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**RADIATION PASTEURIZATION OF
FRESH MEATS AND POULTRY**

Annual Report, February 15, 1967—February 14, 1968

March 15, 1968

Department of Food Science
Michigan State University
East Lansing, Michigan

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RADIATION PASTEURIZATION OF FRESH MEATS AND POULTRY

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Division of Isotopes Development
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Annual Report

For the Period February 13, 1967 through February 14, 1968

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III. PREFACE

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

The material included in this first 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 of different 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.

During the first contract year, research efforts were focused on the three principal deteriorative processes that limit the salable life of fresh meats and poultry: 1) fluid exudation (drip), 2) color deterioration, and 3) microbial spoilage. Although the work is incomplete, indications from the first year's effort are that the combination of ionizing radiation in the dose range of 50 to 150 Krad, plus refrigeration, polyphosphate treatment and effective packaging, and handling will provide the means for a centralized fresh meat cutting and distribution system.

V. INTRODUCTION

A. General:

Meat is sold as several types: fresh, cured, processed, and canned. By far, the largest percentage is sold as fresh meat. Fresh meat may be considered as meat from the dressed and chilled carcass which is not subjected to further treatment, except cutting and sometimes grinding. Steaks, chops, roasts, and hamburger are examples of fresh meat.

One of the objectives of curing, processing, and canning is to make such meats keep without spoilage. While the modern use of refrigeration and packaging has reduced the need for such preservation, the advantage of the inherent longer life of these meats is utilized in their distribution. Because they have sufficient stability, they can be prepared at the packing plant or other centralized points, and packaged for retail sale.

Fresh meats are much more perishable, and because of this perishability the distribution of fresh meats has generally followed a pattern in which: a) preservation for limited times is through refrigeration; b) the packing plant ships to the retail selling unit wholesale cuts including carcasses, sides, quarters, and primal cuts; c) conversion to retail cuts or to ground meat, including packaging, is done in the retail unit. Hence, the retail unit performs a part of the manufacturing operation for fresh meats.

It has been proposed to centralize the processing of fresh meats (1)(2). Savings in construction, equipment, and labor costs are anticipated. In addition, there are other opportunities for other savings: " a) better distribution of meat cuts according to market preferences; b) more uniform and efficient cutting methods; c) better control of overhead costs; d) advantages of quantity purchases; and e) a better market for carcass by-products' (1).

The salable life of fresh meats is the maximum length of time that freshly cut and packaged retail cuts can remain in satisfactory salable condition. Under the present marketing system, salable life is limited by any one or combination of discoloration, fluid exudation and microbial spoilage. The perishability of fresh meats as now prepared is such that a two or three day salable life is all that can be obtained (3). It has been stated that improvements in present handling methods could about double this (1)(2)(3). Hence, the most that can be hoped for by this approach is a five or six day total salable life for retail cuts. Such a life is to be compared with a present approximate seven day life for dressed poultry. Cured

meats such as bacon in a conventional package have a two to three week display life, and in a vacuum package approximately 30 days. Vacuum packaged sliced cold cuts also maintain good quality for about 30 days. Haddock fillets keep about 13 days (4).

While opinions as to what is needed may vary, it is clear that the present life of fresh meats in retail units is extremely small, and more importantly, that the expectations for extension of this life using "improved handling" are not in fact much greater. The hoped for life of fresh meats is shorter than that for other meats and even shorter than for fish. It has appeared desirable, therefore, to seek means in addition to "improved handling" to obtain a longer display life and thereby to enable these meats to be processed and distributed in a manner similar to other meats; that is, to permit centralized cutting.

Studies suggest that a product life of from seven to 21 days will be required. This range covers the likely approaches to the distribution of retail cuts from a centralized cutting point (5). The goal of this research is to maintain the salability for refrigerated retail cuts up to 21 days which should be sufficiently long to satisfy a truly centralized retail cutting and distribution operation. Ionizing radiation is expected to be useful in attaining this goal by controlling one of the deterioration processes, namely, microbial spoilage.

In stating the desirability of centralized cutting of fresh meats, it is recognized that there are other problems besides the technical one of product life. These problems lie principally in the areas of economics, merchandising, and government regulations. It is believed that once the needed technical feasibility is attained, adjustments in these other areas will follow and lead to over-all feasibility.

B. Deterioration Process:

In developing an approach to the technical problem of life extension, it was stipulated that: a) meats would have their normal color at the point of retail sale; and b) ingredients and procedures would be such that, if not now approved by regulating agencies, there is reasonable basis for securing such approvals. Specifically excluded from consideration are color-retaining agents such as carbon monoxide gas, sulfites, nicotinamide, etc.

The deterioration that limits the life of fresh meat involves at least three different processes: a) microbial development; b) chemical changes; and c) physical changes.

The outgrowth of bacteria, yeasts and molds, present on and within the carcass, and as a result of exposure of the meat to the environment of the

slaughtering, dressing, storage, transportation, and cutting operations produces changes normally associated with spoilage, namely changes in odor, flavor, appearance and texture. Changes associated with microbial growth are of major importance in the deterioration of quality of fresh meat.

Chemical changes involve primarily changes in the meat pigments leading to discolorations. These pigment changes are frequently associated with the action of microbes, but can also occur independently of this effect. The color of meat as cut is purplish. Exposure to air produces the normal bright red color due to reaction of the pigment with oxygen to form oxymyoglobin. The oxygen is loosely bound and the degree of "oxygenation" is dependent upon the pressure of oxygen in the atmosphere in contact with the cut surfaces. The following equation shows this relationship:

Continued exposure to oxygen causes the formation of metmyoglobin, the oxidized form of the pigment, which is brown. This reaction, therefore, involves a valence change in the iron of the myoglobin porphyrin moiety:

Equation [2] unlike [1] is normally not reversible. Consequently, the reaction of equation [2] eventually leads to total conversion of all of the myoglobin present at the cut surfaces to metmyoglobin.

The work of Brooks demonstrated that there is an optimum oxygen pressure for the oxidation of heme pigments (6). George and Stratmann showed that the first order rate constant for autooxidation of myoglobin in phosphate buffer is maximum at about 1-2 mm Hg oxygen partial pressure (7)(8). Pressures lower than the optimum impede the oxidation reaction, [2] by lowering the concentration of the reactant oxygen. Pressures well above the optimum tend to counteract the formation of metmyoglobin by pushing the equilibrium in equation [1] to the right; that is, toward the formation of oxymyoglobin, while at the same time providing an excess of oxygen for tissue respiration. As a result, respiration cannot reduce the high oxygen concentration to a level favorable to pigment oxidation. Eventually, however, due to changes in the physiological state of the tissue (e.g., loss of reducing capacity) myoglobin would undergo autooxidation at high oxygen pressure.

Thus, at the outset, it was recognized that there are at least two potentially useful fundamental approaches to the problem of avoiding or delaying

red meat pigment oxidation: a) use of a large excess of oxygen in contact with the retail cuts of red meat during storage and handling; and b) complete removal of oxygen from contact with the meat during storage and handling. This second approach would be expected to result in conversion of the oxygenated form (and any metmyoglobin present) to purplish reduced myoglobin at the cut meat surface, and would necessitate reoxygenation of pigment in the shallow layer at the surface by some means prior to displaying the cuts for retail sale.

Retail cuts of fresh meat are presently wrapped in transparent films which are permeable to oxygen in order to attain and/or hold the normal bright red color (9). This availability of oxygen, usually below 160 mm partial pressure (air is 21% O_2), ultimately leads to browning of the meat, which is a major limitation on the display life of the product under present practices (3). As mentioned, such discoloration can be hastened by microbial action, but also occurs independently.

Other chemical changes in the meat, such as fat oxidation, primarily affect flavor. Ordinarily, such effects are relatively minor and, under present practices, are usually overwhelmed by microbial outgrowth. However, it was also recognized at the outset that lipid oxidation could potentially take on new importance if the combination of ionizing radiation and oxygen were used to extend the salable life of fresh meats.

Physical effects are essentially those associated with exudation of the serum like fluid ("drip" or "weep"), usually somewhat pigmented, from the cut lean surface. The amount of exudate is a function of the amount of cut surface, the type and cut of meat, and the time and temperature of holding the fresh cuts. As the fluid accumulates in the package it becomes unsightly, and unless hermetically sealed, the package will leak liquid. Purchase resistance can develop. Declared net weight can also be a problem, since some state regulatory agencies do not include the released liquid in the weight of the meat.

The water binding properties of meats have been studied by many workers. The use of phosphates for reducing the shrink of cooked cured meats is well established (10). Similar usage for poultry (11) and fish (12) has been investigated. The method has recently been approved for poultry. The use of phosphates to solve the drip or exudate problem with fresh meats seems to be indicated. Additional benefits might be some degree of control of 1) microbial outgrowth (13),

2) pigment oxidation, and 3) any tendency toward rancidity development (14).

C. The value of ionizing radiation:

The value of ionizing radiation in preserving refrigerated fresh meats has been the subject of many studies (15). Doses of 50 to 250 Krad cause significant reductions of microbial populations and, with respect to microbial spoilage, are effective in extending product life (16)(17)(18).

The research in this area in the United States was conducted about a decade ago and more or less came to a halt with the recognition that irradiation did not provide all that is needed (15). Of the three deteriorative processes operating on fresh meats, radiation controls only the microbial. As previously mentioned, chemical and physical changes also occur and, although in good condition microbiologically, meat cuts lose salability with time.

To summarize, the experimental plan was and is to accomplish salable life extension of refrigerated retail cuts of fresh meats through: a) control of microbial spoilage with ionizing radiation; b) prevention of pigment oxidation with the assurance of normal color at the point of retail sale by means of effective packaging and by controlling the atmosphere in contact with the surface of the retail cuts; and c) prevention of fluid exudation with phosphates (which may also have a beneficial effect on a and b).

By using these agents in combination, a full solution of the salable life extension problem is anticipated and a technical obstacle to centralized meat cutting and distribution would thus be removed. Extension of the approach to poultry is also included in the plan.

VI. RADIATION SOURCES AND DOSIMETRY

Three sources of ionizing radiation were employed during the contract year. Two of the three are Cobalt-60 gamma emitters, and the third is an electron generator. All three are batch type as contrasted with continuous type irradiators.

The first, and the source used exclusively during the first half of the contract year, is the 10 kilocurie Cobalt-60 research irradiator consisting of encapsulated Co-60 slugs, arranged in a cylindrical configuration. It is housed in the Phoenix Memorial Building, North Campus, University of Michigan, Ann Arbor, Michigan. Samples are arranged around the outside of a hollow cylindrical source shield at distances corresponding to dose rates predetermined in the usual manner by means of the Fricke standard chemical dosimeter. In this manner, total dose absorbed by the meat samples can be estimated* knowing 1) the position of the

* The term "estimated" absorbed dose is used because it is neither possible nor necessary to know exactly the quantity of energy absorbed by exposed meat samples. In food irradiation studies a quoted absorbed dose is understood to be an estimated figure derived from supporting dosimetry measurements and standard calculations.

meat samples in relation to the source, and 2) the length of time of exposure. After the samples have been properly positioned, the source is raised from its water pool for a pre-set period of time. Temperature during irradiation is held at 50°F (10°C).

The second source is the new 51 kilocurie Cobalt-60 research irradiator housed in its water pool in the basement of the new Food Science Building on the Michigan State University campus. This source became available for research during Autumn, 1967, after installation was completed and dosimetry studies were sufficiently far enough along to allow estimation of absorbed dose in the manner outlined above. The Michigan State University Cobalt-60 irradiator is basically of the same type as the University of Michigan source, and samples are similarly arranged about the outside perimeter of the source shield. Since it consists of a greater number of curies of Cobalt-60, the M.S.U. irradiator provides a much higher dose rate for a given distance than does the U. of M. irradiator. Thus exposure times are generally of a smaller duration. The air conditioning system accompanying the M.S.U. irradiator is as yet not completely functional and cell temperature during irradiation has been variable. Thus, irradiation temperature will be noted where appropriate in the body of the report. It is anticipated that exact temperature control down to 34°F (1°C) will be possible within six months.

The third source of ionizing radiation, used to only a limited extent to date, is the General Electric 1 MeV resonant transformer electron beam generator housed in the M.S.U. Agricultural Engineering Building. The generator is expected to be used to an increasing extent for high dose rate (megarads per minute range) and shallow surface irradiation studies. Both accelerated electrons and secondary bremsstrahlung X rays contribute to absorbed energy, but the latter are of only incidental importance. Dosimetry and absorbed dose estimations are accomplished in a manner analogous to that for the cobalt irradiators, but using electron capture devices.

VII. EXPERIMENTAL

A. Introductory discussion of methods and materials:

1. Meat:

Work reported herein was largely limited to the round of beef. This limitation was decided upon since it is felt that beef is more prone to color and drip problems than is the meat of other species (pork, lamb, and poultry). The round was selected since it is convenient to obtain and since there is some

indication that cuts from the round are more sensitive to discoloration and drip than are cuts from other parts of the carcass. With few exceptions, rounds from U.S.D.A. commercial grade beef were employed. This grade is a relatively lean beef. Lamb leg and pork loin were used to a limited extent during the first contract year, and veal, poultry and non-muscle meats not at all. The beef was freshly cut from intact rounds of unknown history received at the meat department of the University Food Store which services the entire campus. Further cutting into retail type units and packaging was done at the Food Science Department. The same holds for fresh lamb rounds. The pork loin that was used was taken from hogs soon after local slaughter.

2. Packaging:

Details of packaging are provided in the results of individual experiments. Packaging material used includes:

a) Laminate ("L" pouches) consisting of a polyester (Mylar) base with a thin coat of polyvinylidene chloride (Saran) applied to the outer surface, and a heavier extrusion coat of polyethylene on the inner surface which acts as a sealant. The gas impermeable pouches were supplied by International Kenfield Distributing Company under the trade name "IKD Super All-Vak #13". Size 7" X 8" pouches were used.

b) Polyethylene: One mil "poly" sandwich bags were procured locally; and 1.5 mil polyethylene fresh meat wrap film was obtained from the Dow Chemical Co. These films are in the moderate oxygen permeability range.

c) Fresh meat cellophane: (Nitrocellulose coated) manufactured by the DuPont Company and sold under the designation 195-MSAD-80. This film is highly oxygen permeable when wet (i.e., when in contact with the cut surface of fresh meat).

d) Plasticized polyvinyl chloride fresh meat wrap film "Resinite RMF-61" manufactured by the Borden Chemical Company, one of several producers of this relatively new and popular, highly oxygen permeable film.

e) Multi-quart "home pressure canners" fitted with vacuum and pressure gauges and modified for bulk packaging under either pressure or vacuum. Oxygen backfill is accomplished using a cylinder of oxygen of 99.6% purity. Vacuum is drawn by either a high vacuum pump or by a high vacuum aspirator filter pump.

