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**RADIATION PASTEURIZATION OF
FRESH MEATS AND POULTRY**

Annual Report, February 15, 1968—February 14, 1969

March 15, 1969

Department of Food Science
Michigan State University
East Lansing, Michigan

UNITED STATES ATOMIC ENERGY COMMISSION • DIVISION OF TECHNICAL INFORMATION

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RADIATION PASTEURIZATION OF FRESH MEATS AND POULTRY

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FOR:

**Division of Isotopes Development
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ANNUAL REPORT

For the Period:

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III. PREFACE:

This annual report was prepared in accordance with the terms of Contract No. AT(40-1)-1689 ... Modification No. 2 between the United States Atomic Energy Commission and Michigan State University.

The material included in this second 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.

IV. SUMMARY

The objective of this contract in the A.E.C. Radiation Preservation of Foods Program is to develop the process of radiation pasteurization of fresh meats and poultry to permit centralized cutting of these products. Work during this contract year has demonstrated the effectiveness of the following integrated approach in solving the discoloration, drip and microbial problems:

- a) Irradiation at 50 to 150 Krad in combination with conventional refrigeration (ca. 35-42°F) to sufficiently delay the onset of microbial spoilage.
- b) Treatment with condensed phosphates (e.g. tripolyphosphate) to control fluid exudation and to aid in preventing pigment oxidation.
- c) Bulk vacuum packing of the individual cuts at the point of cutting, and re-exposure of the cuts to the atmosphere at the point of retail sale in order to provide normal red meat color in the display case.

This combination approach has worked satisfactorily in the laboratory and shows considerable promise. Developmental efforts, particularly in the areas of phosphate application and packaging, are continuing.

V. INTRODUCTION

This second annual report on the contract covers work completed subsequent to that covered in the first annual report (Report No. COO-1689-2) and on up to the end of the second contract year (February 15, 1969). Background information and materials and methods were discussed in considerable detail in the first annual report. Such information is not repeated in this report, and the reader is referred to the first annual report for the same. Supplementary background and materials and methods information are, however, included in this report where appropriate. The reader is also referred to Report No. ORNL-IIC-20, 'Centralized Processing of Fresh Meat and Poultry Including Radiation Pasteurization . . . A Bibliography', which contains an extensive compilation of literature references dealing with all phases of the contract work. This bibliography, dated April, 1969, is published by the Oak Ridge Isotopes Information Center and is available through the National Clearinghouse for Federal Scientific and Technical Information.

The contents of this report are grouped according to primary problem areas although the areas are somewhat inter-related, and some overlap is unavoidable. The emphasis of the first part is on quantitative microbiological data, and is followed by material largely having to do with data of quantitative drip control and lipid oxidation experiments. This is followed by material dealing with quantitative color measurement and the effects of various parameters, including dose rate, on color. A discussion follows of results of a number of bulk packing experiments which are aimed at determining the effect of various bulk packing parameters on product life, and, most importantly, product appearance. The report concludes with results of taste panel evaluations. Tentative conclusions are drawn from the results of the contract work available thus far.

VI. EXPERIMENTAL RESULTS

A. MICROBIOLOGICAL

This section is concerned with completed experiments mainly having to do with the evaluation of various treatment and handling parameters as to their effect on the quantitative microbial picture. Primary reliance was on total plate counts which were carried out as described in the first annual report (COO-1689-2) "Radiation Pasteurization of Fresh Meats and Poultry", dated March 15, 1968, published by the USAEC Division of Technical Information, and available through the Clearinghouse for Federal Scientific and Technical Information. In addition, pH determinations were usually made in order to provide some insight as to the nature of the outgrowing flora and the mode of spoilage. 'Normal' fresh meat spoilage is usually manifested by, among other things, a rise in pH whereas anaerobically-held radiation-pasteurized (50-150 Krad) fresh meat spoilage is usually accompanied by a fall in pH, most likely the result of a spoilage flora dominated by organisms producing short chain organic acids. More definitive qualitative microbiological investigations are presently underway. Whenever a sample was taken for analysis, its color, odor and overall appearance were observed and recorded. The following is a discussion of each experiment included under part (A).

A-1: Comparison of irradiated and unirradiated, phosphate treated and untreated vacuum packaged and aerobically packaged fresh beefsteak:

A U.S.D.A. Commercial Grade round of beef was trimmed of layer fat and separated into individual muscles which were sliced into 1/2" thick steaks. These steaks, without differentiation, were used as follows:

The steaks were first randomly divided into two groups. One group of steaks was individually packaged in oxygen permeable "Lexel" (Dow Chemical Company experimental fresh meat wrapping film), and the other group was individually vacuum packaged in gas impermeable laminate pouches. Prior to packaging, some of the steaks of each group were dipped 30 seconds in a 10% solution of sodium tripolyphosphate (TPP). Some of each group were irradiated to 100 Krad at a rate of about 200 Krad per hour and the remainder were not irradiated. Except for the set which was evaluated immediately after irradiation, all samples were placed in storage at 38°F to await evaluation. Results of the analyses and observations are summarized in Table 1.

Table 1. (Expt. A-1). Comparison of irradiated and unirradiated, phosphate treated and untreated vacuum and aerobically packaged fresh beefsteaks

Variable	3 hrs after irradiation		
	pH	TPC/gm	Observations
A-0	5.58	1.2×10^5	Good color and odor
A-0 dip	5.95	2.0×10^5	'
A-I	5.60	1.0×10^3	"
A-I dip	5.98	1.6×10^3	'
V-0	5.58	1.2×10^5	'
V-0 dip	5.95	2.0×10^5	'
V-I	5.58	2.1×10^3	'
V-I dip	6.00	4.2×10^3	'
7 days @ 38°F			
A-0	5.62	2.7×10^8	Brown, spoiled
A-0 dip	6.03	8.4×10^8	Red brown, spoiled
A-I	5.60	9.5×10^3	Red + brown, good odor
A-I dip	5.92	4.3×10^4	Red, good odor
V-0	5.60	3.1×10^5	Purple,
V-0 dip	5.95	1.2×10^6	"
V-I	5.57	2.8×10^3	'
V-I dip	5.90	7.2×10^3	'
7 days under vacuum plus 5 days in air @ 38°F			
V-0	5.72	6.5×10^8	Red + green + brown, spoiled
V-0 dip	6.08	7.8×10^9	'
V-I	5.50	1.2×10^6	Dull red, trace off-odor
V-I dip	5.85	3.0×10^7	Red + green, sl off-odor

CODE:

A = Packaged in oxygen permeable film.

V = Packaged under vacuum.

O = No irradiation.

I = 100 Krad.

Dip = Dipped 30 seconds in 10% TPP.

Table 1 - cont'd:

Variable	14 days @ 38°F		Observations
	pH	TPC/gm	
A-O	Badly spoiled, discarded		
A-O dip	"	"	
A-I	5.68	8.2×10^8	Discolored, sl spoiled odor
A-I dip	6.15	1.3×10^8	" "
V-O	5.60	4.2×10^7	Brown, purple, trace sour odor
V-O dip	5.96	9.0×10^7	" "
V-I	5.57	9.1×10^5	Purple, tr. off-odor
V-I dip	5.90	2.5×10^6	" "
<u>14 days under vacuum plus 3 days in air @ 38°F</u>			
V-O	5.82	6.6×10^8	Discolored and spoiled
V-O dip	6.40	3.1×10^9	" "
V-I	5.60	8.0×10^6	Brown, sl off-odor
V-I dip	5.85	9.2×10^7	Red, " "
<u>21 days @ 38°F</u>			
V-O	5.56	5.5×10^8	Discolored and sour
V-O dip	5.85	1.6×10^9	" "
V-I	5.48	8.8×10^6	Purple + brown, tr.off-odor
V-I dip	5.78	3.8×10^7	" "
<u>21 days under vacuum plus 3 days in air @ 38°F</u>			
V-O	5.60	1.4×10^9	Discolored and sour
V-O dip	5.88	4.8×10^9	" "
V-I	5.42	1.1×10^8	Brown, sl off-odor
V-I dip	5.76	3.5×10^8	Red + brown, sl off-odor

The three hour post-irradiation analysis data indicate that the magnitude of the total microbial count reduction is essentially the same for comparable samples whether irradiated aerobically or under vacuum. The immediate effect of the irradiation was an overall plate count reduction corresponding to about two log cycles. Unirradiated, aerobically packaged samples became discolored and spoiled within one week whereas irradiated aerobically packaged samples did so within two weeks. Spoilage was accompanied by a rise in pH for both variables. Counts of vacuum packaged irradiated and unirradiated samples rose steadily but more slowly than the aerobically packaged samples, but once open to the air the rise was quite rapid. The vacuum packaged samples, particularly those irradiated, exhibited a tendency to undergo a decline in pH during storage. This tendency reversed itself somewhat, particularly with the unirradiated samples, upon going from vacuum to aerobic storage. This suggests that, for the unirradiated vacuum packaged meat especially, acid producing species dominate during vacuum holding and are overgrown by the aerobic spoilage types during subsequent holding in air. Pseudomonad type colonies were much more prevalent on plates from these samples after a few days in air than was the case immediately upon removal from vacuum. This was much less true with irradiated vacuum packaged samples where irradiation destroyed nearly all of the pseudomonad types. Steaks treated with TPP and irradiated with 100 Krad. were slightly discolored and had a detectable odor difference after storage at 38°F for 21 days under vacuum plus three days in air.

A-2: Effect of polyphosphate dip on total bacterial counts of fresh beefsteak:

A-2-1: Four Commercial Grade beef semitendinosus (eye of round) muscles each were sliced into 3/4 inch thick steaks. One-half of the slices from each muscle were dipped for 30 seconds in a 10% tripolyphosphate solution; the other half was not treated. Half of the TPP treated and half of the untreated steaks were packaged in gas-permeable plasticized polyvinyl chloride fresh meat film, and were stored at 38°F for five days, after which time they were analyzed for total bacterial counts. The other samples of each variable were immediately analyzed (zero time) for total bacterial counts and pH. Results are summarized in Table 2. Radiation was not employed. Zero time pH readings were taken to observe the initial effect of phosphate treatment on the meat pH.

As expected, in all cases dipping in TPP solution raised the pH of the meat significantly. Dipping did not appreciably alter the zero time total bacterial counts. In three of the four cases (muscles 1, 2 and 3) five day total

Table 2. (Expt. A-2-1). Effect of polyphosphate dip on total bacterial counts of fresh beefsteak

	Zero storage time		5 days @ 38°F
	TPC/gm	pH	TPC/gm
Muscle #1, no dip	3.8×10^5	5.56	2.6×10^8
" , dip	3.2×10^5	5.88	9.5×10^8
" #2, no dip	4.5×10^5	5.65	8.8×10^7
" , dip	4.6×10^5	5.92	3.5×10^8
" #3, no dip	2.1×10^6	5.98	1.0×10^8
" , dip	4.0×10^6	6.43	1.6×10^9
" #4, no dip	1.2×10^5	5.62	9.0×10^8
" , dip	9.2×10^4	5.98	6.6×10^8

counts were significantly higher for dipped samples than for their undipped counterparts. This may or may not be entirely due to increased moisture and more favorable pH for outgrowth. It was observed that the numbers of large, smooth, spreading pseudomonad colonies especially were greater on TPC plates from dipped steaks than on plates from comparable non-dipped steaks. Since this is the principal spoilage microorganism of aerobically stored unirradiated fresh beef, it is of particular interest that TPP treatment appears to enhance the pseudomonad outgrowth.

In a similar experiment (A-2-2), a top, bottom and knuckle of the round, each part from a different Commercial Grade beef round, were trimmed and sliced into 3/4 inch thick steaks. Half of the steaks taken from each of the parts of the round were dipped for 30 seconds in a 10% TPP solution; the remaining steaks were not treated with phosphate. After packaging all steaks in plasticized polyvinyl fresh meat film, half of the dipped steaks and half of the undipped steaks were gamma irradiated to 100 Krad; the other steaks were not irradiated. Half of the steaks from each variable were immediately analyzed for total bacterial count and pH. The remaining steaks of each variable were stored at 38°F for five days before evaluation. Results are summarized in Table 3.

As in the previous experiment TPP dip had no great effect upon initial plate counts. In all cases an increase in the pH of the meat occurred immediately. After five days at 38°F all unirradiated samples showed signs of spoilage, whereas all of the irradiated did not. After five days, in almost every case, dipped samples had somewhat higher total plate counts than their undipped counterparts. Pseudomonad type colonies (large, smooth, creamy, spreading surface colonies) were almost non-existent on plates from irradiated samples, whereas they were the predominant type on plates from non-irradiated samples. Again, these colonies were observed to occur in greater numbers on plates from dipped non-irradiated samples than from comparable undipped samples. The 100 Krad dose provided a one to two log cycle reduction in total counts, and this difference in total count between irradiated and unirradiated samples seemed to widen slightly with time.

From these and other data it was concluded that the slightly higher counts obtained from phosphate treated samples are due to accelerated outgrowth during the holding period which may have been influenced by a pH nearer neutrality.

Table 3. (Expt. A-2). Effect of polyphosphate dip on total bacterial counts of fresh beefsteak

Source of samples *	Phosphate dip	Dose (Krad)	Zero storage time		5 days @ 38°F
			TPC/gm	pH	TPC/gm
Top	No	0	3.3×10^4	5.52	1.2×10^8
'	Yes	0	3.0×10^4	6.02	9.6×10^8
'	No	100	8.7×10^2	5.53	1.1×10^6
'	Yes	100	2.2×10^3	5.98	4.6×10^6
Bottom	No	0	5.5×10^5	5.50	6.0×10^8
'	Yes	0	3.8×10^5	5.92	10.0×10^8
'	No	100	2.0×10^3	5.50	1.8×10^6
'	Yes	100	4.7×10^3	5.90	2.7×10^6
Knuckle	No	0	8.2×10^4	5.63	9.2×10^8
'	Yes	0	6.6×10^4	6.15	8.5×10^8
'	No	100	2.4×10^3	5.62	9.8×10^5
'	Yes	100	1.3×10^3	6.20	5.9×10^6

* 'Top', 'bottom' and 'knuckle' refer to three major parts of the 'round'.

A-3: Effect of total dose on microbial flora of phosphate treated vacuum packaged fresh beefsteaks:

A Commercial Grade bottom round of beef was trimmed of loose fat and sliced into 1/2 inch thick steaks. Sixteen of the steaks were immediately individually vacuum packaged in gas impermeable pouches. The remaining steaks were dipped 30 seconds in a 10% TPP solution, allowed to drain for 15 minutes, and vacuum packaged. Phosphated and unphosphated steaks were irradiated at several levels as shown in Table 4. Dose rate was 200 Krad per hour. A cross comparison between phosphated and unphosphated samples occurs at the 0 Krad and 100 Krad dose levels. Samples were post-irradiation stored at 38°F. At the 0, 7, 14 and 21 day post-irradiation times, duplicate samples of each variable were analyzed for total plate count. pH was measured after a 1:10 dilution with water. They were also observed for irradiation odor and for spoiled odor as well as for discoloration and overall appearance. Results are summarized in Table 4.

As usual, phosphate treatment caused a rise in meat pH which may have contributed to the slightly higher plate counts in these samples after storage. In this instance, 50 Krad appeared sufficient to reduce the microbial population enough to obtain 21 days vacuum storage at 38°F. Had these samples been stored for an additional 1 to 3 days in air, a higher dose . . perhaps 100 Krad . . might have been necessary. The decrease in pH of the unirradiated and the lower dose irradiated samples during storage coupled with the sour odor detected from the unirradiated samples after 21 days storage in vacuum at 38°F again suggests that microbial spoilage under vacuum is due to the outgrowth of acid-producing (i.e., lactic acid) bacteria.

A-4: Comparison of total plate counts from the surfaces and interiors of beefsteaks treated with electron and gamma radiation:

Since the microbial contamination is usually confined to the surfaces, it has been suggested that a surface dose of electron (or beta) radiation may, for certain applications such as meat pasteurization, be preferred over penetrating gamma radiation. With electron irradiation, it may be possible to use a greater dose with less flavor change since only the surface will be irradiated. Lower irradiation costs could be anticipated, mainly through reduced shielding requirements and smaller total energy needs.

Table 4. (Expt. A-3). Effect of total dose on microbial flora of phosphate treated vacuum packaged beefsteak

Dose (Krad)	TPP treated	Storage time (days) at 38°F							
		0		7		14		21	
		pH	TPC/gm	pH	TPC/gm	pH	TPC/gm	pH	TPC/gm
0	No	5.58	3.0×10^6	5.56	8.3×10^6	5.42	3.3×10^7	5.23	4.5×10^8
0	Yes	6.42	1.6×10^6	6.45	1.0×10^7	6.22	5.6×10^7	6.00	7.0×10^8
50	Yes	6.23	1.8×10^4	6.20	6.2×10^4	6.10	7.7×10^6	5.98	3.0×10^7
100	No	5.60	1.6×10^3	5.60	2.8×10^4	5.58	1.4×10^6	5.52	5.5×10^6
100	Yes	6.40	8.0×10^3	6.36	3.3×10^5	6.33	3.0×10^6	6.32	9.0×10^6
250	Yes	6.18	1.0×10^2	6.20	2.8×10^2	6.20	8.6×10^3	6.16	9.8×10^3
500	Yes	6.36	60	6.36	1.0×10^2	6.40	2.0×10^2	6.38	2.2×10^2
1000	Yes	6.30	< 10	6.34	< 10	6.34	< 10	6.35	< 10

Surface electron irradiation of fresh meats does have some disadvantages when compared with penetrating gamma irradiation. One disadvantage, as just mentioned, is insufficient penetration to effect possible subsurface microorganisms. Another is that surface irradiation precludes the option of irradiating the cuts after they are packed in a bulk container, again because of lack of penetration. Surface irradiation would necessitate irradiating each prepackaged cut individually and on all sides. Surface irradiation may further eliminate irradiating the cuts anaerobically (individual vacuum packaging is probably too expensive) thus foregoing the advantage, especially with regard to lipid and other oxidations, of having the meat in a vacuum or otherwise inert atmosphere during as well as after irradiation unless individual cut packaging, irradiation, and multi-package bulk packing all could somehow be economically done under vacuum or inert atmosphere conditions. Surface irradiation would, of course, make it mandatory to pre-package the individual cuts prior to irradiating.

