pH and Na_2HPO_4 on the oxidation rate of myoglobin; time vs percentage oxidized pigment.

pH	Time (minutes)	Percentage of oxidized pigment			
		Control	Molar ratio of phosphate to myoglobin		
			1:10	1:1	10:1
5.6	15	25.5	9.5	9.0	8.5
	60	46	30	28.5	31
	120	74.5	52.5	52.5	55
	150	86	63	63	65
6.1	15	17	9.5	8	9.5
	60	23.5	20.5	19.5	20
	120	40.5	37	35.5	34.5
	150	47	44	43	44
6.5	15	6.5	9	8	8
	60	15.5	19	17.5	18.5
	120	24.5	27.5	27	27
	150	29	32.5	31.5	31.5
7.0	15	4	4	5	2.5
	60	8	10	11	8.5
	120	15.5	16	16	13.5
	150	17	18	18.5	15.5

This particular study was an effort to find the role of phosphate in meat color stabilization as reported earlier and especially in Part 4 (page 41) of this report. This study on the isolated meat pigment, myoglobin, shows surprisingly little action by Na_2HPO_4 in decreasing the oxidation rate of the pigment. It may be significant that some effect was observed at pH's which correspond with those which are normal to fresh meat. The findings reported in Part 4 of this report suggest that phosphate may be acting additionally on other factors of importance such as enzyme systems.

That some association does exist between inorganic phosphates and myoglobin can be shown with radioactive phosphate, Table 18. Small quantities of $\text{Na}_2\text{H}^{32}\text{PO}_4$ were added to myoglobin solutions, held overnight at 40°F, and then applied to a Sephadex G-75 column. As shown by the table, some radioactive phosphate was eluted from the column with the myoglobin fraction. If it was not bound, because of its small size, the phosphate ion should have been held back on the column and separated from the heavier myoglobin. This experiment was a qualitative indication of association, but could not be used to determine the interaction ratio or its specificity.

A dialysis method also gave indications that an association existed, Table 19. Again, the experiments were qualitative and did not attempt to show any interaction ratio. The molarity of the phosphate was very low, and the number of molecules of phosphate was much lower than the number of molecules of myoglobin. Figure 2 shows that near equilibrium was reached within two days of dialysis. The amount of radioactive phosphate remaining with the myoglobin was a fairly small percentage of the total after one day of dialysis and decreased very little in an extra day. The count

Table 18. Separation of radioactive phosphate from myoglobin on a Sephadex column ; fraction location vs the count of $\text{Na}_2\text{H}^{32}\text{PO}_4$.

Fraction location		Count/minute (0.1 ml aliquot)
First red color out of column	5 ml	30
	5 ml	32
	5 ml	36
Bright red color	5 ml	39
	5 ml	43
	5 ml	43
Buffer after Mb	300 ml	28

Table 19. Separation of radioactive phosphate from myoglobin by dialysis; time vs count of $\text{Na}_2\text{H}^{32}\text{PO}_4$.

Time (hours after beginning of dialysis)	Count/minute (0.1 ml aliquot)
16	50
20	31
21	36
22	33
28	24
41	13
46	10
52	9
52 (with myoglobin)	1.14×10^4 (2% of added phosphate)

Figure 2. The dialysis to equilibrium of radioactive phosphate from a myoglobin solution.

given by the radioactive phosphate in the myoglobin at the time dialysis was stopped indicated association.

Attempts to quantify the possible ratio of association were not successful, Table 20. If, as theorized, there was a 1:1 association, no radioactive phosphate should have been dialysed from the samples with 1:10, 1:2, and 1:1 ratios of phosphate to myoglobin. The counts for 2:1, 5:1 and 10:1 should have been proportional to the amount of phosphate in excess of myoglobin, i.e., equivalent to 4, 8 and 9 ml of added phosphate, respectively. However, as shown in Table 20, radioactive phosphate was dialysed from all the samples, and thus, under the conditions of the test, it would seem unlikely that a 1:1 association existed that could withstand the forces exerted by dialysis. The test sample counts were almost linear with quantity of radioactive phosphate added, and indicated a definite retention of phosphate when myoglobin was present. This suggested, as did the results from the previous tests, that some form of association occurred between phosphate and myoglobin.

Table 20. Association of radioactive phosphate with myoglobin; count of radioisotope in dialysis water vs molar ratios of phosphate to myoglobin.