Package sealing is accomplished by a Kenfield flexible package sealer with vacuum pump and gas flush attachments.

3) Phosphates: The following kinds were used:

- a) Sodium tripolyphosphate (TPP)
- b) Sodium hexametaphosphate (HP)
- c) Sodium acid pyrophosphate (APP)
- d) Trisodium orthophosphate (TOP)
- e) Disodium orthophosphate (DOP)
- f) Metaphosphate "Glass" 64% P_2O_5 (Glass)

Phosphates were mainly applied to the meat by dipping the cuts in a solution of the phosphate for a definite period of time. Pumping the solution into thick cuts was also investigated. Procedural details are given in the results of individual experiments. Solutions are on a percent weight basis.

4) Storage:

Meat samples were stored in a refrigerator which is set at 38°F (refrigerator temperature gauge occasionally fluctuated a degree or two on either side of the 38°F mark).

5) Evaluation of samples:

a) Color: During the preliminary phase, covering approximately the first six months, color evaluation was generally limited to subjective evaluation employing the following grading scale: 1) uniform bright red color (most desirable); 2) generally bright red but with areas of brown or purple; 3) uniformly red, but not bright red; 4) red color, not bright, with areas of brown or purple; 5) brown in color with essentially no red areas.

During the latter half of the contract year, the method of reflectance spectrophotometry began to be phased into the work as an objective method of measuring relative concentrations of the three pigment forms (oxygenated, reduced, and oxidized myoglobin), and, by correlation with subjective color evaluation, a method of objectively evaluating color.

b) Drip: Early observations included collection of drip and measurement of volume (ml) or noting the presence of a substantial amount of drip or essential freedom from drip. During the latter half of the contract year, objective analytical methods were introduced into this phase of the work.

c) Microbiology: Through the first two-thirds of the contract year, presence of or freedom from spoilage was estimated principally by observing the odor of the samples. The presence of bacterial slime was also noted where observed. Characteristic spoilage odors were taken as an index of excessive microbial growth.

During the remainder of the contract year, objective evaluation of the microbial situation was incorporated into the work. Emphasis was on quantitative aspects by means of total microbial plate counts. Also, preliminary attempts to identify those types eliminated by and surviving irradiation were made, especially types contributing to ultimate spoilage under various conditions.

d) Other: In the course of investigating the above three principal problem areas, other factors such as performance of packaging materials and development of oxidative odor were observed.

B. Microbiological:

As pointed out in the general introduction, during the preliminary probing phase of the work, covering approximately the first six months of the contract year, assessment of samples for microbiological outgrowth and spoilage was limited to sensory detection of spoilage odor and slime formation. Samples exhibiting typical meat spoilage odor, with or without slime, were judged as spoiled, the rationale being that the universal index of microbial spoilage of fresh refrigerated meats is a slimy surface layer of spoilage bacteria usually preceded in time by characteristic odors and flavors of products of their metabolism. Whether or not endogenous enzyme activity contributes to the overall effect, the overt signs of normal microbial spoilage of fresh meats are unmistakable.

Recognizing that radiation pasteurization of fresh meats alone or in combination with various packaging methods alters microbial population and outgrowth patterns, direct methods of evaluating the microbiological picture were phased into the work during the latter half of the contract year. Major emphasis thus far has been on obtaining total plate count information for the treatment variables under study. Attempts were also made to characterize the flora as to: a) species eliminated by radiation treatment; b) species surviving radiation treatment; c) species growing out under aerobic or anaerobic packaging; and d) dominant specie(s) involved in ultimate spoilage. Some detail on methodology follows:

1) Total microbial plate counts:

A 30 to 40 gram portion is aseptically removed from sample and placed in a sterile stainless steel blender jar. Sample is minced for 10 seconds, diluted 1:10 by adding sterile distilled water to blender jar; and blended for three minutes. With a minimum of delay, samples are plated in sterile plastic petri dishes. Decimal dilutions are made by pipetting 1 ml into 99 ml of sterile half-strength nutrient broth (0.15% beef extract, 0.25% peptone) in milk dilution bottles which are then

thoroughly shaken before pipetting either 1 ml or 0.1 ml to plate. Plates are poured with sterile "standard methods agar" (Plate count Agar-Difco) tempered @ 45°C. After solidifying, plates are inverted and incubated for 72 hours @ 21-23°C. A Quebec dark field colony counter is used to count only those plates containing between 30 and 300 colonies. Each dilution is poured in duplicate. If more than one dilution yields plates within the countable range, the set yielding the higher count is used in the final tabulation although all counts are recorded. Plates are not discarded until: 1) representative colonies are picked for microscopic examination, and 2) until slow growing organisms requiring a longer incubation period are given time to form colonies. For instance, there have been occasions when it has taken a week or longer for some gram positive bacteria to develop their tiny subsurface colonies to the point where they are clearly visible. This is especially true of plates from irradiated vacuum packaged samples which have been in storage for a number of days. Obviously, longer incubation periods are advisable in such instances as such colonies may or may not be detectable at 72 hours. In the few instances where this has been encountered thus far in the work, colonies were counted after 72 hours, and again after about a week, at which time it was evident that newly appearing sub-surface colonies would increase no further in numbers. As yet, when counts have increased after 72 hours these have been only slight increases, and the 72 hour counts have been used. However, for future work, the total plate count methodology will be expanded to cover such situations in a more precise manner.

In some instances additional plates were poured with acidified potato dextrose agar as a check for yeasts. None was detected at any time that this was done; however, yeasts are among the common contaminating flora of commercial cutting operations. All equipment and reagents are sterilized by autoclaving for 15 min at 250°F before coming in contact with samples.

2) Identification of microorganisms: has thus far been presumptive in nature. Positive identification to the species level via biochemical tests, etc. was not attempted during the reporting period.

Representative isolated colonies from pour plates were picked and either directly smeared onto micro-slides or first enriched in trypticase soy broth (B.B.L.). Smears were heat fixed, gram stained and examined microscopically under oil immersion. By means of colony and individual cell characteristics, some general conclusions about the microbial population under various conditions are made.

It perhaps should be pointed out that microorganisms encountered in this work do not necessarily completely represent what the microbial situation would be at some future centralized cutting plant, or under each and every existing fresh

meat processing situation. However, there is a fairly large and growing body of literature having to do with microbiology of fresh meats, irradiated and non-irradiated, which indicates that, on the whole, the microbiology of fresh meats is rather constant and well known although there is not complete agreement as to taxonomy. Relative radiation resistance among the principal fresh meat microorganisms is also rather well mapped. Nevertheless, the fact that a good deal is known on the subject does not relieve one of the need to investigate the microbiological side of any contemplated application of radiation pasteurization to raw meat, especially in view of the fact that bacteria constituting a potential public health problem (e.g., salmonellae, staphylococci, and fecal streptococci) are more radiation resistant than the normal spoilage species, particularly the pseudomonas, of untreated fresh meats [(19) through (32)].

pH was measured on the blended 1:10 dilution of each sample, using a Beckman Expandomatic (Model 76) pH meter. pH values so obtained were found to agree closely with measurements on the intact tissue as well as the undiluted juice. Calibration and measurement were always done in the same manner by the same individual. The same is true of the plating of samples and the counting of plates. Thus any potential person-to-person variability is avoided.

Experiment 1:

This was a preliminary survey study of the combined effects of gamma radiation enriched oxygen atmospheres, and a sodium tripolyphosphate dip on the keeping quality of beef. In addition, the effect of the use of oxygen-permeable film was compared with that of an oxygen-impermeable film.

Slices from a beef top round were dipped for one minute in a 10% (by wt) solution of TPP, drained for a few minutes and divided into three lots, one lot each being sealed in 1) polyethylene, 2) fresh meat cellophane, and 3) an impermeable laminate of saran, mylar and polyethylene, under in-package atmospheres as indicated in Table 1. The next day samples to be irradiated were transported to Ann Arbor under refrigeration and were irradiated as indicated. Upon returning to East Lansing, samples were placed in the 38°F refrigerator to await evaluation after 5, 7, 9 and 16 days of storage. Color, odor, slime formation and the amount of drip were observed.

The oxygen pressures given are readings from a manometer which formed a closed system with the packages and a cylinder of oxygen during the packaging operation, and therefore are to be considered as only relative. Color scores are on the 1 to 5 (bright red to all brown) scale.

Table 1. Combined effects of 0 and 100 Krad radiation, oxygen pressure and phosphates on keeping quality of beef

Package	O ₂ **** mm	Press. Dose Krad	Color after days storage**				Drip*** ml	Spoilage odor at 16 days
			5	7	9	16		
PE	Air	0	3.9	5.0	4.0	5	0	Strong
PE	Air	100	4.0	4.5	4.2	5	0	Slight
PE	50	0	2.6	5.0	5.0	5	< 1	Strong
PE	50	100	3.7	4.5	4.7	5	< 1	Slight
PE	300	0	1.0	1.8	5.0	5	0	Strong
PE	300	100	1.8	3.0	2.3	5	1	Slight
PE	700	0	2.5	3.5	5.0	5	< 1	Strong
PE	700	100	3.2	3.2	3.2	5	1	None
L	50	0	5.0	5.0	5.0	5	< 1	Strong
L	50	100	5.0	5.0	5.0	5	2	Slight
L	300	0	2.5	2.0	2.3	5	0	Slight
L	300	100	4.2	2.2	2.0	5	2	Slight
L	700	0	2.5	3.7	2.7	4	2	Strong
L	700	100	3.9	4.3	3.8	4	3	None
FMC	Air	0	1.0	2.3	2.3	4	0	Slight
FMC	Air	100	4.0	3.0	3.2	4	0	None
FMC	700	0	3.0	4.5	5.0	5	0	Strong
FMC	700	100	3.0	3.3	3.3	5	0	None
FMC*	700	0	3.5	5.0	5.0	5	2	Strong
FMC*	700	100	4.5	5.0	4.8	5	5	None

* Not dipped in TPP.

** Average of two observations on three replicates.

*** Maximum amount at any time.

**** Manometer reading.

Conclusions:

Color of all samples deteriorated with time and at 16 days all were of unsatisfactory color. Fairly good color (color grade 2) was obtained at 9 days with the 300 mm samples in some cases. Drip was small for the TPP dipped samples but did not occur in some cases. Irradiation generally prevented strong spoilage odors at 16 days' storage. It was noted that 100 Krad of gamma radiation did not immediately effect color. A slight off-odor, however, was observed immediately after irradiation. The value of oxygen pressure was not clearly demonstrated in this early preliminary experiment; however, the beneficial effects of irradiation and phosphate treatment are evident.

Experiment 2:

This experiment was essentially the same as Experiment 1, except dose was set at 50 Krad. Steaks from a commercial grade beef top round were dipped for one minute in 10% TPP, drained for a few minutes and packaged either in polyethylene pouches or in the gas impermeable laminated pouches. As in Experiment 1, the oxygen pressures given are manometer readings taken during the sealing of the packages. The same 1 to 5 (bright red to brown) visual color scoring scale was used by three judges. Color, drip and spoilage odor results are summarized in Table 2.

Table 2. Combined effects of 0 and 50 Krad radiation, oxygen pressure and phosphates on keeping quality of beef

Package	O ₂ Press. mm*	Dose Krad	Color after storage days**				Drip ml ***	Spoilage odor at:	
			4	8	13	18		13 days	18 days
PE	Air	0	1.1	4.6	5.0	5.0	0	Strong	Strong
PE	Air	50	2.6	4.1	5.0	5.0	2	None	Strong
PE	200	0	1.8	5.0	5.0	5.0	0	Strong	Strong
PE	200	50	2.3	3.3	4.5	5.0	5	None	Strong
PE	400	0	1.8	4.9	5.0	5.0	0	Strong	Strong
PE	400	50	3.3	3.8	5.0	5.0	4	None	Strong
PE	600	0	1.9	4.7	5.0	5.0	0	Strong	Strong
PE	600	50	3.2	2.7	3.0	5.0	3	None	Strong
PE	800	0	1.6	5.0	5.0	5.0	0	Strong	Strong
PE	800	50	2.1	2.3	4.8	5.0	3	None	Strong
L	Air	0	3.1	4.4	5.0	5.0	0	Strong	Strong
L	Air	50	2.7	4.0	5.0	5.0	1	None	Slight
L	200	0	2.1	3.7	5.0	5.0	0	Strong	Strong
L	200	50	3.0	4.3	5.0	5.0	2	None	None
L	400	0	2.7	3.1	5.0	5.0	0	Strong	Strong
L	400	50	2.7	3.8	4.8	5.0	3	None	None
L	600	0	2.0	3.0	5.0	5.0	0	Strong	Strong
L	600	50	3.0	3.9	5.0	5.0	5	None	Slight
L	800	0	1.3	3.2	5.0	5.0	0	Strong	Strong
L	800	50	2.9	3.9	5.0	5.0	3	None	Slight

* Manometer reading, but actual O₂ pressure in package is uncertain.

** Average of one to three observations on three replicates.

*** Maximum amount at any time.

Conclusions:

Color of all samples deteriorated with time and at 13 days all but one were of unsatisfactory color. Samples with higher levels of oxygen concentration retained their color longer. Drip occurred in significant quantities in the irradiated samples, largely due to the fact that during irradiation, due to equipment breakdown, meat temperatures reached 70°F. 50 Krad of radiation delayed spoilage at least 13 days in this instance. Non-irradiated samples in polyethylene discolored more rapidly than the irradiated, perhaps due to higher microbial activity.

Experiment 3:

-Objective: To determine effect of gamma radiation dose-level on microbial spoilage, drip and color.

Materials and Method:

Meat - 1 cm slices of beef (eye of round)
Phosphate - 10% water solution of TPP
Packaging - PE
Irradiation - Dose as specified under Results; dose rate was constant
Method - Meat slices dipped for 60 seconds in TPP solution, drained, and placed in package. Packages placed in Kenfield machine and atmosphere changed to provide manometer reading of 500 mm with O_2 . Packages heat sealed, stored at 38°F, and, next day, transported under refrigeration to Ann Arbor, where they were irradiated. Returned to East Lansing the same day and stored at 38°F. Observations made at 7, 12 and 25 days. Amount of drip, color and odor and slime formation observed.