It was considered desirable, none-the-less, to compare the relative effects of gamma (penetrating) and electron (surface) radiation. Results of an initial exploratory comparison are summarized in Table 5. Further comparisons from the point of view of microbial kill and spoilage delay are planned. Color studies employing electron irradiation are included in Section VI-C of this report.

A bottom round of beef was cut into 1 1/2 inch thick steaks which were packaged individually in 'Laminate' pouches. Unirradiated controls were set aside and the remaining steaks were divided into two lots. One lot was gamma irradiated to 100 Krad at a dose rate of 2 Mrad per hour. The other lot was electron irradiated using a Resonant Transformer (1 million volt peak energy; current = 0.1 milliamp) to 100 Krad at a dose rate of about 9 Mrad per hour.

For the initial evaluation duplicate electron, gamma and control steaks were first aseptically sampled by the 'normal' method. Then a scalpel was used to aseptically remove a thin surface layer a few millimeters thick from surfaces of the irradiated samples. Following this a cork borer was used to aseptically take plugs from the irradiated samples for an interior microbial count. For subsequent withdrawals only the 'normal' sampling method was used. The data indicate that the flora was practically confined to the surface, as expected with this type of cut. Gamma and electrons gave about the same overall kill, but the electrons appeared to give a greater surface kill. This apparent difference could have been an artifact due to sampling. Electron irradiated steaks appeared to reach higher overall counts with storage. More work comparing the two types of irradiation is needed, especially on bulky retail cuts, before any conclusions can be drawn.

Table 5. (Expt. A-4). Comparison of total plate counts from the surface and from the interior of beefsteaks treated with electron and gamma radiation

Variable	Total microbial plate counts per gram of sample*	
	Zero time, immediately after irradiation	8 days in vacuum
Electron (normal)	1.5×10^3	3.0×10^4
Gamma (normal)	1.1×10^3	3.2×10^4
Electron (surface)	6.1×10^2	- -
Gamma (surface)	2.0×10^3	- -
Electron (plug)	< 10	- -
Gamma (plug)	< 10	- -
Unirradiated (normal)	7.4×10^4	8.2×10^5
	<u>15 days in vacuum</u>	<u>23 days in vacuum</u>
Electron (normal)	4.5×10^6	5.5×10^7 Good odor
Gamma (normal)	6.0×10^5	8.4×10^6 ' '
Unirradiated (normal)	5.5×10^7	1.2×10^9 Spoiled odor

Dose = 100 Krad

Storage temp = 38°F

'Plug' indicates sample taken from meat interior.

'Surface' indicates sample taken from shallow surface layer.

"Normal" indicates sampling by the usual method of removing a portion consisting of both surface and interior.

* Averages of duplicate samples.

A-5: Effect of TPP dip, radiation, storage temperature and anaerobic -
followed by aerobic storage on quality of fresh red meats:

To study the effect of the above variables three separate experiments were conducted; two employing fresh beefsteak, and a third employing ground fresh lamb in order to challenge the treatments with highly perishable raw material.

The two beef experiments were each set up as follows: Three commercial grade beef top rounds were trimmed and cut into 1/2 inch thick steaks. The steaks from each top round were kept separate from, but were treated in the same manner as the steaks from the other two top rounds. The steaks from each round were divided into four treatment variable groups:

- Group 1) No phosphate, no irradiation (0-0)
2) 30 second dip in 10% TPP solution, no irradiation (P-0)
3) " " " 100 Krad. (P-I)
4) No phosphate, 100 Krad. (0-I)

All steaks were individually vacuum packaged, and the four groups were further divided into three storage temperature variables: 29, 40 and 50°F for the first beef experiment (Table 6), and 29, 35 and 40°F for the second beef experiment (Table 7). Post-irradiation storage temperatures were precisely controlled by use of special constant temperature storage boxes. Irradiation was carried out at a temperature of about 40°F at a dose rate of 200 Krad/hour.

At the 8, 16 and 24 day withdrawal times, duplicate samples for each variable were removed from storage and taken from their vacuum pouches. After noting appearance and odor, a 40-50 gram portion of each sample was aseptically taken for total plate count and pH analysis. The remaining portion of each sample was placed in a polystyrene tray and overwrapped with plasticized polyvinyl chloride fresh meat film. These samples were returned to their original storage temperatures for an additional four days of storage in air (aerobic) after which they were again evaluated for appearance and odor, total plate count and pH. The zero time (shortly after completion of irradiation) evaluation is a cross comparison of variables from all three of the rounds. All samples for the 8-day evaluation were from round No. 1 of the three rounds employed, and similarly, samples for the 16 and 24 day evaluations were from rounds Nos. 2 and 3, respectively. In this manner, round to round variability was eliminated. Results from the first beef experiment (A-5-1) are summarized in Table 6, and results from the second beef experiment (A-5-2) are summarized in Table 7.

Table 6. (Expt. A-5-1). Effect of phosphate, radiation, temperature and anaerobic followed by aerobic storage on quality of fresh beefsteaks

Variable		Zero time evaluation	
		pH	TPC/gm
Round 1,	O-O	5.53	2.0×10^4
"	O-I	5.57	4.0×10^3
"	P-O	6.25	6.1×10^4
"	P-I	6.20	1.4×10^3
Round 2,	O-O	5.52	8.2×10^3
"	O-I	5.58	5.0×10^2
"	P-O	6.17	2.0×10^4
"	P-I	6.35	3.8×10^3
Round 3,	O-O	5.53	3.2×10^4
"	O-I	5.55	2.2×10^3
"	P-O	6.20	3.3×10^4
"	P-I	5.98	6.0×10^2

CODE:

O-O = No dip, no irradiation.
P-O = 10% TPP dip, no irradiation.
O-I = No dip, 100 Krad.
P-I = 10% TPP dip, 100 Krad.

29, 40 and 50 are constant storage temperatures in °F.

Table 6. Cont'd:

		<u>5 days in vacuum</u>		Observations	
Variable		pH	TPC/gm		
Round 1,	O-0-29	5.58	4.5×10^4	Phosphate treated samples were more purplish than the untreated. Samples stored at 50°F had slight off-odor. Samples stored at 29°F and 40°F had good odor.	
	P-0-29	6.32	7.2×10^4		
	O-1-29	5.48	1.5×10^3		
	P-1-29	6.23	5.0×10^3		
	O-0-40	5.45	1.0×10^2		
	P-0-40	6.12	1.3×10^7		
	O-1-40	5.42	1.5×10^5		
	P-1-40	6.15	1.7×10^5		
	O-0-50	5.23	7.0×10^7		
	P-0-50	5.83	8.2×10^8		
	O-1-50	5.45	7.1×10^7		
	P-1-50	5.95	3.0×10^8		
<u>8 days in vacuum plus 4 days in air</u>					
Round 1	O-0-29	5.58	2.5×10^5	Brown red, good odor	
	P-0-29	6.20	1.4×10^5	Bright red, "	
	O-1-29	5.62	1.8×10^4	Brown red, "	
	P-1-29	6.13	1.2×10^4	Bright red, "	
	O-0-40	5.40	2.2×10^8	Brown red, spoiled odor	
	P-0-40	7.00	7.9×10^8	Red + green, "	
	O-1-40	5.40	1.7×10^7	Brown red, good odor	
	P-1-40	5.73	8.0×10^7	Bright red, "	
	O-0-50	These 50°F samples were discarded at this time since they			
	P-0-50	were extremely spoiled and discolored.			
	O-1-50				
	P-1-50				

Table 6. Cont'd:

Variable		16 days in vacuum		Observations
		pH	TPC/gm	
Round 2,	O-0-29	5.42	5.5×10^4	Brown, good odor
	P-0-29	6.02	3.5×10^4	Purple, "
	O-I-29	5.50	1.0×10^3	Brown, "
	P-I-29	6.24	1.4×10^3	Purple, "
	O-0-40	5.28	1.5×10^7	Brown-purple, sl off-odor
	P-0-40	6.02	1.2×10^8	Purple, "
	O-I-40	5.46	1.1×10^6	Brown-purple, good odor
	P-I-40	6.08	6.0×10^6	Purple, "
	O-0-50	5.10	1.6×10^8	Brown-purple, sour odor
	P-0-50	6.07	1.4×10^9	" " "
	O-I-50	5.10	1.0×10^8	" " sl off-odor
	P-I-50	5.78	3.2×10^8	Purple, " "
<u>16 days in vacuum plus 4 days in air</u>				
	O-0-29	5.60	6.0×10^5	Brown, good odor
	P-0-29	6.12	8.0×10^5	Bright red, good odor
	O-I-29	5.50	7.0×10^4	Brown, " "
	P-I-29	6.28	4.2×10^4	Bright red, "
	O-0-40	5.41	3.1×10^8	Brown + green, spoiled
	P-0-40	6.28	2.2×10^9	" " "
	O-I-40	5.43	1.0×10^7	Brown, sl off-odor
	P-I-40	5.98	1.2×10^8	Red, " "
	O-0-50	Samples stored at 50°F were discarded at this time since they were extremely spoiled and discolored.		
	P-0-50			
	O-I-50			
	P-I-50			

Table 6. Cont'd:

Variable		24 days in vacuum		Observations
		pH	TPC/gm	
Round 3,	0-0-29	5.42	4.1×10^5	Brown, good odor
	P-0-29	6.14	1.5×10^6	Purple + brown, good odor
	0-I-29	5.42	2.0×10^3	Brown, good odor
	P-I-29	6.05	6.0×10^3	Purple, "
	0-0-40	5.25	1.8×10^8	Brown, spoiled
	P-0-40	5.94	4.3×10^8	Purple + brown, spoiled
	0-I-40	5.37	9.0×10^6	Brown, sl off-odor
	P-I-40	6.18	9.5×10^7	Purple, good odor
	0-0-50	5.23	5.2×10^8	Poor color and odor
	P-0-50	5.83	6.5×10^9	" "
	0-I-50	5.00	1.2×10^8	" "
	P-I-50	5.58	4.5×10^8	" "
<u>24 days in vacuum plus 4 days in air</u>				
Round 3,	0-0-29	5.50	1.8×10^6	Brown, good odor
	P-0-29	6.18	3.0×10^6	Bright red, good odor
	0-I-29	5.48	7.5×10^4	Brown, " "
	P-I-29	5.90	5.0×10^4	Bright red, "
	0-0-40	5.25	2.3×10^8	Brown + green, spoiled
	P-0-40	5.72	6.0×10^8	Red + green, "
	0-I-40	5.38	1.3×10^8	Brown, sl off-odor
	P-I-40	5.97	4.7×10^8	Bright red, sl off-odor
	0-0-50	Discarded		
	P-0-50	"		
	0-I-50	"		

Table 7. (Expt. A-5-2). Effect of phosphate, radiation, temperature and anaerobic followed by aerobic storage on quality of fresh beefsteaks

Variable	Zero time evaluation	
	pH	TPC/gm
Round 1,	O-0	5.48
	P-0	6.12
	O-I	5.49
	P-I	5.92
Round 2,	O-0	5.56
	P-0	6.03
	O-I	5.51
	P-I	5.82
Round 3,	O-0	5.39
	P-0	5.97
	O-I	5.41
	P-I	5.97

CODE:

O-0 = No dip, no irradiation.
P-0 = 10% TPP dip, no irradiation.
O-I = No dip, 100 Krad.
P-I = 10% TPP dip, 100 Krad.

29, 35 and 40 are constant storage temperatures in °F.

Table 7. Cont'd:

Variable		8 days in vacuum		Observations
		pH	TPC/gm	
Round 1,	O-0-29	5.43	1.3×10^5	Brown, good odor
	P-0-29	5.87	5.6×10^5	Purple, "
	O-I-29	5.50	1.5×10^3	Brown, "
	P-I-29	5.98	5.0×10^3	Purple, "
	O-0-35	5.45	1.2×10^6	Brown, "
	P-0-35	5.98	6.2×10^6	Purple, "
	O-I-35	5.46	1.5×10^3	Brown, "
	P-I-35	5.87	3.5×10^3	Purple, "
	O-0-40	5.56	8.8×10^6	" "
	P-0-40	5.86	3.0×10^7	" "
	O-I-40	5.46	1.4×10^5	" "
	P-I-40	5.92	1.6×10^6	" "
8 days in vacuum plus 4 days in air				
Round 1,	O-0-29	5.52	1.2×10^6	Red-brown, good odor
	P-0-29	5.78	9.3×10^6	Bright red, "
	O-I-29	5.54	7.0×10^3	Red-brown, "
	P-I-29	6.02	8.5×10^4	Bright red, "
	O-0-35	5.44	2.0×10^7	Brown-red, "
	P-0-35	5.88	1.9×10^8	Red, "
	O-I-35	5.51	1.1×10^5	Brown-red, "
	P-I-35	5.92	2.1×10^6	Bright red, "
	O-0-40	5.65	1.3×10^9	Brown + green, spoiled
	P-0-40	5.92	1.6×10^9	Red-brown + green, "
	O-I-40	5.46	2.0×10^7	Red-brown, sl off-odor
	P-I-40	5.75	4.1×10^8	Red, " "

Table 7. Cont'd:

Variable		16 days in vacuum		Observations
		pH	TPC/gm	
Round 2,	O-0-29	5.43	6.2×10^5	Brown, good odor
	P-0-29	6.08	5.8×10^6	Purple "
	O-I-29	5.40	2.0×10^4	Brown, "
	P-I-29	5.90	8.6×10^4	Purple, "
	O-0-35	5.44	1.0×10^7	Brown, "
	P-0-35	5.82	2.6×10^7	Purple, "
	O-I-35	5.42	4.6×10^5	Brown, "
	P-I-35	6.02	5.5×10^6	Purple, "
	O-0-40	5.47	6.6×10^7	Purple-brown, tr off-odor
	P-0-40	5.82	2.1×10^8	Purple, "
	O-I-40	5.43	9.3×10^6	Brown, "
	P-I-40	5.90	9.0×10^7	Purple, "
16 days in vacuum plus 4 days in air				
Round 2,	O-0-29	5.47	1.8×10^6	Red-brown, good odor
	P-0-29	5.97	2.2×10^7	Bright red, "
	O-I-29	5.43	3.9×10^5	Brown, "
	P-I-29	5.88	7.2×10^5	Bright red, "
	O-0-35	5.51	2.3×10^8	Brown-red, sl spoiled odor
	P-0-35	5.79	6.6×10^8	" " "
	O-I-35	5.45	1.3×10^7	" " good odor
	P-I-35	5.96	5.0×10^7	Bright red, " "
	O-0-40	5.62	3.0×10^9	Brown + green, spoiled odor
	P-0-40	5.82	4.2×10^9	" " "
	O-I-40	5.38	3.3×10^8	Brown-red, sl off-odor
	P-I-40	5.87	1.0×10^9	" " "

Table 7. Cont'd:

Variable:		24 days in vacuum:		Observations:
		pH	TRC/gm	
Round 3,	O-O-29	5.42	2.2×10^6	Brown, good odor
	P-O-29	6.03	2.7×10^7	Purple, "
	O-I-29	5.60	2.0×10^5	Brown, "
	P-I-29	5.98	2.1×10^5	Purple, "
	O-O-35	5.38	4.9×10^7	Brown, "
	P-O-35	5.90	1.5×10^8	Purple, "
	O-I-35	5.46	6.5×10^6	Brown, "
	P-I-35	5.98	8.3×10^7	Purple, "
	O-O-40	5.30	1.3×10^8	Brown, spoiled odor
	P-O-40	5.88	1.0×10^9	Purple, "
	O-I-40	5.52	8.5×10^7	" sl. off-odor
	P-I-40	5.92	2.5×10^8	" "
24 days in vacuum plus 4 days in air				
Round 3,	O-O-29	5.51	1.2×10^7	Brown, good odor
	P-O-29	5.90	1.3×10^8	Bright red, good odor
	O-I-29	5.67	1.4×10^6	Brown, "
	P-I-29	6.03	1.3×10^6	Bright red, "
	O-O-35	5.40	9.0×10^8	Brown-red + green, spoiled
	P-O-35	6.00	7.0×10^8	" " "
	O-I-35	5.55	2.5×10^7	Red-brown, good odor
	P-I-35	5.80	3.0×10^8	Red, "
	O-O-40	5.42	2.7×10^9	Brown, spoiled
	P-O-40	6.00	1.3×10^9	Brown-red, "
	O-I-40	5.52	8.3×10^8	" sl. off-odor
	P-I-40	5.97	9.8×10^8	" " "

On the average, dipping in 10% TPP increased meat pH 0.65 unit in the first experiment (A-5-1) and 0.50 unit in the second (A-5-2). A pronounced pH drop occurred during 50° storage, especially with the irradiated samples. Acid producing bacteria, which would be expected to grow well under such conditions, likely account for the decline in pH. Jaye, Kittaka and Ordal (1) reported that vacuum packed ground beef stored at 38°F experienced a pH drop and a rapid increase in lactic acid type bacteria. A 50°F storage temperature would be expected to further enhance such outgrowth. Similar reports to the same effect appear elsewhere in the literature. The same general trend occurred at 35° and 40°F, but not to as great a degree as at 50°F. This is in contrast to the spoilage of aerobically held unirradiated fresh meats which normally undergo a rise in pH due to the presence of a different spoilage flora which produces different metabolic products.