Condition	Molar ratio of phosphate to myoglobin					
	1:10	1:2	1:1	2:1	5:1	10:1
Quantities of added $^{32}\text{PO}_4$, ml	1	4	5	8	10	10
Quantities of Mb added, ml	10	8	5	4	2	1
Average cpm after 24 hr dialysis	455	1870	2315	3450	4605	4495
Count/minute from control (no Mb present), 24 hr dialysis	675	- -	2460	- -	- -	5295
Theoretical count for the control (100% dialysis)	630	- -	3150	- -	- -	6300

Phosphate buffer, pH 6.4.

Mb, 2×10^{-4} M.

Radioactive phosphate tracer, 1 in 250.

Sample aliquots, 0.2 ml.

CONCLUSIONS

Previous work (1)(2) led to the following conclusions:

1. Radiation at dose levels of 50 to 150 Krad delays microbial spoilage for more than 21 days at 38°F.
2. Total microbial counts of vacuum packed irradiated (50-150 Krad) and unirradiated cuts rise steadily during refrigerated holding, but much more slowly than on similar cuts held in air. Once re-exposed to the atmosphere, upon removal from vacuum, the total counts increase more rapidly, especially on unirradiated cuts which, if not already spoiled, quickly spoil.
3. Phosphate dipping with sodium tripolyphosphate or sodium hexameta-phosphate glass (64% P_2O_5) resulted in a 0.5 to 1 unit rise in meat pH. This treatment reduces fluid exudation, and generally improves pigment stability by a) aiding in the prevention of pigment oxidation, and b) enhancing pigment reduction when oxidation takes place upon vacuum packaging. Phosphate treatment also resulted in a slight overall enhancement of microbial outgrowth on refrigerated cuts held aerobically and anaerobically. This latter effect is not considered to be a significant deterrent to the use of phosphates on fresh meats for control of fluid exudation and pigment oxidation.
4. The approach of bulk vacuum-packing individual retail cuts immediately after cutting and treating with phosphate followed by re-exposure of the cuts to the atmosphere after three weeks or more of refrigerated (40°F) anaerobic holding is effective in yielding normal red meat color at the end of the extended post-cutting holding period, provided irradiation is employed to delay microbial spoilage.

This approach invariably yields excellent results with respect to color and drip control when non-wrapped cuts are tightly packed into the bulk vacuum containers, and are individually packaged after removal from the bulk vacuum containers after two to three weeks or more of refrigerated storage.

5. When the retail cuts are prepackaged in gas permeable fresh meat wrapping film prior to bulk vacuum packing, some discoloration and fluid exudation frequently occur in the individual packages. The major cause of discoloration of the prepackaged cuts appears to be residual air entrapped within the package when the vacuum is drawn in the bulk container.

6. If fresh meat is held at a constant holding temperature of 28-30°F, radiation is not needed to delay microbial spoilage to achieve the needed extension of salable life. Also, a holding temperature just above the freezing point practically eliminates drip and aids in preventing pigment and lipid oxidation. However, this approach is probably not feasible for packer level red meat centralized cutting. At "conventional refrigeration temperatures" (c.a., 35-42°F) irradiation to 50-150 Krad is necessary to delay the onset of microbial "spoilage". Under the conditions of handling and holding being developed in this work, both the irradiated and unirradiated cuts eventually undergo an innocuous spoilage process dominated by short chain organic acid (e.g., lactate) producing species. The radiation treatment at the level proposed does not eliminate recognizable microbial spoilage, it merely delays it. Under conditions of temperature abuse (e.g., holding at 50°F) irradiation at the cited dose range is inadequate, and rapid, frank spoilage occurs with both irradiated and unirradiated cuts.

As a result of work reported herewith, the following conclusions may be added:

With respect to eating quality, phosphate treatment results in increased juiciness and an apparent increase in tenderness of irradiated and non-irradiated beefsteaks. The combination of ionizing radiation and added water in the meat due to phosphate treatment produces a detectable change in the taste and odor of the meat. Determination of the significance of this change, which is typically scored as less than one point difference on an acceptance scale of nine steps requires study with large consumer type panels. Phosphate concentrated at the surfaces of cuts resulting from dipping in phosphate solution may also be contributing to lowered taste, odor and overall quality scores. The data indicate that both the phosphate and the irradiation-water interaction influence taste and odor of cooked beefsteaks. Further study of this area is indicated. It is likely that by minimizing the amount of added water, the undesirable side effect would also be minimized. A similar effect has been observed with irradiated ham containing added water.