Table 3. Effect of gamma radiation dose on microbiological spoilage, color and drip (PE Package)

Dose Krad	Color after storage - days ***				Drip ml*	Spoilage - odor at		
	7	12	21	25		12 days	21 days	25 days
600	3.8	4.0	5.0	5.0	2	None	None	None
450	4.3	4.0	5.0	5.0	2	"	"	"
250	2.7	3.8	4.5	5.0	2	"	"	"
200	2.5	3.8	5.0	5.0	< 1	"	"	"
150	2.3	2.5	5.0	5.0	< 1	"	"	Slight
100	2.2	3.0	4.5	5.0	1	"	"	None
50	1.8	4.0	5.0	5.0	0	"	"	Slight
0	2.2	5.0	5.0	5.0	0	Strong**	Strong**	Strong**

* Maximum at any time.

** Also slimy.

*** Average of three replicates and two observations.

Conclusions:

Up to 21 days spoilage odor development did not occur at any radiation dose level used. Color deterioration, however, occurred at all dose levels before the development of a spoilage odor. Higher dose levels, caused earlier deterioration than lower levels. Amount of drip also increased slightly with radiation dose. As a general conclusion, a dose level above 150 Krad appears excessive. 50 Krad was probably sufficient, in this instance, to achieve a 21 day + life.

Experiment 4:

This experiment was intended to determine the effect of gamma radiation dose-level on microbial spoilage, color and drip. A combination package was utilized which consisted of an inner wrap of fresh meat cellophane in contact with the meat, both enclosed in an outer package of the "L" laminate, with the space between film filled with oxygen to just under the bursting point of the outer pouch during the sealing operation.

Slices from beef "eye of the round" were dipped for one minute in TPP solution (10% by weight) and drained for a few minutes before packaging. Samples to be irradiated were transported to Ann Arbor under refrigeration where they were irradiated. Following irradiation, samples were returned to East Lansing and were placed into 38°F storage to await observation after 8, 16, and 22 days of storage. Samples were observed for color, odor, slime formation and drip. Results are tabulated in Table 4.

Table 4. Effect of gamma radiation dose on microbial spoilage, color and drip (combination package)

Dose Krad	Color after storage ***			Drip ml*	Spoilage odor at:		
	8 days	16 days	22 days		8 days	16 days	22 days
600	3	4.7	5	< 1	None	None	None
300	3	4.6	5	< 1	"	"	"
250	3	4.3	4.5	1	"	"	"
200	3	4.1	4.5	< 1	"	"	"
150	3	4.3	4.5	< 1	"	"	"
100	2.7	3.7	4.5	1	"	"	"
50	2	3.7	4.5	0	"	"	Slight
0	3	5.0	5.0	0	"	Strong**	Strong**

* Maximum at any time.

** Also slimy.

*** Average of three replicates and one to three observations.

Conclusions:

Up to 16 days spoilage odor did not occur at any radiation dose level. At only the 50 Krad level did a spoilage odor occur at 22 days. Color deterioration occurred at all dose levels before the development of a spoilage odor. The data suggest that higher doses cause earlier color deterioration. Amount of drip was negligible at all dose levels. As a general conclusion, in this instance a dose of about 100 Krad was sufficient to delay detection of spoilage odor more than three weeks post-irradiation. As in Experiment 3, color deterioration was the chief obstacle in the way of a completely successful three week life at 100 Krad and 38°F, although oxygen pressure appears to have had some beneficial effect.

Experiment 5:

This was a study of the combined effects of gamma radiation, enriched in-package oxygen atmosphere, and TPP dip on the keeping quality of pork. In addition, oxygen permeable film was compared with oxygen impermeable film with respect to color.

Slices from fresh boneless loin of pork were divided into two lots, one of which was dipped for one minute in a 10% solution of tripolyphosphate. Slices were sealed in fresh meat cellophane only, in the impermeable laminated pouches only, and some were double packaged, first in fresh meat cellophane and, in turn, in laminated pouches to which segments of tygon tubing had been bonded. By means of the tubing, the double packages were individually joined to the cylinder of oxygen. The spaces between the inner and outer pouches were filled with pure oxygen to a pressure just below the burst point of the outer 'L' pouches. The tubings were closed off with pinch clamps. Next day the samples to be irradiated were transported under refrigeration to Ann Arbor. Upon completion of irradiation they were returned to East Lansing and stored at 38°F to await evaluation after 7, 14, and 21 days of storage. Samples were observed for color, odor, slime formation, pH, and drip accumulation. Observations are summarized in Table 5.

Table 5. Combined effects of radiation, enriched oxygen atmosphere and tripolyphosphate on keeping quality of pork

Package	TPP	Irrad. dose Krad	7 days storage			14 days storage		21 days storage		Drip*
			pH	Color	Spoil. odor	Color	Spoil. odor	Color	Spoil. odor	
FMC	No	50	5.48	3	None	5	None	5	None	0
FMC	No	100	5.46	3	None	5	None	5	None	< 1
FMC	Yes	100	5.75	1	None	2	None	4	None	0
FMC	Yes	50	5.78	1	None	2	None	3	None	0
FMC-L**	No	50	- -	1	- -	5	None	5	None	0
FMC-L**	No	100	- -	1	- -	5	None	5	None	< 1
FMC-L**	Yes	50	- -	1	- -	1	None	2	None	0
FMC-L**	Yes	100	- -	1	- -	1	None	2	None	0
FMC	No	0	5.52	1	None	5	Strong	- -	- -	- -
FMC	Yes	0	5.83	1	None	1	None	1	None	0
L	No	0	- -	1	- -	4	None	5	Strong	< 1
L	Yes	0	- -	1	- -	5	Strong	- -	- -	- -

* Maximum at any time.

** Combination package: see Materials and Methods.

Conclusions:

Radiation with a dose of about 50 Krad was effective in controlling microbial spoilage in this instance. Generally, the O₂ enriched atmosphere improved color retention. The TPP dip caused a rise of about 0.3 pH, and in all except the bottom set (Table 5) significantly improved color retention. Normal pork (not P.S.E.^{1/}) is generally considered to be the least susceptible of the major meats to fluid exudation, and the negligible drip experienced in this study supports this view. The dipped, irradiated FMC-L samples and the dipped, non-irradiated FMC sample had excellent appearance at 21 days. (That the latter sample was not spoiled at 21 days can be considered an extraordinary and atypical result, probably due to an extremely low initial microbial load).

^{1/} Pale-soft-exudative.

Experiment 6:

The purpose of this study was to determine the effect of 100 Krad of gamma irradiation on the total microbial count and on the keeping quality of fresh beef slices held in air, under vacuum and under oxygen pressure. Also of interest was the response, with respect to color, to reoxygenation after storage under vacuum.

Steaks were cut from the muscle of fresh commercial grade beef bottom round. Randomly selected slices were double packaged, first in fresh meat cellophane, and then in laminate pouches fitted with tygon tubing. The packages were evacuated by means of a high vacuum pump to which they were connected by means of the tubing. The tubings were clamped off, and in this manner individual vacuum packages were obtained. During evacuation, both the inner FMC and the outer L pouches collapsed about the slices to form a "skin tight" wrap. A rapid shift from red to purple surface color indicated that essentially anaerobic conditions prevailed within the double pouch. Other slices were packaged in polyethylene pouches. Variables studied are the following: A) in polyethylene, in air, 0 Krad; B) double package, under vacuum, 0 Krad; B-0) same as (B) except vacuum replaced by oxygen after 8 days for some and after 10 days for others; C) in polyethylene, in air, 100 Krad; D) double package under vacuum, 100 Krad; D-0) same as (D) except vacuum replaced by pure oxygen at 10 days; and E) same as (D) except vacuum replaced by pure oxygen immediately after irradiation.

Samples B-0, D-0, and E were backfilled with oxygen through the tygon tubing which was clamped off when oxygen pressure reached just short of the bursting point of the outer pouches.

All samples were stored at 38°F. Sets were withdrawn periodically for total microbial plate counts and evaluation of color, odor, and general appearance. For purposes of comparison both Standard Plate Count Agar (Difco) and APT Agar (Difco) were employed for quantitative evaluation of microbial survival and outgrowth. Except in cases where counts were about the same in both media, Plate Count Agar often yielded slightly higher counts than did APT Agar, all other factors being the same. Observations are summarized in Table 6.

Table 6 - Continued -
Storage -
Days after irradiation

Variable	10		15	
	Total count	Observations	Total count	Observations
(A) 0 Krad, in air	2×10^8	Dark red + brown, spoiled odor & slime	4.4×10^8	Brown and spoiled
(B) 0 Krad, vacuum	3×10^6	Purple, no off-odor		
(B-0) 0 Krad, vacuum O ₂ backfill	3.6×10^7 (8 days in vac. 2 days in O ₂)	Bright red, trace off-odor	3.3×10^8 (10 days in vac; 5 days in O ₂)	Dark red + brown spoiled odor and slime
(C) 100 Krad in air	6.5×10^6	Dark red purple; no off-odor	3×10^8	Dark red brown trace off-odor
(D) 100 Krad vacuum	1.8×10^5	Purple, trace off-odor		
(D-0) 100 Krad vacuum O ₂ backfill			3.6×10^6 (10 days in vacuum; 5 days in O ₂)	Bright red, trace off-odor
(E) 100 Krad in vacuum immediately backfilled O ₂	$> 10^8$	Bright red, dark red slight off-odor	2.4×10^8	Dark red, sl. off-odor

The summary of experimental data (Table 6) indicates the following: an absorbed dose of an estimated 100 Krad reduced the total viable cell count by about two to three log cycles. The unirradiated control (A) became spoiled and discolored within 10 days. The unirradiated vacuum packaged samples became spoiled and discolored within five days in oxygen after being stored in vacuum. Similar samples were in very good condition after two days in oxygen preceded by eight days in vacuum. Irradiated samples showed no signs of spoilage during the 15 day period under any of the conditions; however, color generally deteriorated with time. It appears that non-irradiated vacuum packaged meat, upon re-exposure to oxygen after some storage, spoils rather rapidly.

Perhaps the most significant observation to come out of this experiment is that vacuum packaged irradiated samples (D-O) became bright red upon backfilling with oxygen after 10 days vacuum storage, and that this desired color was retained for five additional days, after which time total viable cell count was only in the 10^6 per gram range. Thus it is demonstrated that this approach can work at least under conditions of this experiment. This lends support to the speculation that some form of this basic approach could be made workable in commercial practice. While holding under oxygen throughout, (E) delayed the onset of discoloration, it did not provide as good a color at 15 days as did the use of vacuum followed by reoxygenation. Also, total count was about two log cycles higher for (E) (and for B-O) than for D-O at 15 days.

The data in Table 6 are consistent with comments in the literature to the effect that a total plate count usually associated with slime and spoiled odor of untreated fresh meat may be, and often is, reached and surpassed in radiation pasteurized fresh meat in the absence of typical overt signs of spoilage. This is no doubt due to the fact that 100 Krad of radiation virtually eliminates the rapid growing slime-producing odorous bacteria of the aerobic gram-negative psychrophilic group, notably the Pseudomonads, as strongly suggested in this experiment by the absence of huge creamy spreading colonies on total count plates from irradiated samples. This was the dominant colony type from non-irradiated samples held in air and from non-irradiated vacuum packaged samples which underwent spoilage after exposure to the air. The degree of microbial kill was approximately the same for irradiated vacuum packaged samples as for samples irradiated in air. Vacuum packaging alone, as would be expected, appears to be somewhat (but not indefinitely) effective in retarding microbial outgrowth during storage, but only so long as the meat remains under vacuum.

Experiment 7:

This was essentially a repeat of Experiment 6 and again was an attempt to evaluate the effects of irradiation and packaging by means of subjective observation and total plate counts. As in Experiment 6, uniformly thick slices were taken from component muscles of fresh commercial grade beef bottom round. Here, however, all slices were first placed in polyethylene rather than in cellophane pouches. One-third of the slices were randomly selected and their polyethylene pouches were vacuum heat sealed. That is, during the sealing operation, air was withdrawn by means of the vacuum pump attached to the sealer so that almost no air remained inside of these pouches once sealed. Then, as rapidly as possible, these were in turn sealed inside of the gas impermeable laminated pouches which were then evacuated through tygon tubing fittings by means of a vacuum pump. The vacuum packaging was done in this way because it was earlier noted that, although the polyethylene is moderately gas permeable, air pockets remain inside when a vacuum is pulled from between it and the outer pouch. That is, the polyethylene does not collapse to form a 'skin-tight' inner package as does fresh meat cellophane. In spite of this effort, instead of becoming purple the meat for the most part remained red and gradually became brown.

For the remaining two-thirds of the slices, the polyethylene pouches were merely heat sealed without pulling a vacuum, since they were to be stored either in air or in oxygen after irradiation in air. Half of ^{each} of the two packaging variables was given a dose of approximately 50 Krads of gamma radiation. Irradiated and non-irradiated polyethylene packaged samples to be held under oxygen were packed loosely in a modified 12 quart pressure canner. With the aid of high vacuum grease the canner lid was secured to the pot with lugs so as to make a vacuum-pressure tight seal. The canner was then evacuated to a gauge reading of $28\frac{1}{2}$ " of vacuum, and, after allowing the pump to continue pulling for a few additional minutes, the valve was closed on the canner. The canner was then attached to a cylinder of high purity oxygen from which it received oxygen to an internal pressure of 30 psia or about two atmospheres. The valve was closed and the canner was placed in the 38°F refrigerator along with vacuum packaged samples, and those in polyethylene in air.

Samples were periodically withdrawn and analyzed by total microbial plate counts (Standard Plate Count Agar), yeast count (Acidified Potato Dextrose Agar), pH (using homogenized 1:10 dilution), and observed for off-odor, slime and discoloration. An attempt was also made to characterize eliminated and surviving

flora. Observations are summarized in Table 7.

Variables are as follows:

- OA) Non-irradiated, in polyethylene, in air
- IA) 50 Krad, " " "
- OV) Non-irradiated, vacuum packaged
- IV) 50 Krad, " "
- OO) Non-irradiated, in polyethylene, under two atmospheres oxygen
- IO) 50 Krad, " "
- OVO) Non-irradiated. Sample plated at 21 days had been held under vacuum 18 days followed by three days under the two atmos. of oxygen. Sample plated at 26 days had been held under vacuum for 21 days followed by 1 day under two atmos. of oxygen followed in turn by four days in air.
- IVO) 50 Krad, otherwise same as (OVO)

The very low initial count was due to exceptionally hygienic handling and packaging of samples. Samples stored in air (polyethylene only) rapidly became brown while samples under oxygen pressure browned much more gradually, especially areas of the meat not in direct contact with the polyethylene. These areas were red for several days after areas in direct contact with the film had browned. This and similar observation leads to the conclusion that the polyethylene used in these experiments was not sufficiently oxygen permeable. This likely also explains why vacuum packaged samples did not respond as well colorwise as in Experiment 6 where cellophane was the inner wrap.