Only one set of samples of those held at 29°F (those unirradiated and TPP treated) reached a total plate count in excess of 10^8 per gram, and this was after 24 days in vacuum plus 4 days in air. No signs of spoilage were evident at any time for samples stored at 29°F. There was also no sign of spoilage in any of the samples stored at 35°F as long as they remained under vacuum. At 35°F rapid outgrowth and spoilage took place within a few days after removal from vacuum, and the data indicate that radiation is necessary to achieve up to three weeks salable life at this temperature. Provided phosphate treatment was used to protect color, irradiation at 100 Krad followed by 40°F storage appeared to be a barely adequate dose to provide a salable life in excess of three weeks. A 150 Krad dose would likely have provided a significant protective margin in this instance. At a constant storage temperature of 50°F all samples became noticeably spoiled after removal from vacuum and exposure to air. The pH dropped steadily throughout storage, and, even in vacuum, the samples exhibited recognizable signs of deterioration within the 2-3 week time frame. This is important since 50°F represents a possible storage temperature at which outgrowth of food poisoning and pathogenic microorganisms could occur should any be originally present in sufficient numbers. Such an occurrence would probably be extremely unlikely as long as harmless microorganisms of one type or another survived the irradiation in sufficient numbers to inhibit outgrowth of harmful species by rapid growth and production of obvious spoilage. At the dose range of interest (50-150 Krad) this would most probably be the situation, and the data and observations tend to support this view.

When spoilage was not the limiting factor, phosphate treatment was necessary with all storage temperatures to provide satisfactory bloom regeneration and salable meat color. TPP treatment does, however, have the undesirable effect of slightly increasing general microbial outgrowth. This is most likely due to the resulting increase in meat pH whereas most bacteria grow best in a medium of near neutral pH. Although this outgrowth enhancement is undesirable, its magnitude does not appear to be cause for concern and the beneficial effects of TPP treatment for drip and color appear to substantially outweigh this factor.

Another experiment (A-5-3) similar to the two with beef, was carried out with ground lamb in order to observe the effect of the variables on an extremely perishable raw material. A set of Choice Grade lamb legs from the same carcass was trimmed of excess fat and cut up into chunks. Half of the meat from each leg was dipped 30 seconds in 10% TPP solution and coarsely ground. The undipped portion was similarly ground. The dipped and undipped meat was separately mixed and then each was passed through a fine grind and thoroughly mixed again to insure homogeneous starting material. 200 gm portions of each batch were filled into laminate pouches. After the pouches were vacuum sealed, half of them were irradiated to 100 Krad at a rate of 200 Krad per hour. The other half was not. The preparation and irradiation were done at 40°F. Immediately after irradiation a set of samples was analyzed. The remaining samples were divided into three lots for subsequent storage at 29°, 34° and 40°F. Results are summarized in Table 8.

In this case the TPP dip raised the pH of the meat about 0.65 unit. There was a general reduction in pH with time for all variables. However, with the non-irradiated samples, some pH fluctuation was noted at the earlier withdrawal times between samples stored under vacuum and those stored in air at 40°F. The fluctuation in pH is probably due to the fact that the "normal", aerobic, pH-raising spoilage flora exerted a pH rise in air before the acid-producing types finally became dominant under vacuum as storage progressed. A storage temperature of 29° without irradiation was sufficient to prevent spoilage for 15 days in vacuum plus 4 days in air. For similar storage, but at the higher temperatures of 34° and 40°F, radiation was clearly necessary. After 23 days under vacuum plus 4 days in air, irradiation possibly was necessary for samples stored at 29°F. Judging from total counts and indications of spoilage, 29°F storage without irradiation was, as in the previous beef experiments, superior to 40°F storage with irradiation at 100 Krad.

Table 8. (Expt. A-5-3). Effect of phosphate, radiation, temperature and anaerobic followed by aerobic storage on quality of fresh ground lamb

Variable	Zero time evaluation		Observations
	pH	TPC/gm	
O-0	5.72	2.0×10^4	
P-0	6.43	3.4×10^4	
O-I	5.80	8.6×10^2	
P-I	6.40	1.0×10^3	
<u>7 days in vacuum</u>			
O-0-29	5.81	7.1×10^4	Brown-purple, good odor
P-0-29	6.50	5.0×10^5	Purple, "
O-I-29	5.82	2.0×10^3	Brown-purple, "
P-I-29	6.42	4.6×10^4	Purple, "
O-0-34	5.86	5.2×10^5	Brown-purple, "
P-0-34	6.42	5.0×10^6	Purple, "
O-I-34	5.84	8.8×10^3	Brown-purple, "
P-I-34	6.45	3.0×10^5	Purple, "
O-0-40	5.82	2.5×10^7	" sl sour odor
P-0-40	6.42	6.8×10^8	" " "
O-I-40	5.83	4.0×10^5	" good odor
P-I-40	6.50	3.3×10^7	" " "
<u>7 days in vacuum plus 4 days in air</u>			
O-0-29	5.82	4.6×10^6	Brown-red, good odor
P-0-29	6.42	8.0×10^7	Red + brown, "
O-I-29	5.82	1.1×10^4	Brown-red, "
P-I-29	6.45	6.8×10^6	Red, "
O-0-34	5.82	4.5×10^7	Brown + green, sl off-odor
P-0-34	6.31	2.0×10^7	Red + green, " "
O-I-34	5.86	5.0×10^4	Brown + red, good odor
P-I-34	6.44	1.5×10^6	Red, "
O-0-40	6.05	1.8×10^9	Brown, sour odor
P-0-40	6.56	4.2×10^9	" "
O-I-40	5.86	1.0×10^8	" sl off-odor
P-I-40	6.28	1.5×10^9	Red, "

Table 8 - Cont'd:

23 days in vacuum:

Variable	pH	TPC/gm	Observations	
O-0-29	5.43	2.6×10^7	Brown	SL. sour odor
P-0-29	6.27	2.3×10^8	Purple	" "
O-I-29	5.62	1.2×10^6	Brown	Good odor
P-I-29	6.32	1.0×10^8	Purple	" "
O-0-34	5.52	1.2×10^8	Brown	SL. sour odor
P-0-34	6.28	2.7×10^8	Purple	" "
O-I-34	5.67	4.2×10^6	Brown	Good odor
P-I-34	6.20	2.3×10^8	Purple	" "
O-0-40	5.37	5.0×10^8	Brown	Sour odor
P-0-40	6.30	1.0×10^9	Purple	" "
O-I-40	5.17	7.2×10^7	Brown	SL. off-odor
P-I-40	6.28	5.0×10^8	Purple	" "

23 days in vacuum + 4 days in air:

O-0-29	5.50	7.1×10^7	Brown	SL. sour odor
P-0-29	6.18	5.6×10^8	Brown-purple	" "
O-I-29	5.68	8.6×10^6	Brown	Good odor
P-I-29	6.35	3.0×10^8	Purple	SL. off-odor
O-0-34	5.58	3.4×10^8	Brown	Spoiled
P-0-34	6.20	3.6×10^9	Red-brown	"
O-I-34	5.70	2.7×10^7	Brown	SL. off-odor
P-I-34	6.22	5.3×10^8	Red	" "
O-0-40	5.30	6.0×10^8	Brown	Spoiled
P-0-40	6.32	5.5×10^9	"	"
O-I-40	5.20	8.0×10^8	"	"
P-I-40	6.30	3.9×10^9	"	"

CODE:

O-0	=	No dip, no irradiation.
P-0	=	10% TPP dip, no irradiation.
O-I	=	No dip, 100 Krad.
P-I	=	10% TPP dip, 100 Krad.

29, 35 and 40 are constant storage temperatures in °F.

Table 8 - Cont'd:

15 days in vacuum:

Variable	pH	TPC/gm	Observations	
O-0-29	5.79	5.2×10^6	Brown-purp.	Good odor
P-0-29	6.85	5.7×10^7	Purple	" "
O-I-29	5.77	3.1×10^4	Brown-purp.	" "
P-I-29	6.45	5.0×10^6	Purple	" "
O-0-34	5.80	1.6×10^7	Brown-Purp.	Sl. sour odor
P-0-34	6.33	1.3×10^8	Purple	" "
O-I-34	5.83	5.0×10^4	Brown-purp.	Good odor
P-I-34	6.48	2.1×10^7	Purple	" "
O-0-40	5.48	2.8×10^8	Brown-purp.	Sl. sour odor
P-0-40	6.28	6.2×10^8	" "	" "
O-I-40	5.60	3.8×10^7	" "	Good odor
P-I-40	6.20	2.1×10^8	Purple	" "

15 days in vacuum + 4 days in air:

O-0-29	5.62	2.3×10^7	Brown-purp.	Good odor
P-0-29	6.22	4.8×10^8	Purple	" "
O-I-29	5.72	2.8×10^5	Brown-purp.	" "
P-I-29	6.38	1.5×10^8	Purple	" "
O-0-34	5.72	2.2×10^8	Brown	Spoiled odor
P-0-34	6.32	1.3×10^9	Brown + red	" "
O-I-34	5.73	2.0×10^6	Red	Good odor
P-I-34	6.31	4.5×10^8	Red	" "
O-0-40	5.83	2.3×10^9	Brown	Spoiled odor
P-0-40	6.46	3.5×10^9	"	" "
O-I-40	5.42	4.0×10^8	"	Off-odor
P-I-40	6.32	1.0×10^9	Red-brown	" "

ALL of these results support the view advanced by many meat scientists and meat industry people that: carefully maintaining cut meat temperature continuously at 28-30°F would control microbial spoilage and greatly extend salable life, particularly if this were coupled with rapid chilling of the carcass after slaughter and improved sanitation. If centralized cutting moves to the packer level and it is felt that it is essential to maintain the nature and features of fresh meat as it is presently sold, then either radiation or a 28-30°F handling, storage and display temperature would seem to be the only real alternatives for spoilage control. Observations in this laboratory also support the widely held beliefs that fluid retention and color protection will also be aided by keeping fresh meat cuts just above the freezing point. Should technological advances in refrigeration provide this temperature on a constant and routine basis, then a treatment such as radiation pasteurization would probably not be necessary to provide the additional salable life required of centralized cutting at the packer level. However, the data also suggest that even small deviations upward from the 28-30°F temperature range during the shipping, holding and marketing cycle (and the period between sale and consumption if longer than a day, for example) could become a problem. Whether the necessary temperature control is feasible under a commercial packer-level central cutting system is uncertain. A recent study* indicated that major meat packers do not consider the 'modified conventional' approach (constant 29-31°F plus improved sanitation) as sufficient to meet their salable-life extension needs. If this is correct, then radiation pasteurization would perhaps be the only feasible alternative approach to central cutting at the packer level that would still provide retail cuts at the point of sale that are essentially unchanged in form and appearance from the meat as it is presently marketed.

Regardless of whether controlled temperatures or irradiation is used to delay microbial spoilage, the data presented suggest that a controlled atmosphere during storage must be maintained as well as pre-packaging treatment with phosphate to provide meat of normal red color with little or no drip in the form presently

* Daniel Yankelovich, Inc.. October, 1968. Cost benefit study of selected meats in Atomic Energy Commission's low dose food irradiation program. Contract AT(SO-1)-8831. Report No. NYO3831-1. Page 120. Published by USAEC Div. of Technical Info. (Available from Clearinghouse for Federal Scientific and Technical Information):

available for the required 3 weeks after cutting and subsequent retail display. It is also possible that more than one approach to centralized cutting will be used; therefore, it would be desirable to develop every promising approach.

B. DRIP CONTROL AND LIPID OXIDATION

This section reports work directed primarily toward the analytical evaluation of the influence of various process parameters on fluid exudation and lipid oxidation. Fluid exudation ('drip' or 'weep') is a problem with fresh meats as they are presently commercially handled but lipid oxidation ordinarily is not. Under a system of centralized cutting at the packer level, however, fresh meat would probably not be sold and consumed until two to three weeks or longer after cutting, therefore lipid oxidation could become a problem, especially if simultaneous exposure to the atmosphere and to ionizing radiation were a part of the system. Having the meat in an oxygen-free environment during irradiation and during post-irradiation holding and distribution would be expected to aid in counteracting any tendency toward lipid (as well as pigment, etc) oxidation. One would expect the opposite to be true of holding and/or irradiating the meat in air. As mentioned earlier, this factor could prove to be a drawback for electron or beta radiation which, by necessity due to low penetration, would have to be applied to individual cuts which, if packaged, would be wrapped in an oxygen permeable film (it was previously suggested that individual vacuum packaging is probably too expensive). Although bulk vacuum packing prior to gamma irradiation, holding and distribution would be advantageous from, among others, the oxidation point of view, some lipid oxidation can occur upon re-exposure to the atmosphere at the point of retail sale. Some oxidation, under these circumstances, is no doubt inevitable, and a certain amount is tolerable, but not to the extent that oxidized odor and flavor become objectionable. It is perhaps worth noting that Soviet scientists engaged in radiation pasteurization of raw meat, especially meat of high fat content, have found it desirable to combine vacuum packaging and an antioxidant treatment.

Since lipid oxidation is, in addition to color change, drip and microbial outgrowth, a potential problem with meats undergoing lengthy refrigerated holding, especially if irradiated, a review was made of literature pertinent to this question, including references (2) through (13) of this report. One could list many more references but those cited bring out the following main points which apply to this contract:

1. Irradiation can have a profound effect upon the stability of unsaturated lipids in particular, but also the saturated fats.

2. The magnitude and specific features of the radiation effect depend upon such factors as total dose, dose rate, temperature, presence or absence of oxygen and antioxidants during and subsequent to irradiation, and the nature and state of the lipid, (e.g. pure or part of a multicomponent system). One would expect smaller changes with a lower dose, with a very high dose rate, and with a complex multicomponent system.

3. Antioxidants vary as to their radiation sensitivity. For instance, tocopherols are very sensitive and are readily destroyed while BHA is relatively resistant. Generally, antioxidants have a considerable protective effect against radiation-induced lipid oxidation, and oxidative changes (including pigment oxidation) during refrigerated air storage.

4. Vacuum or an inert atmosphere also protect against oxidation during irradiation and/or during storage but only so long as the air is kept from contact with the meat. Indications are that nitrogen gas may not be satisfactory when radiation is used.

5. Vacuum does not protect against all radiation damage to lipids or other components, but changes in the lipid are both quantitatively and qualitatively more severe if oxygen is present.

6. Reduction of preformed lipid peroxides by radiation in vacuum or inert atmosphere has been reported to occur, and is desirable.

7. If radiation must be carried out in air, post-irradiation storage in vacuum can result in partial recovery of stability and is therefore superior to post-irradiation storage in air.

8. Tripolyphosphate type phosphates are readily broken down by the meat phosphates and therefore probably do not exert much of an antioxidant effect unless applied shortly before cooking. Others such as hexametaphosphate are much more resistant to breakdown. A mixture of the two types is suggested for use in drip control applications.

9. Sufficient lipid oxidation will give rise to oxidative odor in raw beef which is not removed by cooking but can be essentially prevented by vacuum packaging and/or antioxidants.

The amount of lipid oxidation occurring in meat can be measured analytically by several methods including peroxide value, Kreis test, manometric measurement of

oxygen uptake and the thiobarbituric acid (TBA) test in addition to sensory evaluation. The TBA method of Sinnhuber *et al.* (14) as modified by Tarladgis *et al.* (15, 16) was used in this work. It is considered relatively reliable for red meats although irradiation is believed to produce TBA active compounds other than malonaldehydes (17).

Most of the methods employed in the determination of water-holding capacity of meat are empirically based on the measurement of fluid released due to an applied stress (centrifugation, pressing, etc) on a meat sample. The actual amount of loosely bound water in meat cannot be determined by any of these methods. Meat contains several protein components of unknown degree of hydration, and the amount of 'bound' water is affected by laboratory analysis methods. Although it is recognized that water binding is largely due to the myofibrillar proteins (actin, myosin, etc), the relative changes in water holding capacity can be determined by treating the various muscle proteins as a single component and by using the same method under the same conditions.

The method of Howard (18) was used for drip measurement. This involves direct measurement of sample weight-loss resulting from drip only. Phosphate uptake determinations were done by measuring weight gain after phosphate treatment and by analyzing for increase in phosphorus content using the AOAC colorimetric method. Water holding capacity measurement was by the centrifugation method of Sherman (19), which actually measures water holding ability after grinding the samples and swelling them with added water. The following is a discussion of individual experiments.