Off-flavor development during the post-vacuum aerobic phase of storage has been demonstrated to be associated in part with lipid oxidation during this holding period in air. The significance of this problem, which is of a more serious nature than that discussed in the previous paragraph, is underlined by the fact that a minimum of four to five days of refrigerated aerobic holding from the time of placing in the display case to the time of consumption in a commercial situation must be achieved (10). An antioxidant treatment (e.g., ascorbic acid) may be needed. Ascorbic acid was found to

be effective in reducing lipid oxidation during the aerobic phase of holding. The TBA analysis data clearly support this conclusion but additional taste panel data are needed to determine if the flavor is satisfactory.

Data and observations point to the gas entrainment pumping method of phosphate application for large cuts as being markedly superior to the dipping method in improving water holding capacity. Dipping of beefsteaks in phosphate solution, however, seems to provide better surface color stability than does pumping of large cuts prior to slicing into steaks. Perhaps pumping prior to cutting for increasing water holding capacity plus a light surface phosphate solution spray after cutting into steak type cuts for maintaining surface color might together constitute an optimum approach to phosphate application. Further work in this area is also indicated.

Results of the dose rate work indicate that the higher the dose rate above a threshold level (approximately 5 Krad/sec), the greater is the surface discoloration on fresh red meat cuts irradiated in air. A dose rate effect on surface color and reoxygenation ability is insignificant at dose rates up to 50 Krad per sec applied to cuts irradiated in vacuum.

The role of phosphates in protecting against surface discoloration in air may be due to its influence on the pigment oxidation rate. Enzymatic processes associated with metmyoglobin reducing activity (MRA) may also be contributing to color stability by maintaining the pigment in the reduced, oxygen binding state. Phosphate could facilitate such enzymatic processes, assuming they are significant, by raising the meat pH to a level more nearly optimal for the enzyme activity involved, and perhaps also by providing inorganic phosphate.

SELECTED REFERENCES

- (1) Urbain, W. M., G. G. Giddings, P. S. Belo and W. W. Ballantyne. 1969. Radiation pasteurization of fresh meats and poultry. Annual Report to the U.S. Atomic Energy Commission, Div. of Isotopes Development. COO-1689-5 (TID-4500).
- (2) Urbain, W. M., G. G. Giddings, P. S. Belo and W. W. Ballantyne. 1968. Radiation pasteurization of fresh meats and poultry. Annual Report to the U.S. Atomic Energy Commission, Div. of Isotopes Development. COO-1689-2 (TID-4500).
- (3) Anon. 1967. "Now it's Swift tendering of primals with liquid". The Nat. Provisioner, Sept. 9, 1967.
- (4) Anon. 1968. "Internal Spray System Beats Aging". Meat, April, 1968.
- (5) Brown, W. D., and A. Dolev. 1963a. Autoxidation of beef and tuna myoglobin. J. Food Sci. 28:207.
- (6) Brown, W. D., and L. B. Mebine. 1969. Autoxidation of oxymyoglobin. J. Biol. Chem. 244:6694.
- (7) George, P., and C. J. Stratmann. 1952b. The autoxidation of myoglobin to metmyoglobin by oxygen. Biochem. J. 57:586.
- (8) Snyder, H. E., and J. C. Ayres. 1961. The autoxidation of crystallized beef myoglobin. J. Food Sci. 26:469.
- (9) Yamazaki, I., K. Yokota and K. Shikama. 1964. Preparation of crystallized oxymyoglobin from horse heart. J. Biol. Chem. 239:4151.
- (10) Yankelovich, D., Inc. 1968. Cost benefit analysis of radiation pasteurization of meat under centralized cutting conditions. NYO-3831-1. Prepared for the U. S. Atomic Energy Commission, Div. of Isotopes Dev. (TID-4500), pp 71 and 118.

NOTICE: The government publications (References 1, 2 and 10) are available from:

National Technical Information Service
U. S. Department of Commerce
Springfield, Virginia 22151

PRICE: Printed copy \$3.00; Microfiche \$0.65