It is of interest to note that two atmospheres of pure oxygen alone appear to have accounted for an inhibition toward overall microbial outgrowth judging from overall total count data, and absence of typical spoilage odor and slime at 26 days. A stale musty odor, suggestive of oxidation, was observed with samples stored under oxygen pressure, however. Similarly, vacuum packaging alone also accounted for lower total counts during storage, while the greatest reduction in total counts resulted from the combination of vacuum packaging and irradiation. Yeast counts, using acidified potato dextrose agar as the plating medium, yielded no colonies whatever at any time; therefore, it can be assumed that yeasts were not among the natural flora of the samples.

After the completion of colony counting at each evaluation time, representative colony types were picked from the agar plates, smeared and fixed on micro-slides, gram stained, and examined microscopically under oil-immersion. For

each isolate notations were made as to cell morphology, gram reaction, colony morphology and color, etc., and from which treatment variables the organism was either isolated in large numbers or not at all. A dozen distinct colony types were picked which were judged to represent all colonies on all plates.

The overwhelming colony type from, and obvious cause of spoilage of, non-irradiated samples stored in air (samples representing normal commercial handling) was a relatively huge light colored smooth creamy surface spreader composed of aerobic psychrophilic gram negative rods, most likely of the genus *Pseudomonas*. This organism was virtually eliminated by the 50 Krad dose and, interestingly, was inhibited by oxygen pressure whereas it was virtually absent on plates from non-irradiated and irradiated samples so stored.

Surface colonies consisting of short gram negative rods, possibly an *Achromobacter* specie, were isolated from the irradiated samples stored in air. A modest number were also isolated from non-irradiated vacuum packaged samples.

The most frequently encountered types of bacteria on samples other than non-irradiated in air were gram positive cocci. Although present on all variables, they were particularly abundant on plates from all irradiated samples, and plates from non-irradiated samples held both in vacuum and under oxygen pressure. Those forming pinpoint and small lens shaped subsurface colonies were particularly abundant. but a few less prevalent types indicated a preference for aerobic conditions by forming surface colonies. Data from pH measurements do not appear to show any clear-cut trends except with O-A samples which steadily rose in pH during storage and spoilage. pH of vacuum packaged samples, including those reoxygenated, appeared to undergo a slight decrease in pH preceded by a slight rise.

Table 7. Total microbial plate counts (bacteria per gram), pH, and color, odor, and appearance of beef slices, 0 and 50 Krad, stored in air, under oxygen and under vacuum at 38°F

Storage - Days									
Variable	0			7			13		
	Total count	pH	Observations	Total count	pH	Observations	Total count	pH	Observations
O-A	$< 10^3$	5.45	Bright red - no off-odor	5×10^6	5.48	Mostly brown, no off-odor	2.1×10^8	5.62	All brown, spoiled odor
O-V	'			1×10^4	5.55	Brown surface, no off-odor	4×10^6	5.65	Brown surface, no off-odor
O-O	'			5×10^4	5.65	Bright red, no off-odor	1.2×10^6	5.65	Red-brown, moderate stale odor
I-A	$< 10^3$	5.43	Red, slight off-odor	2×10^4	5.50	Mostly brown, sl.off-odor	1.9×10^6	5.50	All brown, sl.off-odor
I-V	"	5.44	Purple, no off-odor	2×10^3	5.48	Brown-purple surface, tr. off-odor	2.5×10^4	5.45	Brown surface, no off-odor
I-O	'			10^3	5.52	Bright red, trace off-odor	3×10^5	5.60	Red-brown, mod. stale odor
O-V-O									
I-V-O									

Table 7 - continued

Storage days - 19				21			26		
Variable	Total count	pH	Observations	Total count	pH	Observations	Total count	pH	Observations
O-A	1×10^9	5.78	Brown & green, spoiled odor & slime			Discarded			
O-V	2.1×10^6	5.63	Brown surface tr. off-odor	2×10^6	5.68	Brown, slight off-odor	1×10^8	5.43	Purple, sl. off-odor
O-O	6.1×10^6	5.60	All brown mod. off-odor				4×10^8	5.32	Brown, stale odor
I-A	1.8×10^8	5.52	All brown, sl. off-odor				1.3×10^8	5.48	All brown, spoiled odor
I-V	8×10^6	5.52	Brown surface no off-odor	1.5×10^7	5.63	Brown, sl. off-odor	1.2×10^6	5.62	Brown, sl. off-odor
I-O	2×10^7	5.72	Red-brown stale odor				2.5×10^7	5.50	All brown stale odor
O-V-O				3.2×10^7	5.65	Brownish red sl. off-odor	3×10^8	5.42	Brown spoiled odor
I-V-O				6×10^6	5.75	" "	4×10^8	5.60	Brown, stale odor

Experiment 8:

The purpose of this experiment was to investigate the same variables as in Experiment 7 but applied to a matched pair of rounds from a U.S.D.A. Choice lamb carcass. The rounds were cut up, trimmed of loose fat and put through a coarse grind followed by a fine grind. The ground meat was thoroughly mixed and then packaged in polyethylene pouches which were heat sealed and randomly divided into six lots, treated and coded as follows:

1. O-A) Non-irradiated, in polyethylene, in air
 2. O-V) " double packaged, under vacuum
 3. O-O) " in polyethylene, under 30 psia oxygen
 4. IA) 50 Krads, in polyethylene, in air
 5. IV) " double packaged, under vacuum
 6. IO) " in polyethylene, under 30 psia oxygen
- OVO) Reoxygenated OV [vacuum broken with 30 psia oxygen for 2 hrs]
 " followed by placing in air at atmos pressure
- IVO) " IV " " "
- IVA) Vacuum broken by filling outer pouch just short of burst point with compressed air, holding for 2 hrs, and then placing in air at atmos pressure
- OVA) " " " "

Samples were gamma irradiated at M.S.U. (irradiation dose rate 200 Krad/hr, irradiation temperature 75°F) and stored in the 38°F refrigerator. Vacuum packaging was by means of double packaging as in Experiment 7. Evaluation procedure was the same as Experiment 7. Observations are summarized in Table 8.

Discussion:

The 50 Krad dose reduced the total microbial population by about one log cycle, which in this instance apparently was not sufficient except possibly in combination with vacuum packaging. Grinding and mixing, which was done to provide uniform sample material, likely caused a hastening of microbial spoilage and discoloration. The pH of all samples increased with time except those irradiated in vacuum which underwent a pH decrease indicating that, for these, acid production accompanied outgrowth of flora surviving irradiation. After removing a sample for plate counting, both the 22 day IV and 40 day IV samples were broiled and informally tasted by one investigator who observed a slight but noticeable sour flavor. This off-flavor plus the observation of detectable (although very slight) spoiled odor of the raw 22 day and 40 day IV samples is of interest since it indicates that spoilage

of vacuum packaged irradiated lamb is readily evident to the senses.

In contrast to the preceding beef experiment wherein oxygen pressure storage appeared to have an inhibiting effect upon microbial outgrowth, lamb samples stored under oxygen pressure appeared to experience enhanced microbial outgrowth. The fact that the lamb was ground may partially explain this effect assuming that it is real and not an artifact. The bacteria on the beef slices would be largely confined to the surface, whereas they would be expected to be dispersed throughout the volume of the ground and mixed lamb samples. If one accepts this, then it is certain that pure oxygen under two atmospheres of pressure will penetrate more deeply into the ground meat and reach more subsurface aerobes than would air-oxygen at its atmospheric partial pressure. Thus it is conceivable that pure oxygen under pressure could be somewhat inhibiting toward a surface flora while the same condition could enhance the outgrowth of a flora dispersed throughout a ground medium. Other factors, however, such as differences in microbial population between the beef and the lamb, and species differences of the meats could cause variation in microbial response to an environmental condition.

Although primarily a microbiological study, as usual color, odor and general appearance were noted at each withdrawal time. Oxygen pressure storage delayed browning but, again, not sufficiently long, while apparently giving rise to a stale oxidative type of odor. It is of interest to note that the greening that became very prevalent on samples spoiling in air did not appear at all on samples that spoiled under oxygen pressure. Samples held in vacuum rapidly became brown upon exposure to either oxygen or air. This is likely at least in part due to insufficient oxygen transmission of the polyethylene wrap and the fact that the meat was ground. Although 50 Krad appeared to be too low, the most effective treatment from the point of view of microbial spoilage was the combination of vacuum packaging and irradiation. Further, if 50 Krad was too low in combination with vacuum packaging, then it follows that irradiation (but at a higher dose) would be necessary with vacuum packaging; that is, vacuum packaging alone is insufficient.

Microscopic examination of gram stained smears from representative isolated colonies on total count plates revealed the following observations: The predominant colony-type isolated from irradiated and non-irradiated samples that spoiled in air and under oxygen was a large smooth creamy spreading type of surface colony made up of gram negative rods, most likely a *Pseudomonas* species. Apparently the 50 Krad dose, instead of virtually eliminating this particular bacterium in lamb, had

practically no effect in delaying its proliferation during low temperature aerobic storage in ground lamb. In the preceding study on beef slices an analagous organism was practically eliminated by 50 Krad and did not grow out under two atmospheres of pure oxygen. It is possible that this reflects a species difference. There were two other principal microbial types isolated from the above samples. One was a gram negative rod, and the other was a gram positive coccoid bacteria which formed tiny white subsurface lens shaped colonies. These latter two bacterial types were the principal organisms isolated from irradiated and non-irradiated samples held in vacuum.

The remaining flora from all of the plates was a mixture of gram positives and gram negatives each of which were present in very small numbers compared to the frequency of occurrence of the three types mentioned above. The lamb seemed to carry a greater variety of bacteria than beef samples that have been plated to date; and the lamb seemed more prone to rapid microbial spoilage.

Table 8. Total microbial plate counts (bacteria per gram), pH, and color, odor and appearance of ground lamb, 0 and 50 Krad stored in air, under oxygen and under vacuum at 38°F

Storage - Days	0			8			13		
	Total count	pH	Observations	Total count	pH	Observations	Total count	pH	Observations
QA	3×10^6	5.92	No off-odor. brown where poly- ethylene touches	1.4×10^8	5.83	Spoiled odor, brown	4.5×10^8	5.95	Spoiled, brown
QO	"	"		5×10^8	5.82	Sl.stale odor, dark red	9.8×10^8	6.00	Spoiled, brown
QV	"	"		4×10^6	5.95	No off-odor purplish surface	1.4×10^8	5.85	Sl.off- odor, purplish
QVO	"	"							
QVA	"	"							
IA	4×10^5	"		3×10^8	6.25	Sl.spoiled odor	1.4×10^9	6.20	Spoiled, brown
IO	"	"		2.4×10^8	5.87	Sl.stale odor dark red	1.3×10^9	6.10	Stale odor brown
IV	5×10^5	5.96	No off-odor brownish purple	3.8×10^7	5.93	No off-odor purplish	4.0×10^7	5.95	No off-odor purplish
IVO									
IVA									

Table 8 - Continued:

Storage -
Days

Variable	18			22			40		
	Total count	pH	Observations	Total count	pH	Observations	Total count	pH	Observations
OA	8×10^8	6.57	Spoiled brown + green				3.8×10^9	6.70	Spoiled, all green, slimy
OO	1.9×10^9	6.58	Spoiled brown	2.5×10^9	6.88	Spoiled brown	10×10^9	7.25	Spoiled, all brown, slimy
OV	2.9×10^8	5.96	Spoiled purple + brown	2×10^8	6.20	Spoiled, discarded			
OVO				$*1.8 \times 10^8$	6.11	Spoiled brown + green			
OVA				$*1.2 \times 10^8$	6.42	Spoiled brown + green			
IA	4.2×10^8	6.85	Spoiled brown				4.5×10^8	6.70	Spoiled, sl. green, sl. slimy
IO	1.8×10^9	6.20	Stale odor brown	1.2×10^9	6.55	Stale odor brown	8.6×10^9	7.03	Spoiled, all brown
IV	2.5×10^8	5.73	Trace off-odor, purplish	1.2×10^8	5.62	Trace spoiled odor, purplish	1.3×10^8	5.56	Trace spoiled, off-odor, purple & brown
IVO				$*2.2 \times 10^8$	5.62	Spoiled odor brown			
IVA				$*1.3 \times 10^8$	5.90	"			

* Vacuum was broken on 19th day.

Experiment 9:

This study was intended to repeat the previous experiment using ground beef, but employing seven radiation doses instead of the previous two in order to obtain a comparative picture of total dose effect in addition to the comparison of air vs oxygen pressure vs vacuum storage.

A commercial grade of beef top, bottom and knuckle of the round (each of the three cuts from a different round so as to increase the randomness of the starting material) were cut into small chunks after removal of loose fat. The chunks were mixed, and then coarsely ground, mixed, fine ground, and then given another thorough mixing. The homogeneous ground beef was then taken to a cold room and spooned into fresh meat cellophane pouches, 175 grams to a pouch. The pouches were heat sealed and pooled into a single lot. One-third of the pouches was randomly removed from the total. These were vacuum-packaged in oxygen impermeable flexible L pouches in the previously described manner. These, and the samples in fresh meat cellophane only, were divided into seven lots and treated with gamma radiation to the following total doses: 0, 50, 100, 150, 200, 300, and 500 Krads, all at a dose rate of 190 Krads per hour. Cell temperature during irradiation was 75°F. Up to 200 Krad, all samples were given uninterrupted irradiation. The 300 and 500 Krad samples were removed along with the 200 Krad samples at 200 Krads and were placed at 35°F for an hour before returning to the cell to receive the balance of the 300 and 500 Krad dose. This was done to re chill the higher dose samples as they were barely cool to the touch after being irradiated for over 1 hour at 75°F. The lower dose samples (up to 150 Krad) were still quite cold upon removal from the cell and had not risen more than 10°F from a starting temperature of about 33-34°F. Although the meat had not been treated with phosphates, drip was not at all severe with the high dose ground meat, in spite of the high irradiation temperature, as it surely would have been with unground meat.