B-1: Effect of phosphate pre-treatment on drip formation, pH and water-holding capacity of radiation pasteurized fresh beef:

Expt B-1-1: The effectiveness of phosphate pretreatment in retarding drip formation in radiation pasteurized beef slices stored in vacuum at 38°F for 21 days was tested in the following experiments. Beef semitendinosus muscles from Commercial Grade rounds were trimmed of adipose tissue and sliced into 1 to 2 cm thick samples. The weight of the samples ranged from 100 to 250 grams. After weighing, some samples were dipped in 10.0% aqueous solution of sodium tripolyphosphate for 30 to 60 seconds. Other samples were not dipped. The dipping solution was at about 72°F for pH of 9.5. The samples were next drained on a wire screen for 10-15 minutes. The non-dipped and TPP dipped samples were then weighed and vacuum

packed in oxygen-impermeable laminate pouches. They were irradiated to 0, 50, 100, 150, 250 and 500 Krad, and then stored at 38°F for 21 days.

The amount of sodium tripolyphosphate solution that was absorbed by the meat, and pH changes caused by the dipping process are given in Table 9. The amount of solution uptake varied between meat samples used in different runs ranging from 0.91 to 2.00 percent by weight. In all cases, the treatment caused the pH of the meat to increase from 0.37 to 0.45 pH unit.

Data in Figure 1 show that in spite of the increase in weight due to the dipping process, percentagewise there was much less drip loss observed for the treated samples than for the control. There appeared to be no noticeable change in the amount of drip due to the variation in dose of ionizing radiation. There was, however, a slight increase in drip in both phosphate treated and the untreated samples, apparently due to the vacuum packaging.

Figure 2 shows the percentage water retention of irradiated meat samples after 21 days of storage at 38°F. Phosphate pretreatment increased the water holding capacity of the beef samples, from 6.40 to 9.00 percent, in spite of the fact that the samples had a substantial net increase in water content after dipping and draining. The percentage water retention or water holding capacity differed among samples used in different runs. No large changes in water retention due to irradiation were observed.

Expt.B-1-2: In Figure 3, the percentage water retention during storage of beef slices treated with sodium tripolyphosphate solution prior to 100 Krad irradiation is compared with that of beef that was not phosphated and/or irradiated. The phosphate treated samples had a relatively high percentage water retention compared with the untreated. No change in water retention due to 100 Krad irradiation was observed. There was, however, a slight increase in water retention for all samples during storage for 21 days at 38°F. Data in Figure 3 represents averages of several samples.

Table 9. (Expt. B-1-1). Solution uptake and pH of fresh beef slices after dipping in 10% solution of sodium tripolyphosphate for 45 seconds and draining for 15 minutes

Replicate	pH before dipping	pH after dipping	Percent solution uptake*
1	5.50	5.95	1.00-1.62
2	5.60	6.03	0.91-1.62
3	5.75	6.12	1.14-2.00

* Range obtained from 18 samples (percent by weight),

Identical replicates performed at different times and on different pieces of meat.

Fig. 1. (Expt. B-1-1) Loss of drip-phosphate dip vs no dip, of fresh beef slices that were vacuum packed, irradiated at various pasteurizing doses of gamma radiation and then stored for 21 days at 38° F.

* Identical replicates performed on different days with pieces of meat.

Fig. 2. (Expt. B-1-1). Percentage water retention of phosphate treated and untreated fresh beef slices that were vacuum packed, irradiated at various pasteurizing doses of gamma radiation and then stored for 21 days at 38°F.

* Identical replicates performed on different days with different pieces of meat.

Fig. 3. (Expt. B-1-2). Water retention during storage at 38°F of fresh beef, pre-treated with sodium tripolyphosphate and untreated, irradiated and unirradiated.

B-2: Effect of phosphate pretreatment, vacuum packaging and radiation on lipid oxidation in fresh beef:

This study was conducted to determine the degree of lipid oxidation occurring in beef samples during 21 days at 38°F, with respect to the effect of phosphate treatment, vacuum packaging and irradiation, and the effect of exposing the meat to the atmosphere after 21 days of vacuum storage.

B-2-1: Beef slices (1 to 2 cm thick) from semitendinosus muscle of commercial grade rounds were either not dipped or dipped in 10.0% aqueous solution of sodium tripolyphosphate and vacuum packed in oxygen-impermeable laminate pouches. The samples were irradiated at 0, 50, 100, 150, 250 and 500 Krad and stored at 38°F. After 21 days storage the vacuum of each pouch was broken. Representative samples were taken from the individual slices which were immediately analyzed for TBA number. The remaining portions of the slices were rewrapped with oxygen permeable film and stored in air at 38°F. Daily measurements of lipid oxidation (TBA number) of the samples were conducted during this time. Results of these experiments as shown in Table 10 indicate that vacuum packaging retarded lipid oxidation up to 21 days of storage at 38°F. Relatively low TBA numbers were obtained at this time. When the samples were exposed to the atmosphere, however, TBA numbers began to increase with storage time. Differences in TBA numbers between the phosphate treated samples and the untreated samples were slight. Further, no large differences in TBA numbers were observed between irradiation doses.

B-2-2: In a separate set of experiments, the progression of lipid oxidation changes was followed during storage under vacuum and during the subsequent exposure to the atmosphere. Both phosphate treated and untreated beef slices were vacuum packed and irradiated at 0 and 100 Krad. After irradiation they were stored at 38°F. At the end of 0, 7, 14 and 21 days, the samples were analyzed for TBA number. On the 21st day the samples were removed from their vacuum package and rewrapped with oxygen-permeable film and stored for three more days in air at 38°F. Daily TBA-number determinations were made during this period. Figure 4 shows that there was a slight increase in TBA values for all variables during the first week under vacuum. TBA values decreased during the one to three week vacuum storage period. No large differences in the degree of oxidation appeared to occur between phosphated and non-phosphated samples, or between irradiated and unirradiated samples.

(Expt. B-2-1)

Table 10. Effect of phosphate pretreatment, vacuum packaging, radiation and subsequent exposure to the atmosphere on lipid oxidation in fresh beef during storage at 38°F

Experiment*	Dose (Krad)	Phosphate treatment**	After 21 days in vacuum	TBA Number ^{a/} Days exposed to the atmosphere ^{b/}		
				1	2	3
1	0	O	0.08	0.36	0.60	1.04
		P	0.09	0.49	0.68	1.10
	50	O	0.13	0.51	0.72	1.13
		P	0.13	0.36	0.57	1.08
	100	O	0.14	0.41	0.61	1.12
		P	0.13	0.53	0.69	1.07
	150	O	0.17	0.46	0.58	1.15
		P	0.22	0.34	0.52	1.07
	250	O	0.27	0.36	0.54	0.79
		P	0.17	0.42	0.49	0.84
	500	O	0.31	0.43	0.51	0.87
		P	0.14	0.33	0.50	0.90
2	0	O	0.33	0.52	0.91	1.71
		P	0.36	0.58	0.78	1.75
	50	O	0.36	0.54	0.78	1.73
		P	0.31	0.67	0.82	1.86
	100	O	0.34	0.56	0.79	1.55
		P	0.28	0.50	0.92	1.61
	150	O	0.26	0.43	0.78	1.53
		P	0.24	0.47	0.85	1.56
	250	O	0.26	0.39	0.79	1.56
		P	0.26	0.41	0.77	1.55
	500	O	0.28	0.39	0.80	1.48
		P	0.28	0.38	0.75	1.48

* Identical experiments performed at different days with different pieces of meat.

** O = Untreated; P = Phosphate treated.

^{a/} Average of two replicates.

^{b/} Number of days exposed to the atmosphere after removal from vacuum.

Fig. 4. (Expt. B-2-2). Lipid oxidation changes in irradiated and unirradiated fresh beef as indicated by TBA values during storage in vacuum for 21 days followed by storage in air for three additional days at 38°F.

upon exposure to the atmosphere, however, there was an immediate increase in TBA values in all cases on the first day which continued through the third day. The magnitude of the TBA values, even after one to three days re-exposure to the atmosphere, suggests that only mild oxidation occurred in the lean beefsteak under the conditions used. The results follow the same general trend suggested in the literature; that is, there is little or no change in the degree of lipid oxidation during irradiation and holding in the absence of oxygen, but there is an increase in oxidation upon subsequent exposure to air. It is most instructive to note that the atmosphere during storage was the determining factor and that the influence of phosphate and radiation on the TBA results was practically non-existent.

Further work is planned using beef cuts of higher fat content as well as pork cuts of varying lipid content. The critical factor in lipid oxidation appears to be the holding period in air after vacuum storage which is necessary to provide normal red color at the point of retail sale and prior to consumption by the purchaser. Under the conditions assumed in this work for the commercial application of centralized cutting, radiation does not appear to be a significant contributing factor to lipid oxidation.

C. COLOR AND PIGMENT MEASUREMENT

The first annual report for the contract (Report C00-1689-2) contains a brief discussion of the chemistry of the red meat pigment myoglobin specifically as regards the color changes involving gaseous oxygen, as well as a discussion of the theory and practice of reflectance spectrophotometry which is the analytical measurement method used in conjunction with subjective color evaluation in this work. On page 60 of this previous annual report, heavy emphasis was placed upon the necessity of providing normal red meat color at the point of retail sale of the fresh red meat cuts regardless of the approach adopted to achieve centralized cutting. The paragraph ended with 'Perhaps the greatest challenge in the technical phase of the overall effort is in achieving success with color'. These statements are no less true now, one year later, and can only be re-emphasized.

Rickansrud and Henrickson (20) state that consumer preference studies have emphasized that the color of the lean is one of the basic factors influencing the acceptance of fresh meat. They conclude from their results that hemoglobin contributes more to total pigment concentration and probably to muscle color than had been previously reported. Fox (21) mentions that even in meat from a well-bled animal, hemoglobin will comprise 20 to 30% or more of the total pigment present. Since both hemoglobin and myoglobin auto-oxidize most readily at low partial pressures of oxygen, Fox concludes that packaging films for fresh meats should be considered with this fact in mind, and should, ideally, have as high an oxygen transmissability as possible. Saleh and Watts (22), in reporting on enzymatic reduction of metmyoglobin in ground beef, point out that lack of substrates (for example, glyceraldehyde-3-phosphate, malate, glutamate, etc) and cofactors rather than loss of enzymes probably limits reductive capacity of meats after slaughter. If so, this factor could likely be favorably influenced in a centralized cutting operation by measures which tend to conserve tissue enzyme substrates such as quick chilling and cutting as soon as is feasible after slaughter along with bulk vacuum packing as quickly as possible after cutting. In this connection, it would be of great value to determine whether a tenderizing process such as that associated with conventional meat aging would proceed satisfactorily in retail cuts if the carcass were broken down into retail units immediately after chilling. If so, this could be of practical value. Saleh and Watts further point out that metmyoglobin is reduced in fresh meats only under anaerobic conditions; they suggest that adding enzyme substrates to cut meat, while somewhat effective,

is impractical. It is conceivable, however, that proper ante-mortem and post-mortem treatment and handling, even selective breeding, could be beneficial in this regard. This may be worth considering in conjunction with large scale packer-level centralized cutting. Greene and Watts (9) observed that wrapping meat in oxygen-impermeable film did not always result in metmyoglobin reduction and that metmyoglobin-reducing enzyme activity was almost completely absent in samples that did not undergo reduction of oxidized myoglobin after anaerobic packaging. They conclude that anaerobic packaging can apparently be useful only if reducing activity is present in meat. They further observed that antioxidants (BHA and PG) offered substantial protection against pigment oxidation as well as against lipid oxidation in aerobically packaged ground beef whether irradiated or not. They established that pasteurizing doses of radiation do not diminish metmyoglobin reducing capacity of ground beef. They also point out that there is a great variability in this most desirable property among different samples of meat.

The following experiments were conducted in which diffuse reflectance (and in one case absorption) spectrophotometry was employed in evaluating the influence of selected treatment parameters on color by measuring the relative amounts of the three major forms of the principal fresh red meat pigment.

C-1: Effect of phosphate pretreatment, vacuum packaging, radiation and subsequent exposure to the atmosphere on color and metmyoglobin formation in fresh beef:

Samples were obtained solely from semitendinosus muscle of Commercial Grade beef. The meat was trimmed of adipose tissue and cut into 1 to 2 inch thick slices. The slices were either not dipped or were dipped in sodium tripolyphosphate solution and drained. Then they were vacuum packed in oxygen-impermeable laminate pouches and irradiated to 0, 100, 200, 300, 400 and 500 Krad. After irradiation the samples were stored at 38°F.

At the end of 21 days, the vacuum of each pouch was broken and the samples were prepared for reflectance measurements. Samples were cut into pieces 1 3/4 X 1 1/2 inches, placed in plastic ice cube holders and each holder was wrapped with oxygen permeable plasticized polyvinyl chloride fresh meat film (Resinite RMF-61). The preparation of the samples and reflectance measurements were done with as little exposure to the atmosphere as possible. Visual observations on the color of the samples were also made. The samples with oxygen-permeable film wrap were further stored at 38°F. After 24 hours reflectance measurements were again made

Experimental data presented in Table 11 indicate a considerable difference in color between phosphate treated samples and untreated samples. The visual observation showed that, in general, phosphate treated samples appeared purple in color while the untreated were usually brownish after 21 days in vacuum, probably indicating low reducing capacity meat. Reflectance measurements showed that reduced myoglobin predominates in phosphate treated samples while considerable amounts of brown metmyoglobin are present in untreated samples. Pasteurizing doses from 50 to 300 Krad at a rate of about 100 Krad/hr did not result in any detectable effect except for a possible slight decrease in metmyoglobin formation. At 400 to 500 Krad, there was a slight discoloration, with the phosphate treated samples appearing purplish brown whereas the untreated appeared brown.

Exposure of the meat to air for a day at 38°F shifted the color of phosphate treated samples from purple to red. Samples without phosphate pretreatment remained brown. In all cases, there was an increase of metmyoglobin during exposure to the atmosphere. Reflectance measurements after exposure to air show that, in all cases, phosphate treated samples had much less metmyoglobin than comparable unphosphated samples. The pigment in the phosphate treated samples appeared to be in oxygenated form as evidenced by the predominant red coloration. Phosphate treated samples that were irradiated at 400 to 500 Krad appeared reddish brown due to the greater metmyoglobin produced at these higher doses.

C-2: Effect of phosphate pretreatment on metmyoglobin formation in irradiated and unirradiated fresh beef during anaerobic followed by aerobic storage:

Studies on the progression of pigment changes during storage were performed at different times using different set of meat samples. Samples with 1 3/4 X 1 1/2 X 3/4 inch dimensions were prepared from Commercial Grade beef semitendinosus. After dipping half of the samples in a 10% solution of sodium tripolyphosphate, all samples were placed in plastic ice cube holders which were vacuum packed in oxygen-impermeable pouches and irradiated at 0 and 100 Krad. After irradiation, all of the samples were stored at 38°F. At the end of 0, 7, 14 and 21 days, reflectance measurements were taken directly on the samples while still in the packages. At the end of 21 days, the vacuum of each pouch was broken. The samples in the ice cube trays were rewrapped with oxygen-permeable film and stored at 38°F for three more days. Daily reflectance measurements were conducted on the wrapped samples during this three day period.

Table 11. Effect of phosphate pretreatment, vacuum packaging, radiation and subsequent exposure to the atmosphere on color and metmyoglobin development on fresh beef during storage at 38°F

Dose (Krad)	Phosphate treatment	21 days in vacuum		21 days in vacuum plus 1 day in air	
		Color (Visual observation)	Percent Metmyo- globin ^{a/}	Color (Visual observation)	Percent Metmyo- globin ^{a/}
0	No	Brown	11.5	Brown	28.2
	Yes	Purple	0.0	Red	2.0
50	No	Brown	9.0	Brown	24.7
	Yes	Purple	0.0	Red	3.2
100	No	Brown	5.0	Brown	18.0
	Yes	Purple	0.0	Red	7.0
200	No	Brown	11.5	Brown	28.0
	Yes	Purple	0.0	Red	3.0
300	No	Brown	10.3	Brown	27.5
	Yes	Purple	0.0	Red	9.5
400	No	Brown	21.2	Brown	43.0
	Yes	Purplish brown	9.0	Reddish brown	11.5
500	No	Brown	47.0	Brown	66.0
	Yes	Purplish brown	10.0	Reddish brown	20.2

^{a/} Average of two determinations.

The progression of metmyoglobin formation during storage in vacuum and during the subsequent exposure to the atmosphere of phosphate treated and untreated beef slices irradiated at 0 and 100 Krad is shown in Figure 5. It is apparent that the percentage metmyoglobin is lower in phosphate treated samples than in untreated ones throughout the storage period in vacuum and during exposure to the atmosphere. There was a rapid increase in metmyoglobin formation beginning between the seventh and the fourteenth days and proceeding on up to the 21st day of storage in vacuum in the case of beef slices without phosphate pretreatment. Again, this likely indicates low reducing capacity meat. In phosphate treated samples, a slight increase in metmyoglobin was observed beginning between the 14th and 21st days in vacuum. At the end of 21 days in vacuum, all samples that did not have phosphate treatment appeared brown while the phosphate treated retained their deep purple color. Upon subsequent exposure to the atmosphere, the purple color of the phosphate treated samples reverted to red. No such reversion was observed with the untreated. There was an increase in all cases in metmyoglobin at the surfaces of the meat samples after three days exposure to the atmosphere. It is also apparent from Figure 5 that irradiation at 100 Krad had no great influence on metmyoglobin formation in phosphate treated and untreated samples.