It was observed that the vacuum-packaged samples became rather brown almost immediately after the vacuums were drawn. It is probable that grinding traps air in the meat and exposed the pigment to oxidation at low oxygen tension upon drawing vacuum. In earlier experiments, beef slices rapidly turned purple instead of brown under the very same conditions. That pigment of ground meat is more labile to oxidation than that of meat slices is to be expected.

It was, however, also observed that the irradiated vacuum-packaged samples changed from the brown surface color to a purplish surface color during irradiation.

This change was very obvious when vacuum samples, immediately after receiving any one of the doses, were placed next to non-irradiated vacuum samples. This is not surprising in view of the fact that irradiating in vacuum tends to generate reducing conditions in meat whereas aerobic irradiation tends to result in oxidative conditions.

It was also noted that the higher doses, particularly 300 and 500 Krads, caused a darkening of the surfaces of the samples (bright red to dark red, which did not return to bright red during subsequent storage) packaged in polyethylene only.

Half of the samples at each dose that were irradiated in air were stored in a modified pressure canner which, after filling, was evacuated to a 28" vacuum gauge reading and then backfilled to 30 psia with pure oxygen.

Thus, the original pool of pouched ground beef samples was divided into two irradiation atmosphere variables (air and vacuum), seven dose variables (0 through 500 Krads), and three storage variables (air, oxygen pressure, and vacuum). All samples were stored at 38°F, and sets were periodically removed for evaluation.

Again, since this was intended to be principally a microbiological study, well mixed ground meat was used rather than slices in order to obtain a more uniform distribution of microorganisms throughout the total raw material. Grinding, however, tends to hasten discoloration and microbial spoilage. Observations are summarized in Table 9.

Discussion:

The initial plate count data indicate that, for a given dose, there is approximately the same degree of total count reduction when irradiating in air that there is when irradiating in vacuum. This is contrary to the widely held view that, for equivalent kill, as much as three times more irradiation is needed in the absence of oxygen as is needed in the presence of oxygen. The conditions which generally have given rise to the latter view (e.g., irradiating a single strain of E. coli suspended in phosphate buffer) are, of course, far removed from the irradiation pasteurization of meats. It is not unreasonable to assume that the radiation sensitive fresh meat bacteria (notably the gram negative) will be destroyed at 50 Krad or more regardless of the surrounding atmosphere. As a general rule, model-system type-experiments do not extrapolate well to food systems. As usual, oxygen pressure storage had a temporary protective effect against discoloration but fell short of providing what is needed. Also, a pronounced stale tallowy odor was observed at 12 days which seemed to be even more intense at 23 days when samples under oxygen were discarded. The oxidative odor was so strong that it completely masked any

indications of spoilage from the 0 Krad samples at 23 days. Further, it rendered all oxygen samples indistinguishable with respect to color, odor and appearance. Control samples with a total count of perhaps 10^9 /gram could not be distinguished from samples irradiated to 500 Krad which contained practically no bacteria because the oxidative odor was so overpowering. All oxygen stored samples had uniform grey brown color at 23 days.

At the outset, when it was felt that two weeks salable life would be sufficient for retail cuts, the hope was that oxidation would not pose a problem, at least during that brief period, if ionizing radiation plus high oxygen tension were to be used, although it was recognized that this combination constitutes an excellent oxidation promoting treatment, especially if applied to lipid containing food. Since then the estimate for required salable life extension has been lengthened by a week or more (5), and experimental evidence now strongly suggests that lipid oxidation alone will preclude the use of this combination. It is planned to confirm this presumption by means of direct analysis.

The pH data support the findings with lamb that, while fresh meat normally undergoes an increase in pH during aerobic spoilage, the pH decreases with spoilage in the case of vacuum packaged irradiated (and non-irradiated in this experiment) fresh meat. Also, it is again demonstrated that, in the dose range being considered for ultimate use (ca 50-150 Krad), vacuum packaged irradiated meat does eventually undergo a recognizable spoilage process. Presumably homofermentative and/or heterofermentative lactic acid producers are involved but this has yet to be proven.

There was some partial regeneration of redness when vacuum-packed samples which were plated at 28 days were exposed to the air at 23 days as evidenced by a shift from brownish purple to a dark red surface color. A very slight stale odor was noted after the samples had been in air for five days following 23 days in vacuum. This suggests that a very mild oxidation process may have taken place since the slight off-taint was similar to (but much, much less intense than) that of samples stored under oxygen pressure.

It is possible that reoxygenation of vacuum packaged irradiated samples may call for use of an antioxidant treatment during processing, but the need has yet to be demonstrated.

Table 9. Total plate count and pH data, and color, odor and appearance observations for ground beef, gamma irradiated, and stored in air, under oxygen and under vacuum at 38°F

Storage -						
Days	1			9		
Variable	Total count	pH	Observations	Total count	pH	Observations
A0	4×10^5	5.60	Bright red	4.2×10^8	6.20	Discolored, spoiled odor & slime
A50	5×10^3	5.72	Sl. dark red	2.6×10^7	5.51	Discolored, slight off-odor
A100	3×10^3	5.60	Sl. dark red	1.3×10^6	5.55	" "
A150	1×10^3	5.63	Purplish red	1×10^4	5.52	" "
A200	4×10^2	5.62	" "	$< 10^3$	5.63	" "
A300	$< 10^2$	5.65	" "	$< 10^3$	5.67	Discolored, moderate off-odor
A500	$< 10^2$	5.65	" "	$< 10^3$	5.68	" "

All A samples became severely desiccated during the third week of storage and were discarded.

Table 9 - Continued:

Storage -						
Days	1			9		
Variable	Total count	pH	Observations	Total count	pH	Observations
VO	4×10^5	5.60	Brown	4.5×10^6	5.70	Brown, good odor
V50	8×10^3	5.55	Purple, became red in air	4.5×10^5	5.72	Purple "
V100	3×10^3	5.80	" "	2×10^4	5.72	" "
V150	3×10^3	5.70	" "	3×10^3	5.68	Purple, trace off-odor
V200	5×10^2	5.68	" "	5×10^2	5.70	Purple, sl. off-odor
V300	$< 10^2$	5.62	" "	$< 10^2$	5.70	" "
V500	$< 10^2$	5.62	" "	$< 10^2$	5.69	" "
Days -	23			28*		
VO	4.2×10^8	5.44	Brown, sl. spoiled odor	3×10^8	5.22	Brown, spoiled odor
V50	1.7×10^8	5.60	Brown-purple sl. off-odor	1.2×10^8	5.60	Brown, sl. stale odor
V100	2.6×10^7	5.58	" "	4.6×10^7	5.58	Dark red, sl. stale odor
V150	1.4×10^7	5.63	" "	5.5×10^7	5.57	" "
V200	3.5×10^6	5.68	" "	1.8×10^7	5.62	" "
V300	$< 10^3$	5.64	" "	$< 10^3$	5.64	" "
V500	$< 10^3$	5.68	" "	$< 10^3$	5.64	" "
Days -	40**					
VO	-	-	-			
V50	3×10^8	4.90	Brown, spoiled odor			
V100	1.8×10^8	5.20	" "			
V150	7.8×10^7	5.40	Brown, trace spoiled odor			
V200	2.5×10^7	5.61	Brown-purple, sl. off-odor			
V300	$< 10^3$	5.63	" "			
V500	$< 10^3$	5.65	" "			

* 28 day V samples were opened to the air at 23 days and remained so for the five subsequent days.

** Held in vacuum until plated at 40 days.

Table 9 - Continued:

Storage -			
Days -	12		
Variable	Total count	pH	Observations
00	6.3×10^8	5.50	Brown-red, stale misty odor
050	7×10^7	5.52	" " "
0100	2.2×10^6	5.52	" " "
0150	1.6×10^4	5.57	" " "
0200	4.6×10^3	5.55	" " "
0300	$< 10^2$	5.58	" " "
0500	$< 10^2$	5.60	" " "

All 0 samples were severely discolored and oxidized at 23 days and were discarded.

The total count data suggest that from 200 to 300 Krad is a critical dose range. It seems that on the low side of this range a sufficient number of microorganisms survive to gradually build up the total count while at the high side of the range microbial outgrowth potential in the refrigerated samples seems, for all practical purposes, virtually eliminated. This observation is supported by results of irradiation of heavily inoculated agar plates which was done to evaluate the usefulness of this method as a source of information. In conjunction with this experiment, flora from 1) fresh beef, and from 2) a piece of beef which was allowed to undergo severe spoilage were enriched in tripticase soy broth. After a few days incubation the broths from both were 1) streaked on agar plates, 2) added to petri dishes and poured over with plate count agar, and 3) put into several 15 X 125 ml test tubes. The streaked and poured plates and the test tubes were divided into groups of three and were irradiated to the same total doses used for the meat in this experiment. A set of controls from each was not irradiated. The irradiated test tube suspensions were transferred to petri dishes and poured with plate count agar. After incubation it was clear from visual observation that, in each case, the number of surviving microorganisms growing out after irradiation decreased greatly but gradually on going from 50 to 200 Krad, and then dropped off sharply to few or no colonies per plate at 300 and 500 Krad.

From the total count plates seventeen representative colony types were picked, gram stained, and examined microscopically. The predominant type from non-irradiated samples held in air (representing present commercial handling) was a short gram negative rod which produced the familiar large smooth creamy pale spreading colonies on the agar surface. Only a very few of these were isolated from non-irradiated samples held under oxygen pressure or vacuum. These conditions apparently inhibit their outgrowth. Occasionally one or two were found on plates from A-50, A-100, V-50 and V-100 samples, but never from the higher doses. None was isolated from irradiated samples held under oxygen.

Others from A-0 samples were chiefly two types of gram positive rods and one gram positive coccoid type which also occurred in all irradiated samples held in air, but in decreasing numbers with increasing dose. The same three gram positives made up practically the total flora isolated from higher dose (150 Krad and up) vacuum samples.

Almost the only colony type in any numbers on irradiated oxygen stored samples were small white subsurface lens-shaped colonies consisting of long slender

gram positive rods. Occasionally a few very flat white surface colonies of gram positive cocci appeared on plates from non-irradiated and lower dose oxygen samples.

It is interesting to note that a single colony-type accounted for practically all of the colonies on all plates from oxygen stored samples whereas ground beef samples stored in air and under vacuum, and ground lamb stored under all three atmospheric conditions, each yielded a variety of bacterial types. These observations strongly suggest that, for beef at least, storage under superatmospheric pure oxygen has a selective bacteriostatic effect upon certain of the microflora.

This experiment again demonstrates that vacuum-packaged irradiated samples (in the projected dose range of ca 50-150 Krad) undergo readily detectable spoilage accompanied by a reduction in pH. This suggests that the spoilage process is very likely harmless, probably an innocuous lactic fermentation which could be delayed until after the desired salable life extension has been achieved.

In this connection it is perhaps worth quoting a section of the FAO-WHO publication "The Technical Basis for Legislation on Irradiated Food . . . Report of a Joint FAO/IAEA/WHO Expert Committee, Rome, 21-28 April, 1964" (Rome, 1965). It is stated on page 33 of this publication in regard to radiation pasteurized refrigerated foods: "Provided the treated food is stored at temperatures below 3°C, no known public health hazard will arise. However, there is one significant peculiarity that arises with food treated in this manner and which could be of particular importance in the event of failure to control the storage temperature as recommended. When spoilage does occur, evidence of the spoilage may be unusual, because the characteristic spoilage flora has been replaced by a more restricted one. Irradiated poultry, for example, does not develop a putrid odor even when it contains very large numbers of bacteria (19). New criteria of spoilage will have to be learned by experience with food treated with this type of process. Failure to control storage temperature as recommended could also provide the favorable environment essential for proliferation of mutant organisms if they should arise."

Elsewhere in the publication the "3°C" temperature maximum is discussed in the context of Type E C1. botulinum which "can form toxin during significant periods of storage (e.g., 4-6 weeks), at temperatures as low as 3.5°C". One would not expect to find this botulinum strain in meats and poultry; thus, the temperature requirement for products therefrom would in all probability not be quite as rigid. The comment on mutant organisms is elsewhere discussed in the context of the risk of "repeated exposure". Since radiation pasteurization of fresh meats and poultry

is to be strictly a single exposure process, radiation induced mutation would not be expected to be a matter of concern. For that matter, it is questionable that there is any mutation hazard at all in multiple exposure of foods to ionizing radiations.

What is highly pertinent to this work are the comments about the possibility of "unusual" types of spoilage and the need to consider new criteria of spoilage. If, as is suggested by the modest amount of microbiological work herein reported, it can be demonstrated unequivocally through future work that meats irradiated in the dose range of interest (ca 50-250 Krad) and held under refrigeration and under vacuum throughout irradiation handling and storage, and exposed to oxygen to restore normal red color prior to the point of sale, do in fact undergo a readily detectable spoilage process subsequent to a satisfactory salable life, then this would probably, in effect, demonstrate the safety of the process in the context of the preceding quotation while satisfying storage life requirements.

C. Drip control:

Water is by far the largest single component of fresh raw meat, and most of this water is only loosely bound or immobilized throughout the lean tissue. Exudation of fluid (water plus solubilized and suspended material) from meats and meat products is directly related to water "binding" or water holding capacity (WHC) within the lean tissue. This important property of meat influences color, flavor, tenderness and juiciness, and meat quality in general (10)(33)(34)(40). Water holding capacity is in turn directly and indirectly influenced by a number of factors. Improvement of WHC is possible by certain treatments of, and ways of handling of the live animal, and by a number of post-mortem treatment and handling possibilities. In this work we have limited our efforts to application of phosphates to the cut meat.

It is a well known fact that incorporation of phosphate compounds into meats and fish increases water holding capacity of the tissues (35)(36)(37)(39)(40)(50). It has also been asserted that pyro-, tripoly-, and hexameta-phosphates have a protective effect against rancidity of cooked meats, and that they reduce cooking losses (14).

Several postulates have been advanced to explain the nature of the action of polyphosphates with meat proteins. For instance, polyphosphates, due to their anionic nature, are believed to increase water holding capacity through a lowering of the isoelectric pH of muscle proteins, and that the reverse effect is caused by divalent cations. Grau et al. (41) and Mamm (42)(43)(44) advanced the theory that

sequestering action of polyphosphates on calcium, magnesium and zinc increases the WHC of raw meats. Swift and Berman (38) found positive correlations between water retention of beef muscle and its pH and zinc content. They also reported that water retention is inversely proportional to the calcium and magnesium content of meat. On the other hand, Bendall (35) and Sherman (37) are of the opinion that sequestering ability of calcium and magnesium does not account for the effectiveness of polyphosphate. Swift and Ellis (39) have further shown that small amounts of magnesium chloride slightly improved the water binding capacity of beef. Wierbicki *et al.* (45) demonstrated that calcium chloride and magnesium chloride increase the fluid retention of beef heated at 70°C. Mahon (36), however, suggested that sodium chloride is the key to the increased meat volume and that sodium chloride and tripolyphosphate exhibit a strong synergism when used within well defined limits.