C-3: Sensory, color and odor evaluation of phosphate treated and untreated beef irradiated to several doses and stored in vacuum followed by exposure to air:

The combined effect of phosphate treatment, vacuum packaging, radiation and the subsequent exposure to the atmosphere on color and odor of the meat was evaluated by a sensory panel. Beef slices from the semitendinosus muscles of a Commercial Grade round were phosphate treated, drained, vacuum packed and irradiated to 0, 50, 100, 250 and 500 Krad, and stored at 38°F. At the end of 20 days the vacuum of each pouch was broken. The samples were then rewrapped with oxygen-permeable film and exposed to the atmosphere for an additional day at 38°F. Similar treatments were given to samples which did not receive phosphate pretreatment. Sensory evaluation of color and odor was made after exposure to the atmosphere. Results are shown in Table 12.

The data of Table 12 show that the panelists found the color of phosphate treated samples clearly superior to the color of the untreated. The mean color scores of phosphate treated samples ranged from fairly good to good (5.9 to 7.0),

Fig. 5. (Expt. C-2). Effect of phosphate pretreatment on metmyoglobin formation in irradiated and un-irradiated fresh beef during storage for 21 days in vacuum followed by an additional three days in air at 38°F.

Table 12. (Expt. C-3). Color and odor evaluation of phosphate treated and untreated beefsteaks irradiated at various pasteurizing doses of gamma radiation and stored for 20 days in vacuum followed by one day in air at 38°F

Test No.	No. of judges	Dose (Krad)	Mean color score		Mean odor score	
			Phosphated	No phosphates	Phosphated	No phosphates
1	10	0	6.9	4.0	5.6	5.4
		50	6.5	4.5	7.4	7.0
		100	7.0	4.1	7.3	6.9
		250	6.8	4.1	6.8	6.2
		500	5.9	3.7	5.8	5.5
2	12	0	7.1	5.4	5.6	5.5
		100	7.2	5.3	7.0	7.2

SCORING CODE

Acceptable range

9 - Excellent
 8 - Very good
 7 - Good
 6 - Fairly good

Unacceptable range

4 - Fairly bad
 3 - Bad
 2 - Very bad
 1 - Poor

5 - Marginal

while those of the untreated ranged from fairly bad to marginal (3.7 to 5.4). There were no significant differences in odor scores between phosphate treated and untreated samples. Unirradiated samples and those that were irradiated to 500 Krad received lower ratings than those irradiated to the lower doses. The low odor-score for the unirradiated sample probably was due to a slight spoilage odor, while the 500 Krad sample had an obvious irradiated odor which the panel found somewhat objectionable. Apparently color became significantly affected by radiation only at the 500 Krad level, which further indicates that the pasteurizing doses under consideration (50-150 Krad) do not materially affect color unless, as suggested by the next experiment, a very high dose rate is used.

C-4: Effect of total dose and dose rate on the pigment and color of fresh beefsteak.

The semitendinosus muscle of a Commercial Grade bottom round of beef was trimmed and cut into 1/2 inch thick steaks. After allowing all of the steaks to reach full 'bloom' in the air at 38°F., a Bausch and Lomb 505 recording spectrophotometer with reflectance attachment was used to determine the concentrations of the three pigment forms (oxy-, reduced and met-myoglobin) at the cut surfaces of the steak samples. Following this, each steak sample was individually wrapped in highly gas-permeable plasticized polyvinylchloride fresh meat wrapping film. Some of the samples were electron irradiated with the General Electric Company Resonant Transformer (1 MeV peak energy) to a total dose of 200 Krad on each side of each irradiated steak. Two dose rates were used, 5 Krad per second and 50 Krad per second.

Immediately after irradiation, pigment concentrations were measured on surfaces of both irradiated and unirradiated steaks. For these and subsequent measurements, the wrapping film was removed just prior to, and replaced immediately after taking the measurements. After these initial post-irradiation measurements were completed, the polyvinyl-chloride wrapped samples were immediately individually vacuum packaged in laminate pouches and were placed into 38°F storage. Samples were taken from storage at 10 days and at 21 days at which times visual observations and reflectance measurements were made, both immediately upon removal from vacuum and again after one day of exposure to the atmosphere. During the period of exposure to the atmosphere the samples remained wrapped in polyvinyl-chloride, except while reflectance measurements were being taken. Results are given in Table 13.

Table 13. (Expt. C-4). Effect of dose (0 vs 200 Krad) and dose rate on the pigment and color of fresh beefsteak

Evaluation time	Percent myoglobin			Percent oxymyoglobin			Percent metmyoglobin		
	0 Krad	200 Krad @ 5 Krad/sec	200 Krad @ 50 Krad/sec	0 Krad	200 Krad @ 5 Krad/sec	200 Krad @ 50 Krad/sec	0 Krad	200 Krad @ 5 Krad/sec	200 Krad @ 50 Krad/sec
Immediately before irradiation	4	4.5	2	93.5	93	95	2.5	2.5	3
Immediately after irradiation	4	25	35	96	56.5	13	0	18.5	52
10 days in vacuum	56.5	51	46	1	1.5	3	42.5	47.5	51
10 days in vacuum + 1 day in air	18.5	10.5	6	52	61	64	29.5	28.5	30
21 days in vacuum	64	49	36	13	0	1	23	51	63
21 days in vacuum + 1 day in air	9	19	12	74	41.5	39	17	39.5	49

The percentages of the three pigment forms calculated from the reflectance values indicate that the immediate effect of electron irradiation in air at the relatively high dose rates employed is the conversion of some of the oxymyoglobin to both reduced and metmyoglobin. The conversion of oxymyoglobin was much greater at the 50 Krad per second than at the 5 Krad per second dose rate which demonstrates an interesting dose-rate effect. The effect upon color due to irradiation, and the difference in magnitude of the effect between the two dose rates were both visually observable as well. This is in contrast to earlier work using Cobalt-60 gamma radiation where no visual observable change in color due to irradiation was noted. In these instances, beefsteaks were similarly wrapped and were given about the same total dosages as the electron irradiated. With gamma irradiation, however, the dose rates were much lower (about 50 rads per second).

At the ten day withdrawal period the data indicate considerable reduction of metmyoglobin for all three variables during the one day holding period in air. Reduced myoglobin also decreased as exposure to the air brought about a large increase in oxymyoglobin for each variable but more so for the irradiated samples. The dose rate effect noted immediately after irradiation was practically gone after 10 days vacuum storage. The data suggest a large increase in metmyoglobin during this storage period for both the unirradiated and for the samples irradiated at 5 Krad per second, but not for samples irradiated at 50 Krad per second.

The data for the unirradiated samples indicate that at 21 days there was about half of the metmyoglobin that was present at 10 days, and considerably more oxymyoglobin after 21 days in vacuum and a day in air than after 10 days in vacuum and a day in air. The higher dose rate irradiated samples show somewhat higher met- and somewhat lower reduced myoglobin than do the lower dose rate irradiated samples. In contrast to the unirradiated samples, both irradiated variables showed a good deal less oxymyoglobin regeneration at 21 plus 1 days than at 10 plus 1 days.

C-5: Absorption spectrophotometric evaluation of the effect of total dose and dose rate upon the pigment of fresh beefsteak and fresh beef drippings:

The effect of the two radiation parameters was further studied using an ultra violet absorption spectrophotometric method developed by Zukal and Hamm (23). This is a simple, accurate method of measuring the percentage metmyoglobin of total myoglobin which avoids the major difficulties of similar absorbance methods. The study employed both intact fresh Commercial Grade beef top round and undiluted drip (normal fluid exudate from freshly cut beef rounds). The top round was cut into one inch thick steaks. Half of the steaks were packaged in individual oxygen-permeable polyethylene pouches, and half were vacuum packaged in individual laminate pouches prior to irradiation. A quantity of the very fresh beef drippings was oxygenated by bubbling in oxygen gas for 15 minutes after which the bright red drippings were dispersed into 5 ml glass vials.

The vials containing the oxygen saturated drippings were irradiated at various dose rates and to three selected doses. Immediately after irradiation, the irradiated and unirradiated drippings were analyzed for percentage metmyoglobin based on total myoglobin present with a Beckman DU spectrophotometer. The aerobically and vacuum packaged beefsteaks were likewise irradiated at various dose rates and to three selected doses. A total dose of 100 Krad was used at each dose rate in the dose rate study. Dose rate was a constant 100 Krad per hour for the total dose study. For analysis of the beefsteaks, expressed juice was used and this was extracted from each steak sample by applying pressure to the sample in a stainless steel funnel from which the expressed juice was collected and immediately analyzed. It was found that for all readings it was necessary to dilute the samples (one part expressed juice or drippings plus twenty parts pH 6.6 phosphate buffer) in order to obtain absorbance readings within the 0.3 to 0.9 range. Readings were made at the 540 nm wavelength. Results of the dose rate experiment are summarized in Table 14, and results of the total dose experiment are tabulated in Table 15.

The data indicate that the higher the dose rate, the greater was the metmyoglobin production in the fresh beefsteaks. The effect was much more pronounced in aerobically packaged steaks than in vacuum packaged steaks. Data on the effect of dose rate on irradiated fresh beef drippings demonstrate that there is considerable protection against radiation induced myoglobin oxidation in irradiated beef that does not occur in irradiated beef drippings. Metmyoglobin content of

(Expt. C-5)

Table 14. Effect of dose rate on metmyoglobin content of expressed juice from irradiated and unirradiated fresh beefsteak, and on metmyoglobin content of irradiated and unirradiated oxygenated fresh beefdrip

Expressed juice from irradiated and unirradiated fresh beefsteaks ^{1/}	
Variable	Percent metmyoglobin of total myoglobin
Unirradiated in air	28.1
in vacuum	26.1
100 Krad per hour in air	32.8
in vacuum	30.6
2000 Krad per hour in air	48.3
in vacuum	36.0
180,000 Krad per hour in air	51.7
in vacuum	35.5

Irradiated and unirradiated oxygenated fresh beef drip ^{2/}	
Variable	Percent metmyoglobin of total myoglobin
Unirradiated	39.2
100 Krad per hour	94.3
500 " "	98.5
2000 " "	100

^{1/} The expressed juice was extracted by pressure from each beefsteak sample immediately prior to being analyzed after the steaks were irradiated.

^{2/} The fresh beef drip (normal fluid exudate from freshly cut beef rounds) was irradiated in glass vials after being saturated with oxygen gas, and was analyzed immediately after irradiation.

Note: A 100 Krad total dose was constant for all irradiated variables.

Table 15. (Expt. C-5). Effect of total dose on metmyoglobin content of expressed juice from irradiated and unirradiated fresh beefsteaks and on metmyoglobin content of irradiated and unirradiated oxygenated fresh beef drip

Expressed juice from irradiated and unirradiated fresh beefsteaks ^{1/}		
Variable		Percent metmyoglobin of total myoglobin
Unirradiated in air		28.1
" in vacuum		26.1
100 Krad in air		32.8
" in vacuum		30.6
500 Krad in air		54.4
" in vacuum		39.0
1000 Krad in air		68.4
" in vacuum		39.7

Irradiated and unirradiated oxygenated fresh beef drip ^{2/}		
Variable		Percent metmyoglobin of total myoglobin
Unirradiated		39.2
100 Krad		94.3
500 Krad		100
1000 Krad		100

1/ The expressed juice was extracted by pressure from each beefsteak sample immediately prior to being analyzed after the steaks were irradiated.

2/ The fresh beef drip (normal fluid exudate from freshly cut beef rounds) was irradiated in glass vials after being saturated with oxygen gas, and was analyzed immediately after irradiation.

Note: A 100 Krad dose rate was constant for all irradiated variables.

the drippings is well over 90% of all three dose rates although, here as well, there appears to be a slight dose rate effect.

The total dose data for the beefsteaks indicate that the higher the dose the greater the radiation induced metmyoglobin production. As with the dose rate experiment, the increase in metmyoglobin with dose was much greater for steaks irradiated in air than for steaks irradiated in vacuum. The vacuum packaged steaks had a purplish reduced myoglobin color prior to irradiation which darkened somewhat during irradiation. It is quite possible that a pigment reduction occurred during irradiation of the beefsteaks in vacuum, and that the increase in metmyoglobin content indicated by the data occurred after irradiation during expression and preparation of the juices for analysis.

Finally, as would be expected, the higher the total dose given to fresh beef drippings, the greater the metmyoglobin production. Again, due to the absence of the protective effect which occurs in the intact meat, irradiating the undiluted drip to 100 Mrad oxidized nearly all of the myoglobin to metmyoglobin.

D. BULK PACKING

From a practical standpoint individual retail cut packaging and bulk vacuum packing are the most critical component of the overall process. This is because success with color hinges almost entirely upon a satisfactory methodology and materials for both individual cut packaging and multi-cut anaerobic bulk packing. There are two basic approaches to this requirement. The most desirable is to centrally package the retail cut in the final package (that in which it is displayed and sold to the retail consumer). The second is to delay the final packaging until the meat is to be put in the retail store display case. This second method can be regarded as only a partial solution to the centralized preparation of retail cuts.

The following is a discussion of observations and experiences with bulk packing of both unwrapped and prewrapped retail cuts of beef. Commercial Grade beefsteak and roast-sized cuts were used in every instance except one in which Choice Grade beefsteaks for taste panel tests were employed. Three oxygen-permeable meat wrapping films were employed in the individual cut packaging. These are fresh meat cellophane, plasticized stretch-polyvinylchloride, and a new shrink film ('Lexel') manufactured by Dow Chemical Company. They were used both with and without polystyrene meat trays. The bulk vacuum containers were large impermeable transparent laminate bags (15 lbs of meat per bag) or rigid vacuum containers. The samples were evaluated by visual observation for any gross differences in color and overall appearance of the meat, and the degree of drip accumulation in the bulk containers. Also, where appropriate, odor of bulk packed meat was evaluated and noted, and accumulated drip was measured volumetrically.

D-1: Comparison of unwrapped bulk packed steaks and prewrapped bulk packed steaks:

This first exploratory bulk packing test merely compared the two previously discussed fundamental approaches to individual packaging and bulk packing. A Commercial Grade top and bottom round of beef was trimmed and sliced into steaks of varying sizes but of approximately equal thicknesses. All steaks were simultaneously dipped thirty seconds in a 10% TPP solution and drained. Several of the steaks were randomly selected and were individually packaged in polystyrene trays with an oxygen permeable stretch polyvinylchloride film overwrap. The PVC film was heat tacked underneath each tray. The prepackaged steaks were

loosely packed in a 25 quart pressure canner such that there was essentially no pressure applied by one package upon another. Six layers of packages, four packages to a layer, were put in the rigid bulk container. The cover was sealed on with high vacuum grease and lugs, and a vacuum was drawn for several minutes after the gauge registered the ultimate 28.5 inches of vacuum. The remaining steaks were tightly packed into a second modified pressure canner without having been prepackaged. Since these steaks were unwrapped, essentially the entire volume of the bulk container could be filled with the tightly packed meat. After sealing and evacuating, both containers were irradiated to a dose of about 100 Krad. By rotating the containers at intervals during irradiation, an attempt was made to achieve a uniform dose distribution within the containers.

After three weeks storage at 38°F, the bulk containers were opened and the meat was laid out on wire screens. It was immediately apparent that unwrapped steaks from the tightly packed container were all uniformly purple in color, having the appearance of freshly cut meat, while the prepackaged steaks from the loosely packed container were generally unsatisfactory in appearance (dull purple to brownish purple, with areas of brown mainly but not exclusively where meat and film were in direct contact). A very watery pigmented drip was present in all of the individual packages varying from about one ml to about six ml. In contrast, only a few ml of a rather viscous, coagulated fluid remained behind in the tightly packed bulk container from which some 30 pounds of unwrapped beefsteaks were removed. The most probable explanation for the very small amount of drip loss from so large a quantity of cut meat is that by tightly packing the unwrapped cuts the surface to volume ratio of the cut meat is, in effect, drastically reduced. Put another way, the tightly packed bare steaks performed essentially as a single, uncut large piece of meat since the cut surfaces were pressed against one another. Upon removal from the bulk container several of the unwrapped steaks were immediately individually packaged in the same manner as the prepackaged steaks had been, and they proceeded to bloom rapidly and extensively as though freshly cut. Excellent color prevailed until the second day of aerobic storage whereupon some of the bright red color had become dull red or brown. The prepackaged steaks (those that were individually packaged prior to bulk packing) did not undergo satisfactory bloom regeneration and they became practically all brown within one day in air after removal from the bulk container. As previously mentioned, these packages also contained varying amounts of drip which had accumulated during bulk storage.

It is noteworthy that there was no drip accumulation whatever after four days in the trays in which the unwrapped steaks were packaged upon removal from the bulk vacuum container. Again, there was less than five ml of drip remaining in the bulk container after the 30 lbs of unwrapped steaks were removed. The little that was present was considerably more viscous than the usual free drip in an individual package.

The results of this first bulk packing trial yielded two important indications. One is that from a color and drip point of view the method of tightly bulk vacuum packing unwrapped cuts prior to irradiation and storage (and handling and shipping) appeared very promising. The only real criticism of these samples in this trial was that after individually packaging and storing in air at 38°F the steaks showed noticeable discoloration after about two days which is a day or two sooner than is usual with beefsteak as it is presently retailed. The other important indication was that under the conditions employed, steaks packaged by the method of choice (individually prepackaging prior to bulk vacuum packing) did not yield satisfactory color or drip results. Subsequent bulk packing studies were therefore largely aimed at determining the reasons for the unsatisfactory performance of this approach, and at investigating potentially more promising modifications of the procedure used in this test. Also, the alternate or non-prewrap approach was repeated, sometimes with slight modifications, to confirm the good results and to gain additional experience.