In general, however, it is agreed that the tissue contractile proteins, actin and myosin in particular, are largely responsible for the water binding capacity of muscle meat, and that the increase of water binding is the result of the inter-actions between these and other fibrillar proteins with polyphosphates. The diversity of opinions on the mode of action of polyphosphates have been partially resolved by the finding of Yasui *et al.* (46, 47) in studies on the reactions of rabbit actomyosin with various phosphates. They showed that there are two types of phosphate bindings of polyphosphate to myosin. The first of these is a direct binding of highly polymerized polyphosphates such as hexametaphosphates, and the other type is the preferential cation binding followed by di- or tripolyphosphate binding. The second type of reaction is greatly enhanced by the presence of univalent and divalent ions, whereas the first type is inhibited under this condition. According to Yasui, improvement of water retention by ion binding is the result of the changes in the charge of protein induced by the ions. Under conditions in which ions were not bound, polyphosphates increased the ionic strength and solubilized the protein.

While differences in opinion as to the exact mechanism of polyphosphates action in meat still exist, there is no doubt of their beneficial effects on fluid retention. It is the object of this phase of research to determine the effectiveness of a variety of phosphate compounds in extending the shelf life of fresh meat in terms of drip control, and their possible influence on color retention, microbial outgrowth and flavor.

Experimental:

Work in this area principally involved studies on the following: 1) the type of phosphate, solution strength and dipping time for satisfactory control of drip; 2) phosphate uptake and distribution in fresh meat slices; 3) use of an injection method as a means of applying phosphate solution to thicker meat cuts; and 4) physical characteristics of phosphate treated meat.

Efforts in these areas during the first contract year are described and discussed on the following pages.

Materials and Methods:

Meat: Fresh commercial grade beef cuts were used in all experiments. Samples were freed from external fat and sliced to the desired thickness.

Phosphates: The following phosphates were used: a) sodium tripolyphosphate. b) sodium hexametaphosphate (glass, 64% P_2O_5); c) sodium hexametaphosphate (67% P_2O_5); d) "Kena" FP-28 (Calgon); e) sodium acid pyrophosphate; f) trisodium orthophosphate; and g) disodium orthophosphate.

All phosphate solutions used were prepared on percentage weight basis.

Drip measurements: During the first half of the year, observations on drip loss were by visually noting the volume of drip and/or observations on the absence or presence of drip. In the latter half of the year a more quantitative and reproducible method of measurement of drip based on the method of Howard (48) was employed.

In the work employing thin slices, the slices were 5 X 5 X 1 cm, the shortest dimension being in the direction of the muscle fiber. They were dipped in phosphate solution, drained and placed in aluminum dishes approximately 8 cm in diameter and 5 cm deep. In each aluminum dish was placed a glass grid which served to keep the samples out of contact with the exuded fluid. Sample and container were weighed immediately, and sealed with Scotch tape to prevent moisture loss through vaporization. Samples were then stored in the refrigerator at 38°F. Periodic determinations of drip loss were made by measuring loss in weight of samples. This was done by transferring the sample to a tared container and weighing. The loss in weight was calculated as percent drip loss.

For larger sized samples an alternative method was used. Samples were packed in moisture impermeable Saran and stored in the refrigerator. Loss of weight was determined at the end of storage by draining the samples on a wire screen. Loss of weight was calculated as drip loss, and is based on the weight of the samples after dipping and draining.

Phosphate absorption determination: Phosphate absorbed by fresh meat was determined, after dipping and draining, as total phosphorus. Meat samples, after blending to obtain a uniform mixture, were analyzed for phosphorus according to the AOAC colorimetric method (49). The percentage phosphate absorbed is based on the weight of the samples after dipping and draining.

An injection method of phosphate application using beef knuckle of the round was studied in this work as an alternative to application by dipping.

Aqueous solutions containing 16% by weight of phosphate compounds were introduced into the whole knuckles, using a hand pump injector. Phosphate solutions were added to about 3% of the weight of the knuckles. After draining for several minutes, the knuckles were stored at 38°F for 24 hours, after which the knuckles were separated into their component muscles which were then sliced, weighed, packaged, and stored. Drip losses were determined after three weeks of storage. Samples for total phosphorus analysis were taken at different locations of each muscle in order to determine the phosphate uptake throughout the muscle.

Samples to which analytical methods were applied during the latter half of the year often were not irradiated, and thus were usually quite spoiled at the end of a three week storage period. This did not, however, influence measurements since pH and phosphorus analysis were done before and immediately after phosphate treatment, and prior to placement into storage. Drip was allowed to accumulate under conditions which prevented its disappearance via vaporization or re-absorption. Irradiation is now being included in this phase of the total program.

Results and Discussion - Experiment 10:

This was a preliminary probe which compared the drip control effectiveness of different concentrations of tripolyphosphate and three other phosphates on slices from a beef top round. Dipping for thirty seconds was done in the following solutions:

2.5%	Sodium tripolyphosphate	pH = 9.5
5.0%	"	9.5
7.5%	"	9.5
10.0%	"	9.5
7.5%	hexametaphosphate	6.2
7.5%	Trisodium orthophosphate	12
7.5%	Sodium acid pyrophosphate	3.5
100 %	Distilled water (no phosphate)	

Samples were drained on a wire screen for a few minutes, and then sealed in polyethylene pouches. Observations were recorded at five days and at 14 days. Radiation was not employed. Results are summarized in Table 10.

Table 10. Effect of various phosphates on amount of drip

Phosphate	Concentration Weight %	Drip* (ml)	
		5 days storage	14 days storage
Sod. tripolyphosphate	2.5	< 0.5	3
" "	5.0	< 0.5	3
" "	7.5	0	< 0.5
" "	10.0	0	< 0.5
Sod. hexametaphosphate	7.5	1	2
" orthophosphate	7.5	< 0.5	3
" acid pyrophosphate	7.5	1	3
Water only	- -	6	4

* Average of three samples.

Conclusion:

Of the phosphates tested, sodium tripolyphosphate was best for drip control. Its solubility also was excellent. It was noted that lower pH's caused discolorations. A dipping solution of at least 7.5% TPP appears advisable.

Experiment 11:

This was also a preliminary probe which was intended to determine the effect of pH of phosphate solution on meat color and on the amount of drip. Slices from a beef top round were dipped in 10% (by weight) solutions of sodium acid pyrophosphate (APP) and/or sodium tripolyphosphate (TPP). All of the dipping solutions (Table 11) except the plain water control, were 10% total phosphate. APP and TPP each ranged from 0 to 100% of the total 10% so as to achieve a range of pH in the series of solutions while holding total phosphate constant. In each solution a set of slices was dipped for 30 seconds. They were then drained for a few minutes and individually sealed in polyethylene pouches. Samples were observed frequently and were evaluated and scored after five and nine days of storage. The volume of drip was measured, and the color was graded by visual estimate. Radiation was not employed. Results are summarized in Table 11.

Color was visually graded by three investigators as percentages of the total sample surfaces that were red, purple, and brown. Sample pH was taken on a blended homogenate of 100 grams of sample plus 300 grams distilled water.

Table 11. Effect of pH of phosphate solution* on amount of drip and on color

Percent of total phosphates		Solution pH	Color of exposed surfaces			pH of blended	
% acid pyro	% TPP		% Red	% Purple	% Brown	Approx. ml drip	meat after 5 days @ 38°F**
100	0	4.0	20	5	75	6	5.5
			40	10	50	8	
			25	5	70	10	
80	20	5.1	20	-	80	8	5.5
			30	10	60	10	
			15	5	80	10	
75	25	5.4	15	5	80	9	5.6
			25	15	60	4	
			25	5	70	3	
66 2/3	33 1/3	5.5	15	5	80	5	5.6
			20	10	70	4	
			15	10	75	2	
50	50	6.2	20	40	40	0	5.6
			40	10	50	1	
			20	65	15	4	
33 1/3	66 2/3	6.7	30	20	50	2	5.7
			20	30	50	2	
			10	30	60	3	
25	75	7.2	30	40	30	< 1	5.8
			30	40	30	< 1	
			15	40	45	0	
20	80	7.4	40	20	40	5	5.7
			40	30	30	4	
			30	30	40	< 1	
0	100	10.0	35	30	35	0	5.7
			35	30	35	1	
			20	40	40	< 1	
0	0	(Not dipped)	40	-	60	5	5.5
			30	-	70	3	

* 10% total phosphate.

** Nine day observations were little different from those given for five days.

Conclusions:

Higher pH solutions (6.2 and above) caused smaller amount of drip and resulted in better colors (i.e., less oxidation) than did the lower pH solutions. In spite of the fact that dipping resulted in increased water content (net weight gain) samples dipped in high TPP solutions exhibited greater water holding capacity than did non-dipped samples.

Experiment 12:

The effect of type and amount of phosphate compound on drip loss from fresh beef stored at 38°F was also studied in this experiment.

Fresh beef slices (5 X 5 X 1 cm) were dipped in various concentrations of different phosphate compounds for 30 seconds and drained for several minutes on wire screens. In this study provision to prevent reabsorption of exuded fluid during storage was employed using the method of Howard (48).

Table 12 shows the amount of drip loss that occurred during storage at 38°F. Drip formation is significantly retarded only when the concentration of the dipping solutions exceeded 5.0%. In all cases phosphate treated samples showed less drip than the untreated control samples in spite of their moisture contents having been increased as a result of dipping.

Fig. 1 shows the amount of drip formed during storage of fresh beef samples treated with 10% solutions of the various phosphate compounds. As shown, drip formation is retarded when samples were dipped in 10% solution of sodium tripolyphosphate. A more or less comparable effect is observed in 10% sodium hexametaphosphate (glass, 64% P_2O_5). Disodium orthophosphate has the least effect on retarding drip formation, followed by trisodium orthophosphate, sodium acid pyrophosphate, Kena FP-28 and sodium hexametaphosphate in that order.

Fig. 2 shows the relationship between the amount of drip that is formed during storage at 38°F and the concentration of sodium tripolyphosphate. It is shown that drip formation is effectively prevented to a greater degree when samples are dipped in 7.5% and 10.0% sodium tripolyphosphate solutions than when they are dipped in less concentrated solutions.

Table 12. Drip loss during storage at 38°F of fresh beef pre-treated with various concentrations of phosphates by dipping

Phosphate compound	Conc. percent by wt.	Percent drip loss*		
		1st week	2nd week	3rd week
Control	- -	2.53	4.50	5.40
Sodium tripolyphosphate	2.5	1.20	3.20	4.13
	5.0	0.90	1.53	2.20
	7.5	0.28	0.57	0.85
	10.0	0.09	0.10	0.41
Sodium hexameta-phosphate (glass, 64% P_2O_5)	2.5	1.26	3.48	4.05
	5.0	0.98	1.86	2.35
	7.5	0.92	0.48	0.65
	10.0	0.18	0.25	0.45
Sodium hexameta-phosphate	2.5	1.34	4.54	5.41
	5.0	1.33	2.00	2.76
	7.5	0.35	0.78	1.20
	10.0	0.22	0.58	0.95
Kena FP-28	2.5	2.63	3.75	4.48
	5.0	1.78	2.25	3.28
	7.5	1.20	2.00	2.73
	10.0	0.90	1.28	1.73
Sodium acid pyrophosphate	2.5	2.22	3.10	3.83
	5.0	1.82	2.45	3.12
	7.5	1.33	1.88	2.52
	10.0	0.88	1.33	2.30
Trisodium ortho-phosphate	2.5	2.30	3.22	4.07
	5.0	2.47	3.05	3.97
	7.5	1.72	2.12	2.95
	10.0	1.31	1.88	2.15
Disodium ortho-phosphate	2.5	2.42	3.94	5.14
	5.0	2.13	3.59	4.83
	7.5	1.91	3.07	4.30
	10.0	1.92	2.68	3.69

* Average of three replicates (% by weight).

Fig. 1. Drip loss during storage at 38°F of fresh beef slices dipped in 10% solution (% by wt) of various phosphate compounds for 30 seconds.

Fig. 2. Drip loss during storage at 38°F of fresh beef slices dipped at various concentrations of sodium tripolyphosphate for 30 seconds.

Experiment 13:

In this experiment phosphate absorption by fresh beef from 10% solutions of sodium tripolyphosphate, hexametaphosphate glass (64% P_2O_5), and sodium hexametaphosphate was studied.

The amount of phosphate solution absorbed by fresh beef samples was determined by weighing the meat samples before and after phosphate treatment. Dipping 5 X 5 X 1 cm fresh beef samples for 30 seconds in 10% solutions of the three phosphate compounds resulted in 0.5-2.0% gain in weight due to solution absorbed and held.

Total phosphorus content of both treated and untreated fresh beef samples was obtained by analysis to determine the amount of phosphate absorbed. Data are summarized and illustrated in Table 13 and Figure 3.

Of the three phosphates, results show that sodium tripolyphosphate is more readily absorbed and held by the meat. Sodium hexametaphosphate (glass) shows an almost linear relation between the amount absorbed and the concentration of the dipping solution.

Table 13. Phosphate absorbed by fresh beef dipped at various concentrations of sodium tripolyphosphate, sodium hexametaphosphate (67% P_2O_5) and sodium hexametaphosphate (glass, 64% P_2O_5) for 30 seconds

Phosphate compound	Conc. wt %	* Phosphate absorbed %
Sodium tripolyphosphate	2.5	0.20
	5.0	0.23
	7.5	0.31
	10.0	0.46

Sodium hexameta- phosphate (glass, 64% P_2O_5)	2.5	0.08
	5.0	0.17
	7.5	0.26
	10.0	0.39

Sodium hexameta- phosphate	2.5	0.09
	5.0	0.15
	7.5	0.22
	10.0	0.47

* Average of three replicates (% weight basis).

Fig. 3. Phosphate absorbed by fresh beef slices dipped in various concentration of sodium tripolyphosphate, hexametaphosphate (glass) and sodium hexametaphosphate (67% P_2O_5) for 30 seconds.