D-2: Comparison of unwrapped bulk pack steaks and bulk packed steaks pre-wrapped with three different fresh meat films:

This study was essentially a repeat of the foregoing (D-1). The only difference was that one-third of the prewrapped steaks was overwrapped with fresh meat cellophane (Dupont MSAD-80), another third of the steaks was overwrapped with plasticized stretch polyvinylchloride film (Borden 'Resinite' RMF-61) and the last third was overwrapped with the new polyolefin shrink fresh meat film (Dow 'Lexel'). All packages were sealed by heat-tacking underneath the individual polystyrene trays.

The results were similar to those reported for D-1. Prepackaged steaks were extensively discolored upon removal from vacuum with only patches of satisfactory color, and bloom did not proceed uniformly or extensively during subsequent aerobic holding. There did not appear to be any differences attributable to the different

packaging films used. Steaks wrapped with all three films showed about the same degree of discoloration. Varying amounts of drip up to several ml were present in the prepackaged steak packages upon removal from the bulk container. As in D-1, the packages in the bottom layers had the greater amounts of drip although there appeared to be very little pressure on these samples from the packages toward the top of the container. Unwrapped steaks were uniformly purple upon removal from the vacuum container. Again, only a few ml of quite viscous, rather coagulated fluid remained in the container upon removal of the 25-30 pounds of tightly packed unwrapped steaks. These steaks were immediately placed in trays and overwrapped with either the cellophane or the PVC or the "Lexel" film. Regardless of the film used, color response was essentially that of freshly cut steaks with bloom developing rapidly, uniformly and extensively. After two days at 38°F in air, noticeable browning had taken place on these steaks although they were perhaps still "salable" colorwise. As in D-1 drip accumulation in these packages during four days of 38°F storage in air was nil. Besides reinforcing the results of the previous test, observations in this experiment suggest that the particular gas permeable fresh meat film used does not significantly influence the color results under the conditions employed.

D-3: Comparison of gas permeable film vs no film, and vacuum vs nitrogen flush plus vacuum on color of irradiated stored beefsteaks:

For this experiment both prewrapped and unwrapped phosphate dipped steaks from a Commercial Grade beef bottom round were loosely packed into two bulk containers. The prewrapped steaks were placed in polystyrene trays and overwrapped with either stretch PVC or "Lexel" polyolefin shrink film. The unwrapped steaks were placed in trays with no overwrap. One-half of the steaks of each packaging variable (PVC, "Lexel", no film) was placed in each of the two bulk containers such that each layer of trays in each container consisted of all three packaging variables. After clamping on the lids, one bulk container was evacuated for several minutes after the vacuum gauge reached to ultimate 28.5 inches of vacuum, and the other container was alternately evacuated and flushed with nitrogen gas five times before drawing the final vacuum. After irradiating to 100 Krad the containers of meat were stored for 21 days at 38°F, after which they were opened and the contents removed. In this instance all steaks, prewrapped and unwrapped, nitrogen flushed and not flushed, exhibited about the same purple color. Varying

quantities of drip, up to several ml, were evident in all of the trays.

Immediately after noting the initial color, some of the unwrapped steaks were overwrapped with either stretch PVC or Lexel. Others of these remained unwrapped. Also, the PVC or Lexel wrap was removed from some of the steaks which had been prewrapped prior to vacuum storage. Others of these remained wrapped. All steaks were then returned to 38°F storage in air where they were frequently visually observed throughout the day for differences in degree of and rate of bloom development. The following statements summarize these observations.

1. All steaks ultimately bloomed to what could be termed a satisfactory degree although there were differences in the rate and extent.
2. All steaks without film overwrap during the post-vacuum aerobic storage (some had never been wrapped and others had been overwrapped in PVC or Lexel during vacuum storage) bloomed extensively within minutes, and were uniformly bright red well within one-half hour.
3. All steaks with film overwrap during aerobic storage (some had previously been overwrapped, and others were not wrapped until the termination of vacuum storage) bloomed extensively but a good deal more slowly than, and to a somewhat lesser extent than, the unwrapped steaks. Previously unwrapped steaks that had been newly wrapped seemed to bloom more rapidly and to a somewhat greater extent than did steaks which had been wrapped throughout the three week period. The newly wrapped steaks were closer in appearance to the unwrapped steaks than to the steaks which had been wrapped all the time.
4. The packaging film variable had no apparent effect on color results. Steaks wrapped in PVC and Lexel performed about the same colorwise. Also, multiple nitrogen flushing and evacuation did not appear to improve upon the results obtained from evacuation alone in this instance.
5. Unwrapped steaks maintained excellent color for a day or so until surface drying caused a darkening. Noticeable browning did not appear on the wrapped steaks until the third day of aerobic 38°F storage at which time they were still reasonably good colorwise. These showed no signs of drying out after five days storage in air which attests to the moisture barrier property of the films employed.

Perhaps the most important indication from this test is that, in contrast to the two previous bulk packing experiments, reasonably good results can sometimes be obtained with prewrapped beefsteaks under the conditions employed. The good color results of this test seemed to be largely due to the favorable physiological

condition (i.e., sufficient metmyoglobin reducing capacity) of the meat employed. Its ability to develop and hold good color seemed to greatly surpass that of the meat used in the two previous bulk packing experiments. Although high reducing capacity was not established experimentally here, it usually can be recognized by empirical observation when one handles and observes large quantities of red meat and becomes familiar with the usual indications such as: 1) rapid and extensive development of deep purple color upon removal of atmospheric oxygen; 2) rapid and extensive blooming upon later re-exposure to air; and 3) ability to hold and maintain purple (reduced) color under vacuum, and red color in air.

D-4: Comparison of packaging film vs no film in contact with meat surface as to effect on meat color:

Results and observations thus far in the contract work have pointed toward the biochemical condition or "freshness" of the meat and the method of packaging and bulk packing (with respect to availability of oxygen) as being possible factors governing color results under the conditions employed here. In addition, there is that possibility that constituents of the packaging material might be of such a nature as to migrate in sufficient quantities into the meat so as to affect the meat pigment in the vicinity of the meat surface and thus contribute to discoloration.

To examine this latter possibility, stretch polyvinylchloride film was chosen for the first test because it is highly plasticized, and an additive such as a plasticizer is much more likely to migrate from film to food than are the basic film components. Also, certain plasticizers are metabolizable by some fresh meat microorganisms. Steaks from Commercial Grade beef semitendinosus muscle were cut to the diameter of plastic petri dish bottoms. The thickness of each steak was one-eighth of an inch greater than the depth of the petri dishes. All of the circular steaks were placed in petri dish bottoms, and one-third of these were loosely packed, unwrapped, in a modified pressure cooker. Another third was placed in the container after first covering the top of each steak with two thicknesses of plasticized stretch PVC film and then a plate glass disc. Each of these units was wrapped with masking tape in such a manner that the film and glass disc would press down on the meat and be in contact with the rim of the dish when sealed in place with the tape. This insured anaerobic conditions and a tight, uniform contact between film and meat surface under a slight mechanical pressure.

The last third of the dishes of meat were handled in the same manner except that the PVC film was excluded and the meat surface was in direct contact with the dichromate cleaned glass discs. After all dishes were loosely packed into the bulk container it was sealed, evacuated, irradiated (200 Krad internal) and stored for three weeks at 38°F. Then the container was opened and the contents inspected visually for color differences.

Meat surfaces in contact with glass and in contact with PVC film were uniformly dark purple and identical in appearance. Both underwent red color regeneration at the same rate and to the same high degree. Samples with uncovered top surfaces were distinguishable in color from the covered samples as being slightly lighter purple. However, these also bloomed in air to about the same degree as the covered samples. The three variables were essentially the same in appearance throughout five days of aerobic storage after which they all were brown. They all had been wrapped in PVC after blooming to prevent against surface drying. The results indicate that the PVC film had no effect on color over and above that of the glass in this instance. It would seem reasonable to expect that, were the film constituents capable of exerting an observable effect on the meat color, they would have done so under the conditions employed here. If this is a factor, the meat in contact with the film should have been to some degree discolored and different in appearance from the meat in contact with dichromate cleaned glass. In fact the colors of both were very good and indistinguishable.

D-5: Comparison of wrapped and unwrapped, irradiated and unirradiated bulk vacuum packed beefsteaks:

This experiment was essentially the same as other previously discussed bulk packing tests, the only departure being the inclusion of the unirradiated variable. A Commercial Grade beef round was trimmed and cut into steaks of assorted sizes and shapes. After phosphate dipping and draining, two bulk containers were filled with tightly packed unwrapped beefsteaks, and another pair of bulk containers were loosely filled with beefsteaks which were first individually prepackaged in polystyrene trays with stretch polyvinylchloride overwrap. The four containers were sealed and evacuated to 28-29 inches of vacuum. Then one container tightly packed with unwrapped steaks and one container loosely filled with prepackaged steaks were irradiated to 100 Krad internal. As usual the containers were rotated so as to achieve a uniform dose. Following irradiation, the two irradiated

containers were stored along with the two unirradiated containers for 21 days at 40°F at which time they were opened and the contents inspected.

It was immediately apparent that the irradiated and unirradiated meat were quite different odorwise, the former having essentially a fresh meat odor while the latter had an objectionable, sour spoilage odor. The difference in odor was rather marked. Interestingly, the irradiated and unirradiated unwrapped steaks were uniformly purple, and identical in color. Both bloomed rapidly and extensively in air whether they remained unwrapped or whether they were trayed and overwrapped with fresh meat film. After storage for a day in air at 38°F the irradiated steaks which had continually been unwrapped, or had been unwrapped upon removal from vacuum storage, remained bright red while their unirradiated counterparts showed extensive browning and further spoilage.

Prewrapped irradiated and unirradiated steaks were also identical in color upon removal from the bulk vacuum container, but they, in contrast to the unwrapped steaks, were brownish purple indicating that some pigment oxidation had occurred. The prewrapped steaks partially bloomed in air but to a lesser extent and at a slower rate than did the unwrapped steaks. After a day the prewrapped steaks had discolored further. The unirradiated discolored more extensively than the irradiated. As before, the only negligible amounts of fluid remained behind in the containers which held the tightly packed unwrapped steaks. Further, these steaks exuded no drip after being individually packaged and held an additional three days in air. In contrast, the loosely bulk vacuum packed prepackaged steaks exuded varying amounts of watery pigmented drip. Typically, this resulted in unsightly packages at the end of storage.

In general, the color results obtained in this test demonstrate that unirradiated, unwrapped, tightly bulk-packed steaks can have excellent color and at the same time show obvious signs of spoilage. Although these steaks bloomed equally as well as the irradiated unwrapped steaks, their greater microbial load apparently caused them to discolor more rapidly than the irradiated steaks during subsequent aerobic refrigerated holding. The unsatisfactory color results with prepackaged bulk vacuum packed steaks (irradiated and unirradiated) again demonstrate the importance of the method of individually packaging and bulk vacuum packing with respect to controlling color and drip.

D-6: Effect of the method of prepackaging upon color of bulk vacuum packed beefsteak:

Thus far in the bulk vacuum packing studies, the individually prepackaged steaks were packaged in the conventional manner of placing the meat in trays and overwrapping with a gas permeable, flexible fresh meat film which was heat tacked or otherwise sealed. This method usually results in substantial free air space within the individual packages. While this is of no consequence with present fresh meat retailing, it could be a problem under conditions of extended holding in a bulk vacuum container. It is reasoned that although the common fresh meat films are oxygen permeable, in comparison to no film at all they are, to some degree, a gas barrier. Putting conventionally prepackaged red meat cuts in a bulk container and drawing a vacuum in the container could result in a low oxygen tension condition within the free air space of each individual package. This could lead to pigment oxidation, especially with meat of modest reducing capacity. It is felt that this may be the principal reason for the frequent unsatisfactory color results with meat so handled in previous tests. The following test was run to check this point:

A Commercial Grade round of beef was trimmed and cut into steaks of assorted sizes and shapes. Some of the steaks were dipped 30 seconds in 10% tripolyphosphate solution and drained. The remaining steaks did not receive a phosphate treatment. All of the steaks were then divided into the variables described below. The results obtained are also given below:

1. Some phosphated steaks were vacuum sealed either in oxygen permeable fresh meat cellophane or in "Lexel" pouches so that no free air space remained inside of the pouches. The pouches formed a "skin-tight" fit around the individual steaks. These prepackaged steaks were tightly packed into a bulk gas impermeable film bag and loosely packed into a rigid bulk vacuum container and vacuums were drawn on each. After 21 days of post-irradiation vacuum storage, these steaks were allowed to bloom in air either in their original pouches, or after removal from the original pouches and being trayed and over-wrapped with fresh film, or after removal from the original pouches and being placed in trays with no overwrap. Both the fresh meat cellophane and Lexel wrapped steaks responded identically colorwise whether tightly or loosely bulk packed. They were uniformly purple with no discoloration at the termination of vacuum storage. Those that were allowed to bloom with the film overwrap removed became bright red in a very short period of

time. Those that were removed from the original pouches and rewrapped in new film bloomed to the same degree as the unwrapped but at a somewhat slower rate. Those allowed to bloom in their original skin-tight pouches did so at a slower rate and to a lesser extent than the above unwrapped and rewrapped samples. This suggests that post-vacuum storage reoxygenation is favored by either no overwrap film being present or by rewapping with a fresh new film, but is hampered by the presence of the original overwrap film which has been in direct contact with the meat for a period of time in bulk vacuum storage. Steaks which remained in their original pouches after vacuum storage also discolored sooner than did those which were oxygenated without an overwrap or which were rewrapped with fresh film.

2. Steaks were placed in individual trays and overwrapped with either fresh meat cellophane, "Lexel" or plasticized PVC film in such a way that air was trapped in the free space within the packages. These were loosely packed in the same rigid bulk container that was previously used for samples so packed in (1) above. While not as discolored as some of the steaks similarly packaged and packed in other tests, these samples showed some discoloration. They were not as uniformly purple as the steaks in the skin-tight individual pouches in the same bulk container. Upon exposure to air they bloomed somewhat, except in discolored areas, and became only fair in appearance. After a day in air the color of these steaks had deteriorated noticeably. Meat packaged in each of the three films reacted the same as far as color was concerned. The results strongly suggest that air trapped in the packages and not removed by evacuating the bulk containers caused whatever discoloration was present at the end of vacuum storage. The film itself did not appear to contribute to any discoloration, only the presence of air within the packages.

3. Unwrapped steaks were tightly bulk packed into two large gas-impermeable flexible film bags. Phosphate treated steaks were placed in one bag and unphosphated steaks in the other bag. Also included in each of the bags was a roast size cut taken from a semitendinosus ("eye of the round") muscle. The two bags were vacuum sealed, irradiated and stored three weeks at 38°F. The phosphate treated steaks became purple more rapidly and to a greater extent than the unphosphated steaks upon vacuum sealing. The subsequent darkening which occurred during the irradiation nearly equalized the purple color of the two variables. The phosphated steaks were more purplish than the unphosphated and upon removal from vacuum storage they bloomed more extensively than the unphosphated samples. The unphosphated steaks were somewhat discolored at the surfaces which were adjacent to

air pockets along the walls of the vacuum bag, but were quite purple in the interior of the bag where the meat surfaces were against one another. The phosphated and unphosphated steaks, whether they remained unwrapped or were trayed and over-wrapped, bloomed extensively except they were already discolored as noted above. The unphosphated steaks discolored much sooner than the phosphated during storage in air. The roast-size cuts were sliced into halves and the newly cut surfaces quickly changed from purple to bright red, appearing slightly fresher looking than the "old" (original) cut surfaces. This was especially true for the original unphosphated surfaces. These observations point to the fact that phosphate treatment aids in the retention and/or regeneration of color, even with unwrapped tightly bulk vacuum packed meat.

D-7: Bulk vacuum packing of phosphated and unphosphated Choice Grade beefsteak:

U.S.D.A. Commercial and U.S.D.A. Choice grade beef were used during the course of the contract work. The latter grade was used for taste panel tests. In general, based upon experience with the beef used in this work, it appears that the Choice Grade is the more likely of the two to be pale, watery, low pH (a condition analogous to pale, soft exudative pork). This would tend to make it more likely to undergo discoloration due to pigment oxidation and give unsatisfactory color results with respect to ability to remain in the reduced pigment state in vacuum and to reoxygenate to a bright red upon removal from vacuum. On the other hand, any 'dark-cutter' beef obtained in connection with this work has invariably been of the Commercial Grade. It should be noted, however, that all of this meat has been obtained entirely from one source (Michigan State University Food Store) and doesn't necessarily represent industry-wide experience. The beneficial effect of phosphate treatment upon meat color and drip have been the most pronounced on occasions when pale watery low pH (below 5.5) beef has been so treated. In such instances the phosphated cuts exhibited markedly improved initial color, as well as greatly improved water retention and color stability. Unphosphated cuts from the same piece of meat invariably changed from pale red to brown when vacuum packaged, and exuded excessive drip. Phosphate treatment resulted in a shift from pale red to bright red in air which became purple upon vacuum packaging. The following test was done in order to observe the performance of phosphated and unphosphated Choice Grade beefsteaks irradiated and stored in bulk vacuum containers.

The top and bottom of the round were selected from an obviously pale, watery Choice Grade round of beef and were trimmed and cut into one-half inch thick steaks. One-third of the steaks was dipped in a 10% tripolyphosphate solution for 30 seconds and then drained before tightly bulk vacuum packing the steaks in a large (15 pound capacity) transparent flexible laminate bag. Another third of the steaks was dipped in a solution containing 10% tripolyphosphate plus 2.5% culinary grade sodium chloride, and similarly packed. The remaining steaks were bulk vacuum packed in the same manner without any phosphate treatment. Very soon after drawing the vacuums, the untreated vacuum packed steaks became completely brown, which indicated the high susceptibility to pigment oxidation and the lack of "metmyoglobin" reducing capacity typical of pale, watery low pH (5.38 in this case) meat. The phosphate, and phosphate plus salt treated meat became mostly purple soon after vacuum sealing. Only a small number of scattered brownish patches were observed. All three bags of steaks were irradiated to 100 Krad at a rate of 2 Mrad per hour. During irradiation, brown areas became purplish and purple areas became a darker purple. However, during the 21 day 38°F storage period, the unphosphated meat reverted to brownish again at the surfaces adjacent to the bag. At the end of the storage period the steaks were removed from the bags and placed in polystyrene trays which were then overwrapped with oxygen permeable fresh meat film.

The unphosphated steaks were a pale purplish color at the surfaces where meat had been in contact with meat and they bloomed slightly to pale red. In a matter of hours, however, the pigment had completely oxidized and all of these steaks were uniformly brown. Considerable fluid exudate remained in the bag in which the untreated steaks had been stored.

The steaks treated only with tripolyphosphate had the best color and the least drip of the three variables. These were uniformly purple upon removal from vacuum and quickly bloomed to a bright red which began fading after a day. These became somewhat discolored after two days. Only a small amount of drip was associated with this variable.

The steaks treated with tripolyphosphate-plus-salt solution did not have quite as good color as those treated with TPP alone and they exuded somewhat more drip. They were decidedly superior to untreated steaks in both respects, however. From this test it appears that treatment with phosphate only is slightly better with respect to color and drip than treatment with salt-plus-phosphate. Both treatments are markedly superior to no treatment at all under the conditions used.

D-8: Phosphate dip vs surface dusting with dry phosphate and their effect on color and drip:

While phosphate dipping increases water holding capacity, drip usually occurs to some degree. As shown in Section VI-B, dipping in phosphate solution results in a net increase in fluid content after draining. It is felt that this additional burden upon the water holding capacity of the phosphate dipped or sprayed meat is the principal cause of any drip released by such meat during storage. It is reasoned that the incorporation of phosphate into the meat with a minimum of added moisture would even further reduce drip. This test compared dipping in phosphate solution, and application of dry phosphate directly on the cut surfaces (substantial net increase in water vs no added water).

A Commercial Grade top and bottom round of beef was trimmed and cut into steaks of assorted sizes and shapes. The steaks were divided into three equal lots, one of which was dipped in 10% TPP for 30 seconds and drained. Another of the lots remained untreated. The third lot of steaks was laid out on wire screens and given a light surface dusting with dry tripolyphosphate. As nearly as possible all cut surfaces were equally treated. Half of the steaks from each of the three lots were individually packaged in skin-tight "Lexel" oxygen permeable film pouches. These were then tightly packed into three separate large transparent gas impermeable laminate bags which were vacuum sealed. The other halves of the three treatment lots were not individually prewrapped in film before being likewise tightly vacuum packed in laminate bags.

The meat in all six bags soon became purple, the surface dusted meat being the darker of the three variables. All six bags were irradiated to an internal dose of 100 Krad prior to storage. It soon became apparent that the surface dusting with the dry phosphate resulted in fluid from the interior of the meat being drawn to the exterior where it accumulated as excessive drip. After 21 days vacuum storage at 38°F the bulk vacuum bags were opened and some of the unwrapped steaks were trayed and over-wrapped. Some of the steaks that had been prepackaged in Lexel were either simply unwrapped and allowed to bloom without film, or the original film was removed and then they were trayed and rewrapped with fresh new film.

The undipped meat had undergone moderate fluid exudation during vacuum storage. The surface dusted meat exuded an unusually excessive amount but the phosphate dipped meat exuded very little drip. Clearly the untreated were superior

with regard to fluid retention than those which were surface dusted. Dipping in phosphate solution was superior to both no treatment and surface dusting with respect to moisture retention. Steaks from all six bulk vacuum bags were purple upon removal from vacuum with the surface dusted steaks being unusually dark (much like severely "dark-cutter" beef). The phosphate dipped and non-dipped non-wrapped steaks that either remained non-wrapped or were trayed and over-wrapped, and the phosphate dipped and non-dipped prepackaged steaks that were simply unwrapped or were first unwrapped and then rewrapped with fresh film all bloomed rapidly and extensively. (In this test the unphosphated steaks bloomed about as well as the dipped steaks, no doubt because the meat had high reducing capacity and was tightly bulk vacuum-packed). The steaks which were prepackaged and remained in the original individual packages did not bloom satisfactorily and they discolored much sooner than their counterparts that were non-wrapped or rewrapped in fresh film. This indicates again that, under the conditions employed, better results are obtained with regard to control of color and drip when the original wrapping of the prepackaged cuts is removed at the end of vacuum holding and the cuts are rewrapped as opposed to leaving them in the original packages. It could be that the lengthy close contact between film and meat causes the film to lose some of its gas permeability and become insufficiently oxygen permeable to support post-vacuum bloom regeneration.

All of the surface dusted steaks were very dark purple upon removal from vacuum and they never bloomed beyond the dark red plus purple stage. Thus it would appear that surface dusting with dry phosphate is not a useful method of phosphate application for aiding in the retention of color and fluid. Perhaps some method intermediate between immersion into phosphate solution and application of the dry powder would provide the benefits of phosphate treatment and yet keep added moisture to a practical minimum.

Some tentative, general conclusions can be drawn from the results of the bulk packing work. The method of tightly bulk vacuum packing unwrapped retail sized cuts and delaying individual cut packaging until the end of bulk vacuum storage and handling invariably yields good results with respect to color and drip control and overall individual package appearance. Again, however, it is only a partial solution to the centralized preparation of retail cuts in that it provides only for centralized cutting while leaving the packaging to the local retail outlets.

The method of choice, performing the final individual cut packaging at the cutting point, has yielded variable results in these laboratory simulated centralized cutting and packaging investigations. The results with color and drip control have been for the most part unsatisfactory when the cuts have been individually prepackaged prior to bulk vacuum packing and allowed to undergo post-vacuum bloom regeneration in the original packaging film. Work is continuing to be devoted to overcoming this problem.

It does not appear to matter which fresh meat film is used for pre-packaging. The three films used in this work yielded identical results in instances where they were compared. Although the phosphate treatment does markedly reduce the degree of fluid exudation, individually prepackaged phosphate treated cuts do release varying amounts of drip which often results in unsightly packages. A method of phosphate application which involves a minimum of concomitant water uptake is desirable. It is believed that this will further reduce drip loss.

Applying dry phosphate powder to the cut meat surfaces is totally ineffective since it causes moisture to be drawn to the surfaces from the interior, thus increasing drip. Also the resulting alkalinity at the surfaces causes the meat to take on an objectionable "dark-cutter" appearance.

Based on results discussed herein, the principal reasons for unsatisfactory color results with individually prepackaged irradiated cuts appear to be due to the following: 1) meat of substandard biochemical condition, and, therefore, prone to pigment oxidation and lacking in pigment reducing activity. An extreme example is very pale, watery, low pH meat; 2) trapped air within the individual packages which is not removed during bulk vacuum packing, thus creating a low oxygen tension condition in the packages which is optimal for pigment oxidation; 3) loss of some oxygen permeability with packaging film that is in direct contact with the meat during extended vacuum holding; 4) absence of a phosphate treatment which frequently makes the difference between satisfactory and unsatisfactory color.

Future work will be directed toward more clearly defining the cause or causes of the problem, and in finding a solution. This will largely involve package development and will hopefully result in a package that will meet all of the needs of the centralized cutting and packaging of fresh red meats.

E. SENSORY EVALUATION

During this contract year a number of taste panel evaluations were conducted. U.S.D.A. Choice Grade beef top round and eye-of-round (semitendinosus) steaks were used which provided the opportunity to gain some experience with this important grade of beef (studies discussed in previous sections employed the Commercial Grade). Untrained panelists were asked to score color, odor, flavor, texture, moistness or juiciness, and overall quality using a 1 to 9 balanced acceptability scale. Page 71 is a copy of the score sheet. In this manner the effect of selected treatment parameters on the above listed eating quality attributes of the cooked steaks were subjectively evaluated.

For each test all steak samples were cooked simultaneously on the same large rack in a forced air oven operating at 325°F. Each steak was uniformly surrounded by hot air, and the cookout juices dripped into a shallow pan below the rack. Each sample served to the panelists was on the order of about 1 1/2 inches square and the panelists received all samples (three or four per test) together on one large paper plate. No embellishments of any kind (e.g., salt, etc) were served with the samples, nor was anything other than convection plus radiant heating used during the cooking.

The method of conducting the tests was such that differences between samples were accentuated and any masking of differences was avoided. It is believed that where differences between samples were detected in these tests, they would be reduced, and in some instances probably eliminated from detection through normal culinary methods. Thus the samples served in these tests were being evaluated under the most revealing conditions. Controls were either freshly cut steaks or untreated steaks cut from the same piece of meat as the treated steaks. In a sense, these optimum quality controls placed the treated steaks at an extra disadvantage whereas steaks handled by one of the alternative approaches to centralized cutting (e.g., freezing) might perhaps be a more proper basis for comparison.

Since essentially fresh cut meat is what is being replaced by centralized cutting, it was felt that meat representing that presently commercially available should be used as the control for the present. For each of the following taste panels, "overall quality" average scores were compared by means of analysis of variance and multiple range testing.

SENSORY EVALUATION OF QUALITY FACTORS:

For each sample, please evaluate each quality factor. Score each factor with the number corresponding to the most descriptive comment. For example, a color score of 5 would indicate that you consider the sample to have an overall color of marginal quality (i.e., "on the fence" between slightly acceptable or fairly good and slightly unacceptable or fairly bad).

Sample code	Color	Odor	Flavor (taste)	Texture	Moistness or juiciness	Overall quality	Comments

SCORING CODE:**Acceptable Range:**

9 = Excellent
 8 = Very good
 7 = Good
 6 = Fairly good

Unacceptable range:

4 = Fairly bad
 3 = Bad
 2 = Very bad
 1 = Poor

5 = Marginal

E-1: Effect of phosphate and irradiation, separately and in combination, on eating quality of beefsteak:

The design for this series of tests is a simple two by two factorial where the factors are phosphate and irradiation. The lower levels are no phosphate and no irradiation, and the upper levels are thirty second dip in 10% TPP, and 100 Krad of gamma radiation. Three separate panels were conducted using this design.

In order to provide samples for all variables from the same piece of meat and yet achieve three weeks of storage without allowing microbial growth, particularly on the unirradiated samples, to influence the results, all samples were held at 29°F until the time of the tests. It had previously been demonstrated (see Section VI-A) that at 29°F microbial outgrowth on beefsteaks is very slow and total counts remain at relatively low levels through three or more weeks of storage.

For each of the three panels, four Choice Grade beef semitendinosus muscles ("eye of the round") were trimmed and then sliced into steaks of uniform thickness (1/2 inch each) using an electric meat slicer. The steaks from each muscle were evenly divided into four treatment variable groups:

1. No phosphate - no irradiation (O-O)
2. Phosphate - " " (P-O)
3. No phosphate - 100 Krad (O-I)
4. Phosphate - " " (P-I)

One quarter of the steaks from each muscle was given each of the above treatments. Phosphate treated samples were dipped for 30 seconds in a 10% tripolyphosphate solution after which they were allowed to drain on a wire screen. Irradiated steaks were all given 100 Krad of gamma radiation at a dose rate of 200 Krad per hour. All steaks were individually vacuum packaged in laminate gas-impermeable pouches after dipping and draining and prior to irradiation. All steaks were stored for 21 days in vacuum at 29°F plus an additional three days in air at 29°F. For the air storage each sample was removed from its vacuum pouch, placed on a polystyrene tray and overwrapped with highly gas-permeable polyvinylchloride fresh meat film.

At the completion of the 24 day storage period and prior to cooking, the raw appearance of the samples was observed. It was noted that phosphate treated samples were generally bright red and appealing in appearance whereas

those not treated with phosphate were generally dull red to brownish red. All steaks, irradiated and unirradiated, had an essentially fresh odor with no indications of spoilage, although a faint trace of off-odor was noted with the irradiated samples.

After noting the raw appearance and odor, the steaks were laid out on one large wire cooking-rack, grouped according to treatment. The rack of steaks was placed in the large, preheated 325°F hot air oven. The steaks were removed for portioning and serving upon reaching a medium done condition (approximately 170°F internal). In order to eliminate the influence of muscle-to-muscle variability, care was taken to insure that the four samples given each panelist were all from the same muscle. The data are summarized in Table 16.

Although significant differences in overall quality scores are revealed in Panel 1 by analysis of variance and multiple range testing, they are obviously small. Scores for the five quality attributes are similarly close with two noteworthy exceptions. One is the relatively low juiciness score received by the O-I which is likely due to the substantial amount of drip released by these unphosphated steaks during storage. At the time of cooking these steaks had already lost a fair amount of fluid. Cooking, of course, further reduced the moisture content. The other notable exception is the relatively low flavor score received by P-I.

Overall quality scores in Panel 2 show a much greater spread than in Panel 1. Again, variable O-I received low texture and juiciness scores. The two irradiated variables were scored relatively low in all respects and no single quality attribute stands out as the deciding factor. The low juiciness score for P-I is inconsistent with Panel 1.

The most notable inconsistency in the Panel 3 data is the relatively low overall quality score for the untreated control (O-O). The data suggest that this result may be attributed to low juiciness and unsatisfactory texture indicating that the panelists place considerable value upon these two attributes. Again, variable P-I received a relatively low flavor and odor score. This apparent trend suggests a possible unfavorable interaction between irradiation and greater than normal moisture (data in section VI-B indicate that phosphate dip results in a net increase in sample fluid content). It is also possible that phosphate treatment itself is imparting an additional flavor note to the meat independent from that of irradiation. In this series storage is assumed to be a constant where-

Table 16. Effect of phosphate, irradiation, and phosphate plus irradiation on eating quality of beefsteak

Treatment	Cooked color	Odor	Flavor	Texture	Moistness or juiciness	Overall quality	Significantly different @ 95% level*
PANEL 1 (24 panelists):							
Ave	6.5	6.4	6.2	5.9	5.7	6.1	
0-I							0-0
Range	4-9	4-9	4-8	3-8	3-9	4-8	
Ave	6.2	6.1	6.0	6.2	6.1	6.0	
P-0							0-0
Range	4-8	2-8	3-9	4-8	3-8	4-8	P-I
Ave	6.3	6.3	5.8	6.8	6.7	6.2	
P-I							0-0
Range	4-8	2-8	3-8	5-9	4-9	4-8	
Ave	6.4	6.3	6.4	6.4	6.4	6.4	
0-0							
Range	3-9	4-8	3-8	4-8	3-8	4-8	
PANEL 2 (20 panelists):							
Ave	5.7	5.9	5.3	4.8	4.7	4.8	P-0
0-I							P-I
Range	3-8	4-8	4-8	1-7	1-7	2-7.5	0-0
Ave	6.4	6.2	6.2	6.6	6.4	6.3	
P-0							0-0
Range	4-8	3-9	4-9	4-9	3-9	3-9	
Ave	5.6	5.6	5.6	5.5	5.6	5.3	P-0
P-I							0-0
Range	3-8	1-8	3-8	2-9	3-9	3-7.5	
Ave	6.8	6.5	6.4	7.2	6.9	6.6	
0-0							
Range	5-9	2-9	2-9	5-9	5-8	2-8	
PANEL 3 (31 panelists):							
Ave	6.9	5.9	5.9	6.3	6.5	6.2	
0-I							
Range	5-9	3-9	3-9	3-8	4-9	3-8	
Ave	6.2	6.2	5.9	5.9	6.0	5.8	
P-0							0-1
Range	3-8	4-8	1-8	3-8	2-8	3-8	
Ave	6.2	5.6	5.4	5.8	5.9	5.4	0-I
P-I							P-0
Range	3-9	2-8	1-8	3-8	4-8	2-8	
Ave	5.8	6.0	6.0	5.4	5.0	5.5	0-I
0-0							P-0
Range	2-8	4-8	3-8	2-8	3-8	3-8	

* Refers to 'overall quality' scores only. Variables appearing in this column had significantly higher 'overall quality' scores than the variables for that row.

as all samples for all variables were identically stored at just above the freezing point of the meat.

At this time one can only speculate from the data as to the reasons for any differences and no firm conclusions can be drawn. Further work will be aimed at elucidating the precise causes of differences where they exist, and at determining corrective measures for improvement or overcoming deficiencies wherever possible. The instances where the between-panel data contradict one another caution against easy generalizations in interpreting taste panel data.