Experiment 14:

This experiment was concerned with the effect of dipping time on drip, phosphate uptake, and the pH of fresh meat. The optimum dipping time needed to effectively retard drip formation using 10% solution of sodium tripolyphosphate was studied.

Results of this experiment are shown in Table 14 and Figure 4. The data show that there is a rapid uptake of phosphate from 10 to 60 seconds dipping time during which the relationship between time and uptake is almost linear. At two minutes, phosphate uptake was about the same as for one minute dipping time. There is a considerable retardation of drip formation when samples are dipped for from 30 seconds to two minutes.

pH changes of the meat samples were determined immediately after dipping and draining. Results show that the increase in pH due to dipping is quite marked with the relatively small samples.

Dipping fresh meat samples for from 30 seconds to one minute resulted in a significant retardation of drip through three weeks of storage at 38°F. The amount of sodium tripolyphosphate absorbed ranged from 0.44 to 0.53% by weight.

Table 14. Phosphate content of fresh beef dipped for various times in 10% sodium tripolyphosphate, and drip loss measured after three weeks of storage at 38°F

Time of dipping	% * Phosphate sodium tripoly- absorbed	% * Drip loss	pH * of samples
Control (not dipped)	- -	5.19	5.65
10 seconds	0.32	4.32	5.85
30 "	0.44	1.06	5.85
1 min	0.53	0.13	6.05
2 min	0.57	0.12	5.95

* Average of four replicates.

Fig. 4. Phosphate uptake by fresh beef slices dipped for various time intervals in 10% sodium tripolyphosphate (% by wt.) and drip loss after three weeks of storage at 38 F.

Experiment 15:

Phosphate pretreatment of larger meat samples. The objective of this study was to determine the effectiveness of phosphate (by dipping) on larger meat slices.

Approximately 0.3 to 0.5 lb meat slices of 1-2 cm thickness from beef rib eye were dipped in 10% solution of sodium tripolyphosphate and sodium hexametaphosphate (glass) for from 30 seconds to one minute. After draining for 20 minutes, the slices were packaged in oxygen impermeable Saran and were stored for three weeks at 38°F. Drip loss and phosphate uptake were determined.

Results are shown in Table 15. Drip loss was largely prevented during three weeks of storage by sodium tripolyphosphate and hexametaphosphate (glass) pretreatment. The glass type sodium hexametaphosphate is as effective as sodium tripolyphosphate in retarding drip formation. Solution uptake determined after dipping and draining ranged from 0.5-2.0% by weight, and the amount of phosphate compound absorbed (as phosphorus) ranged from 0.36-0.52%. A significant increase in pH after dipping of the meat was observed. This was 0.30 pH units for TPP, and 0.36 pH units for the hexametaphosphate glass.

Table 15. Solution and phosphate uptake, pH, and drip loss of fresh meat dipped in 10% solutions of sodium tripolyphosphate and hexametaphosphate (glass) for 30 seconds

Dipping solutions	Samples	Wt. of samples before dipping (gms)	Wt. of samples after dip & draining (gms)	% soln. uptake		pH Av.	% drip loss (after 3 wks)		% phosphate uptake	
				Samples	Av.		Samples	Av.	Samples	Av.
Control (not dipped)	1	201.0	- -	- -	- -		6.01		- -	
	2	188.0	- -	- -	- -		5.89		- -	
	3	162.0	- -	- -	- -	5.65	5.50	5.79	- -	
	4	152.0	- -	- -	- -		5.46		- -	
	5	175.5	- -	- -	- -		5.65		- -	
	6	191.5	- -	- -	- -		6.25		- -	
10% sodium tripolyphosphate	1	154.0	156.0	1.30			0.92		0.44	
	2	152.0	154.0	1.31			0.85		0.43	
	3	185.0	188.0	1.62			1.02		0.50	0.47
	4	197.0	198.0	0.51	1.10	5.95	1.13	0.88	0.48	
	5	159.0	161.0	1.26			0.75		0.52	
	6	163.0	164.0	0.61			0.60		0.47	
10% sodium hexameta-phosphate	1	197.0	198.5	0.76			0.95		0.39	
	2	163.0	164.5	0.92			1.25		0.42	
	3	160.5	162.0	0.93			1.16		0.38	
	4	149.0	150.0	0.67	0.85	6.01	1.08	1.05	0.52	0.41
	5	152.0	153.0	0.66			0.85		0.36	
	6	175.0	177.0	1.14			0.98		0.38	

Experiment 16:

The injection method as a means of applying phosphate to thick cuts of meat was evaluated.

Pressure injection of phosphate solution with the use of a hand pump injector was performed on fresh beef knuckle of the round. A 16% (by wt) aqueous solution of sodium tripolyphosphate, and one of sodium hexametaphosphate (glass) were introduced via an injection needle (a slender hollow needle having several holes or ports along its length) to about 3% of the weight of the knuckle cuts. After a 24 hour holding time the knuckle cuts were separated into their component muscles. Each muscle was then evenly sliced, and the uniform slices were packaged and stored for three weeks at 38°F. Drip losses were determined after three weeks. Phosphate uptake of each individual muscle was determined after draining and holding for 24 hours post-treatment.

As shown in Table 16, there is a considerable variation in drip loss between slices within a muscle, and between muscles as shown by phosphate distribution analysis. It was further observed that some slices had "alkaline spots" most likely due to uneven distribution of phosphate solution throughout the bulk of the knuckle.

Average drip loss for the untreated knuckle was over 7% of the total weight. In spite of a weight gain of over 3% due to pumping of treated knuckles, much less percentage drip loss was observed with them as compared to the control which, of course, did not receive any added moisture. Muscle M_6 (Table 16) from the knuckle pumped with 16% TPP showed an average drip loss comparable to that from untreated muscles; however, it also showed a relatively low average phosphate uptake. Muscle M_8 showed a relatively low average phosphate uptake and drip loss. Muscle M_9 showed the suggestive combination of highest phosphate uptake and lowest drip loss. In general, there appeared to be more variability between muscles than between the two phosphates used. Nevertheless, it is clearly indicated for pumping, as it was for dipping, that, in spite of the added moisture resulting from the pumping treatment, the treated meats demonstrated considerably more water holding capacity than did the untreated meat. It is very likely that, should a method of phosphate introduction be used which does not involve the concurrent addition of moisture to the meat as was the case here, phosphate improvement of water holding capacity would be even more pronounced.

Table 16. Drip loss and phosphate uptake of meat slices from individual muscles of beef round knuckles treated with phosphate by a pressure injection method

Phosphate solution	Wt. of knuckle		% of solution added	muscle	Slices	% drip loss*	% phosphate uptake
	cut before injection (gms)	after injection & drain. (gms)					
				M ₁	1	8.22	- -
					2	7.68	- -
					3	8.59	- -
					Ave.	8.16	
Control (not treated)							
	3391.4			M ₂	1	7.18	- -
					2	7.28	- -
					3	7.12	- -
					Ave.	7.19	
				M ₃	1	6.19	- -
					2	6.50	- -
					3	7.00	- -
					Ave.	6.56	

					1	1.01	0.37
					2	1.44	0.61
16% sodium tripolyphosphate				M ₄	3	0.94	0.40
			3.79		4	0.75	0.39
	4785.2	4966.8			5	0.81	0.35
					Ave.	0.99	0.42
					1	3.15	0.38
					2	3.58	0.25
				M ₅	3	0.06	0.47
					4	2.02	0.43
					5	0.60	0.62
					Ave.	1.88	0.43
					1	4.46	0.34
					2	9.80	0.33
				M ₆	3	5.20	0.13
					4	5.99	0.07
					5	0.26	0.11
					Ave.	6.94	0.20

Table 16 - continued:

16% sodium hexameta- phosphate (glass)	4122.8	4285.5	3.30	M_7	1	1.11	0.34
					2	0.64	0.49
					3	0.81	0.52
					4	5.72	0.34
					5	6.55	0.34
					Ave.	2.97	0.41
				M_8	1	1.59	0.26
					2	1.08	0.15
					3	1.11	0.17
					4	2.99	0.18
					5	3.50	0.12
					Ave.	2.05	0.18
				M_9	1	0.15	1.11
					2	0.34	0.88
					3	0.70	0.74
					4	0.96	0.21
					5	1.49	0.40
					Ave.	0.73	0.67

* After three weeks storage at 38°F, M_1 through M_9 were individual muscles from pumped fresh beef round knuckles.

D. Color and pigment measurement:

The importance to centralized cutting of providing normal red meat color at the point of retail sale cannot be overstated. The fact that red meat color is an aesthetic rather than a nutritional or eating quality attribute does not diminish its supreme importance in the eyes of the buying public. In addition to imparting an attractive appearance, good color is generally looked upon as an index of freshness of red meats. Thus, delaying microbial spoilage and controlling drip, both of which at this point seem to be rather straightforward, are to no avail without a means of assuring typical red meat color in the retail display case. It follows then that a critical part of the work under this contract involves the handling of the red meat pigment in such a way as to have it in the desired bright red (oxygenated) state after two or more weeks of processing and distribution. Put another way, the aim is to provide retail cuts having essentially the same "eye appeal" and attractive appearance as freshly cut meat in spite of being two to three weeks old. The essence of attractive appearance of red meats is color. Perhaps the greatest challenge in the technical phase of the overall effort is in achieving success with color.

This section concerns itself with methods of evaluating red meat color and the changes that it undergoes as a result of treatment, handling and storage. Color can be evaluated subjectively and/or objectively. It is usually desirable to employ both approaches and attempt to achieve a correlation between the two. During approximately the first two-thirds of the contract year, the subjective approach to color evaluation was used exclusively. Specifically, this involved visual observation of the color of meat samples during concurrent evaluation for spoilage and drip loss. Much of this earlier work, including judgments of color, is covered under VII B- Microbiological. During the latter part of the contract year, a reflectance spectrophotometric method for the objective measurement of pigment was phased into the program. The reflectance spectrophotometric method is desirable for objective measurement of pigment (and therefore, indirectly, of color) because it is a rapid non-destructive technique for measuring the relative concentrations of oxy-, reduced, and met-myoglobin at the meat surface. Thus one can correlate reflectance data with subjective color judgments of sensory panels such that the two approaches will supplement each other in the monitoring of color and the chemical changes that may take place, and in the reporting of results therefrom.

Considerable effort has been directed toward developing and adapting the reflectance technique to the specific needs of the total project. Part of this was the development of standard curves from known model type systems. It has also been necessary to evaluate the factors that affect reflectance measurements under the conditions which the technique is to be used in this work. Adaptation of the technique is reasonably far enough along so that it is now being used to establish a quantitative relationship between pigment form and factors which may influence the pigment, and thus color.

The basis for the reflectance method is the fact that isobestic points exist for the three forms of the major red meat muscle pigment. These are wavelengths at which the reflectance spectral curves of oxymyoglobin reduced myoglobin and oxidized (met) myoglobin intersect as shown in Figure 5. The wavelength 525 m μ is isobestic for all three of the pigment forms and may be used as a reference for total pigment content. That is, a measurement at 525 m μ will give a reflectance value related to the total pigment present; MbO₂ + Mb + Mb⁺. The two reduced (ferro) forms of the pigment, Mb and MbO₂, are isobestic at 571 m μ , therefore the ratio of reflectance at 571 m μ and 525 m μ will be a measure of the relative percentage of oxidized and total pigment. Similarly, comparison of reflectance at 474 m μ and 525 m μ will give the relative proportion of myoglobin (Mb) to the isobestic MbO₂ and Mb⁺. Other wavelengths could be employed, however, the above three are the ones commonly used by meat color investigators.

The measurement of reflectance, or reflectivity (R_{∞}) when an infinitely thick sample is used is usually done with a spectrophotometer and diffuse reflectance attachment with the percentage transmittance scale used for readout. The reflectance value obtained is converted to the ratio K/S where K is the absorption coefficient and S is the scattering coefficient. These values account for the fact that a fraction of the light is scattered by the opaque meat surface, and that the proportion of light absorbed by the pigment to that scattered by the sample decreases with increasing reflectivity. The total light reflected, R_{∞} , varies with K/S according to the Kubelka-Munk equation: $K/S = \frac{(1-R_{\infty})^2}{2-R_{\infty}}$. The wavelengths used for pigment determinations must give a linear response when relating K/S ratios to the proportions of the three pigment forms. Evidence supporting the linear relationship has been obtained using a model system consisting of 5% non-fat dried milk in water to which is added known amounts of a pigment. Linearity is, of course, assumed in the meat system.

Fig. 5. Reflectance spectra of fresh beef samples treated to contain predominantly Mb, MbO₂, and Mb⁺ respectively.

Ratios of reflectance, hence K/S at 571 m μ to K/S at 525 m μ , and K/S at 474 m μ to K/S at 525 m μ are determined for meat samples, the pigment of which is in the all oxygenated (bright red), all reduced (purplish) and all oxidized (brown) state. In this way 0% and 100% values are obtained for standard curves.

The ratio $\frac{K/S \text{ @ } 571 \text{ m}\mu}{K/S \text{ @ } 525 \text{ m}\mu}$ is used to determine relative percent oxidized (Mb⁺) myoglobin. If all of the pigment is in the oxidized state, this ratio will give a value for 100% Mb⁺ (Mb + MbO₂ = 0). If all of the pigment is in the reduced form this ratio will give a value for 0% Mb⁺, (Mb + MbO₂ = 100%). Thus the two ratio values can be used as the 0% and 100% values on a graph of $\frac{K/S \text{ @ } 571 \text{ m}\mu}{K/S \text{ @ } 525 \text{ m}\mu}$ vs percent Mb⁺.

Similarly, values of $\frac{K/S \text{ @ } 474 \text{ m}\mu}{K/S \text{ @ } 525 \text{ m}\mu}$ can be used for the determination of percent myoglobin (Mb) present, all oxidized pigment would give the ratio value for 0% Mb, and all reduced pigment would give the value for 100% Mb. The percentage oxymyoglobin (MbO₂) is determined by difference: % MbO₂ = 100% - (% Mb + % Mb⁺).

In this manner the relative percentages of the three pigment forms can be determined for a sample of red meat, and can be correlated with visual evaluation of the same sample.

Materials and Methods:

Reflectance values are being taken on the Beckman D.U. spectrophotometer with reflectance attachment. The samples are contained in plastic ice cube holders of dimensions great enough to cover the light port opening of the reflectance attachment and having a depth of 3/4". A freshly scraped block of magnesium oxide is used as the reference standard. Readings are determined as absorbance, converted to reflectance (transmittance scale), and then converted to K/S values by using Table D of Judd and Wyszecki (51). Meat samples have mainly been taken from the "eye" (semitendinosus) muscle of commercial grade beef bottom round since samples from this cut tend to be very lean and uniform in color.