E-2: Effect of phosphate dip and/or irradiation and storage at 40°F on eating quality of beefsteaks:

Four separate replicate taste panels are included in this series. For each panel a Choice Grade beef top round was trimmed and sliced into steaks of uniform 1/2 inch thickness. One-half of the steaks was given a 30 second dip in 10% tripolyphosphate solution and the other half was not treated. All steaks were individually vacuum packaged in laminate gas-impermeable pouches after which they were given 100 Krad at a rate of 200 Krad per hour. The steaks were stored under vacuum for 21 days at a constant 40°F, after which time they were rewrapped in oxygen-permeable fresh meat film and held an additional two days at 40°F. At the end of the 23 day storage period the steaks were cooked and served to the panels as described under E-1. Because of the high storage temperature an unirradiated control could not be stored along with the irradiated samples without undergoing spoilage. Therefore the control steaks for this series were freshly cut from a Choice Grade top round the day before the tests and they were merely wrapped in polyvinylchloride fresh meat film and held in air at 38°F for the one day prior to cooking. Thus each test consisted of three variables:

1. No phosphate, 100 Krad, 23 days storage (0-1)
2. Phosphate, " " (P-1)
3. Control (no phosphate, irradiation or storage) (0-0)

Results are summarized in Table 17.

In three of the four panels, the control was judged to have significantly higher "overall quality" than the treated samples.

In Panel 1 the data suggest that the difference in overall quality between the treated samples and the control is largely if not entirely due to superior flavor of the latter. The especially low flavor score for P-1 might

Table 17. Effect of phosphate dip and/or irradiation and storage at 40°F on eating quality of beefsteaks

Treatment	Cooked color	Odor	Flavor	Texture	Moistness or juiciness	Overall quality	Significantly different at 95% level*
<u>PANEL 1 (20 panelists):</u>							
Ave	6.4	5.2	4.9	6.8	7.2	5.5	0-1
P-I							0-0
Range	4-8	1-7	3-7	5-8	6-8	3-8	

Ave	6.8	6.2	6.0	6.5	6.6	6.3	
0-I							0-0
Range	5-8	3-8	4-8	5-8	5-8	4-8	

Ave	7.1	6.6	6.8	6.6	6.6	6.8	
0-0							
Range	5-8	5-8	5-8	5-8	4-8	5-8	

<u>PANEL 2 (25 panelists):</u>							
Ave	6.6	5.7	5.1	6.7	6.6	5.4	0-1
P-I							0-0
Range	4-9	3-7	3-8	3-9	4-9	3-8	

Ave	7.0	6.2	6.1	6.8	6.6	6.3	
0-I							
Range	6-9	8-9	3-8	5-9	5-8	5-8.5	

Ave	6.8	6.5	6.8	6.2	6.2	6.3	
0-0							
Range	5-9	4-8	4-9	3-8	3-8	4-8	

Table 17 - Cont'd:

Treatment	Cooked color	Odor	Flavor	Texture	Moistness or juiciness	Overall quality	Significantly different at 95% level*
<u>Panel 3 (32 panelists):</u>							
Ave	6.3	6.0	5.8	6.4	6.2	5.9	
P-I							0-0
Range	4-9	3-9	3-8	4-8	4-8	3-8	

Ave	6.2	6.1	5.6	6.3	6.2	5.9	
0-I							0-0
Range	3-9	4-9	3-8	2-9	4-8	4-8	

Ave	6.9	7.1	7.0	6.8	7.0	7.0	
0-0							
Range	5-9	5-9	3-9	4-8	5-9	4-9	

<u>Panel 4 (28 panelists):</u>							
Ave	6.7	6.3	5.6	6.4	6.3	6.0	
P-I							0-1
Range	3-9	3-9	1-8	4-9	3-9	3-8	0-0

Ave	6.9	5.8	5.8	6.8	7.0	6.2	
0-I							0-0
Range	4-9	3-8	2-8	5-9	5-9	4-8	

Ave	7.0	6.6	6.8	6.5	7.0	6.7	
0-0							
Range	5-9	3-9	4-9	4-8	5-8	5-8.5	

* Refers to 'overall quality' scores only. Variables appearing in this column had significantly higher "overall quality" scores than the variable for that row.

possibly be due to either an undesirable flavor note originating from the phosphate salt or the interaction between irradiation and increased moisture due to dipping, or to both or other reasons. Further work will attempt to elucidate the reasons for differences and steps that may be taken to correct deficiencies.

In Panel 2 where O-I and O-O were judged to be of equivalent overall quality flavor and odor were slightly lower for O-I, but this was balanced by slightly higher texture and juiciness scores. Objectionable flavor and odor appear to be the reasons for the low "overall quality" score given to P-I.

In contrast to Panel 2, O-I and P-I have equivalent "overall quality" scores in Panel 3, both being significantly lower than that of O-O. In this panel the two treated and stored samples were generally lower on all counts than the fresh control, but here again flavor appeared to be the chief influencing factor. This time, however, P-I apparently had slightly better flavor than O-I.

As with Panels 1 and 2, Panel 4 indicated P-I significantly lower in overall quality than both O-I and O-O, although only slightly lower than O-I. Surprisingly, P-I was judged to be significantly lower in juiciness than both O-I and O-O. Here again, however, flavor appears to have exerted the greatest influence. As a general conclusion, it may be stated that the two treated and stored samples, but moreso P-I than O-I, were inferior in flavor and overall quality as compared to freshly cut untreated steaks.

E-3: Effect of phosphate dip, and salt plus phosphate dip on eating quality of irradiated beefsteak;

There is evidence reported in the literature that the combination of salt (sodium chloride) and condensed phosphates is more effective in retarding drip and oxidation than are the phosphates alone. This point is being investigated in this work; therefore it was felt advisable to study the influence of this supposedly synergistic combination upon eating quality as well. For the first two panels conducted in this series there were three variables per panel. In order to focus on the effect of phosphate alone versus salt plus phosphate, all samples were irradiated. This also provided a good opportunity to observe the panel response to a test lot of samples for which they had come to expect an unirradiated control but which in fact did not include one. Through repeated experience coupled with some awareness of the nature of the project, many panelists became quasi-expert and conditioned to expect an unirradiated sample in each test. The panelists were

not informed that the first two tests included only irradiated samples; this appeared to have the effect of generally raising the scores of the irradiated variables slightly above what might have been expected based upon results of previous panels where fresh unirradiated samples were available for comparison.

The first two tests were conducted in the same manner as those under E-2, except that the unirradiated control (0-0) was omitted and a new variable (S-P-I) was added. Samples involving these three variables were individually vacuum packaged, given 100 Krad of radiation and stored for 20 days in vacuum, then rewrapped in a gas permeable film and stored in air at 40°F for two additional days. Variable 0-0-I was not treated with phosphate. Variable 0-P-I was dipped as usual in 10% tripolyphosphate. Variable S-P-I was dipped 30 seconds in a solution containing 7.5% tripolyphosphate and 2.5% culinary grade sodium chloride. Results are tabulated in Table 18.

The data in Panel 1 indicate a slight overall preference for S-P-I over 0-0-I and 0-P-I; however, the reverse is true of Panel 2 data. Interestingly, S-P-I scored lowest in juiciness in both panels. The expectation was that, if anything, the opposite would be the case.

Two additional taste panels comparing no dip vs TPP dip vs salt plus TPP dip were conducted. Three Choice Grade beef top rounds were trimmed and cut into one-half inch thick steaks. One-third of the steaks from each round was dipped 30 seconds in a 10% tripolyphosphate solution. Another one-third of the steaks from each round was similarly dipped in a solution containing 10% tripolyphosphate plus 2 1/2% culinary grade sodium chloride, and the final one-third remained untreated. Steaks for each of the three variables were bulk packed into large laminate bags (one bag per variable, and about 15 pounds of meat per bag) which were vacuum heat sealed and then held overnight at 38°F before being irradiated to 100 Krad at a rate of about 2 Mrad per hour. Typically, the meat became noticeably darker during irradiation under vacuum. Following irradiation the three bags of meat were stored for 21 days at 38°F at which time the steaks were removed from the bags, placed on polystyrene trays and over-wrapped with polyvinylchloride fresh meat film. The steaks were then returned to the 38°F refrigerator. Some of the steaks of each variable were removed after two days in air for Panel 1, and the remaining steaks were removed after three days in air for Panel 2. Freshly cut steaks from a fresh Choice Grade top round were used as controls for both panels. Cooking and serving were done in the previously described manner. The

Table 18. Effect of phosphate dip, and salt plus phosphate dip on eating quality of irradiated beefsteak

	Cooked				Moistness	Overall	Significantly
Treatment	color	Odor	Flavor	Texture	or juiciness	quality	different at 95% level *
Panel 1 (27 panelists):							
Ave	6.5	6.2	6.3	6.8	6.8	6.3	
O-Q-I							S-P-I
Range	4-8	5-9	4-8	5-9	3-9	5-8	

Ave	6.6	6.1	6.2	6.6	6.7	6.3	
O-P-I							S-P-I
Range	4-8	3-9	4-8	3-9	3-8	4-9	

Ave	7.0	6.2	6.5	6.6	6.2	6.5	
S-P-I							
Range	4-9	2-9	5-8	3-9	3-9	4-9	

Panel 2 (21 panelists):							
Ave	6.2	5.7	6.0	6.6	6.7	6.1	
O-Q-I							
Range	4-8	3-7	4-8	5-8	5-9	5-8	

Ave	6.7	6.0	5.9	6.3	6.9	6.2	
O-P-I							
Range	4-8	3-8	4-7.5	4-8	4-8	4-8	

Ave	6.2	5.8	5.8	6.2	6.3	5.9	
S-P-I							O-Q-I
Range	4-8	4-7	3-8	3-8	5-9	4-7.5	O-P-I

* Refers to 'overall quality' scores only. Variables appearing in this column had significantly higher 'overall quality' scores than the variable for that row.

results of the two panels are summarized in Table 19.

In both tests the fresh control was judged to be inferior in overall quality to the three treated variables. Apparently the control came from a carcass that was not typical of the Choice Grade in that it was unusually tough and flavorless. There were essentially no overall quality differences among the three treated variables in Panel 2, whereas in Panel 1 a preference was indicated for the samples treated with salt plus tripolyphosphate. The flavor data in Tables 18 and 19 indicate an overall acceptance of salt plus phosphate treatment. This suggests that the sodium ion contribution to flavor of sodium tripolyphosphate treated steaks is not objectionable and that substituting the potassium salt probably would not improve flavor scores for the variable. The polyphosphate could still be influencing flavor in a negative manner however, although there is no proof of this. In Panel 1, but not in Panel 2, S-P-I apparently was found to have better moisture and texture. As with previous panels, the results are somewhat contradictory and firm conclusions probably cannot be drawn as yet.

Looking at all of the taste panel data together one can observe some apparent trends. Because comparable data sometimes are contradictory, however, caution must be exercised in interpretation. Every effort was made to cook all samples to the same degree of "doneness". For this reason, in interpreting the data the cooking process was not regarded as a source of variability.

Written and verbal comments by several panelists together with some of the data indicate that phosphate treatment appears to improve juiciness (and apparently texture) by increasing water content and/or reducing cookout fluid. This logical conclusion is somewhat tempered by the fact that other of the data indicate equivalent juiciness for phosphated and unphosphated steaks, and still other data indicate higher juiciness for the latter. One might expect phosphated steaks to be superior in juiciness than their untreated counterparts; however, a conclusion to that effect is not unequivocally supported by the data.

The flavor data generally indicate a tendency toward scoring unphosphated-unirradiated steaks (both freshly cut, and stored at 290°F) superior in flavor to unphosphated-irradiated stored steaks which in turn are judged to be superior in flavor to phosphated-irradiated stored steaks. Here again, however, the data do not allow an unqualified conclusion. At best, the data indicate an overall trend. This suggests that 100 Krad alone, under the conditions employed, can cause a detectable negative effect upon flavor which is enhanced somewhat by the combination

Table 19. Comparison of undipped, phosphate dipped, and salt plus phosphate dipped irradiated beefsteaks with freshly cut untreated control beefsteaks

	Cooked				Moistness	Overall	Significantly
Treatment	color	Odor	Flavor	Texture	or juiciness	quality	Different at 95% level*
<u>Panel 1 (24 panelists):</u>							
Ave	5.9	6.5	6.0	5.4	5.3	5.8	
O-I							
Range	4-8	5-9	3-9	2-8	4-8	3-8	S-P-I

Ave	6.2	5.7	5.5	6.0	5.8	5.6	
P-I							
Range	3-8	3-7	3-8	3-8	2-8	3-8	S-P-I

Ave	6.4	6.1	6.0	6.6	6.3	6.3	
S-P-I							
Range	3-9	3-9	1-9	4-8	4-8	4-8	

Ave	6.4	6.0	5.6	5.2	5.7	5.4	O-I
Control							P-I
Range	4-8	2-8	1-8	3-8	2-8	2-8	S-P-I

<u>Panel 2 (25 panelists):</u>							
Ave	5.8	5.4	5.4	6.4	6.3	5.8	
O-I							
Range	3-9	2-8	1-8	4-9	4-9	1-8	

Ave	6.2	6.0	5.8	6.1	6.3	5.8	
P-I							
Range	4-9	4-8	2-8	3-9	4-8	3-8	

Ave	6.5	5.6	6.0	6.1	5.9	5.7	
S-P-I							
Range	2-9	3-9	3-8	3-8	3-8	2-8	

Ave	6.1	6.0	5.8	4.8	5.5	5.5	
Control							O-I
Range	3-9	4-9	3-8	2-7	2-9	3-8	P-I

* Refers to "overall quality" scores only. Variables appearing in this column had significantly higher "overall quality" scores than the variable for that row.

of phosphate dip plus irradiation. The slight off-flavor can perhaps best be described as aged or merely different, but is not the classical irradiation flavor associated with meat processed with greater than pasteurizing doses. The panel rating differences, while real, are not large differences. They very likely would be greatly diminished and possibly rendered undetectable by some culinary practices.

That phosphate treatment plus irradiation plus anaerobic holding followed by aerobic holding might affect taste and odor, etc., to some degree is not an unreasonable expectation.

Some form of change associated with the period of holding the meat and not specifically related to the treatment given the meat might be expected to occur. Evaluation of the meaning of such a change in terms of consumer acceptance probably will require a large-scale consumer-type panel, possibly best employed under true marketing conditions.

VII. CONCLUSIONS AND GENERAL DISCUSSION

Based upon findings from work completed during this and the previous contract year, the following conclusions may be drawn:

1) 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.

2) Phosphate dipping 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 if not considered to be a significant deterrent to the use of phosphates on fresh meats for control of fluid exudation and pigment oxidation.

3) 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.

4) 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 pre-packaged cuts appears to be residual air entrapped within the packages when the vacuum is drawn in the bulk container.

5) 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 to the cited dose range is inadequate, and rapid, frank spoilage occurs with both irradiated and unirradiated cuts.

6) TBA test data indicate that little or no lipid oxidation occurs during anaerobic irradiation and anaerobic refrigerated holding. Upon subsequent exposure of irradiated and unirradiated, phosphated and unphosphated cuts to the atmosphere, all treatment variables undergo lipid oxidation at about the same rate and to about the same extent. During three days post-vacuum exposure to air the extent of lipid oxidation with relatively lean cuts does not appear to be very great, and the need for an antioxidant treatment has not as yet been demonstrated under the test conditions being employed.

7) Reflectance spectrophotometric measurements and visual observations indicate that phosphate treated cuts do not undergo pigment oxidation to any significant extent during anaerobic holding, but do undergo a relatively slow gradual pigment oxidation (much like freshly cut meat) upon post-vacuum re-exposure to the atmosphere. In contrast, individually prepackaged cuts not treated with phosphate frequently undergo extensive pigment oxidation upon bulk vacuum packing and/or upon subsequent re-exposure to air. Thus phosphate treatment appears to have a pigment stability promoting effect as well as a drip reducing effect.

8) Both total dose and dose rate have an observable effect upon red meat color. Total dose effects are of no apparent concern until the dose is several times greater than the required dose, as determined in this study. Dose rate effects become apparent at moderate to high (e.g., 1 Mrad or more per hour) rates. It was found that metmyoglobin production during irradiation of aerobically packaged fresh beefsteaks increased with increasing dose rate. Conversely, irradiation of vacuum packed cuts seems to exert a beneficial effect on color through conversion of any oxidized (brown) color that may be present to the desired purple during the irradiation. This noticeable positive effect is consistent with the view that radiation applied anaerobically promotes reducing conditions in material such as

meat. Since the effect is more pronounced at higher dose rates, it may be stated that, from the color standpoint, lower dose rates are advisable with aerobic irradiation of meat and higher dose rates are advisable with anaerobic irradiation of meat. This point is of particular significance in the context of electron irradiation where bulk vacuum packing is ruled out due to inadequate penetration.

Finally, some tentative conclusions may be drawn with respect to eating quality of beefsteaks treated and held under the test conditions. Phosphate treatment appears to reduce cooking losses and yield a juicier, apparently more tender cooked steak. The combined effects of vacuum packaging, irradiation, and refrigerated holding under vacuum for three weeks followed by a period of holding in air result in a flavor, and sometimes an odor, which is slightly different from that of nonphosphated, nonirradiated steaks when the cuts are cooked and rated by sensory panels under the most revealing conditions. The atypical flavor note is not so-called irradiation flavor but is frequently described as "slightly old" or "aged" as compared to very fresh and may be connected with lipid oxidation. While it does not appear to be a serious problem, this area needs further study with consumer panels in conjunction with chemical testing (e.g., TBA test) to characterize the cause or causes and nature of the off-flavor.

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