To develop standard curves, measurements were taken with meat samples in the 100% reduced (Mb), 100% oxygenated (MbO₂) and 100% oxidized (Mb⁺) form. The "reduced" (Mb) readings were attained by either measuring immediately after cutting or measuring after sodium hydrosulfite treatment or after drawing a vacuum around an oxygenated sample and holding it long enough to allow a shift from MbO₂ to Mb. The "oxygenated" (MbO₂) readings were attained by exposing the freshly cut sample to pure oxygen for a sufficient period and then immediately taking a

measurement. The oxidized (Mb^+) readings were attained either after potassium ferricyanide treatment or after causing the pigment to oxidize naturally.

Average values for K/S ratios have been established from experimental data and are given in Table 17.

There are a number of factors that can influence reflectance measurement, some of which are controllable and others which will tend to limit the use of the method.

The method assumes an infinitely thick sample; one that is thick enough to change the "backing" from white to black without affecting the reflectance value. This thickness is about 1 cm for meat samples and therefore is of no practical concern with regard to measurements made in this work.

When monitoring color change and effects of treatments on color, the measurements should be at the same location on the same sample. Simply rotating the sample can introduce a variability. Since the reflectance method is non-destructive, this criteria can usually be met without difficulty.

It is desirable that the sample surface from which the measurement is taken be flat and smooth. To obtain this a glass slide or cover slip is placed on top of the meat surface.

If a sample is packed too tightly into a holder, this may condense the surface and may, in effect, increase pigment concentration which could result in misleading readings.

"Marbling" and patches of fat or gristle (i.e., non-pigmented material) can give rise to spurious results. What is desired is to reflect light off of pigmented muscle tissue only; therefore, a desirable sample is very lean and free of fat and connective tissue. Commercial grade beef "eye of the round" satisfies this requirement quite well.

Another source of variability is loss of moisture from samples between readings. As the surface of red meats become dry, darkening also takes place. Moisture impermeable packaging is very important if samples are to be held for a period of time between readings during which surface desiccation could occur to an extent sufficient to cause darkening.

Upon removal of a sample from storage, readings should be made as quickly as possible. This is especially true of vacuum packaged samples which begin to oxygenate as soon as they are exposed to the atmosphere. Since the spectrophotometer is stationary, samples are packaged such that they may be brought to the

Table 17. Average K/S values for the three forms of myoglobin

Samples containing myoglobin as:	Average K/S value for:	
	571 $m\mu$ 525 $m\mu$	474 $m\mu$ 575 $m\mu$
100% Mb	1.45	0.5
100% MbO ₂	1.45	1.0
100% Mb ⁺	0.55	1.0

Fig. 6. Curve relating the ratio of K/S @ 571 mμ to K/S @ 525 mμ vs. the percentage of oxidized pigment, metmyoglobin.

Fig. 7. Curve relating the ratio of K/S @ 474 mμ to K/S @ 525 mμ vs. the percentage of reduced pigment, myoglobin.

instrument before being removed from their storage atmosphere.

Tests conducted thus far have employed both gamma and electron irradiation. Samples have been stored in air, under vacuum, and under two atmospheres of oxygen using techniques described elsewhere in the report. In all cases samples have been held at 38°F. Visual observations by one or two technologists were recorded at the same times that reflectance measurements were made. (Future plans include attempts at correlation with sensory panel color scores). Since the same set of samples is used throughout a storage study, it is advisable not to remove samples from storage any more often than necessary. In most cases, two or three sets of readings appear sufficient over a three week storage period.

Preliminary measurements taken for purposes of developing methodology and calibration are not included in the report. Otherwise, a modest amount of reportable data has been compiled thus far, and is covered in the following pages.

Results and Discussion:

Experiment 17:

Cubes were cut from the eye muscle of a commercial grade beef bottom round and were placed in individual sample holders. The samples were immediately gamma irradiated to 100 Krad in air at a rate of about 190 Krad per hour. Following irradiation the samples were randomly divided into two lots; one lot was stored under vacuum at 38°F and the other lot was stored in two atmospheres of pure oxygen at 38°F. Samples were wrapped in PVC (Resinite) film during storage. Reflectance measurements and visual color observations were made after 14 days of storage, and again after 23 days of storage. Vacuum packaged samples were exposed to the air for 24 hours prior to taking the 23 day measurements. Data and observations are summarized in Table 18.

The reflectance data agree reasonably well with visual observations. Reflectance measurements on samples held in oxygen for 14 days and 23 days indicate higher percent MbO_2 than is suggested by visual observation. Color of vacuum packaged samples changed from purplish to dull red upon exposure to the atmosphere at 22 days; however, extensive pigment oxidation took place during the subsequent 24 hour period of holding in air prior to taking measurements. In general, both oxygen and vacuum had some beneficial effect on color, but neither was fully satisfactory. Vacuum yielded better results than oxygen, since the color of the vacuum packaged samples was fairly good for a brief period following three weeks of storage.

Table 18. Reflectance measurements and visual observations on 100 Krad irradiated beef stored under vacuum and in oxygen at 38°F

	Pigment form	14 days storage (%)	23 days storage (%)	Visual observations
V	Reduced Mb		24	Rapidly became purple after drawing vacuum, and remained purple during storage. Became red upon exposure to air at 22 days. Brown color was predominant at the time measurements were taken.
A	Oxygenated		25	
C	MbO ₂			
U	Oxidized		51	
U	Mb ⁺			
M				
<hr/>				
O	Mb	0	0	Initially were bright red. Appeared half red and half brown at 14 days, and completely brown at 23 days.
X	MbO ₂	75	20	
Y	Mb ⁺	25	80	
G				
E				
N				

Experiment 18:

This was essentially a repeat of Experiment 17. Samples held in two atmospheres of oxygen were examined after 14, 21, 22 and 23 days of storage. Samples stored under vacuum were examined; a) after 21 days in vacuum followed by one day in the atmosphere, b) after 21 days in vacuum followed by one day in two atmospheres of oxygen, and c) after 21 days in vacuum followed by two days in two atmospheres of oxygen. Data and observations are summarized in Table 19. As in the previous experiment, results indicate that vacuum followed by reoxygenation yielded better color after three weeks of storage than did storage in oxygen. In this instance, slightly better color regeneration results were obtained with oxygen pressure than with air at atmospheric pressure although neither method provided as good color results as has been shown to be possible by other studies. Reflectance data in Table 19 indicates that color is better than visual observations might suggest. Apparently a fairly high percentage of MbO_2 , as indicated by reflectance measurements, does not necessarily correlate with bright red color (visual) if Mb and Mb^+ are intermixed with the MbO_2 .

Table 19. Reflectance measurements and visual observations on 50 Krad irradiated beef stored under vacuum and in oxygen at 38°F

	Pigment form	14 days storage	21 days storage	22 days *		23 days **	Visual observations
				air	oxygen		
V	Reduced	--	--	10%	10	13	Samples in air for one day after 21 days in vacuum appeared dull red. Samples in oxygen for one day after 21 days in vacuum appeared part red and part brownish purple; after two days in oxygen, at 23 days, they appeared to be a mixture of light and dark red.
A	Oxygenated						
C	MbO ₂	--	--	66	68	76	
U	Oxidized						
U	Mb+	--	--	24	22	11	
M							

O	Mb	11%	14	0		0	At 14 days, samples stored under oxygen appeared dull red. At 21 days they appeared red-brown, and 22 and 23 days brown was the dominant color.
X	MbO ₂	59	42	31		37	
Y	Mb+	30	44	69		63	
G							
E							
N							

* One set of vacuum packaged samples was held in air and a second set was held in two atmospheres of oxygen (held for 24 hours prior to evaluation at 22 days).

** Vacuum packaged samples evaluated at 23 days were held in two atmospheres oxygen for two days following 21 days under vacuum.

Experiment 19:

This study was similar to Experiments 17 and 18, except that a set of samples was stored in air at atmospheric pressure as well as in oxygen at two atmospheres, and under vacuum. Cubes were cut from the "eye" muscle of a fresh commercial grade beef bottom round. The cubes were placed in holders and, after packaging as outlined above, were gamma irradiated to a dose of 50 Krad (190 Krad per hour). The samples were then held at 38°F to await evaluation at 14, 21, 23 and 24 days. Vacuum packaged samples were measured: a) after 21 days under vacuum, b) after 21 days under vacuum followed by two days in two atmospheres of oxygen, and c) after 21 days under vacuum followed by three days in two atmospheres of oxygen. Reflectance data and visual observations are tabulated in Table 20. Again, there seemed to be reasonably good correlation between reflectance data and visual observations, and use of the two approaches to color evaluation in tandem appears to have merit.

In this instance oxygen pressure storage yielded exceptionally good results colorwise through three weeks. As a rule, storage in oxygen has maintained good color for two weeks at best. As usual, samples held in air at atmospheric pressure underwent extensive discoloration ahead of the other two storage variables although samples in air also maintained fairly good color longer than is usually the case. The meat used in this experiment may have had exceptionally good capability for color maintenance (e.g., strong reducing potential). Vacuum packaged samples brightened considerably upon exposure to oxygen; however, the desirable very bright red color was not achieved in this case except possibly for a short while after their metal vacuum-pressure container was filled with two atmospheres of oxygen. When reflectance measurements were made, after 2 and 3 days in oxygen, color was largely red, but not bright red.

Table 20. Reflectance measurements and visual observations on 50 Krad irradiated beef stored in air, under vacuum and in oxygen at 38°F

	Pigment form	14 days storage	21 days	23 days*	24 days**	Visual observations
V	(Reduced)					
A	Mb	- -	56%	34	39	Samples appeared purplish throughout storage while under vacuum. Those in oxygen for 2 and 3 days following 21 days under vacuum appeared deep red when reflectance measurements were taken.
C	Oxygenated MbO ₂	- -	0	40	56	
U	(Oxidized)					
U	Mb ⁺	- -	44	26	5	
M						
O	Mb	27%	29	- -	- -	Samples appeared mostly bright red at 14 days. At 21 days they were largely red with some bright red and some red-brown areas. At 23 days all samples were all brown, and were not measured.
X	MbO ₂	71	63	- -	- -	
Y	Mb ⁺	2	8	- -	- -	
G						
E						
N						
A	Mb	42	19	- -	- -	Samples appeared somewhat discolored at 14 days but were largely red. Samples were completely brown in appearance at 21 days.
I	MbO ₂	41	0	- -	- -	
R	Mb ⁺	17	81	- -	- -	

* 23 day vacuum samples had been in two atmospheres of oxygen for 2 days following 21 days under vacuum.

** 24 day vacuum samples - same as 23 day except 3 days in oxygen.

VIII. CONCLUSIONS AND DISCUSSION.

The findings of the work reported herein suggest that the following conclusions may be drawn:

- 1) Radiation at dose levels of 50 to 150 Krad delays microbial spoilage for more than 21 days at 38°F. This conclusion is based on laboratory experiments with beef, lamb and pork. It presumably applies also to veal and poultry.
- 2) Color retention is aided by an enriched oxygen atmosphere. Up to two atmospheres of pure oxygen, however, do not consistently provide a stability of color for periods longer than 14 days. This is short of the project goal of 21 days. Further, the meat in the presence of oxygen undergoes a change presumed to be oxidative in nature which produces undesirable flavors and odors. The approach of using an enriched oxygen atmosphere, therefore, is concluded to be ineffective in attaining the objective of 21 day preservation.
- 3) An alternate procedure exists to attain the project objective; this procedure involves: a) vacuum packaging the meats prior to irradiation, b) storing under vacuum for the greater part of the storage period (e.g., 18 days), c) breaking the vacuum and oxygenating the meat pigments by exposure to pure oxygen or to air (which converts the purple color of the vacuumized meats to the normal red), and d) storing the meats in air for the remainder of the storage period (e.g., three days). This procedure, based on laboratory results, provides normally red-colored meats at 21 days (i.e., for three days at retail level after 18 days refrigerated vacuum storage).
- 4) Drip control is obtained through the use of phosphates in an amount approximately 0.5% by weight. The most effective phosphates are sodium tripolyphosphate and sodium hexametaphosphate glass (64% P_2O_5). The latter has slightly better solubility than the former and both are of comparable effectiveness in controlling drip. This amount of phosphate is in accord with present U.S.D.A. regulations for those meats to which the addition of phosphates is permitted. (These meats presently do not include fresh meats).

The manner of introducing the phosphates has not been worked out fully. For relatively thin slices, dipping or spraying is satisfactory. For thicker cuts, it appears that an injection procedure will be needed. This will be further studied.

Phosphates appear to have effects on meats in addition to drip control. In some cases, color retention is improved. Other possible actions include an antioxidant effect and inhibiting effects on microbial outgrowth.

The three agents, radiation, phosphates and atmosphere control based on bulk vacuum packing, used in combination extend the refrigerated life of fresh meats to a total time of about 21 days. There is good indication that 50 Krad may be adequate; if not, doses up to about 150 Krad may be considered. The atmosphere-control procedure envisaged in the approach described in (3) above involves a quite simple and practical approach. The phosphate treated retail cuts can be packaged in an oxygen permeable film as they are now. A number of such cuts (e.g., 50 lb) can be grouped and placed in a rigid or a flexible container and a vacuum drawn. The outer container is then sealed and irradiated. This container can be the wholesale unit and the meats can move in it through distribution channels to the retail outlet. At the retail store the wholesale unit can be opened, the retail packages exposed to air or oxygen to restore color, and then displayed for a period equal to that of present practice.

Not only would this approach have the practicality needed, but properly carried out, it should involve costs that would be relatively small. No figures on costs are available at this time, but it is believed that they lie in the area of one cent per lb (irradiation, phosphate and extra packaging).

Vacuum packaging has some effect on the outgrowth of the initial microbial population. The findings of this work indicate, however, that this effect is not sufficient to accomplish the needed life extension of the meats. It is necessary, therefore, to include irradiation to attain the desired life of 21 days.

Because of the changes in the normal spoilage flora, concern exists about the kind of spoilage development that will occur and the ability of the consumer to identify it as spoilage. The indications of this work are that the spoilage of vacuum-packaged irradiated meats is innocuous and involves acid production.

Further work is indicated in order to provide more precise information to guide the process development. The process will need evaluation under commercial conditions.

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